

Neuroendocrine Differentiation in the Progression of Prostate Cancer: An Update on Recent Developments

Valérie Perrot

Laboratory M.E.R.C.I.-EA 3829, Institute of Research and Biomedical Innovation (IRIB), University of Rouen, Rouen, France
Email: valerie.perrot@univ-rouen.fr

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ABSTRACT

Neuroendocrine (NE) differentiation, either benign or malignant, is the hallmark of prostate cancer (PCa). Clusters of malignant NE cells are found in most prostate cancer cases. NE differentiation is among the non-mutually exclusive theories proposed to explain the progression to androgen independence of PCa. NE differentiation is usually associated with an increased aggressivity and invasiveness of prostate tumors and a poor prognosis. This review aims to present an overview of current knowledge on neuroendocrine differentiation in PCa to improve our understanding of tumour progression and androgen independence. The NE component represents an important therapeutic axis. Development of new generation of drugs that selectively target NE-like cells may lead to the development of new therapeutic modalities for advanced and hormone-refractory PCa.

Keywords: Prostate Cancer; Hormonal Therapy; Neuroendocrine; Neuroendocrine Differentiation; Neuropeptides; Chromogranin A; Tumorigenesis; Adenocarcinoma

1. Introduction

Prostate cancer (PCa) is a leading cause of cancer-related deaths among men in the Western countries. Its incidence is increasing annually worldwide due to better and earlier detection and also because of general aging of the world's population [1]. PCa tumours are heterogenous, most men harbour slow-growing tumours while others have neoplasms rapidly progressing to metastatic disease [2]. When PCa metastasizes, the patient 5-year survival rate drops to near 30% from virtually 100% when disease remains localized (confined to primary site) or regional (spread to regional lymphnodes) [3]. PCa depends on

androgens in the early stages. Therefore, androgen ablation is the most common therapy, aiming to deprive the androgen-receptive cells of their growth stimulus. Although most patients respond initially to this treatment, the tumour eventually recurs and enters an androgen-independent stage for which treatment options are few and generally ineffective [4].

A lot of progress has been made in understanding the mechanisms which drive the development and the progression of PCa, and in particular factors leading to the development of androgen independence (**Figure 1**). In this regard, evidence has emerged that androgen-resistant

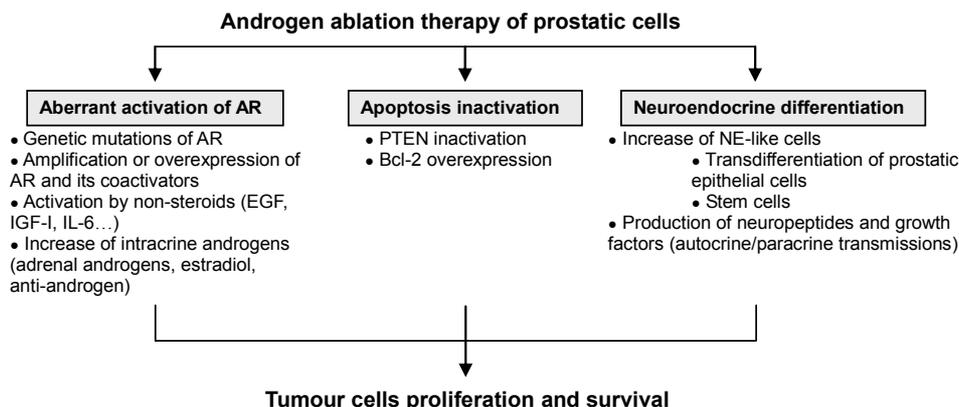


Figure 1. Molecular mechanisms of the transition from androgen-dependence to androgen-independence of PCa. AR: Androgen receptor; EGF: Epidermal Growth Factor; IGF-I: Insulin-like Growth Factor-I; IL-6: Interleukin-6.

