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CASE REPORT

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Unusual prostatic adenocarcinoma with endocrine basophilic FSH-immunoreactive cells

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Abstract We report an unusual variant of prostatic adenocarcinoma with marked endocrine differentiation (mixed endocrine-exocrine adenocarcinoma). Endocrine cells accounted for 60% of the tumour cells, were positive with silver impregnation and for chromogranin A, synaptophysin, and neuron-specific enolase, and coexpressed the exocrine antigens prostatic acid phosphatase and prostatic-specific antigen. Most of the endocrine cells were basophilic with haematoxylin-eosin and proved immunoreactive for alpha subunit of human chorionic gonadotropin and follicle-stimulating hormone. The remaining endocrine cells were represented by eosinophilic cells positive for serotonin, and by calcitonin and serotonin-immunoreactive cells not identifiable in haematoxylin-eosin-stained sections. On ultrastructural analysis, two types of endocrine cells were identified. The most frequent cell type showed abundant cytoplasmic round, electron-dense neurosecretory granules, either small (212±44 nm) or large (471±114 nm), resembling those of gonadotropic pituitary cells. The second type of endocrine cells contained irregular electron-dense granules similar to those of serotonin-storing enterochromaffin cells.

Key words Prostate adenocarcinoma · Endocrine cells · Immunohistochemistry · FSH · Electron microscopy

Introduction

Focal endocrine differentiation in prostatic carcinoma is a very frequent, if not ubiquitous, phenomenon, with an incidence which has steadily increased from 10% in the first reports [6] to 100% in more recent reports [1, 3, 8]. However, tumours with marked endocrine differentiation are rather rare and constitute less than 10% of all prostatic cancers [13]. Such tumours are reported to be more aggressive and resistant to hormonal therapy [7, 10].

Endocrine cells are normally not apparent with haematoxylin-eosin and can be identified only by histochemical and immunohistochemical stainings for general endocrine markers and/or specific peptides. In some cases endocrine cells may display bright eosinophilic granularity of the cytoplasm after formalin fixation, strikingly resembling normal intestinal Paneth cells. Such features of endocrine differentiation are reported in 10% of prostatic adenocarcinomas, and particularly in those showing a cribriform growth pattern [2].

In this paper, we report the histological, immunohistochemical and electron microscopic findings recorded in a prostatic adenocarcinoma with pronounced and unusual endocrine differentiation. The endocrine component was largely represented by tumour cells with large basophilic granules, and, to a lesser extent, by dispersed cells with large eosinophilic granules. To our knowledge, this pattern of endocrine differentiation has not previously been described in prostatic adenocarcinoma.

Clinical history

A 58-year-old man with obstructive urinary symptoms was found to have an elevated prostate-specific antigen (PSA) level of 29.7 ng/ml. Rectal examination revealed a palpable tumoral induration in both prostatic lobes, confirmed by the presence of a hyporeflective area measuring 28×10×17 mm on transrectal ultrasonography. A prostate needle biopsy showed a poorly differentiated adenocarcinoma. The patient underwent radical retropubic prostatectomy with bilateral lymphadenectomy. The postsurgical pathological staging was pT4 pN2 Mx, and the Gleason score was 9 (5+4). The patient was treated by pelvic radiotherapy and is currently alive after 32 months of follow-up, albeit with progressive and metastatic disease.

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Materials and methods

The surgical specimen included the prostate, seminal vesicles and regional lymph nodes. The prostate measured 4×5×3.5 cm and weighed 49 g. It was inked and fixed in 10% buffered formalin for 48 h, serially sectioned at 2- to 4-mm intervals and wholly processed. In the lymphadenectomy specimen, 28 lymph nodes were found.

