Luca Cindolo^a
 Monica Cantile^b
 Francis Vacherot^{c, d}
 Stéphane Terry^{c, d}
 Alexandre de la Taille^{c, d, e}

^aUrology Unit, 'G. Rummo' Hospital, Benevento, and ^bDepartment of Clinical and Experimental Medicine, 'Federico II' University Medical School, Naples, Italy; ^cINSERM, E 0337, Faculté de Médecine, ^dUniversité Paris 12, Faculté de Médecine, and ^eGroupe Hospitalier Henri Mondor, Service d'Urologie, AP-HP, Créteil, France

Neuroendocrine Differentiation in Prostate Cancer: From Lab to Bedside

Key Words

Prostate cancer • Neuroendocrine differentiation • Chromogranin A • NeuroD1 • LNCaP

Abstract

Objectives: To discuss the current knowledge on induction, production, sustenance and promotion of neuroendocrine differentiation in human prostate cancer. **Methods:** Review of the literature using PubMed search and scientific journal publications. **Results:** Morphological evidence explains some functional relationship between neuroendocrine and neoplastic surrounding cells. Transdifferentiation phenomenon and new biochemical pathways could be included in the development of androgen independence and prostate cancer progression. **Conclusion:** Multiple evidence seems to confirm that a synergistic functional network between epithelial PSA secretory cells and neuroendocrine intraprostatic system is the main trigger for the induction and sustenance of neuroendocrine differentiation. The development of new antineoplastic molecules should consider the multiple interference of the intercellular network.

Copyright © 2007 S. Karger AG, Basel

Introduction

Human adult prostatic gland contains three epithelial cell phenotypes: secretory, basal and neuroendocrine (NE) [1]. Secretory cells (luminal) are the most abundant and require androgens for growth and survival. Conventional adenocarcinoma of the prostate (PCa) shows features common to this cell phenotype. Basal cells are the principal epithelial proliferating cell type and are androgen-insensitive. NE cells are endocrine and sensory cells sharing structural, functional and metabolic properties with neurons normally present in human prostate, prostatic intraepithelial neoplasia (PIN) and in PCa [2, 3]. Although their origin is still debated, NE cells, initially considered as derived from progenitor neural crest cells, probably differentiate from a common epithelial stem cell [1]. These cells are rare in the normal prostatic gland (<1%), and show cellular extensions [4] usually referred to as neurites that may evoke a communication network between epithelial and NE elements [5, 6]. These cells produced a wide range of neuropeptides and neurotransmitters, they are postmitotic and highly differentiated, and mostly found as AR-negative cells [7]. These cell populations also exhibit an anti-apoptotic phenotype partially explained by the observation of augmented survivin expression [8, 9] and further stimulate neoangio-

genesis (via VEGF) [10]. Pure NE cell prostate tumors are very rare (<0.1%) and highly aggressive (35% survival rate in 2 years) [11, 12], while focal NE differentiation (NED) represents a common feature of PCa occurring in 30–100% of the cases [1–6].

Because the higher number of NE cells is associated to an increased neoplastic progression rate, the neuroendocrine differentiation phenomenon is considered of general oncological interest. An increased number of overt NE cells was also observed in patients with hormone-refractory prostate cancer (HRPC) following long-term androgen deprivation therapy, even though the question that NED is attributable to a condition of hormone refractoriness or long-term hormonal therapy needs to be further studied [13, 14].

Thus, the great interest in this field is high and the prevalence of NED in PCa is steadily being confirmed. In contrast, knowledge of the pathophysiology of this phenomenon is fragmentary, also considering the limited number of NE cell biomarkers available to score NE features.

The aim of this review is to discuss the current status on induction, production, sustenance, and promotion of NED in human PCa.

New Morphological and Immunohistochemical Issues

NE cells were identified in every stage and grade of PCa, described as ‘freely dispersed throughout the tumor’ by Varschenko and Abrahamson [4]. In particular, two types of NE cells were described: the open cells with extensions at their apex that connect with the lumen, and closed cells with dendritic processes that extend between adjacent cells, resting on the basal lamina and in close topographical relationship with the nerves [4].

Immunohistochemically, Hirano et al. [15] have shown differences in NE cell distribution in PCa. This group found that morphological features of NED in low-grade PCa can be observed in ChrA-positive areas or occasionally in single ChrA cells. In moderately differentiated PCa, NE cells were organized as cell clusters or focal agglomerates; whereas in poorly differentiated PCa diffuse areas of NED morphologically similar to surrounding neoplastic cells could be observed [15].

Interestingly, Grobholz et al. [16] have proposed a quantification method with a cut-off value of 30 NE tumor cells per standard area to facilitate the classification of a high and low NE differentiation, also considering the

distinction between isolated and clustered NE cells (poorer prognosis).

By electron microscopy, Hirano et al. [15] evidenced NE tumor cells as containing extremely pleomorphic granules in the apical supranuclear zone, often with a glandular lumen localization. The same author described dendritic-like NE cell extensions stretched out adjacent to neoplastic epithelial cells.

Moreover, based on ChrA immunogold labeling techniques, Fossmark et al. [17] achieved a good classification of cells with few NE granules and suggested these techniques as an important supplement to standard diagnostic tools in the examination of poorly differentiated tumors, at least in enterochromaffin-like cell carcinoids.

These lines of evidence underlined the strict functional paracrine connections between the NE and the epithelial compartments. This morphological evidence also gives new light to the comprehension of a functional relationship between NE and neoplastic surrounding cells. Finally, the recent observations of the increased proliferation activity and expression of anti-apoptotic genes of epithelial prostatic tumor cells in the proximity of NE cell foci [18, 19] provide evidence to support the notion that inductive stimuli generated directly from NE cells could explain at least in part the positive effect on the proliferation of tumor cells.

