Review Article
Neuroendocrine differentiation in prostate cancer

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Abstract: As any organ in the body human prostate is composed of many different types of cells as well as extracellular components. During prostate development, reciprocal cellular interactions between stromal cells and prostate epithelial cells ultimately lead to the development of a mature prostate. Normal prostate is composed of repeating cellular units that contain stromal and epithelial compartments. The epithelial compartment contains luminal epithelial cells, basal cells and a minor component of neuroendocrine cells whose function may be to regulate the growth, differentiation and secretory function of the prostate gland. Neuroendocrine cells are also evident in prostate cancer and numerous studies showed that its number increases in high grade and high stage tumors, particularly in hormonally treated and hormone-refractory (androgen-independent) prostate cancer. Although androgen withdrawal reduces the secretion of the andromedins from the prostate stromal cells that are critical for the survival for prostate epithelial cells, there is clear evidence that androgen receptor is also required for the tumorigenesis of human prostate cancer, and therefore androgen deprivation therapy likely works through inhibition of androgen receptor in the prostate epithelium. Because neuroendocrine cells lack androgen receptor and are likely androgen-independent, it is conceivable that hormonal therapy for advanced/metastatic prostate cancer, which consists of inhibiting androgen production and/or blocking androgen receptor function, will not eliminate neuroendocrine cancer cells. Instead, these cells may be enriched after the therapy and they may establish paracrine networks to stimulate androgen-independent proliferation of prostate cancer, leading to tumor recurrence. In this article, we will review the known functions of the neuroendocrine cells in prostate cancer, including stimulation of cancer proliferation and invasion, apoptosis resistance and angiogenesis as well as molecular pathways involved in neuroendocrine differentiation.

Key Words: Prostate cancer, hormonal therapy, neuroendocrine

Introduction

The normal human prostate is a tubular-alveolar gland composed of a stromal compartment surrounding glandular acini comprised of a two-layered (i.e. basal and secretory luminal) epithelium (**Figure 1**). The stromal compartment contains a variety of cells including nerves, fibroblasts, infiltrating lymphocytes and macrophages, endothelial cells, and smooth muscle cells. Scattered throughout the epithelial compartment are occasional neuroendocrine (NE) cells. Ontologically, the epithelium is composed of multiple stem cell units [1-6] where the stem cell that has unlimited self-renewal capacity but only rarely proliferates to provide progeny that differentiate to become either transit-

amplifying or NE cells [2, 3] as shown in **Figure 1**. The transit-amplifying cells subsequently differentiate into either the luminal secretory cells or basal cells which can be easily distinguished by light microscopy. Neuroendocrine (NE) cells were first systematically characterized in the 1980's and has been increasingly recognized as an important component of the prostate. NE cells do not show distinguishing features on H&E-stained sections under the light microscope but can be identified by electron microscopy or immunohistochemical staining with antibodies against NE markers chromogranin A, synaptophysin, neuron-specific enolase (**Figure 2**) or other specific NE products. Prostatic NE cells are intraglandular and intraductal cells with hybrid epithelial, neural and endocrine
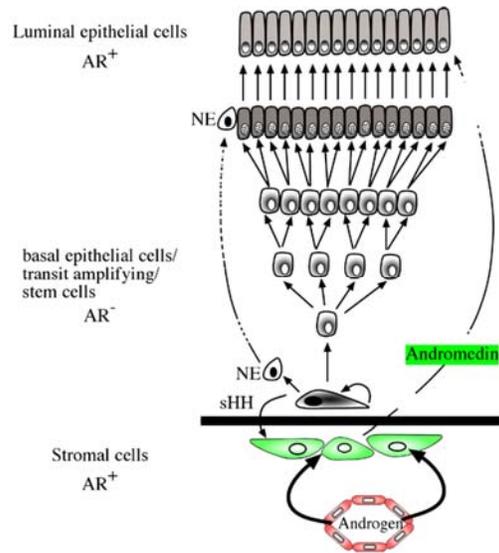
Figure 1. Prostate developmental process. Epithelial cells can promote differentiation of stromal mensenchymal cells while stromal cells through andromedin promote epithelial cell survival.