PCa are often associated with tumour enrichment in neuroendocrine (NE) cells. In the past years a growing body of literature showed increased interest in the NE differentiation phenomenon that occurs in PCa. In contrast, less information is available on the specific role that NE cells play in the pathophysiology and prognosis of PCa. This review aims to present an overview of the current knowledge on NE cells and differentiation in PCa to improve our understanding of tumour progression and androgen independence. Better knowledge of PCa initiation and progression will help for developing new strategies for tumour prevention and treatment.

2. Neuroendocrine Cells of the Normal Prostate: Origin, Localisation and Function

Normal prostatic tissue is composed of stromal and epithelial compartments. The stroma not only acts as a supporting tissue, but also participates to the endocrine and paracrine microenvironment that controls prostatic epithelium growth and differentiation.

Three types of epithelial cells are found in adult prostatic gland: secretory, basal and neuroendocrine (NE). Luminal secretory cells are the most abundant and require androgens for growth and survival. They synthesize and secrete products of the seminal plasma, including prostatic-specific antigen (PSA). Basal cells are the principal epithelial proliferating cell type and are androgen-insensitive. They give rise to pluripotent cells, which are responsive to androgens, and can differentiate into basal cells, differentiated luminal cells, and possibly also NE cells. NE cells are ubiquitously present throughout the body and constitute a minor epithelial cell population widely distributed in normal prostatic acini and ducts. NE cells do not express the proliferation associated Ki 67 and MIB-1 antigens [5]. These post-mitotic cells are highly specialized cells, which share structural, functional and metabolic properties with neurons [6]. NE cells do not express androgen receptors [7,8], suggesting that they are androgen-insensitive [9].

The origin of NE cells in normal prostate is still under debate. The fact that NE cells do not express cytokeratin, a basal cell layer marker, suggests that they originate differently from other prostatic epithelial cells. NE cells may be derived either from undifferentiated basal cells of the prostatic epithelium [10] or represent an independent cell lineage derived from a neurogenic origin [11]. Further investigations will be necessary to clearly establish the origin of NE prostatic cells.

Histological studies revealed that in normal prostate gland, NE cells exhibit two distinct morphologies: 1) open cells with extensions at their apex that connect with the lumen, and 2) closed cells with dendritic-like processes that extend between adjacent cells and do not have

contact with the lumen [12]. Both subpopulations of NE cells participate to a communication network, in particular with the prostatic stroma, through their various secretory products.

In normal prostate, NE cells regulate prostatic growth, differentiation and secretion in an androgen-independent manner. NE cells contain neurosecretory granules rich in various peptide hormones and biogenic amines such as calcitonin [13], parathyroid hormone-related protein (PTHrP) [14], NE markers like chromogranins (CgA, CgB) [15] and neuron-specific enolase (NSE), serotonin [16], bombesin [17] and somatostatin [18] (**Table 1**).

The majority of these products can be either released into the blood stream or act locally. Relatively high levels of peptides are also found in the seminal fluid, suggesting that they may regulate sperm function. Therefore, the secretory products of NE cells affect target cells by endocrine but also paracrine and/or autocrine transmissions in an androgen-independent fashion due to the lack of androgen receptor.

The prostatic epithelium contains cells expressing a continuum of biological properties, differentiation markers, and variable degrees of androgen-dependence. It is assumed that the prostatic epithelium is under hormonal control of androgens for growth and survival. Consequently, androgen deprivation, which is the most common PCa therapy, elicits massive loss (up to 90%) of prostatic epithelial cells and their androgen-dependent precursors [19], and a concomitant increase of androgen-independent cells, such as NE and basal cells.

3. Neuroendocrine Differentiation, Hormone-Independence and Tumour Progression in Pca

Androgens play an important role in the development of normal prostate as well as in the carcinogenesis of PCa through the activation and signaling of the nuclear androgen receptor (AR). During prostatic tumorigenesis, a progression from normal prostate to prostatic intraepithelial neoplasia (PIN) to adenocarcinoma, and finally to small-cell carcinoma of the prostate is observed. It is generally admitted that NE differentiation is part of the oncogenic process.