Serial paraffin sections 4 µm thick were cut from all the tissue blocks containing tumour and stained with haematoxylin-eosin (H&E), Alcian blue pH 2.5, periodic acid–Schiff (PAS) reagent, and by Grimelius' silver impregnation. The histological architecture of the tumour was evaluated according to the WHO-Mostofi classification [21] and the tumour pattern, graded according to the Gleason grading system [15]. Prostatic zones involved by the tumour, surgical margin status, seminal vesicle invasion, and number of lymph nodes with metastases were recorded, and extension of the tumour was assessed in agreement with the TNM classification [24]. To assess the mitotic index the tumour was examined in high-power fields (HPF, ×400) and the number of mitoses/10 HPF was counted. The neoplasm was immunohistochemically evaluated with a panel of monoclonal and polyclonal antibodies using a universal streptavidin–biotin complex (L.V. LSAB2 Kit, HRP, Dako, Carpinteria, Calif.) [16]. The primary antibodies included: prostatic-specific antigen (PSA; Dako, Glostrup, Denmark; rabbit, 1:1000), prostatic acid phosphatase (PAP; Dako; rabbit, 1:800), chromogranin A (CgA; Dako; mouse, 1:800), neuron-specific enolase (NSE; Dako; mouse, 1:200), synaptophysin (Syn; Dako; mouse, 1:100), serotonin (Ser; Dako; mouse,1:20), calcitonin (Cal; Dako; rabbit, 1:500), alpha human chorionic gonadotropin (α-hCG; Biogenex Laboratories, San Ramon, Calif.; mouse, 1:80), β-human chorionic gonadotropin (β-hCG; Biogenex; mouse, 1:200), somatostatin (Som; Dako; rabbit, 1:500), parathyroid hormone (PTH; Biogenex; rabbit, undiluted), vasoactive intestinal peptide (VIP; Biogenex; rabbit, undiluted), gastrin (Gas; Dako; rabbit, 1:300), glucagon (Glu; Dako; rabbit, 1:50), thyroid-stimulating hormone (TSH; Biogenex; mouse, undiluted), adrenocorticotrophic hormone (ACTH; Biogenex; rabbit, undiluted), follicle-stimulating hormone (FSH; Dako; mouse, 1:20), luteinising hormone (LH; Serotec, Oxford, England; mouse, 1:100), MIB1 (Biogenex; mouse, 1:20), androgen receptor (AR; Novocastra, Laboratories, Newcastle, UK; mouse, 1:25), bcl2 (Biogenex; mouse, 1:20), and CD34 (Biogenex; mouse, 1:20). The sections for Syn, PTH, Gas, FSH, MIB1, AR and bcl2 were pretreated with microwave irradiation before application of the primary antibody.

To determine the simultaneous expression of MIB1 or AR and CgA in the same cell, double-labelling immunohistochemistry was performed by sequential method using a polyclonal antibody for CgA (Dako; rabbit, 1:300), and two separate enzyme substrate systems (peroxidase and alkaline phosphatase).

Suitable controls were employed to confirm immunoreactivity, and negative controls were performed by omitting the primary antibody. In addition, internal positive controls for NSE (prostatic nerves), PSA, PAP and AR (prostatic epithelium) and CD34 (vessels) were examined.

The percentage of MIB1 (Ki67) or AR-positive cells was determined with a microscope holding a ×100 oil immersion objective by scoring a minimum of 1,000 tumour cells in areas with the highest immunostaining. Perineural and vascular microinvasion were evaluated in sections stained for NSE and CD34, respectively. Neural microinvasion was assessed by the finding of tumour cells in the perineural space or within nerve fibres identified by NSE. Vascular microinvasion was defined as the presence of neoplastic thrombi within CD34-positive endothelium-lined spaces present at the periphery of the tumour.

Material for ultrastructural examination was retrieved from areas of the paraffin blocks which proved rich in endocrine cells in adjacent sections. The specimens were processed in Epon, and semithin sections (0.5–1 µm) were cut and stained with toluidin blue. Areas of interest were trimmed and then sectioned (60–100 nm) with a Sorvall ultramicrotome. Ultrathin sections were counterstained with uranyl acetate and lead citrate and then viewed in a Zeiss EM 103 electron microscope.

Results

Histopathological findings

Microscopic findings in this prostatic cancer were consistent with a primary origin in the peripheral zone. The tumour largely involved both lobes extending beyond the prostate capsule in periprostatic soft tissue, and was invading both seminal vesicles and bladder neck. The prostatic ducts and urethral urothelium were normal.