Figure 2. NE cells in benign prostate. Immunohistochemical staining with an anti-chromo-granin A antibody identifies scattered NE cells (brown staining) in a benign prostate.

features and express and secrete serotonin and many other peptides/neuropeptides. They are present as scattered individual cells or small nests of cells among the more abundant basal and luminal epithelial cells throughout the prostate with only an occasional cell per gland/duct. They are not evenly distributed in the prostate and are most consistently found in the periurethral ducts and verumontanum [7]. Their numbers are higher in the transition zone and peripheral zone than in the central zone in the human prostate, suggesting that they may be involved in disease processes associated with these areas, such as nodular prostatic hyperplasia and prostate cancer (PC) [8]. The number of NE cells varies from individual to individual with a small percentage of human prostates containing numerous NE cells.

The prostate NE cells can be categorized as open and closed types by electron microscopy studies. The open type NE cells have an apical cytoplasmic process which extends to the glandular lumen and has long specialized surface microvilli; while the closed type NE cells are surrounded by other epithelial cells and do not have direct contact with the glandular lumen. Both types of NE cells have
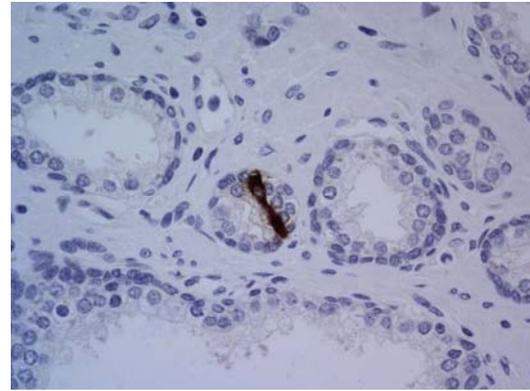
long branching dendrite-like processes which extend between nearby epithelial cells. A wide range of neurosecretory granules with various sizes and morphologies have been revealed by electron microscopic studies which correlate with the large number of products secreted by the NE cells, including serotonin, histamine, chromogranin A and other members of the chromogranin family of peptides, calcitonin, calcitonin gene-related peptide, katacalcin, neuropeptide Y, vasoactive intestinal peptide (VIP), bombesin/gastrin releasing peptide (GRP), somatostatin, alpha-human chorionic gonadotropin (aHCG), parathyroid hormone-related protein (PTHrP), thyroid stimulating hormone-like peptide, cholecystokinin, adrenomedullin and vascular endothelial growth factor (VEGF) [9]. Some of these NE cell products have been detected in seminal fluid raising the possibility that they may be actively secreted into the seminal fluid and regulate sperm function. Receptors for some of the NE products have been found in the benign prostate and/or PC including serotonin (5HT1a) [10], bombesin/GRP (GRPR) [11, 12], neurotensin [13], somatostatin (SSTR1-5) [14-16], cholecytokinin [17], Neuropeptide Y [18] and calcitonin [19]. Hence, it is proposed that the NE cells may regulate the growth, differentiation and secretory activity of the prostatic epithelium, possibly through a paracrine mechanism. On the other hand, the activity of the NE cells may be regulated by the neural network, the contents of the glandular lumen or endocrine, paracrine or autocrine signals.

NE differentiation in PC

NE cells are also present in PC. Some prostatic tumors are composed entirely or nearly entirely of tumor cells with NE differentiation, such as the very rare carcinoid tumors and small cell carcinomas. More commonly, some human tumors show components of conventional adenocarcinoma with a component of small cell carcinoma or very rarely, carcinoid tumor. In general, however, the term neuroendocrine differentiation (NED) in PC refers to the presence of scattered NE cells singly or in small nests in conventional prostatic adenocarcinomas.

Small cell carcinoma and carcinoid tumors

Pure small cell carcinomas of the prostate are rare and accounts for no more than 1 percent of all carcinomas of the prostate. Similar to small cell carcinoma of other organs, they are aggressive tumors and often present as locally advanced or metastatic diseases [20]. Occasionally they are associated with paraneoplastic syndromes [21]. Sometimes, small cell carcinomas can occur in patients who have received hormonal therapy for conventional adenocarcinomas of the prostate [22, 23].

