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Histology and Histopathology

Serotonin-producing pancreatic endocrine tumour. Histological, ultrastructural and immunohistochemical study of a case

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Summary. Serotonin-producing pancreatic endocrine tumours are rare neoplasms which in most cases exhibit malignant biological behaviour. These tumours, in the majority of the well-documented cases, are composed of argyrophil- and argentaffin-positive cells which contain large pleomorphic neurosecretory granules. In contrast, argyrophilic non-argentaffin pancreatic endocrine tumours with tumour cells containing round neurosecretory granules are exceptional. In this study we describe such a tumour not associated with clinical evidence of carcinoid syndrome in a 60-year-old woman. Histological examination revealed tumour extension in pancreatic lymphatic vessels and veins but no evidence of locoregional or distant metastases. Ten months after surgery the patient showed no recurrence of the disease. Immunohistochemistry revealed cytoplasmic serotonin production in the tumour cells which were negative for anti-gastrin, insulin, glucagon, somatostatin, pancreatic polypeptide (PP), vasoactive intestinal peptide (VIP) and ACTH. This study emphasizes the usefulness of combined ultrastructural and immunohistochemical investigations in order to identify and characterize the rare pancreatic endocrine tumours with serotonin production.

Key words: Pancreatic endocrine tumours, Immunohistochemistry, Serotonin, Electron microscopy

Introduction

Serotonin-producing endocrine tumours of the pancreas are extremely rare tumors, which are sometimes associated with carcinoid syndrome and exhibit malignant biological behaviour in the majority of cases (Dollinger et al., 1967; Gordon et al., 1971; Persaud and Walrond, 1971; Patchefsky et al., 1972, 1974; Wilander et al., 1981; Ordonez et al., 1985; Khorsand et al., 1987; Cartens and Cressman, 1989).

Most of the well-documented (with immunohistochemistry and/or electron microscopy) serotonin-producing pancreatic endocrine tumours displayed positive argyrophil and argentaffin reactions and contained pleomorphic cytoplasmic neurosecretory granules (Patchefsky et al., 1974; Wilander et al., 1981; Ordonez et al., 1985; Khorsand et al., 1987).

Conversely, non argentaffin serotonin-producing pancreatic endocrine tumours with round neurosecretory granules are exceptional and, to our knowledge, only one well-documented case, designated as malignant oncocytic carcinoid of the pancreas, has been reported so far (Cartens et al., 1989). In the present study, we describe the histological, ultrastructural and immunohistochemical features of an non-oncocytic pancreatic endocrine tumour of this type which was not associated with clinical evidence of carcinoid syndrome. This tumour displayed extension in pancreatic lymphatic vessels and veins without evidence of metastases.

Materials and methods

A 60-year-old woman experienced a gradual onset of abdominal pains during the 18 months preceding her admission to Lariboisiere Hospital (March 1988). Physical examination showed no abnormalities. Abdominal ultrasonography revealed an 18 mm nodule located in the ante-inferior segment of the pancreas and arteriography evidenced the rich vascularity of this nodule. Fiber optic oeso-gastro duodenal examination and hepatic tests were normal. Laboratory evaluation showed that serum serotonin, gastrin, glucagon, insulin, 5-HIA, C-peptide, and somatostatin levels were within normal limits.

At surgery (September, 1988), resection of the body and the tail of the pancreas with conservation of the head of the pancreas and pancreato-jejunal anastomosis were

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performed. Ten months after surgery (July 1989), the patient showed no recurrence of the disease.

For light microscopy three micron paraffin sections were stained with hematoxylin-eosin-safran (HES), Grimelius and Fontana-Masson stains. For electron microscopy fresh tissue samples were immediately fixed in 3% cacodylate-buffered glutaraldehyde, post-fixed in 1% osmium tetroxide, dehydrated in graded acetone and embedded in epoxy-araldite. Ultrathin sections were stained with uranyl acetate and lead citrate.

Immunohistochemical studies of formalin-fixed paraffin-embedded sections were performed by the peroxidase-antiperoxidase method (Sternberg, 1979).

The panel of antibodies used included anti-insulin (rediluted 1/2), VIP (rediluted 1/2), somatostatin, ACTH and gastrin (ORTHO), pancreatic polypeptide and glucagon (Bolyon, France), and serotonin (diluted 1/5) (Immunotech, France). Positive and negative controls were performed.

