publication (3 years after the workshop), however, may limit its value as an up-to-data reference book.

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Letters

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Vasoactive Intestinal Polypeptide (VIP) and Neuropeptide Tyrosine (NPY) in Prostate Carcinoma

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UP TO 50% of all prostate carcinomas contain neuroendocrine (NE) differentiated cells secreting serotonin and/or regulatory peptides [1]. Abrahamsson and associates were the first to examine systematically the occurrence of NE differentiated cells and their peptide and biogenic amine content both in normal, hyperplastic and neoplastic glands [2]. We report the presence of two neuropeptides not yet reported in prostate carcinoma cells.

Tissue specimens from 20 cases of prostate carcinoma (average age 65 years, range 52–78 years) were obtained from radical prostatectomy operations. They were routinely fixed in neutral 4% phosphate-buffered formaldehyde. Paraffin sections 5 μm thick were adhered to APES (aminopropyltriethoxisilane)-coated glass slides. Haematoxylin and eosin staining was used for routine histopathological examination and tumour grading, according to Mostofi [3].

Tissue sections from representative areas of the peripheral zones of the carcinoma were immunostained for NE markers, i.e. chromogranin A (CgA), synaptophysin, neuron-specific enolase (NSE), and protein gene product 9.5 (PGP-9.5), to screen for NE differentiation, and for a series of neuropeptides using the streptavidin–biotin–peroxidase complex (S-ABC) and the immunogold–silver staining (IGSS) method with silver acetate autometallography [4, 5]. Antigen retrieval techniques were applied using microwave irradiation or autoclaving [5, 6]. Specificity tests also included absorption controls carried out on antibodies to peptides with immunologically related synthetic peptides in concentrations of 10 nM and 100 nM per ml of optimally diluted primary antibody. Analysis of staining results included differentiation between parenchymal cells in apparently normal and hyperplastic areas (as defined by nodularity of the parenchyma), as well as in carcinoma, all of which were sometimes present within the same large tissue section (on average 2–3 cm in diameter).

CgA immunoreactive carcinoma cells were found in 15 cases (75%), NSE in 17 cases (85%), synaptophysin in 6 cases (30%), and PGP-9.5 in 10 cases (50%). Cytoplasmic labelling of scattered, sometimes even numerous, tumour cells was obtained with antibodies to vasoactive intestinal polypeptide (VIP) in 10 cases (50%) and to neuropeptide tyrosine (NPY) in 15 cases (75%). 7 cases expressed both NPY and VIP, sometimes even in the same tumour cells. It was found that most cases expressing either VIP and/or NPY also expressed CgA, PGP-9.5 and NSE, and a few also expressed synaptophysin. However, one VIP-immunoreactive carcinoma did not show any of the broad-spectrum NE markers investigated. In all other peptide-immunoreactive cases, at least one of these general markers was positive.

Other peptide antisera applied, such as peptide histidine methionine (PHM), prepro-vasoactive intestinal polypeptide (prepro-VIP), somatostatin, substance-P, helodermin, helospectin, galanin, bombesin, met-enkephalin, and calcitonin gene-related peptide (CGRP) gave no immunostaining.

CgA-immunoreactive parenchymal NE cells were found in approximately three-quarters of the cases containing hyperplastic and normal areas, and PGP-9.5-immunoreactive NE-differentiated cells were found in one-third of those containing hyperplastic areas, but only in one containing normal glandular areas. NSE-immunoreactive cells were not detected in normal parenchyma, but in 10% of cases containing hyperplastic glandular areas. No synaptophysin immunoreactivity was found. NPY-immunoreactive cells were not present in normal areas, but in one case containing hyperplastic areas. VIP-immunoreactive cells were rarely present in the surrounding normal glandular tissue (one case), but was demonstrated in one-third of cases containing hyperplastic areas.

A number of studies have revealed that NE differentiation in prostate carcinomas occurs frequently [1, 2, 7–9]. In prostate hyperplasia and carcinomas, immunostainings for calcitonin, CGRP, bombesin, somatostatin and thyroid stimulating hormone have been demonstrated [1, 2]. In our study, NPY and VIP cells in prostate carcinoma were detected.