More commonly, small cell carcinoma is seen as a component of mixed tumors which also contain conventional adenocarcinoma. Histologically, small cell carcinomas of the prostate are similar to the more common small cell carcinomas of the lung and are characterized by a solid, sheet-like growth pattern. Tumor necrosis is common. Tumor cells are small, with fine chromatin, scant cytoplasm, and nuclear molding. Mitotic figures, apoptotic figures and crush artifact are frequently observed (**Figure 3**) [24].

The diagnosis of small cell carcinoma of the prostate is usually not difficult if the above histologic features are observed. However, the solid growth pattern of small cell carcinoma of the prostate is similar to that of Gleason grade 5 adenocarcinomas, which makes their differential diagnosis difficult at times, for which immunohistochemical study may be helpful. Like their lung counterpart, small cell carcinoma cells often show dot-like cytokeratin staining pattern and are often positive for TTF-
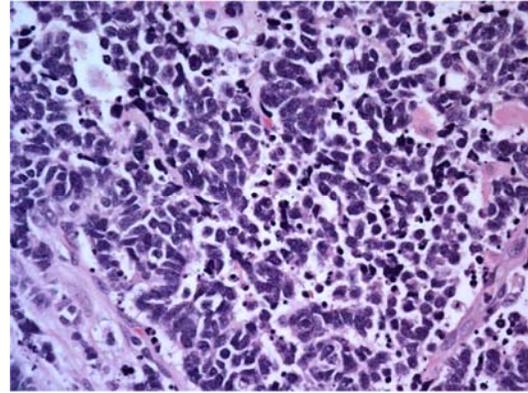

Figure 3. Small cell carcinoma of the prostate. In comparison to adenocarcinoma, such tumor shows sheet-like growth pattern with frequent mitotic figures and apoptotic figures. Tumor cells have scanty cytoplasm, high N/C ratio, fine chromatin pattern and nuclear molding.

1 and CD56. They are often positive for NE markers chromogranin A, synaptophysin and NSE. However, it should be noted that immunohistochemical profiles are not always consistent and one or more of these markers may be negative in any given case. In contrast to prostatic adenocarcinoma, tumor cells of small cell carcinoma are generally negative for androgen receptor (AR) and PSA [24] but exceptions exist [21]. As immunohistochemical profile varies from case to case, morphology remains the gold standard for pathological diagnosis of these tumors.

Small cell carcinoma is a rapidly growing tumor which disseminates early and the prognosis is poor [25]. Hormonal therapy is not effective in treating such tumors while chemotherapy may have some value [26].

Carcinoid tumors are very uncommon. They show complete NE differentiation and are morphologically similar to carcinoid tumors of the lung or GI tract. In comparison to small cell carcinomas, the tumor cells have more abundant cytoplasm, rare mitotic figures and no tumor necrosis (**Figure 4**). Like small cell carcinomas, carcinoid tumor is often present as a component of mixed tumors also containing conventional adenocarcinoma [27].

Focal NED in PC

NED occurs focally in conventional prostatic adenocarcinomas. Similar to NE cells in benign
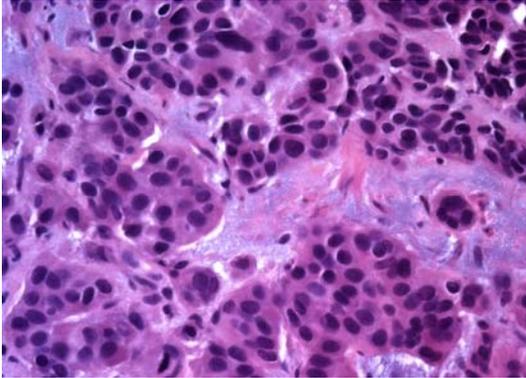
Figure 4. Carcinoid tumor of the prostate. Tumor cells have round, regular nuclei with fine chromatin pattern and low N/C ratio. No mitotic figures, apoptotic figures or necrosis are identified.
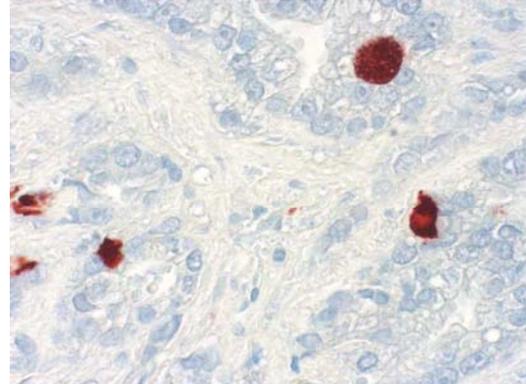

