Review Article

Neuroendocrine differentiation of prostate cancer: a review

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Abstract: Neuroendocrine cells are one of the epithelial populations in the prostate. Neuroendocrine differentiation (NED) has been observed in prostate cancer. In addition to small cell neuroendocrine carcinomas and carcinoid tumors of the prostate, prostatic adenocarcinomas may have NED. The incidence and clinical relevance of NED in prostatic adenocarcinoma is not clearly understood because of conflicting results in the reported studies, and evaluation of NED is not routinely performed in clinical practice. This review is an overall synthesis with an aim to develop a more comprehensive understanding and practical approach towards the current knowledge of neuroendocrine differentiation. In this review we are stratifying these lesions into separate subtypes based on histologic parameters such as tumor morphology, neuroendocrine cell density and distribution and clinical parameters. We also want to identify current controversies and confusing issues not totally resolved in this topic for further investigations. Eventually a clearer understanding of this phenomenon and appropriate handling NED in prostate cancer will benefit clinical practice.

Keywords: Neuroendocrine differentiation, prostate

Benign prostate histology

A variety of epithelial and stromal cells comprise normal adult prostate. The epithelial cells include secretory, basal, and neuroendocrine cells as well as urothelial and ejaculatory duct cells. In addition, there are pluripotent stem cells which cannot be identified by conventional H&E staining. Prostatic epithelial cells share some common markers. For example, both secretory and basal cells are positive for pan keratin markers such as cytokeratin AE1/AE3, CAM5.2 (CK 8/18) and epithelial membrane antigen (EMA). Furthermore, each cell type preferentially expresses relatively unique proteins which can be identified by various modalities such as immunohistochemistry. Prostatic stromal cells are comprised chiefly of smooth muscle cells (positive for smooth muscle actin) and fibroblasts. Along with secretory glandular epithelium, prostatic stromal cells are androgen sensitive and their proliferation, differentiation and regression are directly regulated by androgens via transforming growth factor beta 1 (TGFβ) and androgen receptor (AR). Additionally epithelial cells have been suggested to regulate stromal cells by a paracrine effect through estradiol. Secretory cells are positive for AR, prostate specific antigen [1] (PSA), P501S, prostate specific membrane antigen [2] (PSMA), prostate specific acid phosphatase [3] (PSAP), and prostate specific androgen regulated homeobox gene protein [4, 5] (NKX3.1). Basal cells exclusively express p63 [6, 7] which is essential for prostate stem cell function and high molecular weight cytokeratins (HMWCKs) such as 34βE12 and CK5/6.

Neuroendocrine cells in the normal prostate

Neuroendocrine cells (NEC) are scattered in prostatic glands in all anatomic zones and comprise less than 1% of benign prostatic glandular epithelium. They show characteristic lateral spreading of dendritic processes however these cells cannot usually be recognized within benign prostatic glandular tissue on routine H&E staining. Because of their inconspicuous nature on routine H&E staining, NEC are best recognized by using immunohistochemistry (IHC) [8, 9] with specific NEC markers (Figure 1).
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NEC density in peripheral prostatic acini is highest in neonatal and post-pubertal age groups and this is possibly mediated by androgenic hormones [10]. NEC are believed to play an important role in neuronal and endocrine regulation of benign prostate. However, their precise function is not entirely clear.

NEC markers

A number of markers (Table 1) are used in clinical practice or research for detecting neuroendocrine differentiation. Typical neuroendocrine markers used in clinical practice to elucidate NEC are chromogranin, synaptophysin, neuron specific enolase (NSE), and CD56. Neuron specific enolase (NSE) is considered a generic marker for both neurons and NEC [11] and although it has high sensitivity, its specificity is low [12]. Chromogranin is a specific NEC marker as it is one of the common constituents of NEC secretory granules [13, 14]. Synaptophysin is also a well-established marker for NED. CD56 labels neuronal cells, although the specificity of this marker for NED in the prostate is not clear.

Other NEC markers have been reported in the literature, although they are not typically used in clinical practice. These markers include Cytochrome b561 [15] (Chromomembrin B) and synaptic vesicle elements include synaptophysin [16, 17], synaptic vesicle protein 2 (SV2) [18, 19], vesicular monoamine transporters (VMAT) [20], SNARE complex [21, 22] (synaptobrevin, syntaxin and SNAP-25), Rab3 [23] and vesicle associated membrane proteins (VAMP) [24, 25]. Additionally, some unique lympho-reticular antigens expressed by NEC such as CD57 [26-28] (Leu 7; HNK-1) and neural cell adhesion molecules (NCAM) are known for their role in cell to cell recognition, migration, differentiation and proliferation of prostatic adenocarcinoma cells through paracrine effect. Although some of these markers are promising, more studies are necessary before they can be used in clinical practice for detecting NED of prostatic lesions.

