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Case report

Solid-cystic tumour of the pancreas

An endocrine neoplasm?*

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Summary. Immunohistochemical studies and DNA flow-cytometric investigations were performed in a case of solid-cystic tumour of the pancreas in a 35-year-old woman. All tumour cells were immunoreactive for the neuroendocrine cell markers chromogranin A and neuron-specific γ-enolase. Moreover, about 10% of tumour cells were immunoreactive for insulin, while hypoglycaemia was absent. Few tumour cells (less than 1%) were immunoreactive for somatostatin, and no cells were found to be immunoreactive for pancreatic polypeptide or glucagon. No immunoreactivity was present for duct cell marker carcino-embryonic antigen and only individual cells were reactive for α_1-antitrypsin. Nuclear DNA content of the tumour cells was diploid and the proliferative activity was low. In confirmation of some reports on neuroendocrine markers in solid-cystic tumour of the pancreas, our findings support the theory that the lesion is a hormonally inactive neuroendocrine pancreatic tumour.

Key words: Pancreatic tumour – Immunohistochemistry – Flow cytometry

Introduction

Solid-cystic tumour (SCT) of the pancreas (Morohishi et al. 1987), synonymously termed papillary-cystic tumour (Frantz 1959; Cubilla and Fitzgerald 1984) or papillary-cystic neoplasm of the pancreas (Boor and Swanson 1979; Bombi et al. 1984; Learmonth et al. 1985), is an uncommon pancreatic neoplasm of uncertain histogenesis. Although SCT is not included in the WHO classification of exocrine pancreatic tumours (Gibson and Sobin 1978) it is accepted as a clinicopathological entity and is included in new classifications by Cubilla and Fitzgerald (1979) and Morohishi et al. (1983). SCT has a characteristic predilection for young, fertile women.

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* Dedicated to Professor Dr. K. Goerttler on the occasion of his 65th birthday

Based on the presence of zymogen-like granules in electron microscopy (Klöppel et al. 1981) and on preliminary immunocytochemical findings of immunoreactivity with antisera to acinus-cell enzymes amlyase and lipase (Klöppel et al. 1981), its histogenesis was suggested to be from acinus cells and the term "solid-cystic acinus cell tumour" was proposed (Klöppel et al. 1981). However, since this immunoreactivity was not reproducible, the theory of acinus-cell origin was abandoned (Morohishi et al. 1987). Currently, the histogenesis of SCT is open to discussion.

We report a further case of SCT with the immunohistochemical and DNA flow-cytometric features of a benign neuroendocrine tumour.

Case report

A 35-year-old woman was investigated for vague upper abdominal discomfort. At sonography, a large tumour was found in the left upper quadrant. Hormonal syndromes, especially hypoglycaemia, were absent. At surgery, the mass was located in the retroperitoneum. It was separated from the pancreatic tail by a thick fibrous capsule, and its visceral origin was unclear to the surgeon.

Grossly, the tumour measured $12 \times 10 \times 6$ cm. It was encased by a fibrous capsule, including some remnants of exocrine pancreatic acini and endocrine islets on its deep aspect, while the outer capsule consisted of pure fibrous tissue. The cut surface was solid, soft and contained haemorrhagic areas and multiple cysts up to 3 cm in diameter (Fig. 1).

At light microscopy, tumour cells at the periphery showed a solid growth pattern (Fig. 2a) with fine vascular stalks. A definite tumour stroma was lacking. More centrally, microcystic spaces were formed lined by pseudopapillary epithelial formations (Fig. 2b). The nuclei were vesicular, with only slight anisomorphism. The walls of grossly visible cystic spaces were usually lined by fibrous tissue and a tumour-epithelial lining was rare.

Immunohistochemical studies were performed on formalin-fixed paraplast-embedded tissue by means of an indirect streptavidin-peroxidase method. Sources of the antibodies and antisera used are presented in Table 1.

All tumour cells were immunoreactive for the neuroendocrine cell markers neuron-specific γ-enolase (NSE) and chromogranin A (Fig. 3a, b). Immunoreactivity for NSE resulted in a diffuse cytoplasmic staining, which was enhanced in some cells with broader cytoplasm (Fig. 3a). In contrast, immunoreactivity for chromogranin A featured a polar and granular pattern (Fig. 3b). No immunoreactivity was found for synaptophysin.