Figure 5. Focal NE differentiation in an adenocarcinoma of the prostate. Immuno-histochemical staining with an anti-chromo-granin A antibody identifies scattered NE cells (brown staining) in a case of adenocarcinoma of the prostate.

prostate, the NE cells are indistinguishable from the non-NE cancer cells on H&E-stained sections under light microscope, but can be identified by immunohistochemical staining for NE markers (**Figure 5**). Chromogranin A is the most commonly used marker and is considered to be sensitive and specific. It has been reported that all adenocarcinomas of the prostate contain some NE cells [28] when antibodies for multiple generic NE markers and/or specific peptides/neuropeptides are used and tissue preparation is controlled. About 5-10% of prostatic adenocarcinomas contain abundant NE tumor cells. NE cells are also present in prostatic intraepithelial neoplasia (PIN) [29] and in metastatic PC [30].

NED is increased in high grade and high stage tumors [31] and particularly in hormonally-treated [32] and hormone-refractory tumors [33]. Consistent with the histologic findings, the levels of serum chromogranin A are increased in PC patients than in patients with benign conditions. In PC patients, the serum chromogranin A levels correlate with tumor stage and increased levels are associated with tumor resistance to hormonal therapy [34]. In well- and moderately-differentiated PCs, Chromogranin A positivity by immunohistochemical staining is an independent predictor of tumor progression [35]. In patients with hormone-refractory tumors, elevated serum chromogranin A level is a significant predictor of poor prognosis, independent of serum PSA [33, 34, 36-40]. Intermittent administration of complete androgen deprivation therapy significantly reduces the increase in serum chromogranin A levels in comparison to continuous therapy [41]. The serum levels of NSE may also have prognostic significance [42]. Other serum markers such as chromogranin B, secretoneurin, which is a proteolytic product of secretogranin II (chromogranin C), and gastrin-releasing peptide/ProGRP may serve as additional prognostic and/or diagnostic markers [36, 43-47]. Serum calcitonin appears to be a more specific marker for small cell carcinoma of the prostate [48]. A genomic expression profiling study of PC showed that chromogranin A gene is one of five genes that correlate strongly with Gleason score and expression of these five gene model alone can accurately predict the outcome following radical prostatectomy [49].

Androgen withdrawal and NED in PC

In addition to histologic studies, biochemical studies also support the notion that androgen deprivation may induce NE activity in PC. Neutral endopeptidase 24.11 (NEP) is a cell surface enzyme expressed by prostatic epithelial cells and its functions is to cleave and inactivate a variety of neuropeptides [50]. Androgen deprivation down-regulates NEP which may effectively lead to more extracellular neuropeptides such as neurotensin by the NE cells. Interestingly, only androgen-deprived tumor cells respond to the growth-promoting effect of neurotensin [51].

The expression and catalytic activity of NEP are present in androgen-dependent PC cell lines but lost in androgen-independent cell lines. In vivo, metastatic cancer cells from patients with androgen-independent PC commonly show decreased levels of NEP compared with those from patients with androgen-dependent PC. Growth of androgen-independent cancer cells is inhibited by overexpression of NEP or incubation with recombinant NEP [52].

Animal models of PC recapitulate human diseases and provide more evidence suggesting the importance of NED in PC. In xenograft models of human PC, NED increases markedly after castration [53-55] and precedes the emergence of increased cancer cell proliferation and progression to androgen-independent cancer [56]. Extensive NED is also seen in an allograft model of androgen-independent PC [57]. In a transgenic mouse model of PC (TRAMP), the degree of NED correlates with the degree of tumor differentiation with the poorly differentiated tumors showing significantly more NED. Castration of TRAMP mice leads to aggressive and highly metastatic cancers in the majority of the cases, reflecting androgen-independent growth. NED was detected in the majority of the primary and metastatic tumors in such animals [58]. The tumors in the Pten knockout mice have few NE cells [59, 60] but similarly, their numbers increase in recurrent tumors after castration[60].