Histological examination revealed a conventional acinar adenocarcinoma. About 30% of the tumour was composed of infiltrating medium-sized, irregular glands corresponding to Gleason grade 4. The remaining 70% consisted of solid masses or anastomosing cords of columnar and cuboidal cells with only scattered gland lumina or vacuoles. This pattern has been interpreted as Gleason grade 5. The nuclei were generally uniform, small and medium-sized, with fine dispersed chromatin without prominent nucleoli. Mitotic activity was low (<1×10 HPF) and necrosis absent. A rich lymphoid infiltrate composed of mature lymphocytes and a few plasma cells surrounded the tumour mass and was often intimately admixed with the proliferating cells.

At medium power, about 40% of tumour cells displayed a striking granularity homogeneously distributed throughout the cytoplasm. Most of these cells appeared strongly basophilic at H&E and showed abundant cytoplasm with distinct and coarse granules in both supra- and subnuclear locations (Fig. 1). Granular basophilic cells were present either as isolated elements or, more

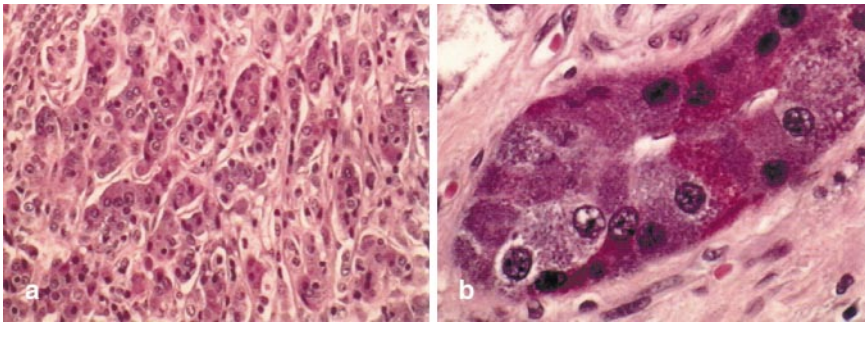

Fig. 1 **a** Prostatic adenocarcinoma with endocrine cells. H&E, ×100 **b** Basophilic and eosinophilic granulated cells in malignant acinus. H&E, ×400

frequently, as solid clusters scattered between exocrine cells. Sometimes chains of endocrine cells lined entire tumour acini. In addition, foci of scattered cells displaying bright eosinophilic granularity were identified as described in an earlier paper [2] in which they were referred to as Paneth-like cells.

Metastases were found in six pelvic lymph nodes. The histological pattern and the distribution of granular cells were similar to those observed in the primary tumour, although one lymph node location showed hardly any cells but basophilic granular cells.

Histochemical findings

Only a few tumour acini were filled with PAS-Alcian blue-positive mucin, and occasional mucinous cytoplasmic vacuoles were rarely seen. The stroma did not contain mucinous pools. Basophilic and eosinophilic cells were negative with the PAS stain and strongly argyrophilic on Grimelius' silver impregnation.

Immunohistochemical findings

The large majority of tumour cells reacted with PAP and PSA antibodies, and the staining was diffusely cytoplasmic, varying from weak to strong. About 60% of the tumour cells were strongly positive for endocrine markers, including CgA, Syn and NSE, and coexpressed PAP and PSA. Comparison of immunohistochemical preparations with consecutive H&E showed that immunoreactivity for endocrine markers was not restricted to granular cells only. However, basophilic and eosinophilic granular cells usually exhibited greater staining intensity.

Most basophilic endocrine cells proved FSH immunoreactive (Fig. 2). Some of these were also positive for α-hCG. By contrast, eosinophilic endocrine cells were generally reactive to Ser antiserum. In addition, immunoreactivities for Cal and Ser were observed in a few scattered cells that were not identifiable in H&E sections. Tests for β-hCG, Som, PTH, VIP, Glu, Gas, TSH, LH and ACTH were all negative.