Results

Gross pathology

The surgical specimen consisted of pancreatic tissue measuring 4.5 cm x 2.5 cm x 2.5 cm and containing a 2.5 cm x 1.5 cm x 2 cm non encapsulated white-yellow mass showing foci of haemorrhage (Fig. 1).

Histology

Histological evaluation of the surgical specimen revealed tumour proliferation infiltrating the pancreatic parenchyma and the peripancreatic fat tissue. The tumour cells were arranged in trabeculae, nests and sheets within fibrohyalin vascular stroma (Fig. 2). Occasionally acini formation was found. Cytologically, the medium-sized tumour cells were rather uniform and contained round, oval or slightly irregular nuclei with one or two small nucleoli. Mitotic figures were rare. Tumour cells were identified in numerous perineural lymphatic vessels and in many small and medium-sized veins. The lymph nodes adjacent to the right gastroepiploic artery, to the common hepatic artery and to the bifurcation of coeliac trunk were not affected by tumour. The tumour cells were argyrophilic-positive (Grimelius stain) but argentaffin-negative (Fontana-Masson stain).

Immunohistochemistry

The majority of tumour cells (70%-80%) were positive for anti-serotonin (Fig. 3) but negative for anti-gastrin, insulin, glucagon, somatostatin, pancreatic polypeptide (PP), VIP and ACTH.

Electron microscopy

The tumour cells were round or oval with round, oval or slightly irregular nuclei containing one or two small nucleoli. The moderately abundant cytoplasm contained many round, usually homogeneous neurosecretory granules consisting of an electron-dense body surrounded by a narrow clear halo and a limiting membrane (Fig. 4). The diameter of these granules varied between 100 to 300 nm. Some mitochondria, poorly developed granular endoplasmic reticulum and many glycogen grains were also observed within tumour cells.

Discussion

We present a primary tumour of the pancreas with a typically endocrine histological aspect (Klöppel and Heitz, 1988). The tumour cells were argyrophil-positive but argentaffin-negative. Immunohistochemistry evidenced cytoplasmic serotonin production by tumour cells which did not react with anti-insulin, gastrin, glucagon, somatostatin, PP, VIP and ACTH antibodies. This immunophenotype allowed us to rule out the diagnosis of an islet-cell tumour of the pancreas. At ultrastructural level, tumour cells contained round cytoplasmic-neurosecretory granules. Taken into consideration the histological, histochemical, ultrastructural and immunohistochemical features of our case, this suggests the diagnosis of a serotonin-producing pancreatic endocrine tumour, similar to that reported by Carstens et al. (1989).

Serotonin-producing pancreatic endocrine tumours which have been previously designated as pancreatic carcinoids, may be derived either from multipotential stem cells of the pancreatic duct epithelium (Wilander et al., 1981) or from the rare enterochromaffin (EC) cells which in human, were found within the duct epithelium and the acinar tissue of the pancreas (Capella et al., 1977). According to current concepts (Grube, 1986) all endocrine cells of the digestive system, including EC cells, are broadly referred to as gastro-entero-pancreatic endocrine system (GEP) and are believed to be derived from the endoderm rather from the neuro-ectoderm (Le Douarin, 1978). The rare well-documented cases of serotonin-producing pancreatic endocrine tumour (Patchefsky et al., 1974; Wilander et al., 1981; Ordonez et al., 1985; Khorsand et al., 1987) are composed of argyrophil-and argentaffin-positive tumour cells containing pleomorphic granules ultrastructurally. In contrast, to our knowledge, only one well-documented case of non-argentaffin serotonin-producing pancreatic endocrine tumour with round neurosecretory granules closely related to our case, was found in previous literature (Carstens et al., 1989). This tumour was further designated as oncocytic because of the presence of abundant granular eosinophilic cytoplasm containing numerous mitochondria at ultrastructural level. Besides oncocytic endocrine tumours, islet cell oncocytomas have also been reported (Radi et al., 1985). The diagnosis of such a tumour, as demonstrated by Carstens et al. (1989), can be eliminated by the absence of staining of tumour cells with antibodies against glucagon, insulin, somatostatin and gastrin. Some of the serotonin-producing pancreatic endocrine tumours are associated

Fig. 1. Macroscopic view of the pancreatic tumour.