Ultrastructurally, NEC typically contain membrane bound large dense core secretory granules measuring 50-500 nm diameter, which represent storage sites for several types of peptide and amine hormones [29]. In addition many NEC contain small synaptic vesicles that are responsible for the release of neurotrans-

**Table 1. Neuroendocrine markers**

<table>
<thead>
<tr>
<th>Commonly used in clinical practice</th>
<th>Chromogranin</th>
<th>Synaptophysin</th>
<th>Neuron specific enolase</th>
<th>CD56</th>
</tr>
</thead>
<tbody>
<tr>
<td>Typically not used in clinical practice</td>
<td>Cytochrome b561</td>
<td>Synaptic vesicle protein 2 (SV2)</td>
<td>Vesicular monoamine transporters (VMAT)</td>
<td>Synaptobrevin</td>
</tr>
</tbody>
</table>

Figure 1. Generally, neuroendocrine cells cannot be recognized in a benign prostatic gland with routine H&E staining (A). However, the neuroendocrine immunostains such as chromogranin (B) or synaptophysin (C) can highlight the neuroendocrine cells which are typically situated in the basal cell compartment with cell processes projecting into the layer of luminal cells.
mitters like gamma amino butyric acid, glutamate and glycine. These synaptic vesicles are regulated by cAMP, cGMP and calcium. NEC are classified into two classes - neural type which contain neurofilaments, a major intermediate filament, and epithelial type which contain cytokeratin with or without neurofilaments.

Prostatic adenocarcinoma

Prostatic adenocarcinoma is the most common malignancy in men and accounts for the second most common cause of cancer death in the United States. Nearly a quarter million men will be diagnosed with PCa every year, out of which close to 33,720 die according to 2011 Surveillance, Epidemiology and End Results Program (SEER) data. Positive digital rectal examination for prostatic induration and elevated serum PSA levels > 4 ng/ml are often followed by transrectal ultrasound guided prostate needle core biopsy, which currently is the gold standard for diagnosis of PCa. As a brief overview, the key histologic features of prostatic adenocarcinoma include haphazard infiltrating patterns, small glandular structures, cribriform patterns with poorly-formed glands, or solid sheet/individual cells with no obvious glandular formation. The cytologic features of prostatic adenocarcinoma include loss of basal cells, nuclear atypia and prominent nucleoli. Other diagnostic features are perineural invasion, glomeruloid formation (protrusion of one gland into another lumen), collagenous micronodules (mucinous fibroplasias), pale pink secretions, luminal crystalloids, blue luminal mucin and thin intra-luminal collagen fibers. Localized PCa (stages T1 and T2) can be successfully treated in a majority of cases by radical prostatectomy (RP) or radiation without additional adjunctive therapies. For patients with locally advanced and metastatic PCa, other treatments, androgen deprivation therapy (ADT) or hormonal therapy may be applied. The most commonly used drugs for complete androgen blockade are luteinizing hormone-releasing hormone (LHRH) agonists (Leuprolide and Goserelin) and antiandrogen agents (Flutamide and Bicalutamide). The relationship between neuroendocrine differentiation and medical treatment is not well-understood and has garnered recent interest due to their potential relation to castration resistant disease [30, 31].

Neuroendocrine prostate carcinomas

Even though the definition of neuroendocrine prostatic carcinomas is still emerging, in this review from a morphologic standpoint, neuroendocrine prostatic carcinomas is considered as a special type of neuroendocrine differentiation of prostatic epithelial neoplasms (Table 2). Neuroendocrine carcinoma of the prostate may represent a subset of prostate cancer phenotypes which may be linked to resistance to androgen receptor (AR) signaling inhibition with aggressive tumor characteristics and a largely dismal prognosis. Neuroendocrine prostatic carcinomas (NEPC) are often diagnosed on primary prostate needle biopsy or on biopsies of metastatic lesions with negative or low PSA levels. Based on morphological characteristics, proliferative index and grade, this spectrum tumor phenotypes can be subdivided into: small cell carcinomas, large cell neuroendocrine carcinomas and carcinoid tumors. Neuroendocrine differentiation within conventional PCa will be discussed below separately. In general, NEPC differ from conventional PCa histologically by presence of neuroendocrine cells which do not express generic PCa markers.