Mechanisms of Action: Transdifferentiation or Autocrine/Paracrine Interaction – A Dynamic Intraprostatic Microenvironment

Recent reviews [2, 20, 21] and papers based on in vitro models evidenced the life and function of the NE cell in human prostate. Today, the mechanisms through which NE cells act in prostate homeostasis are (1) transdifferentiation, and (2) autocrine-paracrine epithelial interactions.

Transdifferentiation

A growing body of literature show increased proportion of overt NE cells that is concomitant with increased levels of NE-derived peptides such as neuron-specific enolase (NSE) and chromogranin A (ChrA) in the serum of advanced, and HRPC patients. This occurrence seems, in most cases, correlate with a poor prognosis [22–27]. Aside from these observations, another intriguing and clinically relevant aspect of PCa is the propensity of neo-

Table 1. New genes involved in NED and related intracellular pathways

Gene	Pathways	Ref.
gp130 expression	IL-6 signaling	36
Protocadherin-PC	Wnt signaling	53–54
Foxa1 and Foxa2	transcription factors	62
Hox D locus	transcription factors	71
NeuroD1	transcription factor	72

plastic cells to undergo a ‘transdifferentiation’ process in which they acquire characteristics of NE-like cells also referred to as NE tumor cells. An extensive body of literature documents now elaborates that PCa cells could transdifferentiate in NE-like phenotype under various growth conditions (androgen-depletion), after exposure to different molecules (epinephrine, VIP, bombesin, calcitonin, growth factors and cytokines) [1] and through intracellular multipathways action [28] (table 1).

Transdifferentiation-Inducing Conditions

It is well known that an androgen-depleted medium induces LNCaP cell transdifferentiation via a dramatic increase of intracellular cAMP levels [29, 30]. Other investigators have confirmed this finding as well as showing that chronic exposure of LNCaP cells to IL-6 or NS-398, a Cox-2 specific inhibitor, would also induce NE transdifferentiation [30–34]. More recently, using a steroid-reduced medium, Yuan et al. [35] described the establishment and the biochemical characteristics of three NE subclones in vitro derived from LNCaP. Interestingly, these cells appeared to be transdifferentiated in cancerous NE cells. They (a) are terminally differentiated, (b) cannot be reverted to LNCaP phenotype, and (c) lack AR and PSA expression but acquire and retain multiple NE markers.

Using transfection of LNCaP cells with a constitutively active subunit of gp130 (gp130act), Palmer et al. [36] induced a NED ligand-independent transdifferentiation mechanism. These data confirm that NED pathways could be initiated and sustained by an IL-6 signaling cascade; on the other hand, this model evidenced the presence of alternative signal mechanisms in NE transdifferentiation of LNCaP. Collectively, these observations suggest that the increased NE cells found in advanced, aggressive and hormone-refractory prostate tumors are likely transdifferentiated PCa cells and clinical observations showing increased numbers of NE cells in prostate tumors from patients following hormonal therapy strong-

ly support this idea. Importantly in malignant lesions, NE tumor cells, at least to a certain extent, are phenotypically similar to NE cells present in the normal epithelium in terms of expression of neuropeptides and growth factors. However, they should be distinguished from the latter as they could share morphologic and genetic features with the surrounding cancer cells. A very recent paper nicely decrypted some genetic features of NE tumor cells and concluded for their relationship with tumor cells [37].

Even if certain of the above-mentioned in vitro models specifically described a kind of terminal differentiation in several isolated subclones that are useful in understanding this complex process, it seems likely that this is not the complete story regarding the well-known heterogeneity of PCa and the previous observations of the dual epithelial characteristics in certain prostate tumor cases marked by PSA and AR expression together with NE markers such as CgA, likewise few xenograft models demonstrate a possible heterogeneity of tumors [38–40].

This information strengthens the idea that different and intermediate phenotypes, possibly reversible, are displayed by tumor cells and that they might influence the clinical outcome of the disease [1, 7, 41]. With regard to the progression of PCa, the idea that some cells can directly undergo a transdifferentiation process that gives them properties of NE cells has a number of critical implications since evidence has been accumulating that non-NE human PCa cell lines have specific cell surface receptors for many of these peptides [42–45]. Further support of the role of NE cancer cells came with compelling evidence from contemporary animal models of prostate cancer [(human tumor xenografts and in transgenic mice (TRAMP and LADY mice)] wherein tumor progression is associated with the acquisition of NE characteristics by the tumor cells [39, 47–49]. The existence of the augmented number of NE and/or NE cancer cells within tumors begs the question of how some environmental factors or genetic alterations mediate establishment and maintenance of this phenotype along progression of the disease.

Clearly, a means to regulate NED, as mentioned before, is a persistent exposure to some molecules and factors. Likewise, there are good reasons to assume that the resulting downstream signaling pathways activated by these factors may have strong implications by driving this process [1]. It is also of interest that fresh studies based on genomic and gene expression analyses further helped to decipher some events that may be implicated or engender the NED.

In different cell culture conditions (e.g. supplementation by the dual 5- α reductase inhibitor, dutasteride) and using microarray gene expression analysis, Schmidt et al. [50] showed that growth, proliferation and viability of LNCaP were strongly reduced by dutasteride treatment. However, at gene expression level analysis they obtained a complex pattern with an increase of neurogenic genes expression (MDK, APLP1, BIRC1 and NOTCH3) hypothesizing that a chronically maintained androgen-deprivation condition could result in a rise of androgen-independent LNCaP cell clones (NED).