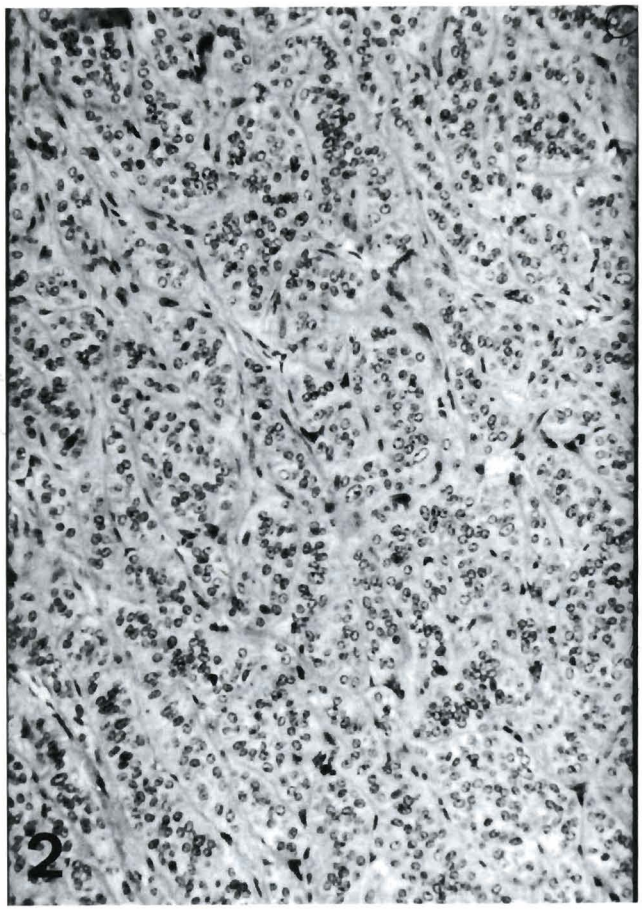

Fig. 2. Tumour cells are arranged as trabeculae and nests within a fibrohyalin vascular stroma HES. × 300

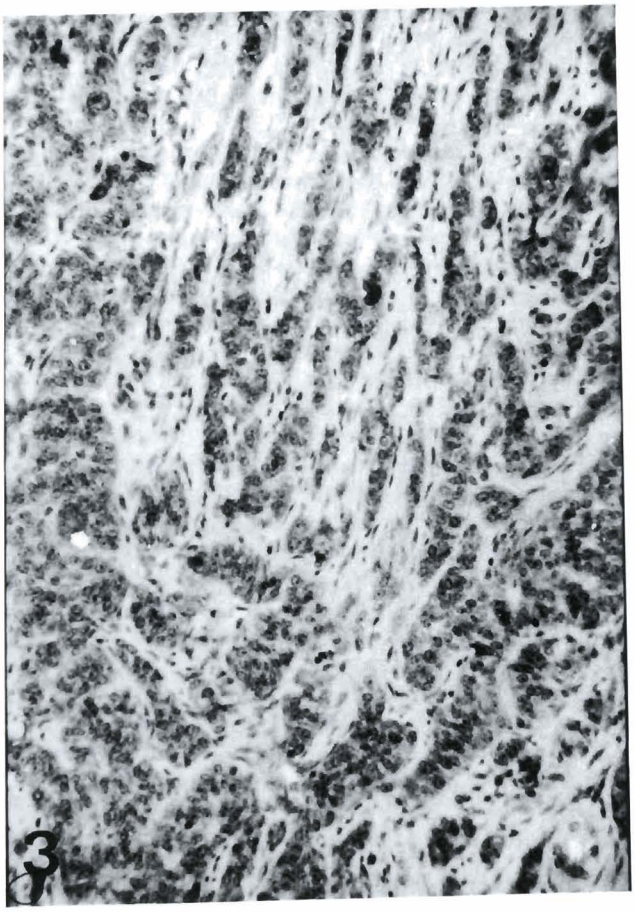

Fig. 3. Tumour cells showing positive staining for anti-serotonin antibody. × 300