MIB1 antibody revealed the presence of 3% of Ki67-positive cells with nuclear staining, and in double-labelling immunohistochemistry such cells were negative for CgA. About 15% of tumour cells were positive for AR, but negative for CgA.

No bcl2 staining was observed in tumour cells. No vascular invasion was found in sections stained for CD34, and high density of small vessels was observed in tumour areas rich in endocrine cells. By contrast, perineural microinvasion was easily identified, particularly in the vicinity of the endocrine part of the tumour.

In lymph node metastases, tumour cell immunoreactivity for exocrine and endocrine antigens was similar to that observed in primary tumour.

Ultrastructural findings

At ultrastructural investigation, abundant cytoplasmic endocrine granules were observed (Fig. 3). The round, occasionally haloed, electron-dense granules were either small (212±44 nm) or large (471±114 nm), sometimes showing a coarsely granular, cerebroid core. Granules of different sizes were stored in different cells. In addition, occasional cells showed strongly electron-dense, irregular granules with variable size and shape, similar to typical serotonin-storing enterochromaffin granules. No

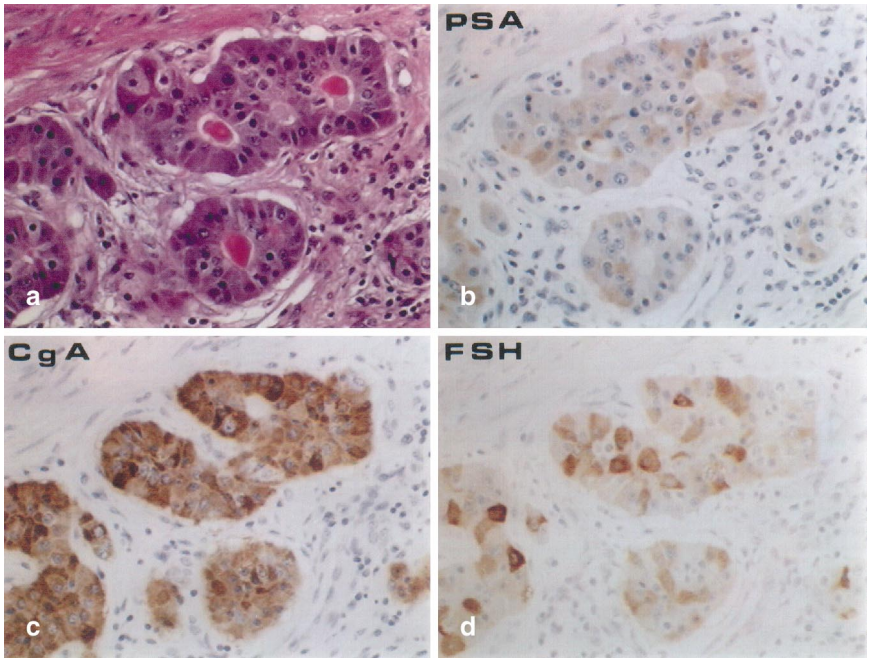

Fig. 2 Immunohistochemical stainings for prostate-specific antigen (PSA), Chromogranin A (CgA) and follicle stimulating hormone (FSH) in consecutive sections of a focus of carcinoma. ×250