The origin of NE cells in PC

In comparison to secretory epithelial cells, the NE cells, whether benign or malignant, do not express AR and PSA [61]. NE cells are considered terminally differentiated and post-mitotic [62, 63]. The origin of NE cells in PC is still controversial, but it is becoming increasingly clear that NE cells in PC are different from normal prostate NE cells in their differential expression of proteins. For example, normal NE cells are positive for cytokeratin 5, a basal cells marker; while the PC NE cells exhibit characteristics of luminal secretory cells by being positive for cytokeratin 18, a luminal cell-type cytokeratin [64, 65]. We have also shown that CgA-positive NE cells are positive for luminal secretory cell-associated cytokeratins; while negative for basal cell markers, including high molecular weight cytokeratin and p63 [61]. More

recently, we found that alpha-methylacyl-CoA racemase (AMACR), an enzyme involved in the ß-oxidation of fatty acids, is expressed in PC NE cells as well as non-NE tumor cells but not in normal prostatic NE cells [61], suggesting that the PC NE cells are part of the tumor. Finally, normal NE cells exhibit two distinct morphologies which have a complex appearance with irregular dendrite-like processes extending between adjacent epithelial cells [66], while some PC NE cells may lack the typically neuron-like processes and are morphologically similar to the surrounding carcinoma cells [67]. These data together suggest that PC NE cells are clearly distinguishable from NE cells in the normal prostate. At the moment, the origin of the PC NE cell is not clear. It is proposed that PC NE cells share the same origin with normal NE cells and are differentiated from the intermediate stem cells [63, 68]. Alternatively, accumulated evidence suggests that adenocarcinoma cells can undergo a transdifferentiation process to become NE cells, acquiring a similar phenotype to normal NE cells and expressing NE markers. In support of this latter model, it has been found that LNCaP cells, an androgen-dependent cell line, can be induced in vitro to show NED by androgen deprivation [69] or agents that increase intracellular levels of cAMP. In addition, NE differentiation is more prevalent in the bone metastatic lesion than within the primary tumor foci, suggesting the occurrence of transdifferentiation process in metastatic PCa cells [70, 71]. More importantly, a genetic analyses of clinical archival specimens further reveal that PC NE cells share essentially identical allelic profiles with non-NE PC cells, but not with normal prostatic epithelium or normal NE cells [72], although studying more clinical samples can make the conclusion stronger. These results collectively provide the evidence that PC NE cells are likely originated from cancerous luminal epithelial cells through a trans-differentiation process.

The mechanism of tumorigenesis for tumors composed entirely of NE cells is unclear. One possibility is that mutagenic events occur in the normally quiescent NE cells in that normal prostate or in prostatic adenocarcinoma make such cells hyper-proliferative and lead to the development of pure NE tumors such as small cells carcinoma or carcinoid tumor. This would explain why such tumors are so rare, as the frequency of the mutagenesis events should

be low in the NE cells that are normally quiescent. Alternatively, a recent report shows that in a case of mixed tumor containing both adenocarcinoma and small cell carcinoma, the two components share similar genetic alterations and may be clonal, suggesting that adenocarcinoma can progress to become the more aggressive small cell carcinoma [73].