Protocadherin-PC

Other genes have recently been identified as potentially implicated in the NED process such as the protocadherin-PC (PCDH-PC) gene [51]. Interestingly, expression of PCDH-PC increases in patients with HRPc and has been suggested to be a relevant factor to promote androgen independence of tumor cells [52]. Moreover, a previous examination demonstrated the importance of this gene for the acquisition of apoptosis resistance [53]. More striking for this review is the observation that chronic culture of LNCaP cells cultivated in androgen-free serum or in a medium containing db-cAMP or NS-398 were all shown to induce PCDH-PC expression. This induced overexpression coincided with transdifferentiation of LNCaP cells towards a NE phenotype, as evidenced by the fact that alteration of the PCDH-PC overexpression by RNA interference was sufficient to block the expression of NE markers by the cells. Importantly, it was also shown in this model that a critical event in the process was its dependency toward the canonical Wnt signaling [53, 54]. Indeed, transient expression of a β -catenin mutant mimics the effect of the PCDH-PC expression in terms of expression of NE markers by the cells and RNA interference against β -catenin as well as competition against the final effector of the canonical Wnt signaling named TCF/Lef strongly affected the process. In this respect, these in vitro data identify the PCDH-PC expression and the activation of Wnt signaling as new and atypical means for tumor cells to start NED. Although the implication of Wnt signaling has been depicted more in other malignancies such as melanomas, colonic carcinoma or hepatocarcinoma [54], accumulating evidence obtained in vivo and in vitro suggests a critical role of Wnt signaling in prostate cancer [55–58]. In other circumstances it should be noted that Wnt signaling is critical in differentiation and normal development including the limb bud, kidney and neural crest cell derivatives that could give certain relevance to

our findings [59, 60]. Nonetheless, caution should be exercised in considering the complex regulation of the Wnt pathway, as reviewed recently [61], and therefore future work should address the link between Wnt signaling and NED.

Transcription Factors

Other candidate genes for PCa progression potentially associated with NED could be some members of the Forkhead box (Foxa) superfamily of transcription factor. This gene family is commonly known for its role in hepatocytic differentiation and function. Recently, a study illustrated the potential role of some Foxa members in high-grade tumors and HRPc by in vivo and in vitro studies [62]. In particular, it seems that while Foxa1 was consistently expressed in benign epithelial cells and tumors cells of both androgen-dependent and androgen-independent PCa, Foxa2 was solely expressed in some high-grade, poorly differentiated tumors, and in small cell carcinoma, typically referred to as pure NE carcinoma type and considered an androgen-independent disease. In addition, Foxa2 expression was always correlated with synaptophysin expression. Further in vitro experimental data showed that forced expression of Fox2a in AR-negative HeLa cells or in androgen-sensitive LNCaP cells could activate the expression of PSA gene in an AR and ligand-independent manner. In agreement with the androgen-independent status of the Foxa2 expression is the observation that the forced enhancement of the AR protein level by co-transfection reduced the co-activation effect of Foxa2 on the PSA promoter construct, suggesting that this gene product may be involved in strictly androgen-independent gene regulation.

In one of the first papers on prostatic NE cancer functional genomics (DNA microarray data set analysis), Hu et al. [63] demonstrated a wide gene number (32 genes) defined as 'neural/endocrine', actively expressed in CR2-TAg prostate tumors compared to normal prostate tissue. Among mRNAs showing a ≥ 2 -fold difference between neoplastic and normal prostate tissue, the authors reported many transcription factors (mASH1, members of the Sox and Six families), neural differentiation factors, secretory granule associated genes (ChrA and B), neuropeptide-related genes and neurotransmitters. NeuroD1 mRNA expression was evidenced in this paper for two reasons: (1) its increase of >200 -fold in advanced NE prostate cancer cell (24 weeks CR2-TAg) versus normal prostates by qRT-PCR, and (2) its expression could be positively regulated by mASH (another critical gene in NED, overexpressed in NE neoplastic cell). NeuroD1 is a

transcription factor originally identified in beta pancreatic cells. Following its hetero-dimerization with the helix-loop-helix protein E47, NeuroD1, originally described as able to regulate insulin gene expression by binding to an E-box motif on the insulin promoter, has been successively shown to be transiently expressed in specific groups of neurons both in the central and peripheral nervous systems at the time of terminal differentiation [64, 65]. Lee et al. [64] have reported that ectopic expression of NeuroD1 in *Xenopus* embryos causes conversion of skin precursor epithelial cells into neurons. Recent findings show that NeuroD1 is a neuronal differentiation factor [66], involved in gut endocrine differentiation [67–69] and able to interact with Rb to activate pro-opiomelanocortin expressing cells [70].

Cantile et al. [71], investigating HOX genes network expression in epithelial, fibroblastic and neoplastic prostate cell lines, revealed that their exposure to cAMP significantly alters the lumbosacral Hox D locus located on chromosomal region 2q31–33. This region contains cAMP effector genes (CREB1, CREB2, CREB3 and cAMP-GEF), a global cis-regulatory DNA control region, a prostate-specific noncoding RNA (PC-GEM1) and a series of genes involved in epithelial-neuronal cell conversion such as NeuroD1, neurexin and *dlx1* and 2. Among these, the authors performed the NeuroD1 in vivo expression analysis, finding a possible association with the Gleason score sum. The same group suggests that the above-mentioned chromosomal area might be involved in epithelial-neuroendocrine differentiation characteristic of human advanced PCa. On the basis of these molecular data, an immunohistochemical analysis intended to test NeuroD1 in human PCa and to compare its expression with ChrA, SNP, CD56 was performed, showing that NeuroD1 tissue reactivity is more frequent than ChrA in PCa and strongly correlates to increased indicators of malignancy in moderately-to-poorly differentiated PCa [72]. Thus, the suggestion is that NE cells present at the early stages of neoplastic development are rare and typical NE cells (ChrA+, serotonin+, probably AR+) less responsive to differentiation-driven transcription factors (e.g. NeuroD1) and more responsive to classic proliferative stimuli (peptide hormones, growth factors, autocrine-paracrine regulatory loops, and stromal-epithelial interactions). The acquisition of neuroendocrine characteristics occurs gradually in response to the cell phenotype influenced by environmental clues [3, 73]. It is reasonable to assume androgen deprivation therapy, cAMP induction or adrenergic agents (such as stress) are able to modify epithelial secretory prostate cells expressing tran-

scription factors (such as NeuroD1, FoxA, Brn-3a) and to induce differentiation or trans-differentiation towards the NE phenotype [73, 74]. This is a possible explanation of the increased number of NE malignant differentiated cells still capable of secreting growth factors and of stimulating surrounding tumor cells.