A focal NE differentiation is present in almost all conventional prostate adenocarcinoma occurring in 30% - 100% of the cases [20,21]. In addition, NE differentiation can be found in highly aggressive small cell neuroendocrine prostate carcinoma, which are rare variants (0.5% - 2%), as well as in certain carcinoid and carcinoid-like tumours. NE cells are also observed in PIN and in metastatic PCa [22]. At all stages and grades of PCa, scattered NE tumour cells, singly or in dispersed clusters, are observed in the vicinity of non-endocrine dividing cells.

Table 1. General characteristics, functional roles, products and receptors of normal NE and NE tumour cells in prostate.

General characteristics	
Normal NE cell	Tumour NE cell
Androgen-receptor negative	Androgen-receptor negative
Non/low-proliferating activity	Non/low-proliferating activity
PSA-negative	PSA-negative
Bcl-2-negative	Bcl-2-positive
AMACR-negative	AMACR-positive
Intermediate and basal cell markers (Cytokeratin-5)	Luminal secretory cell markers (Cytokeratin-18)
Non-aggressive	Highly aggressive
Functional roles	
Regulation of cell growth and differentiation	
Regulation of homeostasis	
Regulation of prostatic secretion	
Products	
Adrenomedullin	
Bombesin/gastrin releasing peptide (GRP)	
Calcitonin, calcitonin gene-related peptide	
Cholecystokinin (CKK)	
Chromogranins (CgA, CgB)	
Gastrin-releasing peptide	
Histamine	
-human chorionic gonadotropin (HCG)	
Katacalcin	
Neuron-specific enolase (NSE)	
Neuropeptide Y	
Parathyroid hormone-related protein (PTHrP)	
Proadrenomedullin N-terminal peptide	
Serotonin	
Somatostatin	
Thyroid stimulating hormone (TSH)-like peptide	
Vascular endothelial growth factor (VEGF)	
Vasoactive intestinal peptide (VIP)	
Receptors	
Bombesin/GRP (GRPR)	
Calcitonin (hCTR-2)	
Cholecystokinin	
c-erbB-2	
Gastrin releasing peptide (GRPR)	
Neuropeptide Y	
Neurotensin	
Serotonin (5HTR1A, B)	
Somatostatin (SSTR1-5)	
Vasoactive intestinal peptide	
PTHrP receptor	

A recent report, from Hirano *et al.*, [23] described differences in NE cell distribution in PCa. In low grade PCa, morphological features of NE differentiation can be observed in CgA-positive areas or occasionally in single CgA cells. In moderately differentiated PCa, NE cells are mostly organized in clusters of focal agglomerates, whereas in high grade PCa, diffuse areas of NE differentiation morphologically similar to surrounding carcinoma cells can be observed.

The origin of NE tumour cells in PCa

The origin of NE cells in prostate tumoral lesions and the underlying molecular mechanisms of enrichment remain controversial. As proposed by Bonkhoff *et al.* [5, 24], NE tumour cells can arise from the intermediate stem cells, under pathological conditions such as androgen deprivation, contributing to increase NE cell population beyond to normal. Alternatively, PCa cells can undergo a “transdifferentiation” process to become NE-like cells, which express NE markers and acquire a NE phenotype. They begin synthesizing their own growth factors, becoming more autonomous and less dependent on the tissue microenvironmental control. These cells still retain some epithelial characteristics [25], in particular cytokeratin K8/18 [26] and prostatic acid phosphatase [27] luminal cell markers, suggesting that they originate from cancerous luminal epithelial cells. Thus, the increase of NE tumour cells as a result of malignant transformation of epithelial/basal cells is a common characteristic of PCa progression. Further investigations will, however, be necessary to clearly establish the origin of NE tumour cells in prostate adenocarcinoma.

