Review

Neuroendocrine pathogenesis in adenocarcinoma of the prostate

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Summary

Background: In the prostate, the importance of sex hormones for its normal development and function is well known. However, it has been proposed that various neuroendocrine (NE) hormones and growth factors may be involved in the pathogenesis of prostatic carcinoma (CaP). Neuroendocrine differentiation appears to be associated with tumour progression and the androgen-independent state, for which there is currently no successful therapy. Therefore, we need to improve our understanding of NE cells, their regulatory products and influence on the prostate gland. Finally, new therapeutic protocols need to be developed.

Methods: Information is presented on prostatic NE cells and neuroendocrine differentiation (NED) in prostatic carcinoma. Neuroendocrine secretory products and interactions with epithelial prostate cells are investigated in order to understand their significance for the pathogenesis of the prostate gland, prognosis and therapy.

Results: Recent research suggests that NE-secreted products, such as serotonin, somatostatin and bombesin, may influence growth, invasiveness, metastatic processes and angiogenesis in CaP. During recent years, new experimental models for NED have been developed to provide evidence that NE products may promote proliferation and confer antiapoptotic capabilities on non-neuroendocrine cells in close proximity to NE cells. Cancerous epithelial cells may become more responsive to NE factors by upregulation of receptors for neuropeptides, or may induce NE cells to upregulate the secretion and synthesis of NE factors. In the androgen independent state, neuropeptides and their intracellular signals may activate the androgen receptor. Furthermore, androgen ablation may lead to downregulation of neural endopeptidase 24.11 (a zinc-dependent metalloproteinase) and PSA, which would lead to increased levels of NE products becoming available. These studies confirm that NE cells and NED may have a significant impact on prostate cancer, especially in the androgen independent state.

Conclusions: Recent developments in molecular biology and pathophysiology of prostate cancer have increased our understanding of the NE regulatory mechanisms. Hopefully, this will lead to the development of entirely new therapeutic modalities. For example, somatostatin agonists may suppress angiogenesis and proliferation, and simultaneously promote apoptosis in prostate cancer cells. Somatostatin may thus have an important role in tumour biology, and in the future there may be a potential role for somatostatin analogues in the treatment of prostate cancer, but also for serotonin and bombesin receptor antagonists. However, a review of the accumulated knowledge in this field suggests that we still need to improve our understanding of NE cells and their regulatory products and influence on the prostate gland, and that clinical trials are needed, to test drugs based on neuroendocrine hormones and their agonists/antagonists.

Key words: growth factors, neuroendocrine, neuropeptides, prostatic carcinoma

Introduction

A tumour is not an autonomous entity, but relies on an ability to respond and to influence the normal cellular microenvironment for proliferation, evasion of the immune system, induction of angiogenesis and an ability to metastasize to distant organs. The formation of a tumour is a multi-step process, involving the accumulation of genetic alterations and aberrant responses to regulatory stimuli and pathways [1]. In recent years, evidence that various hormones and growth factors can be etiological agents and be involved in the pathogenesis of the neoplastic lesion has emerged [2]. At the same time, as growth factors and hormones stimulate the growth and mitosis of cells, hormones increase the risk of mutations solely by increasing the number of cell divisions. Depending on tissue origin, a cancer cell will have different phenotypes and respond accordingly to the hormonal environment.

The importance of sex hormones for the prostate normal development and function is well known. However, it has also become apparent that, as an accessory male sex-organ, the prostate is not exclusively dependent on androgens, but also on additional factors of paramount importance in maintaining normal prostate function and in the development of various pathological conditions. The importance of growth factors, autocrine-paracrine regulatory loops and peptide hormones is now widely recognised. During recent years, a body of evidence has emerged indicating that neuroendocrine cells, which are present in the normal and pathological prostate, have a central role in regulating growth, differ-
entiation and homeostasis of the normal and malignant prostate. Neuroendocrine differentiation in carcinoma of the prostate [3] appears to be associated with poor prognosis, tumour progression, and the androgen-independent state [4] for which there is currently no successful therapy. Therefore, new therapeutic modalities based on neuroendocrine hormones and/or their antagonists need to be developed.

