Neuroendocrine cells in tumour growth of the prostate

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Abstract
The prognostic significance of neuroendocrine differentiation in prostatic malignancy is controversial, but the results of recent studies with markers such as chromogranin A and neurone-specific enolase suggest that neuroendocrine differentiation, as reflected by increased tissue expression or blood concentrations of these neuroendocrine secretory products, is associated with a poor prognosis, tumour progression, and androgen independence. As all malignant neuroendocrine cells are devoid of androgen receptors and the expression of neuroendocrine cells is not suppressed by androgen ablation, clonal propagation of androgen receptor-negative neuroendocrine cells may have an important role in the development of androgen-independent prostatic carcinoma. This has significant implications for the treatment of prostate cancer, because several of the hormones that are secreted by neuroendocrine differentiated, malignant prostatic cells are potential candidates for use in drug treatment. A limited number of hormones have been tested in this context, in particular somatostatin, bombesin, and serotonin. As there is currently no successful treatment for differentiated prostate cancer, new therapeutic procedures and trials need to be developed to test drugs based on neuroendocrine hormones or their antagonists.

Introduction
Diseases of the prostate have a pervasive impact on the health of the male population, as illustrated by the fact that prostatic carcinoma is the most common malignant disease among men in the Western world, and benign prostatic hyperplasia is the most prevalent benign disease (Isaacs 1990, Murphy et al. 1997). It is estimated that, in Western countries, a man has approximately a 10% chance of developing prostate cancer and a 3-4% chance of dying of causes directly related to carcinoma of the prostate (Murphy et al. 1997). The annual incidence of prostate cancer continues to increase, and this type of cancer now accounts for approximately 28% of all cancers in American men, compared with 18% in 1980 (Murphy et al. 1997). Moreover, prostate cancer is the most common cause of cancer-related death among men in northern Europe, and the second most common cause in the United States (Murphy et al. 1997). Prostate cancer is responsible for the rapid increase in the incidence of all malignancies in older men, and the respective death rate is predicted to increase by a further 50% over the next 50 years (Chiarado 1991). In view of the profound impact of prostate cancer on the healthcare sector, our current understanding of the factor(s) responsible for the development of benign or malignant neoplastic changes in the prostate is surprisingly rudimentary.

The widely accepted concept of the human prostate as an organ that is dependent on androgens for its normal development and function has been corroborated over the years by numerous investigators. Thus the identification of androgens and androgen receptors, and of their effects on prostate physiology and biochemistry, have been central themes in prostate research for more than 50 years. Different modes of surgical and drug-related androgen ablation treatments have been developed. However, it has also become apparent that, as an accessory male sex organ, the prostate is not dependent exclusively on androgens, but also on additional factors of paramount importance in maintaining normal prostate function and for the development of various pathological conditions. Currently, there is increased awareness of the vital but complex regulatory function of cell-to-cell communication at the local prostatic level (Davies & Eaton 1991). A recent study by Harper et al. (1998) indicated a high degree of similarity in the expression of androgen receptors and growth factors in high-grade prostatic intraepithelial neoplasia (PIN) and prostatic carcinoma, indicating that the former is a premalignant lesion. Both cell types have a high level of expression of membranous epidermal growth...
factor receptors, c-erbB-2 and cytoplasmic transforming growth factor (TGF)-α and lower expression of fibroblast growth factor-2 compared with low-grade PIN or benign prostatic hypertrophy cells. In addition, there is marked cellular proliferation in high-grade PIN, which is not restricted to the basal layer. In contrast, atypical adenomatous hyperplasia (AAH) lesions show a pattern of growth factors and receptors that reflects a quiescent phenotype: virtually no proliferating cells, markedly reduced levels of androgen receptor and E-cadherin expression and no growth factors or receptors, except for fibroblast growth factor-2.

The importance of peptide hormones, growth factors, autocrine–paracrine regulatory loops, and stromal–epithelial interactions is now widely recognized. In this context, a type of cell commonly referred to as the neuroendocrine cell has attracted attention because of the intriguing link between neuroendocrine cell differentiation and tumour progression in prostate cancer. This paper describes the current state of knowledge of the normal prostatic neuroendocrine cell and neuroendocrine differentiation in prostatic carcinoma and the associated clinical implications in terms of prognosis and treatment.

Historical background
The first description of the neuroendocrine cell, by Pretl more than 50 years ago (Pretl 1944), was followed by Feyrter’s concept of the ‘Helle-Zellen System’, or a diffuse neuroendocrine system (Feyrter 1951). Pease (1969) developed this concept further as the amine precursor uptake and decarboxylation (APUD) system. Neuroendocrine cells have been described and extensively studied in a number of organ systems, including the lungs, gastrointestinal tract, and the pancreas. Most of our present knowledge of neuroendocrine cells derives from these studies, but rapidly growing interest over the past decade has generated specific information about the structure and function of prostatic neuroendocrine cells.

General characteristics of prostatic neuroendocrine cells
Very little is known about the presence of neuroendocrine cells in the developing prostate. They are present in all regions of the prostate at birth, but rapidly disappear from the peripheral regions after birth and then reappear at puberty (Cohen et al. 1993). After puberty, the number of neuroendocrine cells seems to increase until an apparently optimum level is reached, which persists between the ages of 25 and 54 years (Battaglia et al. 1994). Neuroendocrine cells are widely distributed throughout the prostate, but tend to be more abundant in the major ducts and less abundant in acinar tissue (Figs 1 and 2).