Results from Cantile et al. [71] and Cindolo et al. [72] were consistent with the findings of Hu et al. [63], indicating the absence of NeuroD1 in normal prostate and its production by NE normal cells (whole prostatic tissue) [63]. Surprisingly, as compared to data mentioned above, during the acquisition of a more aggressive tumor phenotype, laser capture microdissected (LCM) NE malignant cells show a strong decrease in NeuroD1 production. Because the production of NeuroD1 mRNA is very high in the whole prostatic tissue and is decreased in LCM NE cells, it is possible to assume that the secretion of this neurogenic factor is shared between epithelial, NE prostatic cell lines, glandular stroma and extracellular matrix, in a dynamic microenvironment where signals are shared and others are transmitted from cell to cell [73]. Pooling together this evidence, it is conceivable that malignant epithelial cells in specific growth conditions (such as the presence of AR-negative paracrine NE normal cells) start a slow conversion to the NE-like malignant cell gene program which expresses a complex gene cohort for differentiation, growth and survival of typical neurons, sharing identical allelic profiles with epithelial exocrine prostate tumor cells [37]. On the other hand, the androgen blockade therapy (Gn-RH or dutasteride therapy) (or a cytokine network activation escaping AR signal pathways) could induce LNCaP transdifferentiation towards the NE-like phenotype activating neurogenetic differentiation programs and/or changes in specific chromosomal areas.

Autocrine/Paracrine Interactions

NE cells represent an intriguing liaison between NE cell differentiation and tumor progression in PCa. These androgen-independent neoplastic cells are able to increase the proliferation index (by increasing the Ki-67 positivity) of surrounding non-NE-phenotype cancer cells by the secretion of NE products through a paracrine mechanism (through transactivation of the androgen receptor) [18, 75, 76].

Therefore, cancerous epithelial cells with increased responsiveness to NE factors, or able to induce NE cells to release trophic factors, may have a survival advantage

Fig. 1. Autocrine/paracrine interactions between NE and epithelial secretory prostate cells: new issues.

over their siblings. NE cells in many PCa express growth factors stimulating the growth/progression of the histologically conventional cancers, among these bombesin, calcitonin gene-related peptide, neurotensin, neurotrophin-3, parathormone-related peptide, somatostatin, serotonin, TGF, NGF, KGF and VEGF were well described [4, 73, 74] (fig. 1). However, the gene network/communication program involved with NE cells and PCa cells still remains complex and obscure.

Androgens and Androgens Receptor

Jin et al. [78] described a novel mechanism that allows PCa to escape androgen deprivation therapy. In a NE allograft model (NE-10 tumors) they showed that LNCaP tumors are able to increase AR expression. Indeed in an elegant experiment, the authors showed that implantation of mouse NE-prostate tumors on one flank of castrated immunodeficient mice was sufficient to enable growth of an androgen-dependent human prostate cancer cell line implanted in the opposing flank. They concluded that NE component of the developing PCa appeared to produce factors able to regulate AR activity by enhancing sensitivity to lower circulating androgen levels in a ligand-dependent manner, increasing nuclear AR levels and/or directly activating the AR in a ligand-independent manner of androgen-sensitive LNCaP cells

(fig. 1). In accordance with this latter study, the same group has observed that NE-10 tumor cells promote metastasis of the LNCaP cell line [79]. To our knowledge, these results are the first in vivo evidence that NE tumour cells within aggressive/hormone-refractory human prostate tumors may be 'feeding' adjacent tumor cells not only in a paracrine situation but and also when they are at a distant site from the tumors, highlighting the strong potential of factors (most likely neuropeptides) secreted from NE and NE-like cells.

Also, because an increase in AR level has been reported to be sufficient to account for hormone refractory PCa [80], the NE cell population secretion activity becomes a new key point in increased AR level/activity [80–82].

Consistent with these data, Yuan et al. [35] described three other NE subclones derived from LNCaP. In addition to the previously reported properties, they discovered that the media conditioned by these NE malignant cell lines could upregulate androgen-independent proliferation on PSA secretion by LNCaP cells. They concluded that these transdifferentiated cancerous NE cells (NE-1.3, NE-1.8 and NE-1.9) secrete growth-stimulatory soluble factors directly promoting the proliferation of PCa cells as well as PSA secretion in a steroid-reduced environment, as observed in hormone-refractory PCa.

Chemokines

Other authors investigated the expression of IL-8 and its receptors in PCa, particularly their relationship to the NE and non-NE compartments of cancer. IL-8 is a mitogenic and angiogenic factor for PCa, contributing to the migration and invasion of the PC3 cell line [80]. A recent study has shown that IL-8 may promote androgen-independent growth of PCa [83]. Although there is strong evidence linking IL-8 to the androgen-independent growth of PCa, the source of IL-8 in PCa was unclear. Huang et al. [45] demonstrated that NE cells are the source of IL-8 in human PCa. Although the IL-8 receptor CXCR1 is rarely expressed in benign epithelial cells, its expression is increased in PIN and further increased in invasive tumor, suggesting a paracrine mechanism whereby IL-8 produced by the NE tumor cells may promote the proliferation of the non-NE tumor cells in the absence of androgen. The data concerning the relationship between IL-8 and its receptors, CXCR1 and CXCR2, indicated that IL-8 may play an important role in normal neuronal function. The demonstration of the exclusive expression of CXCR2 in NE tumor cells, but not the non-NE tumor cells of PCa, is consistent with the concept that IL-8 produced by the NE cells of the prostate may regulate the function of NE cells via an autocrine/paracrine mechanism (fig. 1). Probably, these results could provide a novel mechanism by which NE cells in the prostate may promote androgen-independent growth and recruitment of the neighboring non-NE tumor cells.