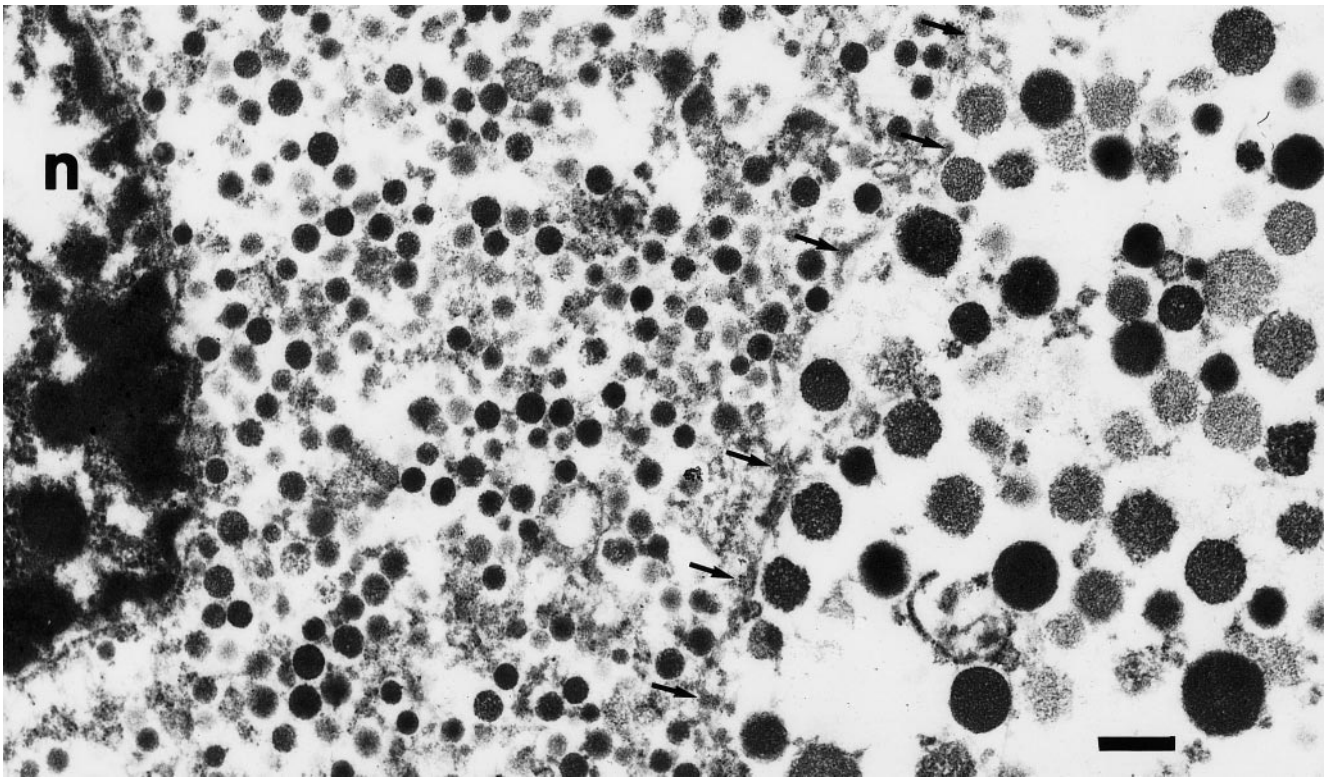

Fig. 3 Ultrastructure of abundant, round, electron-dense granules of small size (*left part* of the micrograph) and of large size (*right part*), belonging to two separate tumour cells. Remnants of the cytoplasm membrane dividing the two tumour cells are indicated by the *small arrows* (*n* nucleus). *Scale bar* 648 nm

further details could be identified owing to the poor conditions of the formalin-fixed, paraffin-retrieved material.

Discussion

Endocrine differentiation is a well-known and rather frequent feature of conventional prostatic adenocarcinoma. Most reports have described tumour cells positive for endocrine markers indistinguishable from exocrine tumour cells [1, 6, 9, 14], or showing large eosinophilic granules referred to as Paneth-like cells [2, 17, 26–28]. The novelty of the current case is the presence, in a prostatic adenocarcinoma, of numerous basophilic and densely granulated cells which represent a light microscopically detectable, unusual, and so far undescribed form of endocrine differentiation, demonstrable by histochemical, immunohistochemical and electron microscopic techniques.

Endocrine cells displaying basophilic granularity of the cytoplasm constitute about 10% of anterior pituitary cells. These cells stain positively with the PAS technique and contain different hormones (FSH, LH, TSH and ACTH). In the present case basophilic endocrine cells were PAS negative, but most of these were immunoreactive for FSH. Some basophilic cells were also immunoreactive to α-hCG. The alpha subunit of hCG is biologically inactive and essentially identical to the alpha subunits of LH, FSH and TSH. In addition, we identified small numbers of two other types of endocrine cells. The first of these corresponded to cells with large eosinophilic granules and showed cytoplasmic immunoreactivity for Ser. The second type with smaller granules is not visible on H&E-stained sections. Such cells were strongly reactive for Ser or Cal.