The neuroendocrine cell

As with other glandular organs, the secretory cells throughout the prostate are separated from the basement membrane and stroma by a flat layer of basal cells. Basal cells are thought to house the stem cells. The secretory cells in the prostate contribute a wide variety of products to the seminal plasma. Prostate-specific antigen (PSA) is produced by the secretory cells in the ducts and acini of all zones of the prostate. The epithelium also contain a small population of isolated cells, seemingly randomly scattered, with dual properties of endocrine cells and neurons; the neuroendocrine (NE) cells. The diversity of secretory products in the normal prostate suggests that the population of NE cells consists of several subpopulations, each with its own set of secreted hormones and probably also with its own mode of regulation.

Morphologically, there are two types of NE cells: the open and closed cell types. The open cell type has extensions at their apex that connect with the lumen, and dendritic processes that extend between adjacent cells, resting on the basal lamina and in close topographical relationship with nerves. It is thought that via a variety of secretory products they form a communication network involved in cell regulation [5-8].

Since NE cells do not contain cytokeratin, commonly found in the basal cell layer and urothelium, it has been suggested that these cells are of a different origin from other prostatic epithelial cells [3, 5, 9, 10]. In a recent study, human prostate NE cells were found to represent a cell lineage of their own, being of neurogenic origin and therefore distinct from the urogenital sinus-derived prostate secretory and basal cells [10]. However, the normal secretory epithelial cell type can differentiate into a NE phenotype in the hyperplastic prostate, and the main NE product, chromogranin A (CgA), is an excellent marker of NE cells and of neuroendocrine differentiated (NED) cells in prostate carcinoma [3]. However, Norlen and coworkers [11] using multiple antisera directed to different regions of rat CgA and to various neuropeptides, observed that individual NE cell types in the rat stomach generate a unique mixture of CgA-derived peptides, probably reflecting cell-specific differences in the post-translational processing of CgA and its peptide products.

Other commonly found secretory products include serotonin (5-HT), neuron-specific enolase (NSE), a thyroid-stimulating-like peptide (TSH), somatostatin (SST), parathyroid hormone-related protein (PTHrP), calcitonin (CT) and other members of the calcitonin gene family, such as calcitonin-gene-related peptide (CGRP) and ketacalcin [12-17]. Neuroendocrine cell products may directly regulate growth, differentiation and homeostasis of the normal and pathological prostate epithelium. The possible autocrine/paracrine regulatory pathways of the prostatic NE cells are illustrated in Figure 1.

As we can see in the figure, the secretory epithelial and neuroendocrine cells may also interact in a paracrine fashion with the stroma [18, 19]. Androgen-dependent tissue growth factors are produced in the stromal cells of the prostate and act on adjacent cells [20-22]. Stromal growth factors include epithelial growth factor (EGF), insulin-like growth factor (IGF), transforming growth factors (TGFα, β), and fibroblast growth factor (FGF). Changes in their relative balance are implicated in the progression of prostate cancer. It appears that, in androgen-independent tumours, autocrine stimulation may become more important and via EGF, could also lead to unrestrained growth [23]. Interestingly, it has been reported that there is an overexpression of EGF receptors in the NE phenotype of tumour cells [24]. The influence of IGF also appears, like EGF, to change because of a move towards autocrine stimulation, with the result that in androgen-independent situations, the cells can proliferate in an uncontrolled manner [25].