Table 1. Antibodies used and immunocytochemical findings

Specificity	Immunoreactivity	Antibody	Source
NSE	++++	BBS/NC/VI-H14	Camon (Wiesbaden, FRG)
Chromogranin A	++++	LK2H10	Camon (Wiesbaden, FRG)
Synaptophysin	−	SY38	Boehringer (Mannheim, FRG)
Insulin	++	HP29-5C	BioGenex (San Ramon, USA)
Somatostatin	+	Rabbit	Amersham (Buckinghamshire, England)
Glucagon	−	Rabbit	Cambridge (Massachusetts, USA)
Pancreatic polypeptide	−	Rabbit	Milab (Malmö, Sweden)
Keratin (KL 1)	++++	KL1	Immunotech (Marseille, France)
Carcino-embryonic antigen	−	Rabbit	Dakopatts (Hamburg, FRG)
α_1-Antitrypsin	+	Rabbit	Dakopatts (Hamburg, FRG)
α_1Antichymotrypsin	+	Rabbit	Dakopatts (Hamburg, FRG)

About 10% of the tumour cells were immunoreactive for insulin (Fig. 4), and very few cells (less than 1%) stained for somatostatin. No immunoreaction was obtained for glucagon or pancreatic polypeptide.

Immunostaining for α_1-antitrypsin and α_1-antichymotrypsin revealed a reactivity only in a few individual cells. Carcino-embryonic antigen as a marker of duct cells was negative. All cells were positive for keratin.

DNA flow cytometry was performed from paraffin-embedded tissue by the method described elsewhere (Haag 1980). The DNA-dependent fluorescence was measured by an ICP 22 cytometer (Phywe AG, Göttingen, FRG), using 365 nm excitation.

The histogram is presented in Fig. 5 (the coefficient of variation was 7.14%). The tumour had a normal diploid stem line (DNA index = 1.0), with 95.6% cells in G_0/G_1 phase, 2.4% cells in S-phase (DNA-synthesis phase), and 2.0% in G_2 and M (mitotic) phase. Thus, its proliferative activity was low.

Discussion

The gross and light microscopic features of this tumour correspond to the current criteria for SCT of the pancreas (Klöppel et al. 1981; Cubilla and Fitzgerald 1984; Morohishi et al. 1987). Although the histological appearance of SCT has been described repeatedly as resembling an endocrine tumour (Klöppel and Heitz 1984), the absence of a hormonal syndrome in patients suffering from SCT is a conventional argument against endocrine origin (Morohishi et al. 1987).

Moreover, several studies of peptide immunohistochemistry in SCT (Klöppel et al. 1981; Morrison et al. 1984; Lieber et al. 1987; Morohishi et al. 1987; Ladanya et al. 1987; Ludvik et al. 1989) yielded negative results for the four pancreatic hormones in all cases ($n=27$) but one (Morrison et al. 1984). Since insulin immunoreactivity of that one case was not confirmed by others, it has been questioned repeatedly. However, our observation now confirms this previous report. In addition, we found very few immunoreactive cells for somatostatin, while glucagon or pancreatic polypeptide-cells were absent.

The previous dogma that endocrine tumours must be hormonally active or at least immunoreactive for peptide hormones was only recently abandoned following the detection of neuroendocrine cell specific markers in some tumours including non-functioning metastastic pancreatic islet-cell carcinoma (Sobol et al. 1989). However, this finding has not been considered in the histogenetic discussion of SCT. In one study, the neuroendocrine cell marker NSE (Tapia et al. 1981) was found in 2 of 16 cases of SCT, but the specificity of this reaction (by polyclonal antisera) was questioned (Morohishi et al. 1987). Negative results were also obtained in 3 of 3 cases by Ladanyi et al. (1987). However, the specificity of positive NSE-reaction was subsequently verified by immunoblotting (Chott et al. 1987). Consequently,