Biogenic Polyamine Pathways and Neurotransmitters

As previously described, NE prostatic cells had cytoplasmic extensions (as neuritis) that are considered part of a communication network between the epithelial and NE compartments. Other morphological studies showed the presence of dendritic-like processes stretched-out towards tumors cells. The normal and neoplastic NE cells produced a wide range of neuropeptides and neurotransmitters. Recent studies discovered a new view of cellular communications in poor-prognosis human NE tumors [84]. The authors combined functional genomics with metabolomics to identify features of NE cancers associated with poor outcome [84]. They showed that a distinguishing feature of poor-prognosis NE tumors is a glutamic acid decarboxylase-independent pathway for production of GABA and a pathway for production of imidazole-4-acetate that involves dopa-decarboxylase and a membrane-associated amine oxidase, amiloride-binding protein 1 (ABP1). In this view, the presence of a membrane-associated amine oxidase (ABP1) allows for

the synthesis of metabolites, such as GABA and imidazole-4-acetate, which act on GABA receptors associated with these and neighboring cells. The repertoire of metabolites produced and the pattern of expressed GABA receptor subunits act in concert to effect the biological behavior of neoplastic NE cells and their neighbors within a given tumor microenvironment through autocrine and paracrine mechanisms (fig. 1). These findings could suggest that noninvasive imaging modalities such as positron emission tomography and magnetic resonance spectroscopy may be useful for phenotyping patient populations with NE tumors based on the activity of key enzymes in these metabolic pathways (e.g. DDC) and their metabolic products (e.g. GABA). Moreover, GABA, imidazole-4-acetate, and indole-3-acetate may only be a subset of products of these pathways; ABP1-derived aldehydes may react with amine-containing compounds in tumor cells to form precursors for alkaloids similar to those found in plants and other organisms [84].

The description of autocrine or paracrine stimulation through biogenic polyamine pathways functionally support the morphological similarities between neural progenitors and NE-differentiated cells. These hypotheses have been supported by the recent findings of Diss et al. [85, 86]. They found that a neuronal transcription factor (Brn-3a) (overexpressed in aggressive NE tumors and in advanced PCa) controls a voltage-gated sodium channel (Nav 1.7) expression in human PCa (upregulation). The Nav1.7 VGSC has been shown to potentiate cellular processes integral to CaP metastasis in vitro and in vivo and it was considered as an element 'necessary and sufficient' for the invasiveness of neoplastic cells [87]. The presence of neurotransmitter biosynthesis pathways and the pattern of GABA receptor and voltage-dependent channels revealed an electrophysiological perspective to the advanced PCa with NE features that explain that some NE tumors may be considered transformed neural stem cell tumors, or that some NE-differentiated adenocarcinomas (e.g. prostate, lung) should be better described as adenocarcinomas with a neural progenitor phenotype [88].

Following these findings, Ippolito et al. [88] published one more view of the metabolism of NE tumor cells. Using magic angle spinning NMR spectroscopy (MAS-NMR) profiling of metabolites, they described that amine oxidases play a role in NE cell metabolism by generating propylene glycol from glycine (a known neurotransmitter), which may increase endogenous pools of energy (NADH) as well as secure exogenous sources (fatty acids). Using a complimentary approach used in silico predic-

tions of possible metabolite biotransformations based upon known features of metabolite structure and combining this strategy with electrophysiological studies, the authors discovered that NE cancer cells use molecules involved in cellular communications to increase their stores of available energy and to promote their proliferation. These results led authors to conclude that a pharmacological intervention could be projected against targeted key enzymes and neurotransmitter receptors using well-established drugs used for other diseases (e.g. amiloride, a diuretic; carbidopa, a drug routinely administered to patients with Parkinson's disease; flumazenil, a benzodiazepine antagonist used to reverse the effects of sedatives). Their preliminary results in mice produced a statistically significant 40% reduction in tumor growth.

Conclusions

The exact pathogenesis of NED in human prostate is still poorly understood, although it is presumed that no single pathway is involved in this phenomenon. A synergistic functional network between epithelial and neuroendocrine intraprostatic systems seems to be the main trigger for the induction and sustenance of NED. From

morphological and immunohistochemical studies, it is possible to appreciate the physical interactions between epithelial and NE cells. From experimental research, it is possible to learn that new and intriguing pathways were implicated in transdifferentiation and in autocrine/paracrine regulatory loops in the intraprostatic microenvironment, suggesting a continuous prostate tissue remodeling process and highlighting the heterogeneity of PCa with neural features.

Thus, the knowledge of these multiple and alternative signaling events may provide new therapeutic targets, but the new therapies could be developed looking at multiple interference of the intercellular communication network (stroma, NE cell, epithelial neoplastic cells, extracellular matrix, vessels, interplay between classical growth factors and neuropeptides) to block or prevent tumor progression.

Acknowledgments

We acknowledge Professor Clemente Cillo for his continuous help in developing ideas and for his critical support. A further thank you goes to Teresa Giovanna Crisci for reviewing the text from a linguistic view point.