On the basis of morphology, two types of prostatic neuroendocrine cells can be distinguished: the ‘open’ cell type that is characterized by its open, flask-shaped form, with long slender extensions reaching the lumen; and the ‘closed’ type that lacks luminal extensions (Fig. 1). Both cell types, but particularly the closed type, have a complex appearance with irregular dendrite-like processes extending underneath and between adjacent epithelial cells (di Sant’Agnese & de Mesy Jensen 1984b, di Sant’Agnese 1992a). Neuroendocrine cells are further characterized by the presence of cytoplasmic dense core granules and ultrastructural studies have shown that these constitutive neuroendocrine organelles manifest marked heterogeneity in size and form, suggesting the existence of a number of cell-type variants (di Sant’Agnese 1992a).

Dense core granules are a characteristic feature of endocrine cells and are involved in the mechanism responsible for the storage and secretion of a variety of endogenously active substances. Some proteolytic processing of peptide hormones is believed to occur in these granules. The intracellular sorting and packaging of different substances destined for export is highly regulated, although little is yet known about these processes (Bean et al. 1994). An identifying ‘tag’ necessary for correct sorting and processing has been found for some substances, but in most cases the sorting signals, if present, have yet to be identified. The extracellular release of substances occurs either constitutively or in response to incoming stimuli. Within one cell, some granules may be released constitutively,
whereas others are released in response to certain stimuli. It is known from studies of neurones that dense core granules may contain either a single hormone or several hormones. The variation in granule structure may thus reflect differences in the secretory products they contain, which in turn further illustrates the complex nature of the prostatic neuroendocrine cell.

**Histogenesis of neuroendocrine prostatic cells**

A number of different hypotheses have been put forward concerning the histogenesis of neuroendocrine cells. Originally, it was believed that neuroendocrine cells in general, being part of the diffuse APUD system (Pearse 1969), derived from migratory neural crest ectoderm (Pearse & Pollak 1971). However, findings for the neuroendocrine cells of the intestine, gut, and pancreas are inconsistent with this concept (Andrew *et al.* 1983, Tutton & Barkla 1987). In these organ systems, both neuroendocrine cells and columnar epithelial cells are believed to originate from a common endodermal pluripotent stem cell. Several groups of investigators have suggested that there is a relationship between prostatic basal cells and secretory epithelial cells (Merchant *et al.* 1983, Cussenot *et al.* 1994). Recent findings suggest that all three cell types comprising the prostatic epithelium (epithelial, basal, and neuroendocrine cells) have a common endodermal origin. Using immunohistochemical techniques, Bonkhoff and coworkers (1994) detected subsets of basal cells that expressed both basal-cell-specific cytokeratins and prostate-specific antigen (PSA), which implies that there are cells in the intermediate stages of differentiation between basal and secretory epithelial cell types. Moreover, neuroendocrine cells of the closed cell type were found to express both chromogranin A and basal-cell-specific cytokeratins. This study demonstrated the high degree of phenotypic plasticity of basal cells.
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(Bonkhoff et al. 1994). The stem cell model, as defined in a more recent paper by Bonkhoff & Remmerber (1996), distinguishes three compartments in the prostatic epithelium (Fig. 1): (i) the stem cell compartment, which is androgen-independent and androgen non-responsive; (ii) the proliferation compartment of androgen-independent but androgen-(or oestrogen-) responsive cells; (iii) the differentiation compartment derived from committed basal cells, which give rise to androgen-independent neuroendocrine cells, androgen-responsive basal cells, and androgen-dependent secretory cells.

Recently, in a collaborative effort between Aumüller and coworkers and my own group of researchers (Aumüller et al. 1999), we presented the first evidence that prostatic neuroendocrine cells are of neurogenic origin. We were able to demonstrate that prostatic neuroendocrine cells follow a certain migration pattern that starts in an early embryonic phase. Periprostatic paraganglia, which derive from the neural crest and display strong chromogranin A-immunoreactivity, concentrate their cells around the urogenital mesenchyme by the end of the 8th week. Later, paraganglionic, neural-crest-derived cells pass through the mesenchyme, eventually reaching the urogenital epithelium (Aumüller et al. 1999). In view of our finding of the neurogenic origin of prostatic neuroendocrine cells, the derivation of neuroendocrine cells from undifferentiated basal cells is difficult to interpret, especially because several recent findings suggest that normal neuroendocrine cells are fully differentiated postmitotic cells. From a purely morphological point of view, and for physical reasons, cells with long processes, such as neurones, are unlikely to divide. Bang and colleagues (1994) showed that terminal neuroendocrine differentiation of two prostate cancer cell lines (PC-3-M and LnCaP) could be induced by increasing the level of intracellular cAMP. In addition to an increased expression of neuroendocrine markers in response to cAMP treatment, there was a decrease in epithelial markers, together with G1 synchronization and growth arrest (Bang et al. 1994). In an immunohistochemical study, Bonkhoff and colleagues (1995a) showed that neuroendocrine cells, defined as cells that possess chromogranin A immuno-reactivity, consistently lacked immunoreactivity for the proliferation-associated MIB-1 antigen, indicating that these cells were arrested in G0. Thus, although there is no generally accepted theory of the cellular origin of prostatic neuroendocrine cells, there is convincing evidence that these cells are of neurogenic origin.