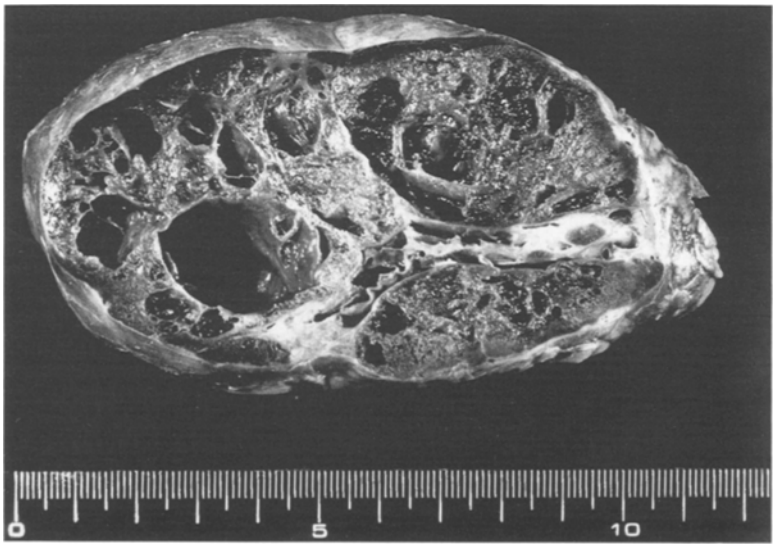
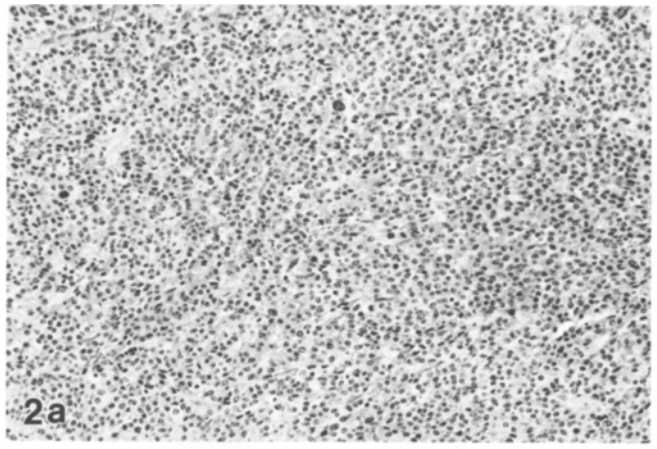
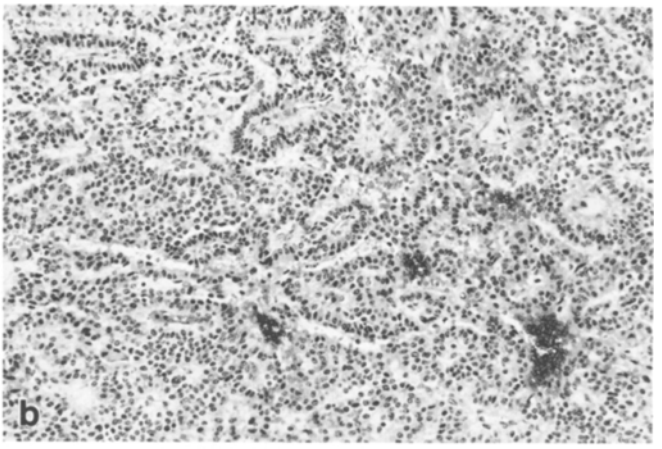
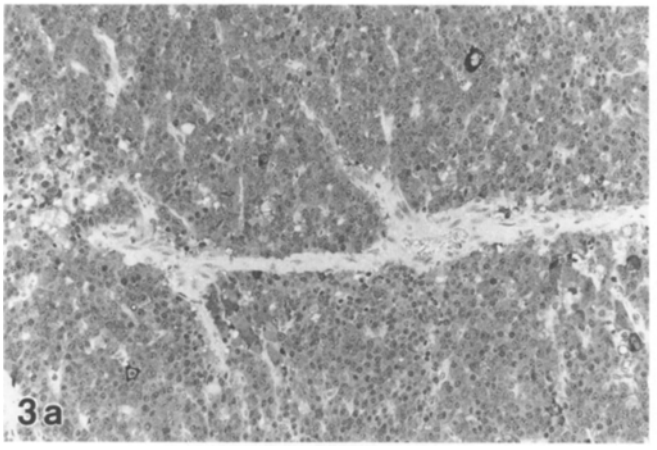
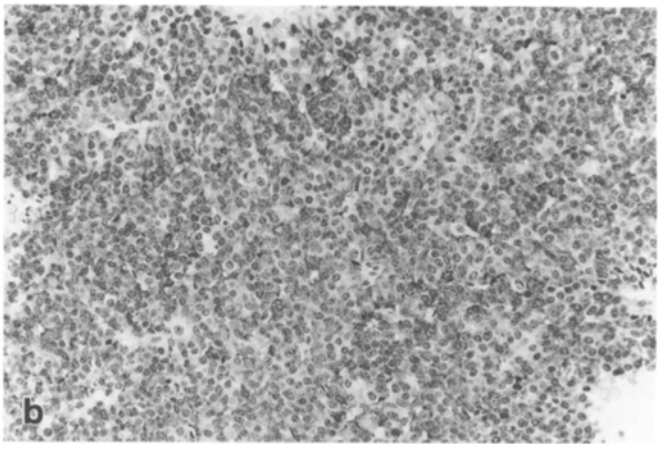