As the prostate is under androgen regulation when a tumour develops, it should be possible to control or inhibit its growth by androgen suppression. Androgen ablation of the prostate results in apoptosis in the secre-
androgen independent population emerges and ultimately predominates. This progression to androgen-independence also appears to involve adaptive up regulation of genes that help cells survive and grow after androgen ablation, together with mutation to the androgen receptor and its interaction with other transcriptional factors. Growth factors and NE secreted neuropeptides may activate the androgen receptor by intracellular phosphorylation, and thus contribute to androgen independent growth [26].

Neuroendocrine differentiation

Neuroendocrine tumour cells are found at all stages of prostate cancer and are ‘freely’ dispersed throughout the tumour. Normal NE cells are believed to be terminally differentiated, postmitotic cells [27], and independent groups of researchers have shown that NE cells lack or do not express the androgen receptor [7, 28–30].

Neuroendocrine differentiation in androgen-independent tumours appears to increase when compared to androgen-dependent tumours, as determined by immunohistochemistry [7, 8] and CgA plasma levels [31]. Exogenously added neuropeptides in androgen-independent prostate cancer cell lines have been shown to induce their growth [32], and cancerous epithelial cells may also become more responsive to NE factors by upregulation of receptors for neuropeptides, or may induce NE cells to upregulate the secretion and synthesis of NE factors. In prostate cancer tissue sections, NE cells have been shown to be located in close proximity to proliferating non-neuroendocrine cells, as demonstrated by Ki-67 immunoreactivity [33].

In recent years, new models for NED has been developed, and evidence that cancerous epithelial cells can be induced to differentiate into the NE-phenotype, and that NE-products promote growth, invasiveness and angiogenesis has emerged. In vitro studies have shown that by increasing intracellular levels of cAMP, prostate cancer cells can be induced to become postmitotic NE-differentiated cells, morphologically similar to normal NE cells [34]. Garabedian et al. [35] have developed a transgenic mouse model of metastatic neuroendocrine prostate cancer that is 100% penetrant, metastasizes to lymph nodes and bone and expresses NE-markers, while Perez-Stable and coworkers [36] have developed a transgenic mouse model where androgen-independent prostate tumours develop, expressing both epithelial and NE-phenotypes. Another very exciting in vivo model of NED is the PC-310 human prostate cancer xenograft model developed by Jongsma et al. [37]. The tumour is androgen dependent, but androgen deprivation induces NED. The PC-310 xenograft does not regress completely after androgen withdrawal, and androgen receptor lacking NE cells express different neuropeptides and VEGF. These studies confirm that NED may have a significant impact in androgen independent prostate cancer, and suggest that secretory NE cell products have proliferative effects on surrounding non-neuroendocrine cells.

Neuroendocrine growth regulation of the prostate

A body of evidence suggests that NE cells have paracrine effects on adjacent cells in both normal and malignant prostatic tissue. Several neuroendocrine hormones are known to manifest growth-factor activities, including serotonin, bombesin, calcitonin and PTHrP. A number of them have also been shown to activate or be activated by a number of oncogenes, as well as being functionally related to oncogenes. For example, the biogenic amine serotonin is associated with malignant transformation [38], and bombesin not only stimulates growth in cultured PC-3 and LNCaP cells [39], but also transiently induces the expression of cellular oncogenes such as c-fos and c-myc in quiescent fibroblasts [40]. The tree known receptors that respond to bombesin-like peptides, i.e. gastrin releasing peptide (GRP) receptor, neuropeptide B (NMB) receptor and bombesin receptor subtype 3 (BRS-3), have been detected in situ in the prostate [41, 42]. Bombesin has furthermore been shown to induce invasiveness of PC-3 and LNCaP cells in Matrigel [43], as has GRP and calcitonin-gene-related-peptide (CGRP) in cultured prostate cancer cells [44]. Calcitonin (CT) and kacalcin are other related products to CGRP that are expressed in NE subpopulations of the prostate [45]. The CT level in normal human prostate tissue has been found to be higher than the values reported for numerous other organs, except for the thyroid gland. Interestingly, we have detected and characterised a TSH-like peptide in NE cells but with unknown functions [46].