The function of NE cells in PC

NE tumor cells, unlike the non-NE tumor cells, do not express AR and are likely androgen-independent. Therefore, they may survive and continue to function in the androgen-deprived environment and establish autocrine and paracrine networks to stimulate androgen-independent growth of prostate cancer. For example, LNCaP xenografts do not normally grow in castrated mice because they are androgen-dependent. However, they can grow in castrated hosts when NE cells from a mouse NE tumor (NE-10) are transplanted on the opposite flank. In such a system, NE cells may directly activate AR in the LNCaP cells, thus promoting growth of LNCaP tumor cells in the absence of androgen [74]. In the presence of androgen, the same NE cells can enhance migration and metastasis of PC cells [75]. Neuropeptides stimulate androgen-independent growth [76] and the invasiveness of PC cell lines [77] in in-vitro assays. Both protein tyrosine kinase and protein kinase C pathways may be required for the activity of neuropeptides [78-81]. Bombesin has a mitogenic function in PC cells and may do so through activation of the transcription factor Elk-1 and the immediate early gene c-fos [82]. Yang et al developed an autocrine neuropeptide model by overexpressing GRP in LNCaP cells and the resultant cell line, LNCaP-GRP, exhibited androgen-independent growth with enhanced motility in vitro. When orthotopically implanted in castrated nude mice, LNCaP-GRP produced aggressive tumors, which express GRP, prostate-specific antigen, and nuclear-localized AR. Chromatin immunoprecipitation studies of LNCaP-GRP clones suggest that GRP activates and recruits AR to the cognate promoter in the absence of androgen [83].

The activity of the type IV collagenase matrix metalloprotease (MMP) is up-regulated by neuropeptides [84]. MMPs are associated with a variety of biological activities, such as tumor invasion, metastasis, and angiogenesis. MT1-MMP protein and mRNA are expressed in androgen-independent PC-3, DU-145 and TSU-pr1 cells but not in the androgen-dependent LNCaP cells. GRP induces the expression of MT1-MMP protein in DU-145 cells and also increases Matrigel invasion by these cells [85]. High-grade tumors are more likely to express MMP-9 and bombesin than low-grade tumors [86]. Bombesin increases the expression of the proteolytic enzyme urokinase-type plasminogen activator (uPA) and plasminogen activator inhibitor-1 (PAI-1) and also stimulates secretion and activation of MMP-9 [87]. Calcitonin affects growth and migration of certain PC cell lines and may play a role in the regulation of prostate cell growth and metastases, especially to the bone [88]. Some neuropeptides regulate intracellular calcium signaling through their respective receptors, which may contribute to the progression of PC [89]. Certain receptors for serotonin may be overexpressed in PC cells, particularly in high grade tumors, further supporting the hypothesis that NE products may promote androgen-independence of PC through a paracrine mechanism [90].

Cytokines may mediate the growth-promoting function of NE cells. IL-8 is a mitogenic and angiogenic factor for many tumors including PC. In in-vitro assays, IL-8 promotes androgen-independent growth and migration of LNCaP cells [91]. We have shown that IL-8 is expressed by NE tumor cells while non-NE tumor cells overexpress IL-8 receptor CXCR1, suggesting that IL-8 signaling may be an important paracrine mechanism in androgen-independent proliferation of PC [92].

NED may confer apoptosis-resistance of PC. While NE cells do not express anti-apoptotic protein Bcl-2 [93], the tissue levels of NSE correlate with Bcl-2, a major anti-apoptotic factor, and the Bcl-2-containing cancer cells are generally in close proximity to the NE cells [94], suggesting that NE cells may confer apoptosis-resistance to neighboring non-NE cancer cells. Bombesin and calcitonin prevent apoptosis of PC cells in-vitro [95-97]. NE cells do not appear to undergo apoptosis [98-100] even though they are negative for Bcl-2 [93]. They express survivin, another anti-apoptotic factor [67], providing a molecular basis for the hypothesis that NE cells may endure stressful conditions and escape from apoptosis during cancer therapy.

NED may promote neovascularization of PC. In addition to producing angiogenic factor IL-8, NE cells are also the major producers of VEGF [101, 102]. VIP, which is produced by autonomic nerves and NE cancer cells of human prostate, stimulates NED and VEGF production in LNCaP cells [103]. Benign prostatic tissue contains low levels of VEGF. PCs stain positively for VEGF and staining intensity correlates with Gleason grade. Complete androgen blockade for three months before surgery decreased the level of VEGF and vascularization, except in the cell areas with NE features [104]. In radical prostatectomy specimens, there is a correlation between NED and neovascularization and both correlate with tumor grade and tumor stage. The number of NE cells was found to be the only predictor of neovascularization [105]. Another study reported similar results and, in addition, found expression of VEGF to be significantly correlated with increased microvessel density, high tumor stage, poor differentiation and shortened disease-free survival [106]. Bombesin stimulates expression of pro-angiogenic factors VEGF and IL-8 in PC-3 cells, possibly through NF-kappa B-dependent pathway [107] while calcitonin may stimulate angiogenesis by directly acting on endothelial cells [108]. However, another study failed to show stimulation of proliferation, migration, or tube formation of human umbilical endothelial cells in vitro by neurotensin and bombesin [109]. VEGF expressed in NE cells of metastatic PC in lymph nodes does not appear to be related to angiogenesis [110].