NPY immunoreactive material is usually found in the central and peripheral nervous system in noradrenergic neurons and nerves [12]. There it functions as a vasoconstrictor, increasing the actions of noradrenalin, and this peptide is considered to be a putative neurotransmitter. In the

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prostate, NPY immunoreactivity has been reported in autonomic nerves, but the functions of NPY in the prostate are currently unknown. It has been suggested that NPY in the prostate could act either to modulate sympathetic stimulation of prostate contraction or to modulate the effects of VIP and/or other neuropeptides/transmitters on prostate epithelial cells [10]. In our study, NPY immunoreactive cells were found in epithelial cells, numerous nerve fibres, and carcinoma and hyperplastic epithelial cells. The role of NPY contained in prostate glandular cells is unknown, but may include a control function in hormone secretion, contractility and/or blood flow.

VIP is another peptide usually found in central and peripheral nervous systems, including the male genitalia, and acts as a potent vasodilator [11]. In our study, VIP was found abundantly in autonomic nerves close to the prostate acini and may act on prostate epithelial cells shown to contain VIP receptors. cAMP is produced in response to VIP in the rat prostate gland. The effects of VIP on normal human prostate epithelial cells have not been studied [10]. In addition to nerve fibres, we found VIP immunoreactive carcinoma cells in normal and hyperplastic glandular cells. It cannot be concluded whether the VIP-like peptide demonstrated in the epithelial cells is identical to the VIP found in nerves, or if it is an immunologically related substance.

The production of regulatory peptides by the prostate is a phenomenon which might have diagnostic, prognostic and therapeutic implications for disease of this gland. The findings of VIP and NPY immunoreactive substances in NE differentiated prostate carcinoma cells may be of significance in understanding the possible roles these cells play in hyperplastic and malignant prostate tissue.

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Carboplatin and 5-Fluorouracil in Squamous Cell Carcinoma of the Head and Neck Previously Responding to Cisplatin and 5-Fluorouracil

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SECOND-LINE CHEMOTHERAPY regimens give only sporadic responses in head and neck cancer, while toxicity is generally high due to the poor conditions of these patients. Therefore, outside clinical trials, only supportive care may be a reasonable choice in this advanced setting.

We treated 13 male patients with a second-line chemotherapy regimen consisting of carboplatin, 100 mg/m^2/day in a 30 min i.v. infusion on days 1, 3, 5 and 5-fluorouracil (5-FU), 200 mg/m^2/day i.v. bolus for 5 consecutive days, every 21 days. Patients' characteristics are reported in Table 1. All patients had previously responded to a cisplatin/5-FU combination (minimum cumulative dose of cisplatin for each patient: 400 mg/m^2) given for relapsed disease (4 patients) or in a front-line chemoradiotherapy programme for unresectable disease (9 patients). The median interval from the end of the first-line chemotherapy was 9 months.

Thirty-nine courses were administered (median 3; range 1–4). The tolerance to the carboplatin/5-FU regimen was good, with thrombocytopenia being the most frequent side-effect (5 out of 13 patients). It was severe (grade III–IV) in 4 patients (31%) but no episode of bleeding was recorded and platelet transfusion was not necessary. 3 patients (23%) had grade I–II leucopenia and 2 (15%) grade II anaemia. Non-haematological toxicity was mild and consisted of grade I–II nausea and vomiting in 2 patients (15%) and grade I transitory renal toxicity in 1 patient (serum creatinine 2.2 mg/dl after the second course). Because of haematological toxicity, 13% (5/39) of the chemotherapy courses were delayed by 1 week, 18% (7/39) by 2 weeks.

A partial response was observed in 3/13 patients (23%). Stable disease was obtained in 8/13 patients (62%), while 2/13 (15%) progressed during the treatment. Median overall time to progression was 9 weeks (range 4–43). The duration of response was 21, 34 and 43 weeks, respectively. The

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