**Phenotypes and functions of neuroendocrine prostatic cells**

Originally, neuroendocrine cells were detected by virtue of their argentaffin/argyrophilic properties (Grasso 1952, Kazzaz 1974, Pollice et al. 1979), but since the advent of immunohistochemical techniques based on validated monospecific antisera, a number of bioactive secretory products have been identified. The predominant product of prostatic neuroendocrine cells is chromogranin A, a member of a family of acidic secretory proteins found in the secretory granules of a wide variety of endocrine cells and neurones, where it is stored together with many different peptide hormones and neuropeptides. The function of chromogranin A remains an open question (Huttner et al. 1991). It may have extracellular bioactivity, or act as an autocrine or paracrine regulatory agent in secretory processes. Consistent with the latter view, pancreastatin, which constitutes a part of the chromogranin A precursor protein, has been shown to inhibit both glucose-induced insulin release from the isolated pancreas (Huttner et al. 1991) and regulated protein secretion from certain cells (Russell et al. 1994). Moreover, chromogranin A may be involved in the packaging of peptides into secretory granules (Huttner et al. 1991). Finally, it may modulate peptide hormone processing, as it has multiple dibasic sites that possibly serve as competitive substrates for proteolytic enzymes (Huttner et al. 1991). Other members of the granin protein family, namely chromogranin B and secretogranin II, are also found in populations of prostatic neuroendocrine cells (Schmid et al. 1994). Another secretory product commonly associated with prostatic neuroendocrine cells is serotonin (5-HT) (Fig. 2), a biogenic amine derived from the amino acid tryptophan (di Sant’Agnese & De Mesy Jensen 1985, Abrahamsson et al. 1986, Davis 1987). Serotonin mediates diverse functions by binding to multiple receptor subtypes (Zifa & Fillion 1992). For example, it may have growth factor activity (Tutton & Barkla 1987, Seuwen & Pouyssegur 1990) and be involved in the regulation of morphogenesis (Lauder et al. 1988), and it regulates the secretion of peptide hormones from endocrine cells (Tinajero et al. 1992). Finally, neurone-specific enolase (NSE) is often expressed by prostatic neuroendocrine cells and is sometimes referred to as a marker of neuroendocrine differentiation. This enzyme is an isoenzyme of the glycolytic enzyme, enolase, and is composed of a γγ homodimer with a molecular weight of approximately 46 kDa. NSE is also widely distributed in neurones and their processes (Schnechel 1985, Carlei & Polak 1986).

In addition to the chromogranins, serotonin, and NSE expressed by the vast majority of neuroendocrine cells, the presence of a number of other hormones has been demonstrated in different subsets of prostatic neuroendocrine cells (di Sant’Agnese 1992a). A number of neuroendocrine cells produce calcitonin (Abrahamsson et al. 1986, di Sant’Agnese 1986, Fetissof et al. 1986), and calcitonin binding sites have been found in the plasma...
membrane fraction of prostate tissue. Calcitonin receptor expression appears to be located in subsets of dispersed neuroendocrine cells, of both the calcitonin-secreting and non-secreting type, indicating that calcitonin may have an autocrine/paracrine regulatory function in the prostatic neuroendocrine system (Wu et al. 1996). In addition, co-expression with other members of the calcitonin gene family, such as calcitonin gene-related peptide (CGRP) and katacalcin, has been demonstrated (di Sant’Agnese & de Mesy Jensen 1989). A similar dual expression has also been demonstrated for C-cells of the thyroid (di Sant’Agnese & de Mesy Jensen 1989). Gastrin-releasing peptide, a mammalian homologue of bombesin, is detected in small numbers of neuroendocrine cells (di Sant’Agnese 1986, Sunday et al. 1988). Also reported are cells immunoreactive for somatostatin (di Sant’Agnese & de Mesy Jensen 1984a, Sasaki & Yoshinaga 1989), α-human chorionic gonadotropin (α-hCG) (Fetissof et al. 1987), a thyroid-stimulating hormone (TSH)-like peptide (Abrahamsson et al. 1986), parathyroid hormone-related protein (PTHrP) (Iwamura et al. 1994c), and cholecystokinin (Cecio et al. 1993). The TSH-like peptide has been characterized as manifesting partial homology with the β-chain of TSH (Abrahamsson & Lilja 1989). Taken together, these findings suggest that there are several populations of prostatic neuroendocrine cells, each with its own set of secretory products. This view may have profound implications for both the diagnosis and the treatment of prostate cancer.

The function of neuroendocrine cells in the prostate is still virtually unknown. However, given their heterogeneous morphology and the diversity of their secreted products, these cells probably exert a number of effects. By analogy with what is known about neuroendocrine cells in the respiratory and gastrointestinal systems, and in the pancreas (Larsson 1980, Cutz 1982, Grube 1986, Yamada 1987, Bishop & Polak 1990, Speirs et al. 1993), it would appear that neuroendocrine cells are essential for both growth and differentiation, and for the homeostatic regulation of the secretory processes in mature prostate glands. This view derives further support from several sources:
Neuroendocrine differentiation in prostate cancer