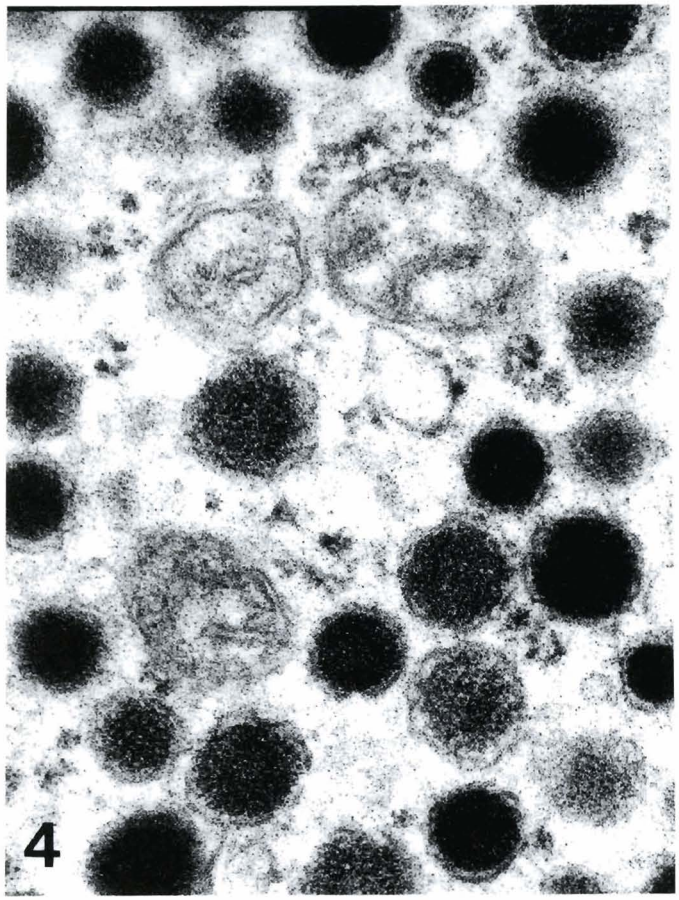

Fig. 4. Tumour cells contained round neurosecretory granules × 33,000

with clinical evidence of carcinoid syndrome (Dollinger et al., 1967; Gordon et al., 1971; Patchefsky et al., 1972; Ordonez et al., 1985; Khorsand et al., 1987) whereas other publications, as well as the present study, describe clinically «non-functioning» serotonin-producing pancreatic endocrine tumours (Patchefsky et al., 1974; Wilander et al., 1981; Carstens et al., 1989). Previous studies suggested that the absence of a recognizable clinical syndrome might be due to the production of biologically inactive substances or to the rapid degradation of potentially active products (De Lellis et al., 1984). Histologically, the serotonin-producing pancreatic endocrine tumours are reported to be composed of more or less uniform cells which are arranged in trabeculae, nests, sheets, acinar formations and/or mixtures thereof (Gordon et al., 1971; Patchefsky et al., 1974; Wilander et al., 1981; Ordonez et al., 1985; Khorsand et al., 1987; Carstens et al., 1989).

In previous literature argyrophil and argentaffin reactions have been used to characterize the serotonin-producing pancreatic endocrine tumours (Gordon et al., 1971; Persaud et al., 1971; Patchefsky et al., 1972, 1974). More recently, immunohistochemistry using anti-serotonin antibodies seems to be a more sensitive method than argentaffin reaction for the identification of tumour EC cells. Indeed, immunohistochemical studies demonstrated cytoplasmic serotonin production in all tested cases of these tumours (Khorsand et al., 1987; Carstens et al., 1989; Ordonez et al., 1989, our case), whereas Fontana-Masson reaction was found negative in our case and only two others (Khorsand et al., 1987; Carstens et al., 1989, our case).

Most of the serotonin-producing endocrine tumours are reported to be malignant (Gordon et al., 1971; Patchefsky et al., 1974; Ordonez et al., 1985; Khorsand et al., 1987; Carstens et al., 1989). The criteria of malignancy in these studies included locoregional or distant lymph node metastases (Gordon et al., 1971; Patchefsky et al., 1974; Carstens et al., 1989), liver and spleen metastases (Gordon et al., 1971), breast metastases (Ordonez et al., 1985) and widespread metastases (Khorsand et al., 1987). In contrast, no metastatic disease was detected in our patient and only histological evidence of tumour extension in numerous pancreatic lymphatic vessels and veins was found. Since the most reliable criterion of malignancy in pancreatic endocrine tumours is the presence of metastasis (Klöppel and Heitz, 1988), the tumour described in the present study cannot be considered as malignant. However, long term follow-up is required for definitive conclusions. The evolution of serotonin-producing pancreatic endocrine tumours was rapid (6-13 months) in some cases (Dollinger et al., 1967; Gordon et al., 1971; Khorsand et al., 1987; Carstens et al., 1989), whereas in one case, despite the presence of breast metastases, the patients survived 18 months after surgery (Ordonez et al., 1985). In our case the patient showed no recurrence of the disease ten months after surgery.

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