Despite controversies regarding their origin, it is becoming increasingly clear that NE tumour cells are distinct from NE cells in normal prostate (**Table 1**). According to histological studies, NE tumour cells are morphologically similar to the surrounding carcinoma cells [28]. Moreover, a recent study deciphered some genetic features of NE tumour cells and concluded to their relationship with carcinoma cells [29]. They also differ from normal NE cells in the overexpression of proteins, such as anti-apoptotic protein B cell lymphoma protein 2 (Bcl-2) [30] as well as-methylacyl-CoA racemase (AMACR), an enzyme involved in the β -oxidation of fatty acids [27]. Additionally, NE tumour cells are positive for cytokeratin 18, a luminal cell-type cytokeratin, whereas normal NE cells rather express cytokeratin 5, a basal cell marker [25,26].

Mechanisms of androgen deprivation therapy-induced NE differentiation

Primary androgen deprivation therapy is conventionally used as a treatment for PCa. The response is usually transient and half of cancers progress to a hormone independent status over a period of 16 to 18 months, for which there is no successful therapy [31].

One major feature regarding prostatic cancer cells is their ability following androgen deprivation to undergo a transdifferentiation process, in which they acquire characteristics of NE cells and express NE markers, thus becoming NE-like cells. As they grow independently of androgen, these subpopulations of NE-like cells give rise to clones that have growth advantage, due to the loss of checks and balances, over their counterparts that retain androgen dependence. They may additionally acquire other alterations at both genetic and epigenetic levels that could contribute to the progression to androgen independence. Thus, through the course of androgen deprivation therapy, NE-like cells gradually substitute the function of stromal cells and allow continued proliferation of cancer cells, contributing to progression and aggressiveness of PCa.

A growing body of literature confirms that NE differentiation is more abundant in PCa after androgen withdrawal, indicating that NE component of PCa is resistant to hormone therapy [32]. Moreover, the increase in NE differentiation also appears to be related to the duration of treatment, including hormone deprivation therapy or chemotherapy [33]. Indeed, a long-term androgen deprivation therapy can induce downstream signaling pathways that drive the acquisition of a NE aggressive phenotype. Acquisition of NE characteristics could also occur in response to the influence of prostatic environment, and in particular the synergistic functional network between epithelial and NE prostatic system that may promote internal cell abnormalities, thus triggering the induction, and maintenance of NE differentiation.

These observations initially made using clinical samples have been confirmed and the underlying molecular mechanisms of androgen depletion-induced NE differentiation studied *in vitro* and in animal models of PCa. In an effort to reproduce NE differentiation of prostatic epithelial cells *in vitro*, a number of human PCa cell lines (i.e. LNCaP, DU145, PC3, PC-82, LAPC-4) as well as stable clones of NE-like PCa cells and co-cultures of prostatic cells which summarize tumour characteristics *in vivo* have now been established. Several studies revealed that androgen deprivation induced NE transdifferentiation of both androgen-sensitive and androgen-insensitive PCa cell lines which acquire NE phenotype and express NE markers and peptides [34]. Androgen depletion induced NE differentiation through the increase of intracellular cyclic AMP (cAMP) levels that in turn induce the activation of protein kinase A (PKA) [35]. Conflicting results, however, have reported the involvement of Erk/Mitogen-Activated Protein Kinase (MAPK) pathway in cAMP/PKA-mediated NE differentiation.

The use of these androgen-sensitive and androgen-insensitive PCa cell lines has led to interesting data and, could still provide insights into molecular mechanisms of

NE differentiation, in addition to identifying novel therapeutic targets. Transdifferentiation processes of these PCa cell lines by various stimuli are, however, transient and cells can fully revert to their original phenotype in the absence of inducers, thus making them different from terminally differentiated human PCa NE cells. A better characterization of expression profiles of NE markers and tumorigenic activity of these different PCa cell lines is still needed.