Neuroendocrine differentiation appears to be more common in prostatic carcinoma than in carcinomas arising in other organs of the male (or female) urogenital tract (di Sant’Agnese 1992a, Abrahamsson & di Sant’Agnese 1993). This may be explained by the fact that the prostate gland has the largest population of neuroendocrine cells of any organ in either the male or female urogenital tract (di Sant’Agnese 1993). Neuroendocrine differentiation is a common feature of prostatic adenocarcinomas, occurring in 30-100% of tumours studied (Fig. 3) (di Sant’Agnese 1992a, b, Abrahamsson & di Sant’Agnese 1993). The discrepancies in prevalence reported in different studies are difficult to explain, but may be attributable to a number of factors such as sample type (e.g., biopsy or prostatectomy specimen), type and extent of fixation, and the antibody used. Neuroendocrine differentiation is usually determined in terms of immunoreactivity for certain neuroendocrine markers, such as NSE (Fig. 3), chromogranin A, or eutopic bioactive hormones (serotonin, somatostatin, etc.) (di Sant’Agnese 1992a, b). Ectopic peptides, including adrenocorticotrophic hormone, leu-enkephalin, and β-endorphin, have been detected in some tumours (di Sant’Agnese 1992a, b). The abnormal secretion of these ectopic substances is believed to account for the paraneoplastic syndromes that are occasionally found in association with prostate cancer. Di Sant’Agnese (1992a, b) has published extensive reviews of the literature on neuroendocrine differentiation in prostatic carcinoma.

Neuroendocrine differentiation as an indicator of poor prognosis

Tissue markers

Neuroendocrine differentiation in tumours is often characterized by scattered clusters of differentiated neuroendocrine cells among a predominant population of non-neuroendocrine malignant cells, except for rare cases of total neuroendocrine differentiation of prostatic carcinomas – that is, small-cell carcinomas or carcinoid tumours. The latter two forms of malignancy usually have an aggressive course (Almagro et al. 1986, Dauge & Delmas 1986, Tetu et al. 1987), although focal neuroendocrine differentiation of an adenocarcinoma is also characterized by a poor prognosis, as reported in studies prior to 1990 (Dauge & Delmas 1986, Abrahamsson et al. 1987, 1989, Cohen et al. 1990). One explanation of the poor prognosis associated with neuroendocrine differentiation is probably related to its manifest correlation with tumour grade (Dauge & Delmas 1986, Abrahamsson et al. 1987, 1989). Another possibility is that most neuroendocrine differentiated tumours are hormone resistant or are characterized by relapse after hormonal treatment (Dauge & Delmas 1986, Tetu et al. 1987, Abrahamsson et al. 1989, Cohen et al. 1990). In some malignancies, neuroendocrine differentiation has been shown to have certain prognostic value, such as somatostatin in medullary thyroid carcinoma (Modigliani et al. 1990), hCG (Yamagushi et al. 1989), and chromogranin A (Hamada et al. 1992) in colorectal cancer, and PTHrP in breast cancer (Vargas et al. 1992). However, evidence of the prognostic implications of neuroendocrine differentiation in conventional prostatic adenocarcinoma is contradictory, with some earlier studies suggesting that there is a strong correlation between neuroendocrine differentiation and prognosis (Abrahamsson et al. 1989, Cohen et al. 1990, di Sant’Agnese 1993), whereas others have failed to demonstrate such a correlation (de Matteis 1992, Aprikian et al. 1993, Paul et al. 1993). In a South African study, the survival time was dramatically different in subgroups with or without neuroendocrine differentiation (Cohen et al. 1994). However, these results have not been replicated in other studies, which may be because of a combination of selection bias, heterogeneous specimens, and prior treatment with androgen blockade or radiation in most patients.

Studies of the prognostic value of neuroendocrine differentiation in biopsy samples obtained after radical prostatectomy have yielded conflicting results. Several investigators, including my own group of researchers, did not find a correlation between the number of neuroendocrine cells and tumour stage, and neither did they demonstrate a significant correlation between neuroendocrine differentiation and tumour grade or between neuroendocrine differentiation and prognosis (Aprikian et al. 1993, Cohen et al. 1994, Noordzij et al. 1995, Bubendorf et al. 1996, Abrahamsson et al. 1998). In contrast, Weinstein et al. (1996) found, in their series of 104 patients, that neuroendocrine differentiation in prostate cancer improved the prediction of progression after radical prostatectomy, but only if the analysis was restricted to the 59 Gleason grade 5 and 6 tumours. In a recent study by McWilliam and coworkers (1997), neuroendocrine differentiation was significantly
correlated with worsening tumour differentiation, the presence of bone metastases and poorer survival. However, no independent effect of neuroendocrine differentiation on survival was found (McWilliam et al. 1997). In other words, tumour grade and the presence of metastases correlated significantly with neuroendocrine differentiation and survival, but a multivariate analysis showed no independent effect of neuroendocrine status on survival.

The biological significance of neuroendocrine tumour cells in prostatic adenocarcinoma is not minimized by the absence of a significant correlation between neuroendocrine differentiation and disease progression (Aprikian et al. 1993, Cohen et al. 1994, Noordzij et al. 1995, Bubendorf et al. 1996, Abrahamsson et al. 1998). Many explanations can be advanced as to why not all studies demonstrated the prognostic significance of neuroendocrine differentiation. The most important is that different cohorts of patients were studied. Other explanations include methodological differences in determining the presence of malignant neuroendocrine cells or differences in interpretation. The present methods for analysis of neuroendocrine differentiation are semiquantitative and standards need to be set using precise tissue imaging techniques to obtain consistency in interpretation. Finally, the rather unequal distribution of neuroendocrine cells in most tumours may cause serious sampling errors if biopsy specimens, or limited tissue samples, are studied (Abrahamsson et al. 1987, 1989, Aprikian et al. 1993, Noordzij et al. 1995).