In situ hybridisation has revealed CT receptors to be present, not on secretory epithelial cells, but on NE cells, a few of them being CT-secreting [47]. CT has a hypocalcemic effect, and in this context, it is noteworthy that CT is able to stimulate the secretion of PTHrP from BEN cells (a cell line derived from human lung tumour) [48]. We have earlier reported PTHrP to be present in normal prostatic NE cells and to be overexpressed in prostate cancer tissue, as demonstrated by immunostaining [49]. Three mRNA splice forms of PTHrP may be generated, and these proteins can further be cleaved into various polypeptides with different biological activities. PTHrP1-34 is known to regulate growth and has hypercalcemic effects, while PTHrP107-141 regulates osteoclast function. Prostate cancer patients often develop bone metastases [50], and malignant epithelial prostate cells can produce IL-6, which may directly or indirectly regulate osteoclasts via stimulation of PTHrP [51, 52]. Both PTHrP and PSA are abundant components of human seminal plasma, and Lilja et al. have shown that PSA proteolytically cleaves PTHrP1-34, disrupting its ability to interact with the PTH/PTHrP receptor and...
thus inhibiting its PTH-like activity [53]. Since PSA is downregulated in CaP, as determined by immunofluorometric procedures [54], it may produce a growth-promoting effect through higher concentrations of uncleaved PTHrP1-34. This may be further accentuated in androgen-ablated patients, since androgen is known to upregulate PSA.

Somatostatin (SST), has been immunohistochemically located in a subset of NE cells, and SST is present in human seminal plasma, in concentrations approximately 200 times greater than in blood plasma [55]. SST has antiproliferative actions in normally dividing cells, e.g. intestinal mucosal cells and immune cells, but also in malignant tumour cells, such as the prostate LNCaP tumour cell line [56]. SST inhibits cell secretion and prevents cell proliferation by inducing cell-cycle arrest and apoptosis. These effects are believed to be directly mediated by somatostatin receptors (SSTRs) on tumour cells, and indirectly by receptors on non-tumour-cell targets which inhibit the secretion of hormones and growth factors involved in promoting tumour cell growth, inhibiting angiogenesis, promoting vasoconstriction and modulating immune cell function. One important growth factor indirectly inhibited by SST is IGF-1, which is downregulated by the hypothalamic-pituitary axis; SST inhibits the secretion of growth hormone (GH), and lowers the serum level of IGF-1 released from the liver, and possibly also IGF-1 locally produced in the prostatic stroma. In the androgen independent state, the influence of IGF-1 seems to be stronger, with the result that the epithelial cells can proliferate in an uncontrolled manner [25]. A remarkable increase in prostatic volume has been noted in acromegalic patients, all with hypersecretion of GH and thus IGF-1. Treatment of these patients with the SST analogue octreotide gives a significant decrease in prostatic volume [57]. Furthermore, transgenic mice expressing human IGF-1 in basal prostate epithelial cells spontaneously develop malignant epithelial growth [58].

The IGF binding proteins (IGFBPs) are important regulators of IGF action in many tissues. It has been shown that androgens may regulate IGFBP-expression. In a study by Gregory et al. [59], IGFBP-5 mRNA decreased by 90% following castration of mice bearing the human prostate cancer xenograft CWR22, while supplying the mice with testosterone reversed the effect. Other groups have shown that IGFBP-3 downregulates in CaP, as compared to normal tissue [60].

In the prostate, secretory epithelial and NE cells express SST receptors in both normal and malignant tissue. Somatostatin may thus not only regulate cancer cell proliferation in the prostate by directly binding to SST-receptors on malignant epithelial cells, but may also inhibit neurotide release from NE cells by auto-crine receptors present on NE cells, and by indirectly lowering IGF-1 levels.