In this regards, stable clones of NE-like PCa cells have been established to elucidate the molecular mechanisms of NE differentiation and its role in PCa progression. These NE-like LNCaP subclone cells obtained by prolonged culturing in androgen-reduced medium, terminally transdifferentiate into NE-like cells and grow to become independent cell lines which exhibit a high tumorigenicity compared to LNCaP parental cells [36,37]. Importantly, they cannot be reverted to LNCaP phenotype and they maintain the expression of various NE markers. Therefore, LNCaP subclone cells resemble to NE-like PCa cells found in adenocarcinomas and may have a better clinical relevance than PCa cell lines previously mentioned.

In addition, co-culture models have been developed in order to reconstitute the complex interplay existing between prostatic epithelial, stromal and extracellular matrix (ECM) components of the prostate gland [38]. Efforts need to be pursued to develop these cell culture models that are taking into account dynamic cellular interactions within the prostatic microenvironment which may influence or promote internal cell abnormalities in the progression towards the acquisition of NE phenotype and the hormone-independent PCa cell growth.

Studies on *in vivo* animal models of PCa (human tumour xenografts and transgenic mice (TRAMP and LADY mice)) validate *in vitro* observations, supporting the importance of NE differentiation in PCa. In these animal models, tumour progression is associated with the acquisition of NE characteristics by cancer cells. Moreover, NE differentiation has been reported to markedly increase after castration in xenograft models of human PCa and to precede the emergence of tumour cell proliferation and progression to androgen independence [39].

Data from *in vitro* and *in vivo* studies collectively support the notion that androgen deprivation induces transdifferentiation of androgen-sensitive adenocarcinoma cells to become NE-like cells in PCa lesions. It remains however to determine whether those transdifferentiated NE-like cells exhibit similar biochemical properties as that of NE-like PCa cells.

In addition to be induced by androgen deprivation conditions, transdifferentiation of prostatic cells in NE-like phenotype can also be initiated by various cues such as cyclic AMP (cAMP), epinephrine, genistein, VIP, cal-

citonin, growth factors and cytokines as well as through the activation of various intracellular signaling pathways, including signal transducer and activator of transcription (STAT-3), MAPKs, cAMP/PKA and phosphatidylinositol 3-kinase (PI3K) [40]. The diversity of the pathways that may promote NE cell transdifferentiation could explain at least in part PCa heterogeneity. It remains yet to determine if NE differentiation induced by agents other than androgen depletion also occurs through inhibition of AR signalling. A better knowledge of these alternative signalling pathways will help to determine what drives acquisition of the NE process and may also allow the identification of new therapeutic targets in order to block or prevent PCa progression.

Paracrine to autocrine shift in tumour cell regulation during malignant transformation of epithelial cells

NE tumour cells do not express androgen receptors [7, 8]; they sustain their function in the androgen-deprived environment by establishing autocrine and paracrine networks to stimulate androgen-independent growth of prostate carcinoma cells. These cells produce a wide range of neuropeptides and neurotransmitters involved in the interactions between the different compartments of the prostate. An ever growing list of prostatic NE cell products have been identified and cell surface receptors for some of these NE products have been identified in non-NE tumour cells [25,41].

The ability of non steroids like growth factors (insulin-like growth factor I (IGF-I), keratinocyte growth factor (KGF)) and cytokines (interleulin 6 (IL-6)) to mimic the effect of androgens through the mitogen-activated protein kinase (MAPK) signaling pathway [25] contributes to the adaptation of NE tumour cells to an androgen-depleted environment, thus increasing their malignant potential. Therefore, the autocrine transmission may become more important in androgen-independent tumours and contribute to CaP progression. Furthermore, during the evolution from normal to malignant cancer cells, the shift to autocrine transmission may also be associated with the acquired regulation of genes involved in proliferation and survival that are not normally expressed in normal prostate epithelial cells.