References

- 1 Nelson EC, Cambio AJ, Yang JC, Ok J-H, Lara PN Jr, Evans CP: Clinical implications of neuroendocrine differentiation in prostate cancer. *Prostate Cancer Prostatic Dis* 2007;10:6–14.
- 2 Shariff AH, Ather MH: Neuroendocrine differentiation in prostate cancer. *Urology* 2006;68:2–8.
- 3 Slovin SF: Neuroendocrine differentiation in prostate cancer: a sheep in wolf's clothing? *Nat Clin Pract Urol* 2006;3:138–144.
- 4 Vashchenko N, Abrahamsson PA: Neuroendocrine differentiation in prostate cancer: implications for new treatment modalities. *Eur Urol* 2005;47:147–155.
- 5 Di Sant'Agnese PA: Neuroendocrine differentiation in carcinoma of the prostate. Diagnostic, prognostic and therapeutic implications. *Cancer* 1992;70:254–268.
- 6 Abrahamsson PA, Di Sant'Agnese PA: Neuroendocrine cells in the human prostate gland. *J Androl* 1993;14:307–309.
- 7 Nakada SY, et al: The androgen receptor status of neuroendocrine cells in benign and malignant prostatic tissue. *Cancer Res* 1993;53:1967–1970.
- 8 Xing N, Qian J, Bostwick D, Bergstrahl E, Young CY: Neuroendocrine cells in human prostate over-express the anti-apoptosis protein survivin. *Prostate* 2001;48:7–15.
- 9 Fixemer T, Remberger K, Bonkhoff H: Apoptosis resistance of neuroendocrine phenotypes in prostatic adenocarcinoma. *Prostate* 2002;53:118–123.
- 10 Chevalier S, et al: Vascular endothelial growth factor and signalling in the prostate: more than angiogenesis. *Mol Cell Endocrinol* 2002;189:169–179.
- 11 Randolph TL, Amin MB, Ro JY, Ayala AG: Histologic variants of adenocarcinoma and other carcinomas of prostate: pathologic criteria and clinical significance. *Modern Pathol* 1997;10:612–629.
- 12 Papandreou CN, et al: Results of a phase II study with doxorubicin, etoposide and cisplatin in patients with fully characterized small cell carcinoma of the prostate. *J Clin Oncol* 2002;20:3072–3080.
- 13 Jiborn T, Bjartell A, Abrahamsson PA: Neuroendocrine differentiation in prostatic carcinoma during hormonal treatment. *Urology* 1998;51:585–589.
- 14 Hirano D, Okada Y, Minei S, Takimoto Y, Nemoto N: Neuroendocrine differentiation in hormone refractory prostate cancer following androgen deprivation therapy. *Eur Urol* 2004;45:586–592.
- 15 Hirano D, et al: Immunohistochemical and ultrastructural features of neuroendocrine differentiated carcinomas of the prostate: an immunoelectron microscopic study. *Ultrastruct Pathol* 2005;29:367–375.
- 16 Grobholz R, Griebel M, Sauer CG, Michel MS, Trojan L, Bleyl U: Influence of neuroendocrine tumour cells on proliferation in prostatic carcinoma. *Hum Pathol* 2005;36:562–570.
- 17 Fossmark R, et al: Ultrastructure and chromogranin A immunogold labelling of ECL cell carcinoids. *APMIS* 2005;113:506–512.
- 18 Bonkhoff H, Wernert N, Dhom G, Remberger K: Relation of endocrine-paracrine cells to cell proliferation in normal, hyperplastic, and neoplastic human prostate. *Prostate* 1991;19:91–98.