**Serum markers**

The measurement of neuroendocrine markers in the blood of patients with prostatic adenocarcinoma certainly constitutes a more representative indicator and a more objective measure of the neuroendocrine differentiation of tumours, because measurements reflect the entire primary tumour cell population and its associated metastases. The first studies of serum concentrations of NSE and chromogranin A in patients with prostatic adenocarcinoma suggested that neuroendocrine differentiation, as reflected by an increase in the serum concentrations of these neuroendocrine secretory products, correlated with androgen independence and poor prognosis (Kadmon et al. 1991, Tarle & Rados 1991). Moreover, the neuroendocrine differentiation of prostatic adenocarcinoma is not suppressed by androgen ablation treatment, in concordance with the results of histological studies with chromogranin A as a neuroendocrine marker (Abrahamsson et al. 1989, Aprikian et al. 1993). A study by Deftos et al. (1996), with serum measurements of chromogranin A in 82 patients with various stages of prostatic adenocarcinoma, and a similar report by Kimura et al. (1997), confirmed the first observations that clearly demonstrated chromogranin A to be a useful serum marker, especially in advanced disease (Kadmon et al. 1991, Tarle & Rados 1991). Among 135 patients with prostate cancer, Cussenot and coworkers (1996) detected increased serum concentrations of chromogranin A and NSE in 23 (17%) and 20 (15%), respectively, before any endocrine treatment, and they speculated that neuroendocrine products may be involved in the progression of prostate cancer independently of androgen withdrawal. However, increased serum concentrations of chromogranin A were more consistently found in patients with androgen-insensitive tumours (Cussenot et al. 1996), in agreement with a report by Hoosein et al. (1995). Both studies showed a correlation between neuroendocrine serum markers and distant metastases, but not between serum markers and locally progressive disease (Hoosein et al. 1995, Cussenot et al. 1996). In a recent study, the number of chromogranin A-positive neuroendocrine tumour cells was found to be correlated with the serum chromogranin A concentration (Angelsen et al. 1997a), supporting the results of an earlier study (Deftos et al. 1996). In a 2-year follow-up study, serum concentrations of chromogranin A, pancreastatin, a breakdown product of chromogranin A, chromogranin B, NSE, and PSA were determined in 22 patients with prostatic adenocarcinoma (Angelsen et al. 1997b). Interestingly, only chromogranin B concentrations showed a statistically significant increase, which may be due to a combination of an increased number of neuroendocrine cells in the tumour (Abrahamsson et al. 1989) and an increased production of chromogranin B in the neuroendocrine cancer cells (Schmich et al. 1994). Thus, not neuroendocrine tissue but, rather, serum markers of the chromogranin family of peptides are promising prognostic markers in prostate cancer. Future studies should confirm, and more accurately define, prognostic markers of neuroendocrine differentiation during the course of the disease.

**Neuroendocrine differentiation and tumour progression**

Neuroendocrine differentiation may be involved in tumour progression in prostate adenocarcinoma. On the basis of a somewhat oversimplified concept of prostatic tumorigenesis, as a progression from normal prostate to PIN to adenocarcinoma to small-cell carcinoma, it could be suggested that neuroendocrine differentiation is part of the oncogenic process. A major issue is whether neuroendocrine cells present in malignant lesions are phenotypically identical to neuroendocrine cells in the normal epithelium. These ‘specialized’ transformed cells differ morphologically from normal neuroendocrine cells in that they usually lack the characteristic cellular processes. Instead, they often have a morphology that is characteristic of cancer cells (Fig. 3). The dual expression of epithelial characteristics, such as prostatic acid
phosphatase (Cohen & Glezerson 1992) or PSA (Aprikian et al. 1993, Bonkhoff & Remberger 1996), and neuroendocrine markers, such as chromogranin A, has been demonstrated in certain cancer cells. Moreover, in a human small-cell carcinoma cell line (UCRU-PR-2), amphicrine cells express a mixture of glandular and neuroendocrine features (Jelbart et al. 1989). Thus the malignant, neuroendocrine differentiated cell should be distinguished from the normal neuroendocrine cell of the prostate.

The progression of prostatic adenocarcinoma is dependent on androgens and, because of this physiological dependence, tumours usually respond well to androgen withdrawal treatment. Antiandrogens or androgen withdrawal induces apoptosis in the prostate, in prostate organ cultures, androgen-dependent prostatic carcinomas, and in rat and human prostate-derived cell lines in vitro or in vivo (Kyprianou et al. 1990, Tenniswood et al. 1992). A prerequisite for the induction of apoptosis in response to androgen withdrawal is the presence of functional androgen receptors. Several specific genes/gene products have been associated with the apoptosis that follows androgen withdrawal. One prominent marker is TRPM-2, which increases within a day after androgen withdrawal (English et al. 1989). Another marker that rapidly responds to androgen withdrawal is TGF-β, and it has been suggested that its induction conveys a paracrine signal for apoptosis (Kyprianou & Isaacs 1989). More recently, it has been shown that several genes are common to the gene programmes induced by effectors of apoptosis in androgen-dependent and androgen-independent prostate cells (Sells et al. 1994). The bcl-2 proto-oncogene prevents apoptosis and is expressed by prostatic basal cells that are known to be androgen independent (Hockenbery et al. 1991). Furthermore, bcl-2 concentrations have been found to increase as prostate cancer becomes androgen independent (McDonnell et al. 1992). Interestingly, there is a proportional relationship between the tissue concentrations of bcl-2 and NSE (that is, neuroendocrine differentiation) in the majority of primary prostate cancers (Segal et al. 1994).