| Table 2. Neuroendocrine differentiation in the prostate |
|---------------------------------|----------------|
| Type                           | Subtypes                        |
| Benign                         | Neuroendocrine cells in benign prostates |
| Neuroendocrine carcinoma       | Small cell (neuroendocrine) carcinoma |
| Carcinoid tumor                | Large cell (neuroendocrine) carcinoma |
| Adenocarcinoma with neuroendocrine differentiation | Carcinoid tumor |
| Conventional adenocarcinoma    | Diffuse |
|                                | Focal |
| Paneth cell differentiation    | Focal |

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like AR, P501S, PSMA, PSAP and PSA but characteristically expresses neuroendocrine markers such as chromogranin A, synaptophysin, CD56, and NSE [32, 33].

However significant overlap and expression heterogeneity in the expression of traditional prostatic epithelial markers can occur in NEPC as evidenced by studies showing that PSA and AR expression are observed in 25% of NEPC [34]. NEPC show histological similarities with the range of neuroendocrine tumors seen throughout the body depending upon the histologic subtype [35-37]. More than 50% of the NEPC cases are associated with PCa [35] and clinical features of NEPC often correlate with AR independence. To this effect, the Prostate Cancer Foundation Working Committee on NEPC in 2013 suggested the terminology of “AR-negative PCa” for NEPC [38]. Also NEPC may not be associated with significant serum elevation of PSA and PSAP levels as PCa. This may be one of the main reasons why close to 50% of NEPC patients present with distant metastasis and have a median survival of 9.8 to 13.1 months [39, 40] compared to 125 months for PCa [41]. Apart from its metastatic propensity, NEPC has high proliferative rates (> 50%) as assessed by Ki67 and this can be used to help grade and prognosticate behavior of a NEPC subtype. In addition several cell cycle specific proliferative proteins such as cyclin D1, AURKA, PLK1 are overexpressed in NEPC [33, 42]. Given the different tumor classes that comprise NEPC, it is unclear whether NEPC arise from resting prostate NEC, from pleuripotent prostate cells or perhaps from poorly differentiated adenocarcinoma (epithelial to neuroendocrine transition) [35-37, 43].

Recent in vitro and in vivo studies on androgen sensitive human PCa cell derived from lymph node metastasis cells demonstrated trans-differentiation of PCa cells upon androgen depletion, cAMP, cytokines and various growth factors [44]. Furthermore NEPC tumors (60-70%) revealed molecular concordance between PCa and NEPC foci in relation to TMPRSS2-ERG gene rearrangement and TP53 [45] suggesting cellular trans-differentiation from an epithelial to neuroendocrine phenotype [33, 39]. These ETS transcription factor family rearrangements are unique to NEPC and are not observed in tumors arising from neuroendocrine tumors of non-prostatic origins. Interestingly no significant survival differences exist in pure NEPC and NEPC with mixed glandular phenotype. Even though NEPC occur de novo, they commonly arise in the setting of post hormone therapy [46]. The 5-year overall survival of NEPC is 12.6% which makes it the most deadly and aggressive subset of prostate cancers.

Small cell neuroendocrine carcinoma

Small cell neuroendocrine carcinoma is a rare high grade epithelial neoplasm of the prostate. The incidence rate of small cell carcinoma (SCC) is about 0.35 cases per million per year [41] and 59% occur in men 70 years and above. SCC constitute the majority of neuroendocrine carcinomas of the prostate. The histologic features of SCC of the prostate include small tumor cells with minimum cytoplasm, indistinct cell borders, nuclear molding, fine “salt-and-pepper” chromatin, lack of prominent nucleoli, extensive tumor necrosis, apoptosis, high mitotic rate and nuclear fragility. SCC may occur in pure form (50-60% of cases), but it may also occur adjacent to or concomitantly with conventional adenocarcinoma in other cases as it is estimated that 40 to 50% of SCC cases have a history of prostatic adenocarcinoma. Although the reported literature states that approximate—
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ly 50% of SCC are associated with conventional PCa, our anecdotal experience shows a higher percentage of association. The median interval between PCa and SCC diagnosis is approximately 25 months. Immunohistochemically, SCC show strong chromogranin and synaptophysin positive expression in the majority of cases (61% and 89%) (Figure 2); 17% and 24% of cases are positive for PSA and PSAP; 24% and 35% of cases are positive for basal cell markers, p63 and HMWCK respectively [47, 48].