Cell-cell interactions are extremely important in maintaining homeostasis between epithelial and stromal compartments of the prostate. Through the course of androgen deprivation therapy, prostatic epithelial cell growth and differentiation can be induced by paracrine growth factors such as epidermal growth factor (EGF), fibroblast growth factor (FGF), KGF, nerve growth factor (NGF) and IGF-I [42,43] that will diffuse from stromal to epithelial compartments, leading to the activation of AR target genes in a ligand-independent manner. Growth factors produced by prostatic epithelial cells can also act in an autocrine manner to stimulate this process.

Aside from these observations, there is considerable evidence indicating that during the transformation process of prostatic epithelial cells some factors are shifted from paracrine to autocrine regulation, as documented for neurotrophins. Classically, NGF, neurotrophin-3 and brain-derived growth factor are involved in the NE regulation of prostatic function. NGF is produced by stromal cells and is not a survival factor for epithelial cells in the normal prostate. Interestingly, reports indicate that in PCa, NGF can be secreted by malignant prostatic epithelial cells and regulate an acquired survival pathway through an autocrine mechanism [44].

Therefore, identification of other growth and survival factors produced by NE tumour cells and a better knowledge of the molecular mechanisms underlying this acquired expression is critical to understand what causes NE cells to become malignant and to effectively treat the disease.

Role of NE tumour cells in PCa

NE tumour cell population is highly aggressive and exhibit tumorigenic activity. Several studies reported that the proportion of NE tumour cells is increased in high grade and high stage tumours [16,45], and in particular in androgen-deprived and androgen-independent tumours. In most cases, NE differentiation is correlated with a poor prognosis [46].

Tumour enrichment in NE-like cells and the consequent increase in neurosecretory products can contribute to the androgen-independent proliferation of PCa through their mitogenic effects on adjacent cancer cells, thus increasing malignancy and reducing responsiveness of cancer cells to androgen ablation therapy. Secretory products of NE-like cells have been shown to increase the proliferation index (Ki-67-positive cells) of neighboring cancer cells through paracrine mechanisms [5]. Some of the peptide products of NE cells in the prostate, like bombesin, calcitonin and PTHrP, can affect prostate adenocarcinoma cell proliferation *in vitro* [9,47].

Moreover, neurosecretory factors produced by NE-like cells may also act by enhancing the sensitivity of prostatic cancer cells to lower circulating androgen levels, consequently allowing PCa to escape androgen ablation therapy. NE-like cells may regulate adjacent tumour cells by paracrine mechanisms, highlighting the strong potential of neurosecretory products [48].

The balance between cell proliferation and cell death is also disrupted in NE tumour cells. The malignant potential associated with NE differentiation is strongly increased by the fact that NE tumour cells are resistant to apoptotic cell death [49,50]. This phenomenon may allow prostatic NE tumour cells to escape radiation and chemotherapy. The apoptosis resistance of NE cells may be partially explained by the overexpression of survival proteins, such as survivin and clusterin. Survivin is a

member of the inhibitor of apoptosis (IAP) family with direct inhibitory action on caspase-3 and caspase-7 activity. Its expression is observed during fetal development but not in normal, terminally differentiated adult human tissues [51]. Additionally, survivin overexpression has been reported in various adenocarcinomas including PCa. Interestingly, increased expression of survivin seems to be associated with PCa progression after radical prostatectomy [52]. Clusterin is a glycoprotein, involved in diverse biologic processes including transport of lipoproteins, modulation of cell-cell interactions, cell death and tissue remodeling [53].

In addition, overexpression of the anti-apoptotic Bcl-2 protein by NE tumour cells may also protect them from apoptotic stimuli, and thereby increase their resistance to androgen deprivation therapy and their ability to progress towards hormone-refractory prostate cancers (HRPC) [49]. Therefore, enhancing the pro-apoptotic potential of PCa malignant cells through targeting key players involved in apoptosis resistance is one strategy to treat androgen-independent PCa.