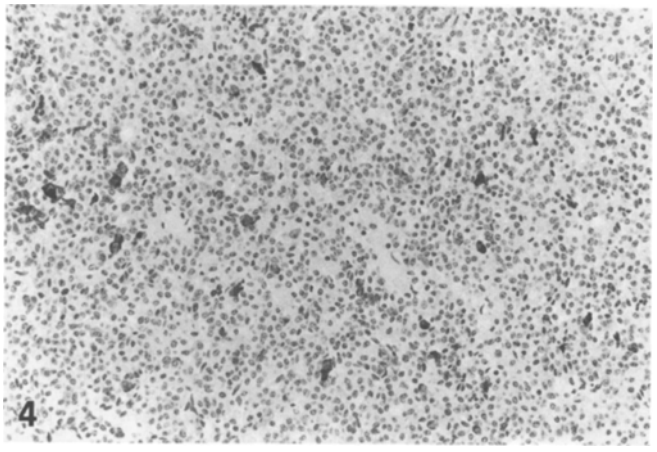
Fig. 1. Solid-cystic tumour of the pancreas: the solid and soft cut surface contains haemorrhagic areas and multiple cysts up to 3 cm in diameter











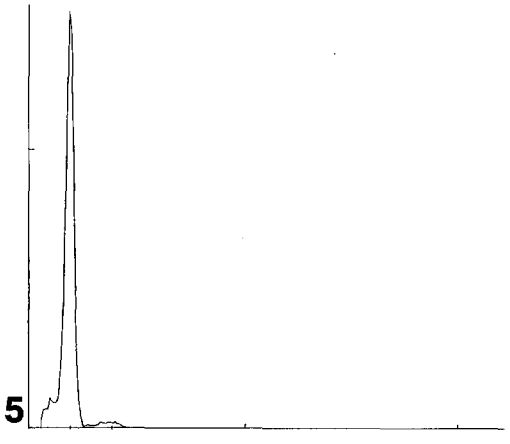


Fig. 2. **a** Solid growth pattern of tumour cells, with only slight nuclear anisomorphism and bright fine granular eosinophilic cytoplasm. H&E, ×245. **b** Pseudopapillary formations of tumour cells with central fine vascular stalks, lining microcystic spaces. H&E, ×245

Fig. 3. **a** Diffuse cytoplasmic immunoreaction for neuron-specific γ-enolase is present in all tumour cells, which is enhanced in some tumour cells with larger cytoplasm. The fibrous stroma in between is negative. Immunoperoxidase, ABC, ×245. **b** Granular cytoplasmic immunoreaction for chromogranin A in all tumour cells. Immunoperoxidase, ABC, ×280

Fig. 4. Positive insulin-immunoreaction of dispersed tumour cells. Immunoperoxidase, ABC, ×245

Fig. 5. DNA-histogram: the first peak corresponds to the diploid tumour cells and to other diploid cells (G_0/G_1 phase). The second small peak represents the particles in the G_2/M phase. DNA index = 1.0. The vertical axis indicates the number of cells measured; the horizontal axis represents the channel number of the cytophometer

NSE is currently accepted as a marker of SCT (Chott et al. 1987; Ludvik et al. 1989) and our results confirm the presence of NSE in SCT, taking advantage of more specific immunoreactivity with monoclonal NSE antibody (Soler-Feddersppiel et al. 1987; Thomas et al. 1987).

In some previous electron microscopic studies electron-dense granules within the cytoplasm of tumour cells were reported. In one report, these were identified as neuroendocrine dense-core granules (Schlosnagle and Campbell 1981). In other studies the authors suggested them to be acinus cell-like zymogen-like granules (Klöppel et al. 1981; Bombi et al. 1984; Learmonth et al. 1985; Ladanyi et al. 1987), or did not find granules at all (Hamoudi et al. 1970). By means of immunoreactivity for chromogranin A in our case, we were able to confirm the presence of dense-core neuroendocrine granules in SCT, since chromogranin A is a 68 kDa matrix glycoprotein of endocrine dense-core granules (Lloyd and Wilson 1983; Wilson and Lloyd 1984). Chromogranin A immunoreactivity of SCT was recently confirmed by Ludvik et al. (1989).

Our negative immunoreaction for synaptophysin, a 38 kDa protein of synaptic vesicles present in neuroendocrine cells (Wiedenmann and Franke 1985), may be due to previous formalin fixation. In our experience, the monoclonal antibody (SY38) used to detect synaptophysin is less effective in routine paraffin material than in frozen tissue.

The normal diploid nuclear DNA content in our case of SCT corresponds to our experience with other benign endocrine pancreatic tumours (Sieg et al., in preparation). Moreover, the proliferative activity of this tumour was within the common range of benign endocrine pancreatic tumours, but was lower than for its malignant counterparts (von Herbay et al. 1990).

In summary, our results support the theory that SCT represents a hormonally inactive neuroendocrine pancreatic tumour.

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