It is thought that SCC represents a heterogeneous immunophenotype and their origin may be from multi-potent stem cells in the prostate (CD44+) [48-50]. Detection of SCC at an early stage of prostate cancer is very rare but is often found in PCa patients treated with first and second line ADT which complicates the cell of origin that gives rise to these tumors. Prostatic SCC often metastasize to the lung and it is important to consider this in the differential diagnosis of primary SCC of the lung when there is a prior history of PCa. Thyroid transcription factor 1 (TTF-1) has limited use in distinguishing the two as both entities express TTF-1, creating a diagnostic dilemma. It is estimated that up to 90% of primary pulmonary SCC express TTF-1 while a smaller percentage (< 50%) of prostatic neuroendocrine tumors including prostatic SCC express TTF-1. Furthermore, prostatic SCC (AR-negative) differs from PCa by lacking ERG protein expression, which is driven by androgens through A. SCC is a systemic disease and often requires chemotherapy. Even with active treatment, SCC has a dismal outcome with an average survival of less than 1 year and 5-year survival rate of 14.3%. Several case reports and clinical trials have been conducted to find whether hormonal manipulation and/or a combination of systemic chemotherapy affect the biologic progression of SCC [51]. None so far has shown any significant effect [52]. It is thought by some clinicians that SCC should be treated as any other small cell carcinoma with systemic chemotherapy alone [53, 54] while others add ADT. Currently targeted therapeutic strategies aimed at c-kit, Bcl-2 and CD56 are being investigated for SCC.

Large cell neuroendocrine carcinoma

Large cell neuroendocrine carcinoma (LCNC) is characterized by cells arranged in nests with peripheral palisading, abundant cytoplasm, vesicular nuclei and prominent nucleoli. The tumor cells themselves are much larger than those of small cell carcinoma and conventional prostatic adenocarcinoma cells. Neuroendocrine features such as fine chromatin in the tumor cells can be appreciated variably. Often geographic necrosis is seen and it is a very helpful histologic feature in this tumor type. Neuroendocrine differentiation in large cell neuroendocrine carcinoma is often demonstrated by extensive staining of at least one NEC biomarker (chromogranin, synaptophysin, etc) (Figure 3). Large cell neuroendocrine carcinomas are extremely rare in pure form and typically follow long term hormonal therapy with an average survival of 7 months [55]. Evans et al

Figure 3. Large cell neuroendocrine carcinoma of the prostate shows typical “salt-and-pepper” chromatin patterns, but the tumor cells are much larger than the ones in small cell carcinoma (A). Neuroendocrine marker such as chromogranin (B) is necessary.
described the only study on LCNC with 7 total cases of which six presented after long standing ADT. LCNC have also been described in association with PCa and SCC. Large cell neuroendocrine carcinoma of the prostate is a high grade malignancy, its aggressive biological is very similar to that of small cell carcinoma of the prostate.

Carcinoid tumor of the prostate

Carcinoid tumor of the prostate is defined as a well differentiated neuroendocrine tumor with limited or no association to PCa histology (negative for PSA) typically arising from prostatic parenchyma. By definition, any prostate cancers with an adenocarcinoma component are excluded from the definition of carcinoid tumor of prostate. Historically, there are rare anecdotal occurrences that fit to this definition. Some authors have suggested terms like “carcinoid-like” when carcinoid tumor is PSA positive. Clinically carcinoid-like PCa behave like conventional PCa. Carcinoid tumor of prostate is graded similar to gastrointestinal neuroendocrine tumors (based on Ki67 proliferative rate). The histologic features seen in this tumor subtype are similar to neuroendocrine tumors in other organs and include variably sized large nests, trabecular or insular architectural patterns of generally round cells with low grade cytologic features and “salt and pepper” chromatin. These tumors stain positive for neuroendocrine markers such as synaptophysin (Figure 4). Compared to SCC, LCNC and PCND, carcinoid tumor of the prostate has the lowest proliferative index. If a carcinoid tumor is seen on prostatic biopsy or resection, differential diagnostic considerations include metastatic neuroendocrine tumors from the gastrointestinal tract and less likely pulmonary origin. Carcinoid tumors of the prostate typically occur in younger adults with locally advanced and metastatic disease but overall have a favorable prognosis compared to the other prostatic neuroendocrine phenotypes.