Molecular mechanisms of NED

The molecular mechanism of NED has been extensively studied. AR signaling may inhibit the NE phenotype [111, 112], providing an explanation for the emergence of the NE phenotype when AR signaling is inhibited, such as in hormonally-treated cancers or in LNCaP cells cultured in androgen-deprived media. Androgen withdrawal is the classical stimulator of NED of LNCaP cells but NED can also be induced by many other means. It has yet to be determined if NED induced by agents other than androgen withdrawal is also through inhibition of AR signaling. Inhibition of FGF signaling by expression of a truncated FGFR2iiib receptor in prostatic epithelium of transgenic mice promotes NED [113], as does expression of a constitutively active

heterotrimeric G-protein subunit alpha [113]. Interaction of IGF-binding protein-related protein 1 with a novel protein, Neuroendocrine Differentiation Factor, results in NED of PC cells [114]. Inhibition of COX-2, a proinflammatory enzyme, induces NF-kappa B and NED of LNCaP C4-2b subline [115]. NED of LNCaP cells can be induced by IL-6, IL-1 [116, 117], interferon gamma [118] and agents that increase the intracytoplasmic levels of cAMP [119]. PKA signaling pathway may be important for NED [120]. In particular, constitutive activation of cAMP-dependent kinase activity results in NE differentiation, and enhanced the tumorigenic phenotype in vitro as well as in vivo with greatest positive impact on androgen-resistant xenograft tumor growth[121]. Papaverine combined with prostaglandin E2 (PGE2) synergistically induces NED of LNCaP cells [122]. NED of LNCaP cells is accompanied by overexpression of an alpha 1H (Cav3.2) T-type calcium channel [123] and changes in intracytoplasmic calcium homeostasis [100]. mAsh1, a basic helix-loop-helix (bHLH) transcription factor, may be involved in NED of PC cells [124]. More recently, it was found that irradiation of LNCaP cells also induces neuroendocrine differentiation, and it does so through differential location of two transcription factors, CREB and ATF2, that act respectively as enhancer and suppressor of NE functions. Radiation induces NE-like differentiation by increasing the nuclear content of phospho-CREB and cytoplasmic accumulation of ATF2 [125]. Silibinin, a flavonoid antioxidant, induces G_1 arrest and NED of LNCaP cells through increasing Rb level, decreasing Rb phosphorylation and inhibition of key cell cycle regulators [126, 127]. Recently, it has been shown that Protocadherin-PC, a member of protocadherin gene family, may play a critical role in NED of PC by activating the Wnt signaling pathway [128].

NED of LNCaP cells induced by heparin-binding epidermal growth factor (HB-EGF) involves mitogen-activated protein kinase (MAPK) signaling pathway [129]. Similarly, ERK has also been reported to be required for androgen-withdrawal-induced NED [130]. The same group has shown that protein tyrosine phosphatase (PTP) alpha, a receptor-type PTP, is involved in this process [130, 131]. We have recently shown that PTP1B, a

cytoplasmic PTP, is also required for androgen withdrawal-induced NED [132].

NED may be induced by autocrine mechanisms. VIP is secreted by NE cells and it induces NED, possibly through ERK, PKA and PI-3-kinase pathways [103, 133]. We have shown that NE cells secret IL-8 and also express IL-8 receptor CXCR2, suggesting a possible autocrine stimulation of NED through IL-8 signaling [92].