Normal neuroendocrine cells are believed to be terminally differentiated, postmitotic cells (Bonkhoff et al. 1995a). By increasing the intracellular levels of cAMP, prostate cancer cells can be induced to become postmitotic neuroendocrine differentiated cells that are morphologically similar to normal neuroendocrine cells (Bang et al. 1994). However, several prostate cancer cell lines manifest a mixture of neuroendocrine and epithelial features, but still proliferate. In a study of neuroendocrine cells in prostatic carcinoma, Angelsen et al. (1995) observed a mitotic neuroendocrine cell in anaphase. This suggests that neuroendocrine differentiation does not necessarily render prostatic malignant cells postmitotic. Perhaps, because of the origin of the premalignant cell from a pluripotent stem cell, a malignant adenocarcinoma cell can mobilize a mixture of genes normally expressed only in basal cells, epithelial cells, or neuroendocrine cells. A prostatic cancer cell may thus gain selected neuroendocrine traits such as secretion of hormones or alternative modes of regulation, or both. In this context, neuroendocrine differentiation should be understood in terms of cells manifesting an altered, abnormal phenotype such that certain neuroendocrine substances are expressed, and not necessarily the complete repertoire of the normal neuroendocrine phenotype.

There are examples of tumours expressing some neuroendocrine products without concomitant expression of the classic neuroendocrine markers, NSE and chromogranin A. In their study, Zhou and coworkers (1995) evaluated several variables in subgroups of American, Chinese, and Japanese patients. Subgroup differences were found in immunoreactivity for chromogranin A, serotonin, and bombesin. We recently detected PTHrP immunoreactivity in otherwise non-neuroendocrine lesions of prostatic intraepithelial neoplasia, which suggests that this peptide hormone is involved early in cellular transformation (Iwamura et al. 1995). Parathyroid hormone may act locally in an autocrine manner to regulate the growth of prostate cancer cells. Immunodetectable levels of PTHrP have been found in the human prostate cancer cell lines, LNCaP, PC-3 and DU-145, and thymidine uptake in PC-3 and DU-145 cells was stimulated by a synthetic peptide of PTHrP under serum-free and steroid-free conditions. Moreover, PTHrP stimulated DNA synthesis in LNCaP cells in the presence of dihydrotestosterone and the effect was neutralized by the mouse monoclonal antibody, 8B12, against PTHrP (Iwamura et al. 1994a).

The phenotypic shift will generate a cancer cell that is more adaptable to environmental changes, including androgen depletion, because neuroendocrine differentiation follows androgen-independent modes of regulation. This important alteration of the malignant phenotype is reflected by the lack of androgen receptors in neuroendocrine differentiated cells, both normal and malignant (Fig. 4) (Bonkhoff et al. 1993, Krijnen et al. 1993, Nakada et al. 1993). The shift from androgen-dependent to androgen-independent modes of regulation may be gradual, but the rate of the process is probably accelerated in the presence of androgen deficiency. Initially, however, while a functional androgen receptor is still expressed, growth factors may functionally replace dihydrotestosterone and mimic the effects of androgens. Culig and coworkers (1994) evaluated the cellular effects of different growth factors in androgen-independent prostate cancer cells (DU-145 cells) cotransfected with an androgen-inducible chloramphenicol acetyltransferase
reporter gene and an androgen receptor vector. Their study provided evidence that insulin-like growth factor-I, keratinocyte growth factor, and epidermal growth factor (EGF) directly activate the androgen receptor in the absence of androgens. Subsequently, androgen-related signalling may be activated by growth factors in an androgen-depleted environment. Theoretically, the androgen-related signalling may also be activated by biogenic amines. In COS cells transfected with steroid hormone receptors, dihydroxyphenylalanine has been shown to activate several steroid hormone receptors in a ligand-independent fashion (Power et al. 1991).

Neuroendocrine products stimulate tumour growth and cell proliferation in an autocrine – paracrine fashion. As already mentioned, neuroendocrine differentiation in prostatic adenocarcinoma is usually manifest as isolated foci or islands of cells expressing certain neuroendocrine-related products. With increasing neuroendocrine differentiation, these areas increase both in number and in volume. This suggests that a local alteration in the microenvironment induces neuroendocrine differentiation in a subset of cancer cells. This initial change may originate from a single malignant cell that has already adopted a secretory, postmitotic neuroendocrine phenotype, perhaps as a result of oncogene activity. Several findings suggest that neuroendocrine cells have a paracrine effect on adjacent cells in both normal and malignant prostatic tissue. In benign hyperplasia, neuroendocrine cells are located in small, immature hyperplastic nodules consisting of proliferating cells (Cockett et al. 1993). In contrast, large mature nodules show a marked decrease or virtual absence of neuroendocrine cells. Neuroendocrine cells are located in close proximity to proliferating cells, as demonstrated by Ki-67 immunoreactivity (Bonkhoff et al. 1991). Similar results were obtained with antibodies against the proliferation-associated MIB-1 antigen (Bonkhoff et al. 1995a). Bonkhoff and coworkers (1995b) have also recently evaluated the expression of the oestrogen-responsive pS2 gene in benign and malignant prostatic tissue. The expression of this gene was significantly related to malignancy and except for the gastric antrum, most normal tissues did not express the gene. In the normal prostate, the pS2 protein was detected on neuroendocrine cells and adjacent cell types within neuroendocrine foci. Whereas pS2 expression was confined to neuroendocrine differentiation in untreated tumours, carcinomas that relapsed after hormonal treatment manifested increased pS2 immunoreactivity, even in the absence of classic neuroendocrine features. Finally, the expression of bcl-2 proto-oncogene seems to be intimately