Prostatic adenocarcinoma with neuroendocrine differentiation

Prostatic adenocarcinomas with scattered foci of neuroendocrine immunohistochemical expression are designated under prostatic adenocarcinoma with neuroendocrine differentiation (PCND) [38, 56, 57]. PCND can present as untreated primary pathology or more commonly as a post ADT and androgen receptor inhibition resistance phenomenon. Almost all PCa show sparse or rare NEC since they are normal constituents of benign prostatic epithelium. Only 5-10% of PCa show characteristic zones or large groups of neoplastic NEC which by definition fall under PCND category [58, 59]. According to the 2004 WHO classification, this differentiation is categorized as “focal neuroendocrine differentiation in conventional prostatic adenocarcinoma” (ICD-O code 8574/3). On H&E fine eosinophilic granules and nuclei with “salt and pepper” chromatin in PCa suggest neuroendocrine differentiation and this can be confirmed by immunohistochemical stains such as chromogranin and NSE. This form of
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Figure 5. Prostatic adenocarcinoma with neuroendocrine differentiation on H&E (A) which is confirmed by strong positive staining for chromogranin (B).

Figure 6. Higher Gleason grade prostatic adenocarcinoma with no obvious histological evidence of neuroendocrine differentiation on H&E (A) but with positive staining for chromogranin (B) and NSE (C).

Figure 7. Paneth cell differentiation of prostatic adenocarcinoma is characterized by the presence of large eosinophilic granules in the cytoplasm of tumor cells (A), which are positive for neuroendocrine markers such as chromogranin (B).

differentiation can be seen in different Gleason grades of tumor (Figures 5 and 6) [60, 61].

Among all the NEC specific biomarkers, synaptophysin is the most sensitive and chromogranin A is the most specific for PCND. Apart from the obvious non-glandular morphology, neuroendocrine differentiation can be seen in tumors with NEC cells with abundant eosinophilic cytoplasm and lack of prominent nucleoli which may prompt a practicing pathologist to utilize NEC markers for cell identification. This
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form of differentiation is known as adenocarcinoma with Paneth cell-like neuroendocrine differentiation (Figure 7).

It is currently thought that the degree of neuroendocrine differentiation increases with PCa disease progression and in response to ADT. This is evident by serum elevation of NSE or chromogranin A in PCa and increased elevation seen with longer duration of ADT [62, 63]. This phenomenon has been suggested as one of the emerging mechanisms of ADT resistance and is more common than previously recognized. The increased incidence of PCND has been attributed to patients living longer, introduction of novel ADT regimens and aggressive follow up biopsy practice of metastatic PCa disease. The significance of the diverse expression of markers such as synaptophysin, NSE, bombesin, serotonin [64], neuroendocrine peptides [65] and vascular endothelial growth factor [66] (VEGF) are yet to be deciphered. PCND clinically behave like PCa in terms of prognosis and outcomes [58]. Several studies were conducted to determine whether neuroendocrine differentiation in primary prostatic adenocarcinoma worsens prognosis - some concluded insignificant prognostic findings [58, 67-72] while others found an independent negative effect on prognosis [73, 74]. Thus far, only neuroendocrine differentiation amongst androgen independent, advanced primary PCa and metastatic PCa concluded poor prognostic outcomes [75-78]. Currently neuroendocrine differentiation is still considered a category III prognostic factor in CAP-1999 consensus statement at any PCa stage.

With respect to treatment options in this broad spectrum and classification of tumors, androgen independent PCa, post androgen therapy PCND and PCND (not associated with post androgen therapy) may benefit from treatment options such as somatostatin and peptide analogues [79, 80], which have been forefront in this endeavor. Of late serum based chromogranin and NSE are routinely used in the detection of post hormone therapy PCND especially in cases where tissue is inaccessible for IHC evaluation. Unfortunately, serum concentrations and tissue based protein expression do not show robust correlation and chromogranin serum concentration does not seem to positively correlate with treatment response to cytotoxic chemotherapy in metastatic NEPC [81]. It has been argued in recently that given the uncertain clinical relevance and importance of neuroendocrine differentiation in prostatic adenocarcinomas, adjunctive immunohistochemical studies to find foci of NE differentiation in conventional PCa is not recommended [82]. However, in our anecdotal experience, we have seen several cases where high Gleason grade PCas (Gleason 8 or 9), particularly with abundant large dense cribriform areas often stain with neuroendocrine markers and they may show diffuse weak positive staining, focally strong positivity, or show more diffuse strong positivity. While the significance of such differentiation is unclear at this time, there may be important clinical relevance with respect to correlation to potential serum tumor marker studies. The possibility of using assays such as serum chromogranin as an adjunct tumor marker for monitoring patients in this category of disease warrants further exploration.