On ultrastructural analysis, we demonstrated the occurrence of at least two types of endocrine cells. The first of these, which represents the major population, is comparable to gonadotropic cells of the human pituitary in size and shape of the secretory granules and most probably corresponds to the FSH-immunoreactive cells observed at light microscopy. The other endocrine cells closely resembled serotonin-producing enterochromaffin cells of the human intestine and correspond to the type 1 cells described by Capella et al. in tumorous and nontumorous prostates [9]. It is thus apparent that basophilic endocrine cells in prostatic adenocarcinoma share some morphological, immunohistochemical and ultrastructural features similar to those of basophilic adenohypophysis cells. In the available literature, FSH-immunoreactive cells have been described only by Hurkadli et al. [19], who observed a focal positive immunocytochemical localisation of FSH in 27 prostatic carcinomas, using a rabbit antiserum. Whether FSH-IR cells have a functional role in the development and growth of conventional prostatic carcinoma remains to be seen. In contrast, the production of Ser and Cal as eutopic hormones in pros-

tatic carcinoma is widely documented [12–14]. In addition, there is strong evidence that Cal is secreted by serotonin-storing cells of the prostate [12].

Our identification of PSA and PAP immunoreactivities in endocrine cells is similar to observations that have already been described [4, 11]. This finding suggests that endocrine and exocrine cells originate from a common multipotent progenitor cell type. The reported presence of cells with combined intracellular endocrine granules and exocrine mucus droplets (the so-called mixed or amphicrine cells) in prostatic adenocarcinoma [9] supports this view. Pure differentiated small cell neuroendocrine carcinomas of the prostate are notoriously negative for PSA, and it has been suggested that they originate de novo from a putative stem cell, though with a lower degree of differentiation [18].

Examination of regional lymph node metastases revealed the presence of extensive endocrine differentiation mainly with the same pattern as observed in primary tumour. The fact that endocrine tumour cell differentiation is not restricted to the prostatic environment indicates that metastatic tumour cells follow a differentiation programme similar to that observed in the primary tumour. In addition, metastasis composed predominantly of endocrine cells was also observed.

The present tumour showed low proliferative activity, and MIB1 immunoreactivity proved to be almost exclusively restricted to the nonendocrine component. Interestingly, a low Ki67 index is typical of well-differentiated endocrine tumour [20]. This feature, together with the diffuse hormone expression, suggests a high degree of tumour cell differentiation in the present case. Notably, high mitotic rate and/or high Ki67 index and low or absent hormone expression are reported for poorly differentiated neuroendocrine carcinomas arising either in the prostate [18] or in other sites, such as lung [25], pancreas [20] and stomach [22]. Similar to normal prostatic endocrine cells, tumour cells in the present case were negative for nuclear AR. This feature further outlines their high differentiation status and supports the concept that endocrine cancer cells represent an androgen-independent cell population refractory to hormonal therapy [7]. This view indicates that prostatic endocrine cells have a different receptor asset than exocrine cells.

It has been reported that bcl-2 proto-oncogene is expressed in prostatic cancers by nonendocrine cells in the vicinity of endocrine cells, suggesting that this apoptosis-inhibiting proto-oncogene is induced by the paracrine action of endocrine cell products [23]. In our case, this feature has not been found. Interestingly, low bcl-2 has been reported in pure endocrine tumours [5].

In summary, we have described the histopathological, immunohistochemical and electron microscopic findings in a distinct form of endocrine differentiation detectable on routine histological examination in a conventional prostatic carcinoma. Endocrine cells were prevalently represented by basophilic granular cells so far not found in normal prostatic epithelium and showed a peculiar resemblance to basophilic adenohypophysial cells. Such cells were all argyrophilic and expressed CgA, Syn, and NSE; most of them were immunoreactive with FSH.

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