Figure 4 Medium-power photomicrography showing a focal area with neuroendocrine differentiation in a case of poorly differentiated prostatic adenocarcinoma. Neuroendocrine tumour cells are stained blue for chromogranin A and the nuclei of the surrounding non-neuroendocrine tumour cells are stained red for the androgen receptor. Note that the neuroendocrine tumour cells do not display the androgen receptor. Original magnification, × 200.
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associated with tumour neuroendocrine cells (Segal et al. 1994). In primary prostatic carcinomas, bcl-2-containing cells were found to be adjacent to single or clustered neuroendocrine cells.

In both normal and malignant prostate tissue, growth is regulated by prostatic neuroendocrine cells by means of hormone secretion. What has previously been said about the regulation and function of the normal prostatic neuroendocrine cell probably also applies to malignant neuroendocrine cells. Several neuroendocrine hormones, including serotonin, bombesin, somatostatin, calcitonin, and PTHrP, are known to manifest growth-promoting activity. A number of these hormones are functionally related to oncogenes, and the biogenic amine, serotonin, is associated with malignant transformation. Introduction of NIH 3T3 cells transfected with the 5-HT1C receptor into nude mice triggered malignant transformation (Julius et al. 1989). On theoretical grounds, serotonin is believed to be related to ras (Tuttun & Barkla 1987). The ras oncoproteins are among the most potent mitogenic polypeptides known, and activating mutations of ras are found in nearly one-third of all human cancers (Lowy & Willumsen 1993). The presence of ras, a guanosine triphosphatase, is a prerequisite for intracellular signal transduction and normally serves to relay mitogenic and developmental signals initiated by cell-surface receptors into the cytoplasm and nucleus (Satoh et al. 1992). More recently, ras has also been shown to be related to serine/threonine kinases by virtue of its function in localizing c-Raf to the plasma membrane, thus acting as a targeting signal for c-Raf (Avruch et al. 1994, Hall 1994). This further emphasizes the paramount importance of ras in the regulation of cell proliferation and differentiation. The induction of PTHrP mRNA by serum, growth factors, and cycloheximide in non-neoplastic cells initiated by cell-surface receptors suggests that the PTHrP gene has features common to a class of immediate early serum-inducible genes (Allinson & Drucker 1992). Some members of this gene family are also induced by oncogenes, and PTHrP was recently identified as a target for ras and src (Li & Drucker 1994). Normal and malignant cells may possess an interesting regulatory feature of malignant transformation

Neuroendocrine differentiation as a feature of malignant transformation

Our findings suggest that, in response to induction and malignant transformation, an epithelial/basal cell may develop dormant neuroendocrine features. During an early phase of cancer progression, only part of the neuroendocrine phenotype is engaged, which may not necessarily reveal itself in the form of neuroendocrine secretory activity (i.e., chromogranin A immuno-
reactivity). More important is that the cell may adopt different modes of regulation, and eventually, or in response to lack of androgens, the malignant cell shifts to an androgen-independent mode of regulation. During progression, the malignant cell may increase its neuroendocrine differentiation, finally becoming a post-mitotic malignant neuroendocrine cell with a morphological appearance resembling that of a non-malignant neuroendocrine cell. The malignant, neuroendocrine differentiated cell should thus be distinguished from the normal neuroendocrine cell of the prostate. This differentiation process may be promoted by factors emanating from normal neuroendocrine cells located in close proximity to the malignant foci, perhaps acting in synergy with products from adjacent malignant cells. Alternatively, part of the neuroendocrine phenotype may be adopted, but the cell retains its ability to proliferate. The proliferation and progression of these malignant cells is then stimulated and sustained by autocrine/paracrine mechanisms. Several neuroendocrine hormones possess growth-promoting activities and may activate, or be activated by, any of a number of oncogenes. Cancer progression is characterized by selective promotion of certain cell clones that are better adapted to environmental demands.