- 19 Segal NH, Cohen RJ, Haffeejee Z, Savage N: BCL-2 proto-oncogene expression in prostate cancer and its relationship to the prostatic neuroendocrine cell. *Arch Pathol Lab Med* 1994;118:616–618.
- 20 Hughes C, Murphy A, Martin C, Sheils O, O'Leary J: Molecular pathology of prostate cancer. *J Clin Pathol* 2005;58:673–684.
- 21 Peehl DM: Primary cell cultures as models of prostate cancer development. *Endocrine-Related Cancer* 2005;12:19–47.
- 22 Kadmon D, Thompson T, Lynch G, Scardino PT: Elevated plasma chromogranin-A concentration in prostatic carcinoma. *J Urol* 1991;146:358–361.
- 23 Tarle M, Rados N: Investigation of serum neuron-specific enolase in prostate cancer diagnosis and monitoring: comparative study of a multiple tumour marker assay. *Prostate* 1991;19:23–33.
- 24 Hvamstad T, Jordal A, Hekmat N, Paus E, Fossa SD: Neuroendocrine serum tumour markers in hormone-resistant prostate cancer. *Eur Urol* 2003;44:215–221.
- 25 Lilleby W, Paus E, Kovelund, E, Fossa SD: Prognostic value of neuroendocrine serum markers and PSA in irradiation patients with pN0 localized prostate cancer. *Prostate* 2001;46:126–133.
- 26 Kamiya N, et al: Pretreatment serum level of neuron specific enolase (NSE) as a prognostic factor in metastatic prostate cancer patients treated with endocrine therapy. *Eur Urol* 2003;44:309–314.
- 27 Isshiki S, Akakura K, Komiya A, Suzuki H, Kamiya N, Ito H: Chromogranin A concentration as a serum marker to predict prognosis after endocrine therapy for prostate cancer. *J Urol* 2002;167:512–515.
- 28 Zelivianski S, Verni M, Moore C, Kondrikov D, Taylor R, Lin MF: Multipathways for transdifferentiation of human prostate cancer cells into neuroendocrine-like phenotype. *Biochim Biophys Acta* 2001;1539:28–43.
- 29 Burchardt T, et al: Transdifferentiation of prostate cancer cells to a neuroendocrine cell phenotype in vitro and in vivo. *J Urol* 1999;162:1800–1805.
- 30 Shen R, Dorai T, Szabolcs M, Katz AE, Olson CA, Buttyan R: Transdifferentiation of cultured prostate cancer cells to a neuroendocrine cell phenotype in hormone depleted medium. *Urol Oncol* 1997;3:67–75.
- 31 Murillo H, Huang H, Schmidt LJ, Smith DI, Tindall DJ: Role of PI3-K signalling in survival and progression of LNCaP cells to the androgen refractory state. *Endocrinology* 2001;142:4795–4805.
- 32 Jimenez N, et al: Peptidylglycine alpha-amidating monooxygenase- and proadrenomedullin-derived peptide associated neuroendocrine deprivation are induced by androgen deprivation in the neoplastic prostate. *Int J Cancer* 2001;94:28–34.
- 33 Meyer-Siegler K: Cox-2 specific inhibitor, NS-398, increases macrophage migration inhibitory factor expression and induces neuroendocrine differentiation in c4-2b prostate cancer cells. *Mol Med* 2001;7:850–860.
- 34 Deeble PD, Murphy DJ, Parsons SJ, Cox ME: Interleukin-6 and cyclic AMP-mediated signaling potentiates neuroendocrine differentiation of LNCaP prostate tumour cells. *Mol Cell Biol* 2001;21:8471–8482.
- 35 Yuan TC, et al: Androgen deprivation induces human prostate epithelial neuroendocrine differentiation of androgen-sensitive LNCaP cells. *Endocrine-Related Cancer* 2006;13:151–167.
- 36 Palmer J, Ernst M, Hammacher A, Hertzog PJ: Constitutive activation of gp130 leads to neuroendocrine differentiation in vitro and in vivo. *Prostate* 2005;62:282–289.
- 37 Sauer CG, Roemer A, Grobholz R: Genetic analysis of neuroendocrine tumour cells in prostatic carcinoma. *Prostate* 2006;66:227–234.
- 38 Rocchi P, et al: Molecular analysis integrating different pathways associated with androgen-independent progression in LuCaP 23.1 xenograft. *Oncogene* 2004;23:9111–9119.
- 39 Huss WJ, Gray DR, Werdin ES, Funkhouser WK Jr, Smith GJ: Evidence of pluripotent human prostate stem cells in human prostate primary xenograft model. *Prostate* 2004;60:70–90.
- 40 de Pinieux G, et al: Clinical and experimental progression of a new model of human prostate cancer and therapeutic approach. *Am J Pathol* 2001;159:753–764.
- 41 Cohen RJ, Gleason G, Haffeejee Z: Prostate-specific antigen and prostate-specific acid phosphatase in neuroendocrine cell of prostate cancer. *Arch Pathol Lab Med* 1992;116:65–66.
- 42 Shah GV, et al: Calcitonin stimulation growth of human prostate cancer cells through receptor-mediated increase in cyclic adenosine 3',5'-monophosphates and cytoplasmic Ca^{2+} transients. *Endocrinology* 1994;134:596–602.
- 43 Sun B, Halmost G, Schally AV, Wang X, Martinez M: Presence of receptors for bombesin/gastrin-releasing peptide mRNA for three receptor subtypes in human prostate cancers. *Prostate* 2000;42:295–303.
- 44 Dizaya N, et al: Expression serotonin receptors and the role of serotonin in human prostate cancer tissues and cell lines. *Prostate* 2004;59:328–336.
- 45 Huang J, et al: Differential expression of interleukin-8 and its receptors in the neuroendocrine and non-neuroendocrine compartments of prostate cancer. *Am J Pathol* 2005;166:1807–1815.
- 46 Huss WJ, Gregory CW, Smith GJ: Neuroendocrine cell differentiation in the CWR22 human prostate cancer xenograft: association with tumour cell proliferation prior to recurrence. *Prostate* 2004;60:91–97.
- 47 Wang Q, Horiatis D, Pinski J: Interleukin-6 inhibits the growth of prostate cancer xenografts in mice by the process of neuroendocrine differentiation. *Int J Cancer* 2004;111:508–513.
- 48 Kaplan-Lefko PJ, et al: Pathobiology of autochthonous prostate cancer in a pre-clinical transgenic mouse model. *Prostate* 2003;55:19–37.
- 49 Masumori N, et al: An allograft model of androgen independent prostatic neuroendocrine carcinoma derived from a large probasin promoter-T-antigen transgenic mouse line. *J Urol* 2004;171:439–442.
- 50 Schmidt LJ, Murillo H, Tindall DJ: Gene expression in prostate cancer cell treated with dutasteride. *J Androl* 2004;25:944–953.
- 51 Yang X, et al: A human- and male-specific protocadherin that acts through the wnt signaling pathway to induce neuroendocrine transdifferentiation of prostate cancer cells. *Cancer Res* 2005;65:5263–5271.
- 52 Terry S, et al: Protocadherin-PC promotes androgen-independent prostate cancer cell growth. *Prostate* 2006;66:1100–1113.
- 53 Chen MW, et al: The emergence of protocadherin-PC expression during the acquisition of apoptosis-resistance by prostate cancer cells. *Oncogene* 2002;21:7861–7871.
- 54 Polakis P: Wnt signaling and cancer. *Genes Dev* 2000;14:1837–1851.
- 55 de la Taille A, et al: β -Catenin-related anomalies in apoptosis-resistant and hormone-refractory prostate cancer cells. *Clin Cancer Res* 2003;9:1801–1807.
- 56 Cheshire DR, Isaacs WB: Beta-catenin signaling in prostate cancer: an early perspective. *Endocr Relat Cancer* 2003;10:537–560.
- 57 Yardy GW, Brewster SF: Wnt signalling and prostate cancer. *Prostate Cancer Prostatic Dis* 2005;8:119–126.
- 58 Verras M, Sun Z: Roles and regulation of Wnt signalling and beta-catenin in prostate cancer. *Cancer Lett* 2006;237:22–32.
- 59 Yanfeng W, Saint-Jeannet JP, Klein PS: Wnt-frizzled signalling in the induction and differentiation of the neural crest. *Bioessays* 2003;25:317–325.
- 60 Vainio SJ: Nephrogenesis regulated by wnt signalling. *J Nephrol* 2003;16:279–285.
- 61 Terry S, Yang X, Chen MW, Vacherot F, Buttyan R: Multi-faceted interaction between the androgen and Wnt signalling pathways and its implication for prostate cancer. *J Cell Biochem* 2006;99:402–410.
- 62 Mirosevich J, Gao N, Gupta A, Shappell SB, Jove R, Matusik RJ: Expression and role of Foxa proteins in prostate cancer. *Prostate* 2006;66:1013–1028.
- 63 Hu Y, Ippolito JE, Garabedian EM, Humphrey PA, Gordon JI: Molecular characterization of a metastatic neuroendocrine cell cancer arising in the prostates of transgenic mice. *J Biol Chem* 2002;277:44462–44474.