Furthermore, NE tumour cells may also block the apoptotic process of prostatic cells in their vicinity through their neurosecretory products, contributing to androgen-independent proliferation of PCa cells and cancer progression. Bombesin and calcitonin have been reported to decrease apoptosis of PCa cells *in vitro* [54].

Several reports indicated that NE tumour cells might also promote neovascularisation of PCa. They may modulate angiogenesis through secreted angiogenic factors such as vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF) and basic fibroblast growth factor (FGF), and thus influence prostatic cell growth and differentiation. This idea is supported by the fact that benign prostatic tissue contains low level of VEGF, whereas VEGF staining intensity correlates with Gleason grade. Complete androgen blockade for three months before surgery, decreased VEGF level and vascularisation, except in areas where cells are NE-like [55]. In radical prostatectomy samples, there is a correlation between NE differentiation and neovascularisation and both correlate with tumour grade and tumour stage.

Increasing knowledge of the role of NE-like cells throughout the course of prostatic carcinogenesis and tumour progression will pave the way to the development of new therapeutic modalities for advanced and hormone-refractory PCa.

4. Pronostic Significance of NE Differentiation in PCa

Discrepancies in reported incidence (30% - 100%) of NE differentiation in PCa mainly occur from the limited number of NE cell biomarkers used to score NE features. Classically, NE differentiation can be assessed by im-

munoreactivity for neuroendocrine markers or bioactive hormones (somatostatin, 5-HT), measurement of serum levels of NE markers or electron microscopy (neurosecretory granules).

Among the different NE markers, most of the attention has been given to the detection of CgA and NSE in PCa clinical specimens. CgA belongs to the granin family of acidic secretory glycoproteins that is found in secretory granules of the regulated pathway of a wide variety of endocrine cells and neuron, including NE tumour cells. Classically, CgA serves as a generic marker of the NE cell population. So far, detection of CgA in neoplastic tissue remains one of the most reliable methods to assess NE differentiation [25]. CgA is considered as one of the PCa progression marker after radical prostatectomy [56]. The use of CgA as a prognostic factor in androgen-sensitive PCa is still controversial.

Despite the fact that PSA is commonly used as a marker for the early detection of PCa as well as monitoring the therapeutic response and tumour recurrence, it is not enough specific and sensitive. A growing body of literature suggests that, independently of PSA, serum CgA is a significant predictor of poor prognosis in patients with advanced and hormone-refractory prostate cancers [22]. Consistent with histological findings, levels of CgA are increased in PCa patients and correlate with tumour stage. A microarray study on PCa revealed that CgA gene is among the 5 genes strongly correlated with the Gleason score, which can predict the outcome following radical prostatectomy [56]. Therefore, serum measurements of NE markers in conjunction with PSA could help screen patients.

In addition to CgA, other serum markers such as CgB, secretoneurin, a proteolytic product of secretogranin II (also known as CgC), gastrin-releasing peptide/ProGRP and NSE, may serve as additional prognostic and/or diagnostic markers [57,58]. Serum calcitonin level has been reported to be a more specific marker for prostatic small cell carcinoma [59].

The possibility to treat NE differentiation in androgen-independent PCa is related to the development of specific and sensitive markers, clinically detectable in patients with PCa. Further investigations will be necessary to strengthen the prognostic significance of NE tissue marker in PCa.