Evidence of this indirect effect of SST on epithelial cells mediated by NE cells includes the fact that SST inhibits the secretion of serotonin in carcinoid neo-plasms [61], that exogenously added IGF-1 to human BON cells induces a marked increase in CgA secretion, and that immunoneutralisation of endogenously released IGF-1 markedly reduces basal CgA release by these neuroendocrine cells [62].

This leads us to the conclusion that if this is also true for NE cells of the prostate, there is a possibility that androgens indirectly may regulate prostatic NE cell secretion and bioavailability of neuropeptides through regulation of IGF by IGF-binding proteins. Furthermore, as we have seen, the androgen regulated PSA proteolytically cleaves PTHrP1-34 and thus should be able to regulate the availability of bioactive PTHrP. Another androgen regulated product that may modulate NE secretary product bioavailability is neutral endopeptidase 24.11 (NEP), a 90–110 kDa zinc-dependent metalloproteinase.

NEP is a cell-surface enzyme expressed by prostatic epithelial cells that cleaves and inactivates neuropeptides. The NEP gene is transcriptionally activated by androgen, and androgen withdrawal results in decreased NEP expression [63]. It has been reported that NEP downregulates in the transition from androgen-dependent CaP to androgen independent CaP [63]. It has also been shown that hypermethylation of the NEP gene promoter, a common phenomenon in prostate cancer, is associated with loss of NEP expression [64].

Examples of NEP substrates are bombesin and endothelin-1 [65–69], and access of these neuropeptides to their cell-surface receptors is negatively regulated by the NEP catalytic domain. The catalytic activity of NEP may thus reduce growth, but also invasiveness and migration. The bombesin-increased motility of PC-3 cells occurs through its receptor, and requires focal adhesion kinase (FAK) tyrosine phosphorylation and active PKC [70]. Sumitomo et al. [71] reported NEP to inhibit FAK phosphorylation and active PKC. The effects of NEP on bombesin and endothelin-1 are not surprising as these neuropeptides induce FAK activation via a cSrc-dependent pathway. Other metalloproteinases are known to be important for increased invasiveness of prostate cancer cells, and have been shown to be upregulated by neuropeptides, e.g. by GRP [72].

Neuroendocrine regulation of angiogenesis and apoptosis

Angiogenesis enables new blood vessels to grow from existing vasculature. This provides the tumour cells with a blood supply enabling them to grow and proliferate. The recruitment process is thought to be triggered in part by activated oncogenes, e.g. EGFR (HER1) and c-erbB2 (HER2/neu). HER1 and HER2 appeared to be colocalised in normal NE cells, and it has also been reported that there is an overexpression of HER1 and HER2 in the neuroendocrine phenotype of tumour cells [24, 73].
Endothelin and bombesin have been shown to transactivate EGF-receptors when activating their own ligand specific receptors [74, 75], and exogenously added ET-1 induces prostate cancer proliferation and enhances the angiogenic effects of such stromal factors as EGF, IGF-1 and FGF [76].

Other blood-vessel recruiting factors are vascular epidermal growth factor (VEGF), tissue growth factor β1 and angiogenin, while angiogenesis is inhibited by thrombospordin, and interferons. The tumour suppressor protein p53 increases transcription of thrombospordin and it is postulated that mutation of the p53 gene may result in a reduced production of thrombospordin and corresponding increased angiogenesis.

The direct involvement of NE cells in angiogenesis has been demonstrated by Grobholz et al. [77], who have shown that there was a significantly higher neovascularisation in high-grade prostate tumours with many NE tumour cells in contrast with high-grade tumours containing few such cells. In archival prostate tumours, VEGF-immunoreactive NE-differentiated tumour cells were significantly correlated with microvessel density [78].

Neuroendocrine products may also decrease neovascularisation. The NE product somatostatin may inhibit angiogenesis directly when SST binds to SSTR-positive blood vessels in the periphery of the tumour, and indirectly by lowering IGF-1 levels, since neovascularisation is enhanced by IGF-1.