Cancer in general is a consequence of accumulated mutations in crucial regulatory pathways. Dominant, activating mutations occur when there is overexpression or constitutive activity of peptide growth factors, their receptors, intracellular tyrosine kinases, transcription factors, and cyclins. In this context, neuroendocrine hormones and the neuroendocrine differentiated phenotype with its androgen-independent regulation play an important part in the progression of prostate cancer. To complicate the matter further, there may be racial differences in the characteristics of prostate cancer, including neuroendocrine differentiation. Histopathological analyses have suggested that the frequency of poorly differentiated cancers is greater among Chinese patients than among American or Japanese patients (Zhu et al. 1995). Furthermore, marked racial differences in prostatic tissue concentrations of bombesin and serotonin (but interestingly not chromogranin A) have been found, which suggests that neuroendocrine differentiation of prostatic carcinomas is significantly more prevalent in Chinese men than in American or Japanese men. Taking all of the above into consideration, our view of the prostate and, in particular, prostate cancer should be revised. Although the prostate is an androgen-dependent organ, other bioactive components, such as growth factors and peptide hormones, are crucial for its normal development and function. Reassessment is particularly important with respect to treatment policy. Consistent with the traditional concept of the prostate as an androgen-dependent organ, treatment strategies involve different forms of manipulation of the hypothalamic – gonadal axis, such as surgical or chemical castration. This treatment has a palliative effect, although in most cases the cancer relapses, often as an incurable, more aggressive form, and thus new treatment strategies are needed. Our present knowledge of neuroendocrine cells and their products could have important implications for the treatment of hormone-resistant prostate cancer. It remains to be seen whether it will be feasible to inhibit the growth of prostate carcinoma by inhibiting the production of these neuroendocrine products. To date, a number of analogues of the neuroendocrine hormones, somatostatin, bombesin and serotonin, have been studied in vitro and in vivo, with encouraging results. However, clinical results with somatostatin analogues have proved disappointing, with only short-lived responses achieved in combination with androgen depletion.

**Somatostatin analogues**

Somatostatin analogues have been used with varying success, to treat a number of neuroendocrine tumours (Wynik & Bloom 1991, Öberg 1994). The hormone is a general inhibitor of neuroendocrine hormone secretion, and its long-acting analogues are effective in treating tumour-related syndromes. There is also evidence that, under certain circumstances, somatostatin analogues inhibit neuroendocrine tumour growth, resulting in a decrease in tumour size (Reubi 1985, Kvols et al. 1986, Pinski et al. 1993). These analogues also affect prostatic carcinoma in a complex manner, with their effects possibly being mediated by the inhibitory action of somatostatin on the release of pituitary growth hormone and prolactin or, more locally, through interference with endogenous growth factors such as EGF (Schally & Redding 1987, Kadar et al. 1988, Pinski et al. 1993). Somatostatin receptors have been found in rat prostatic cancer cell lines (Kadar 1988, Pinski et al. 1993, 1994), which suggests that somatostatin has a direct effect. Indeed, the somatostatin analogue, RC-160, was found to inhibit the growth of the androgen-independent rat prostatic cancer cell line, Dunning R-3327-AT-1 (Pinski et al. 1994), and xenografts of the human prostate cancer cell line DU-145 in nude mice (Pinski et al. 1993). As somatostatin receptors tend to be expressed by both normal and malignant neuroendocrine cells, somatostatin analogues may be effective in combination with androgen depletion, in treating prostatic carcinomas with neuroendocrine differentiation.

**Bombesin**

Bombesin may prove useful in the treatment of prostate cancer because of its potent mitogenic activity. Willey et...
al. (1984) showed that bombesin and the C-terminal portion of gastrin-releasing peptide increased clonal growth rate and colon-forming efficiency of normal human bronchial cells, in the presence or absence of epidermal growth factor. Bombesin antagonists have been shown to inhibit the growth of a human small-cell carcinoma cell line (NCI-H345) both in vitro and in vivo in nude mouse xenografts (Mahmoud et al. 1991). A monoclonal antibody to bombesin has been used to inhibit the growth of small-cell lung cancer (Cuttitta et al. 1985). This approach was later applied to patients and resulted in a partial inhibition of the actions of bombesin without apparent signs of toxicity (Mobley et al. 1988). The presence of bombesin receptors has been demonstrated in prostatic carcinoma in vitro (Bologna et al. 1989, Reile et al. 1994). The bombesin antagonist, RC-3095, has been shown to inhibit the growth of Dunning R-3327-AT-1 cells (Mahmoud et al. 1991). However, the remission produced by RC-3095 was of short duration, probably as a result of downregulation of the bombesin receptor.

Growth of the prostatic carcinoma cell lines, DU-145 and PC-3, was increased in the presence of bombesin and inhibited when an antibody against bombesin was introduced. Similarly, antibody against bombesin inhibited growth of DU-145 cells transplanted into mice. These results indicate that bombesin has an autocrine role in the growth of human prostate carcinoma (Shimoda 1991).

Serotonin

Serotonin is abundantly present in both normal and neoplastic prostate tissue. Increasing evidence indicates that there are specific receptors for serotonin in prostatic tissue (Hoosein et al. 1993). Moreover, subtype-specific serotonin receptor antagonists have been shown to inhibit the growth of human prostatic carcinoma cell lines (Abdul et al. 1994). The 5-HT1A antagonist, pindobind, inhibited proliferation of the androgen-independent cell line, PC-3, in a dose-dependent manner. These findings suggest that serotonin receptor antagonists might prove useful in the treatment of prostate cancer, especially the hormone-refractory form.

Conclusion

Review of the accumulated knowledge in this field suggests that we need to improve our understanding of the intricate and finely tuned activities of neuroendocrine differentiated cells in normal, hyperplastic, and malignant prostate tissue. New therapeutic procedures and trials need to be developed to test drugs based on neuroendocrine hormones and their antagonists, perhaps in combination with traditional cytotoxic drugs. Evaluation of this new approach to the management of prostatic carcinoma with neuroendocrine differentiation, including hormone-refractory cancer, is a matter of urgency, as these tumours are unresponsive to current modes of treatment.

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