Post hormone therapy and androgen resistance

Histologically focal neuroendocrine differentiation ranges from 10 to 100% in prostate adenocarcinomas treated by ADT [82]. It is unclear if the morphologic pattern and appearance of neuroendocrine differentiation varies based on the type of anti-androgen regimen. It has been suggested that after failing primary ADT in metastatic castration-resistant PCa, assessing evidence and the possible degree of PCND may prove valuable in predicting the success of continued androgen receptor targeted therapies [30]. More importantly, given the rise and progress in molecular pathology particularly in fields such as pulmonary adenocarcinomas, it is of great current interest to know any molecular variations in post ADT neuroendocrine differentiation and pure NEPC. This level of molecular analysis could provide a greater understanding of androgen resistance and may help better classify post ADT pathologies.

Conflicting data exists with regards to prior studies on the relationship between neuroendocrine morphology and androgen deprivation. Several studies have demonstrated that prolonged androgen deprivation in androgen sensitive metastatic PCa results in increased neuroendocrine differentiation within the primary PCa. Other studies have proposed that androgen resistant (20-30% of metastatic PCa) pri-
mary adenocarcinomas augment neuroendocrine differentiation. Further studies are needed to clarify the association and it is possible that a molecular approach may provide new information and clues to better understanding the possible relationship between androgens and neuroendocrine differentiation.

**Concluding remarks**

It is of great interest to further study whether PCND is one of the natural outcomes of PCa or a mechanism of resistance to current therapies. With rapid autopsy programs, patient derived matched primary and metastatic xenografts and recent instigation to collect biopsies at various time points of therapy and post treatment biopsies, our understanding of the molecular and phenotypic transformation of drug resistant PCa pathologies may increase significantly. LUCAP 49, WISH-PC2, UCRU-PR-2, WM-4A and MDA PCA 144 are few of the prominent xenografts developed from PCa post ADT, chemotherapy and radiation with NEPC histology and immunophenotype. Of late genetically engineered mouse models like TRAMP, FG-Tag, CR2-Tag and PSP-TGMAP have been successfully developed using SV40 T antigens encasing NEPC phenotype. Others have successfully induced NEPC by conditional knockout of major tumor suppressors P53 and Rb. The specimens from the abovementioned experimental models may provide valuable information in establishing protein loss and novel protein induction, clonal evolution, multi clonal progression and possible follow up of clonal trans-differentiation under the effects of various drug therapies.

Recent advancements in combined parallel sequencing of genomic (DNA) and transcriptomic (RNA) would enable discovery of deleterious cancer variants in NEPC and PCND. Differential gene and splice variants expression analysis together with hierarchical clustering among untreated and post ADT pathologies may provide genomic sub-classification of NEPC. Hierarchical cluster differentiation may shed light on epithelial to neuroendocrine trans-differentiation theory. Furthermore, high-resolution molecular pathology techniques of the future may predict post ADT neuroendocrine differentiation and metastatic potential based on potential primary PCa molecular profiles. As in other cancer types, genomic make up of primary and metastatic tumors vary significantly and therefore it is logical to expect discordant gene and protein expression among primary and metastatic disease.

It is of current interest to study further neuroendocrine differentiation among primary PCa with ADT and metastatic PCa with ADT. These opportunities would address the following questions: 1. Are PCa, PCND and NEPC pathologies in continuum and can transform from one to other? 2. Are PCND and NEPC pathologies of two ends of a spectrum? 3. What might the incidence be of PCND and NEPC among RP and/or ADT? 4. What is the transformation rate of PCa to PCND and PCND to NEPC with and without ADT? 5. Are PCND and NEPC multi-clonal or monoclonal pathologies? Lastly these studies may help elucidate the significance of stem cells and provide better understanding of epigenetic alteration involved in trans-differentiation that may play a significant role within neuroendocrine differentiation in conventional prostatic adenocarcinomas or primary neuroendocrine carcinomas of the prostate.

**Disclosure of conflict of interest**

None.

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