IL-6-induced NED involves the protein tyrosine kinase pathway [4, 134, 135], JAK-STAT signaling [136], induction of cyclin-dependent kinase (CDK) inhibitor p27 (Kip1) and inhibition of CDKs [137]. Androgen and AR can regulate IL-6-mediated LNCaP cell NED via directly modulating the IL-6-PI3-kinase pathway [112]. Interestingly, IL-6-induced NED of LNCaP cells may be qualitatively different from that induced by agents such as epinephrine and forskolin which cause rapid but reversible NED of LNCaP cells by increasing intracellular concentrations of cAMP. The process of IL-6-induced NED takes more time and is permanent [138]. In addition, the behavior of IL-6-treated cells may depend on the concentration of IL-6 used. Long-term exposure of LNCaP cells to low concentrations of IL-6 (5 ng/ml) results in the emergence of a LNCaP variant with more aggressive growth properties *in vitro* and *in vivo* [139, 140] while culture of LNCaP cells in high concentrations of IL-6 (100 ng/ml) for 2 weeks leads to permanent NED and significant loss of the proliferative potential [138]. On a transcript variation level, despite some overlap, each treatment (IL-6 treatment, genistein and epinephrine addition as well as androgen removal) to induce NE differentiation was associated with a changed expression of a unique set of genes [141].

NE differentiation and hormone-refractory prostate cancer

Androgen deprivation therapy is an effective therapy for prostate cancer patients whose tumors can no longer be controlled by local treatments. The therapy shrinks the tumor and leads to significant symptomatic relief in the majority of the patients, suggesting that tumor cell growth is dependent on AR signaling. It has been known for a long time that AR signaling is important for the development and maintenance of normal prostate as castration

leads to involution of normal prostate as well. Interestingly, the molecular and cellular mechanisms for the effect of androgen withdrawal in shrinking normal or cancerous prostate may be different. In normal prostate, androgen action through stromal cells results in secretion of the andromedins that are responsible for the survival and proliferation of luminal secretary epithelial cells that are the majority of the prostatic epithelial constituents [142]. In those cells, androgen receptor has been found to suppress the cell growth through expression of p27^{Kip1} [143-145]. Consistent with this finding, it has recently been shown that AR promotes tumorigenesis in stromal cells of the prostate, and acts as a tumor suppressor in epithelial compartment [146]. However, like estrogen receptor in breast cancer, AR in a small percentage of the prostate cancer has been found to be mutated and flipped its hard-wired role to become an oncoprotein in the prostatic epithelial cells, and likely is required for the growth of the prostate cancer at least in a xenograft animal model [147].

It is assumed that androgen deprivation therapy works in prostate cancer patients because AR is normally growth-promoting. However, as discussed above, the normal function of AR appears to be growth suppression. AR mutation may change its function but only less than 10% of prostate cancer contain mutations in androgen receptor [148]. Moreover, if androgen receptor changes its hard-wired activity from suppression of proliferation of epithelial cells to promoting cancerous epithelial cells, androgen deprivation in principle should continuously work like tamoxifen in breast cancer, yet androgen deprivation therapy eventually fails in the majority of patients with an average time to tumor recurrence of about 18 months. Alternatively, it is possible that androgen deprivation therapy works in a similar manner in prostate cancer as in normal prostate by inhibiting androgen receptor function in stromal cells. After androgen ablation therapy, less andromedins are secreted followed by apoptosis of the cancerous epithelial cells and a period of symptom relief. Since prostatic stromal cells largely support survival and proliferation of epithelial cells in a paracrine fashion, it is possible that NE cells being able to secrete a variety of neuropeptides and cytokines supplant the role of the stromal cells.

Because the NE cells do not express AR, they are likely resistant to androgen deprivation and their number and activity may actually increase in response to androgen deprivation. This scenario is consistent with the hypothesis that androgen deprivation generally works in all patients but eventually fails in the majority as NE cells gradually substitute the function of stromal cells and allow the continued proliferation of cancer cells. The amount of time it takes for the NE cells to substitute for the function of the stromal cells likely determines how long androgen deprivation therapy remains effective. Should this theory be proven, blocking NE function and or NE differentiation will likely prolong the therapeutic window of androgen deprivation therapy.

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