5. Neuroendocrine-Targeted Therapy

One of the most troubling aspects of PCa progression is the conversion from an androgen-dependent to independent state, which at present defies any effective treatment. Different therapeutic approaches, including surgery, radiation therapy, and androgen deprivation therapy, have become the gold standard treatment for hormone-dependent PCa. Until 2010, chemotherapy and in particular

taxane-based chemotherapy (paclitaxel, docetaxel) which is of limited benefit represented the main therapeutic option in the occurrence of hormone-refractory PCa. Novel chemotherapeutics and targeted agents for patients with metastatic hormone-refractory PCa stages have been very recently introduced into clinical practice and include abiraterone acetate (a new androgen biosynthesis inhibitor), cabazitaxel (a novel microtubule inhibitor), MDV-3100 (a novel androgen-receptor antagonist), the radioisotope alpharadin (radium-223), sipuleucel-T (an immunotherapeutic agent) and denosumab (a bone-targeting agent) [60].

Other novel approaches currently being tested in early clinical trials for advanced PCa include immunological therapies (PCa vaccines, PCa antibodies), angiogenesis inhibitors (targeting VEGF, PDGF, PDGF receptor), epigenetic therapy (histone deacetylase (HDAC) inhibitors), pro-apoptotic agents (Bcl-2, survivin modulators), and interference in growth-factor-mediated pathways (mammalian target of rapamycin (mTOR)) [61].

It is now widely accepted that NE differentiation which occurs in prostatic adenocarcinomas is associated with PCa progression and aggressiveness. NE-like cells increase concomitantly with the duration of androgen depletion, thus blocking NE function and/or differentiation will most likely prolong the therapeutic window of androgen deprivation therapy. The NE axis appears to be an important therapeutic target for drug development in advanced PCa. Pharmaceutical agents (somatostatin analogs, bombesin antagonists, serotonin antagonists, mTOR inhibitor, pro-apoptotic agents) able to block the tumour-promoting action of NE-like cells products are under investigation.

NE differentiation is the hallmark of PCa with possible prognostic significance and consequences on therapy response. Detection of focal NE differentiation, using CgA marker, may help to identify patients who are more prone to endocrine therapy failure. In organ-confined disease, assessment of NE differentiation could identify patients that would benefit from more aggressive therapy. Intermittent androgen deprivation or antiandrogen monotherapy could be used to slow down marked NE differentiation, in conjunction or not with NE targeted therapy, to delay the progression towards hormone-independent PCa, while maintaining clinical benefit [62].

The NE axis remains an important therapeutic pathway for drug development in advanced PCa. Development of new generation of drugs that selectively target NE-like cells may help targeting populations of PCa that may be resistant or becoming resistant to traditional therapies.

6. Conclusions

In the past years, much progress has been made towards

better understanding the development and progression of PCa as well as the factors which drive the development of androgen independence. NE differentiation is among the non-mutually exclusive theories proposed to explain the progression to androgen independence of PCa. Although, NE differentiation has been demonstrated in a variety of carcinomas arising in different tissues, making it of oncological interest, very little is known about the role of NE differentiation in PCa pathophysiology.

Recent progress in terms of PCa research highlighted the role of NE differentiation in prostatic carcinomas. Through the course of androgen deprivation therapy, NE-like cells gradually substitute the function of stromal cells and allow the continued proliferation of cancer cells, contributing to the progression and the aggressiveness of PCa. This subset of androgen-independent cancer cells is also associated with a poor prognosis. Interest in understanding the neuroendocrine differentiation in PCa has increased with the identification of several neuroendocrine factors regulating the homeostasis of prostate by autocrine and/or paracrine mechanisms. There is now an ever-growing list of factors that are secreted by these prostatic NE tumour cells which regulate their proliferative activity. As our knowledge of the neuroendocrine factors develops, in the future our focus will be to determine how they interact with other prostate cell types that reside in the dynamic prostatic microenvironment. Investigation of how NE factors and the resulting downstream signaling pathways contribute to the initiation and the progression of PCa should be pursued and will help identifying new therapeutic tools to block or prevent PCa progression.

The NE axis remains an important therapeutic target for drug development in advanced PCa. Development of new generation of drugs directed against NE-like cells may help targeting populations of PCa cells that may be resistant to traditional therapies, thus helping setting up individualized therapy which takes into account the heterogeneity of PCa.

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