Androgen depletion is not usually able to induce apoptosis in all the prostate cancer cells and finally an androgen-independent population starts to proliferate. This might be further accentuated in NED, since it has been found that malignant epithelial cells in close proximity to NE-cells express the antiapoptotic protein Bcl-2, while normal NE cells and NE-differentiated epithelial cells express the protein from a newly found gene encoding another apoptosis inhibitor, designated survivin [79]. NE cells and NE differentiated cells should thus be more resistant to apoptosis, and may also confer antiapoptotic capabilities to surrounding non-neuroendocrine cells.

Somatostatin may promote apoptosis through its subtype 3 receptor, which is associated with dephosphorylation of p53 and induction of the apoptotic promoting protein Bax, while the other four SSTR subtypes are coupled to responses that lead to growth inhibition.

Somatostatin agonists may thus suppress angiogenesis and proliferation, and simultaneously promote apoptosis. It is clear, therefore, that SST has an important role in tumour biology, and in the future there may be a potential role for SST analogues in the treatment of prostate cancer. Other new therapeutics are also needed, and a lot of effort and research is taking place in the development of various pharmacological agents that can antagonise oncogenes, and regulate signal transduction, leading to increased apoptosis and inhibition of angiogenesis.

### Novel therapies

We have seen how recent research in molecular biology and pathophysiology of prostate cancer increases our understanding of the neuroendocrine regulatory mechanisms. Hopefully, this will lead to the development of entirely new therapeutic modalities, to help inhibit tumour cell proliferation.

Newly developed selective non-peptide somatostatin agonists may be useful agents in the treatment of prostate cancer [80, 81], and SST analogues may also be used to scintigraphically localise tumours during surgery. The development of new SST analogues is an important advance, since it could help shed further light on the physiological functions of the different receptor subtypes, and could be the starting point for the development of orally active therapeutic compounds with selective action on specific somatostatin receptor subtypes. Peptide antagonists have also been discovered for SSTR2 and SSTR5 receptor subtypes.

Closely related to SST therapy would be treatment with GHRH antagonists. In vivo studies suggest that such antagonists inhibit the growth of prostatic cancers, in part by a direct suppression of IGF-1 [82]. Serotonin is a well-known neurotransmitter produced by a majority of prostatic NE cells and present in large amounts in the normal and neoplastic prostate alike. Serotonin subtype-specific receptor antagonists have recently been shown to inhibit the growth of a human prostatic carcinoma cell line [83], suggesting that these serotonin-receptor antagonists might prove useful in the treatment of prostate cancer.

Another antagonist that may be effective in treating CaP is bombesin-like antagonists, since bombesin induces growth and invasiveness of malignant prostate cells.

Potential mechanisms of neuroendocrine antitumour action thus include suppression trophic factors and promotion of inhibitory signals, involving autocrine/paracrine mechanisms of the NE cell.

### Conclusions

A great deal of progress has been made towards understanding the development and progression of prostate cancer and the factors which drive the development of androgen independence.

The fact that most cancers eventually produce androgen-independent clones highlights the fact that the genetic changes leading to unrestricted growth may not be uniform in the primary tumour and that this characteristic will result in phenotypically distinct cells with different cellular capabilities, with their own characteristic response to the microenvironment. In the hormone refractory state, neuropeptides and their intracellular signals may contribute to activation of the androgen receptor. Androgen ablation may also lead to downregulation of NEP and PSA, with concomitant increased bioactive levels of NE products, not to mention...
the increased number of NE-differentiated cells so common in this stage of prostate cancer.

Another mechanism which might cause cancerous epithelial cells to become more responsive to neuroendocrine factors involves the upregulation of receptors for neuropeptides, or the induction of NE secreted products.

However, we need to improve our understanding of NE cells, their regulatory products and their influence on the prostate gland in order to develop new therapeutic protocols and trials based on neuroendocrine hormones and their agonists/antagonists.

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