- 64 Lee JE, Hollenberg SM, Snider L, Turner DL, Lipnick N, Weintraub H: Conversion of *Xenopus* ectoderm into neurons by NeuroD, a basic helix-loop-helix protein. *Science* 1995; 268:836–844.
- 65 Liu M, et al: Loss of BETA2/NeuroD leads to malformation of the dentate gyrus and epilepsy. *Proc Nat Acad Sci USA* 2000;97:865–870.
- 66 Chae JH, Stein GH, Lee JE: NeuroD: The predicted and the surprising. *Mol Cells* 2004;18: 271–288.
- 67 Schonhoff SE, Giel-Moloney M, Leiter AB: Development and differentiation of gut endocrine cells. *Endocrinology* 2004;145: 2639–2644.
- 68 Fujii A, Kamiakito T, Takayashiki N, Fujii T, Tanaka A: Neuroendocrine tissue-specific transcription factor, BETA2/NeuroD, in gastric carcinomas: a comparison with chromogranin A and synaptophysin expressions. *Pathol Res Pract* 2003;199:513–519.
- 69 Naya FJ, et al: Diabetes, defective pancreatic morphogenesis, and abnormal enteroendocrine differentiation in BETA2/neurod-deficient mice. *Genes Dev* 1997;11:2323–2334.
- 70 Suhardja A, Kovacs K, Rutka J: Genetic basis of pituitary adenoma invasiveness: a review. *J Neurooncol* 2001;52:195–204.
- 71 Cantile M, et al: cAMP induced modifications of HOX D gene expression in prostate cells allow the identification of a chromosomal area involved in vivo with neuroendocrine differentiation of human advanced prostate cancers. *J Cell Physiol* 2005;205: 202–210.
- 72 Cindolo L, et al: NeuroD1 expression in human prostate cancer: can it contribute to neuroendocrine differentiation comprehension? *Eur Urol* 2006;in press.
- 73 Arnold JT, Isaacs JT: Mechanisms involved in the progression of androgen-independent prostate cancers: it is not only the cancer cell's fault. *Endocrine-Related Cancer* 2002; 9:61–73.
- 74 Mazzucchelli R, Montironi R, Santinelli A, Lucarini G, Pugnali A, Biagini G: Vascular endothelial growth factor expression and capillary architecture in high-grade PIN and prostate cancer in untreated and androgen-ablated patients. *Prostate* 2000;45:72–79.
- 75 Diss JKJ, et al: Brn-3a neuronal transcription factor functional expression in human prostate cancer. *Prostate Cancer Prostatic Dis* 2006;9:83–91.
- 76 Bonkhoff H, Stein U, Remberger K: Endocrine-paracrine cell types in the prostate and prostatic adenocarcinoma are postmitotic cells. *Hum Pathol* 1995;26:167–170.
- 77 Huang J, Yao JL, di Sant'Agnese PA, Yang Q, Bourne PA, Na Y: Immunohistochemical characterization of neuroendocrine cells in prostate cancer. *Prostate* 2006;66:1399–1406.
- 78 Jin RJ, et al: NE-10 Neuroendocrine cancer promotes the LNCaP xenograft growth in castrated mice. *Canc Res* 2004;64:5489–5495.
- 79 Uchida K, et al: Murine androgen-independent neuroendocrine carcinoma promotes metastasis of human prostate cancer cell line LNCaP. *Prostate* 2006;66:536–545.
- 80 Isaacs JT, Isaacs WB: Androgen receptor outwits prostate cancer drugs. *Nat Med* 2004;10:26–27.
- 81 Abrahamsson PA: Neuroendocrine cells in tumour growth of the prostate. *Endocrine-Related Cancer* 1999;6:503–519.
- 82 Wright M, Tsai M, Aebersold R: Androgen receptor represses the neuroendocrine trans-differentiation process in prostate cancer cells. *Mol Endocrinol* 2003;17:1726–1737.
- 83 Lee LF, et al: Interleukin-8 confers androgen-independent growth and migration of LNCaP: differential effects of tyrosine kinases Src and FAK. *Oncogene* 2004;23:2197–2205.
- 84 Ippolito JE, et al: An integrated functional genomics and metabolomics approach for defining poor prognosis in human neuroendocrine cancers. *Proc Natl Acad Sci USA* 2005;102:9901–9906.
- 85 Diss JKJ, et al: A potential novel marker for human prostate cancer: Voltage-gated sodium channel expression in vivo. *Prostate Cancer Prostatic Dis* 2005;8:266–273.
- 86 Diss JKJ, et al: Brn-3a neuronal transcription factor functional expression in human prostate cancer. *Prostate Cancer Prostatic Dis* 2006;9:83–91.
- 87 Bennett ES, Smith BA, Harper JM: Voltage-gated Na⁺ channels confer invasive properties to human prostate cancer cells. *Pflügers Arch* 2004;447:908–914.
- 88 Ippolito JE, et al: Linkage between cellular communications, energy utilization, and proliferation in metastatic neuroendocrine cancers. *Proc Natl Acad Sci USA* 2006;103: 12505–12510.