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Pancreatic endocrine carcinoma with ectopic PTH-production and paraneoplastic hypercalcaemia *

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Summary. In a case of pancreatic endocrine carcinoma hypercalcaemia without bone metastases and normal parathyroid glands prompted our suspicion that there was paraneoplastic production of an osteoclast activating substance by the tumour tissue. This view was further confirmed by bone histology. Immunohistology post mortem revealed the production of PTH in the primary tumour and a liver metastasis. The usefulness of immunohistology in detecting paraneoplastic secretion of hormonal substances is discussed.

Key words: Paraneoplasia – Pancreatic endocrine carcinoma – Parathyroid hormone – Immunohistology

Paraneoplastic hypercalcaemia, a relatively common condition among paraneoplastic syndromes, is only rarely associated with malignant tumours of endocrine origin. Most commonly, this condition is associated with renalcell carcinoma or with carcinoma of the lung and breast (Heitz and Staub 1981; Heitz and Oberholzer 1984; Arps et al. 1984b). If the clinical features of tumour-induced hypercalcaemia include high serum PTH values, hypophosphataemia, and increased urinary cyclic AMP thus imitating the symptoms of hyperparathyroidism, it is called "pseudohyperparathyroidism" (psHPT). Reliable means of diagnosis have been established using RIA (Riggs et al. 1971), immunohistochemical demonstration of iPTH in slices of tumour tissue (Palmieri et al. 1974; Dietel 1982; Arps et al. 1984b), and PTH production by explanted tumour cells (Greeenberg et al. 1973; Dietel 1982; Arps et al. 1984a).

Sometimes adenocarcinomas of the exocrine pancreas are known to produce psHPT (Heitz and Staub 1981; Monno et al. 1984), however, the asso-

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ciation of endocrine pancreatic tumours with psHPT is very rare. To our knowledge, only one report exists describing metastases of a pancreatic islet cell cercinoma as source of paraneoplastic PTH (Palmieri et al. 1974). In the present study we report the clinical and morphological findings of another case of malignant pancreatic endocrine tumour associated with paraneoplastic hypercalcaemia due to ectopic PTH production.

Case report

Clinical data. R.K., a male patient born in 1935 underwent surgery in August 1976 because of obstructive jaundice. The operation revealed an enlarged and indurated head of the pancreas, resembling a pancreatic carcinoma macroscopically. Total excision of the tumour was impossible because the hepatic artery was encircled. Obstruction of the papilla had resulted in dilatation of the bile duct and hydrops of the gall-bladder. To permit the flow of bile a choledochojejunostomy with subsequent cholecystectomy was performed. In 1978 the patient developed diabetes which was controlled by diet and oral antidiabetics. He remained well up to April 1979 when massive duodenal haemorrhage occurred. Iron deficiency and blood glucose levels up to 180 mg/100 ml were the only pathological laboratory values found. Gastro-duodenoscopy revealed compression of the duodenum but did not detect the source of bleeding. Mesentericography showed a hypervascular tumour of 10 cm in diameter in the head of the pancreas. After embolization performed during the arteriography the patient was dismissed without complaints.

Intestinal haemorrhage occurred again in September 1980 with haemoglobin values of 5.8 g/100 ml. The duodenum was infiltrated by tumour masses which were the source of bleeding. Coeliacography performed for a second embolization confirmed the localization of the tumour as previously described and additionally revealed a region in the liver suspicious of metastases.

By April 1982 numerous blood transfusions had proved necessary because of intestinal blood loss. In 1981 the patient acquired transfusion hepatitis. Intra-abdominal tumour masses and liver metastases were detected by sonography. The patient had pain and an increasing demand for insulin. Seven months before death elevated serum calcium levels and decreased phosphate were noticed for the first time. In January 1983, serum calcium levels increased up to 4.2 mmol/l and alkaline phosphatase levels rose to 1290 U/l. The patient died in the same month.

Morphology. At autopsy, macroscopic inspection revealed a tumour measuring $14 \times 10 \times 10$ cm in the head of the pancreas with infiltration into the duodenum. The presence of multiple liver metastases, up to 8 cm in diameter, was verified. In spite of intensive search, skeletal metastases which were suspected clinically were not found. Death resulted from intestinal haemorrhage complicated by dramatic hypercalcaemia. Because of the hypercalcaemic state the parathyroid glands were inspected; all four glands were normal in size and histology without morphological signs of hyperparathyroidism.

Histologically, the tumour showed the pattern of endocrine tissue with polygonal epithelial cells, growing in solid nests without adenoid structures (Fig. 1). The tumour cells appeared to be slightly atypical, uneven in size and shape and containing polymorphic hyperchromatic nuclei. To confirm the endocrine character of the cells, immunohistochemistry using the triple layer method according to Sternberger (1979) was applied. The tumour tissue was tested for neuronspecific enolase, which showed a positive reaction (Fig. 2). The paraffin embedded tissue was processed for re-embedding in Epon 812 and thin sections were obtained for electron microscopy (Dietel et al. 1979). The tumour cells contained typical endocrine secretory granules (Fig. 3) which were a further evidence for the neuro-endocrine origin of the tumour tissue.

Because of the hypercalcaemic syndrome, slices of tissue from the primary tumour and one of a liver metastasis were tested immunohistologically for the presence of PTH as described in detail elsewhere (Dietel and Hölzel 1983). In brief, a sheep anti-bovine PTH antibody was used with specificity for the C-terminal region of the PTH molecule (kind gift by Prof. Dr. R.D. Hesch and Dr. H. Jüppner, MH Hannover). Rabbit anti-sheep IgG and sheep

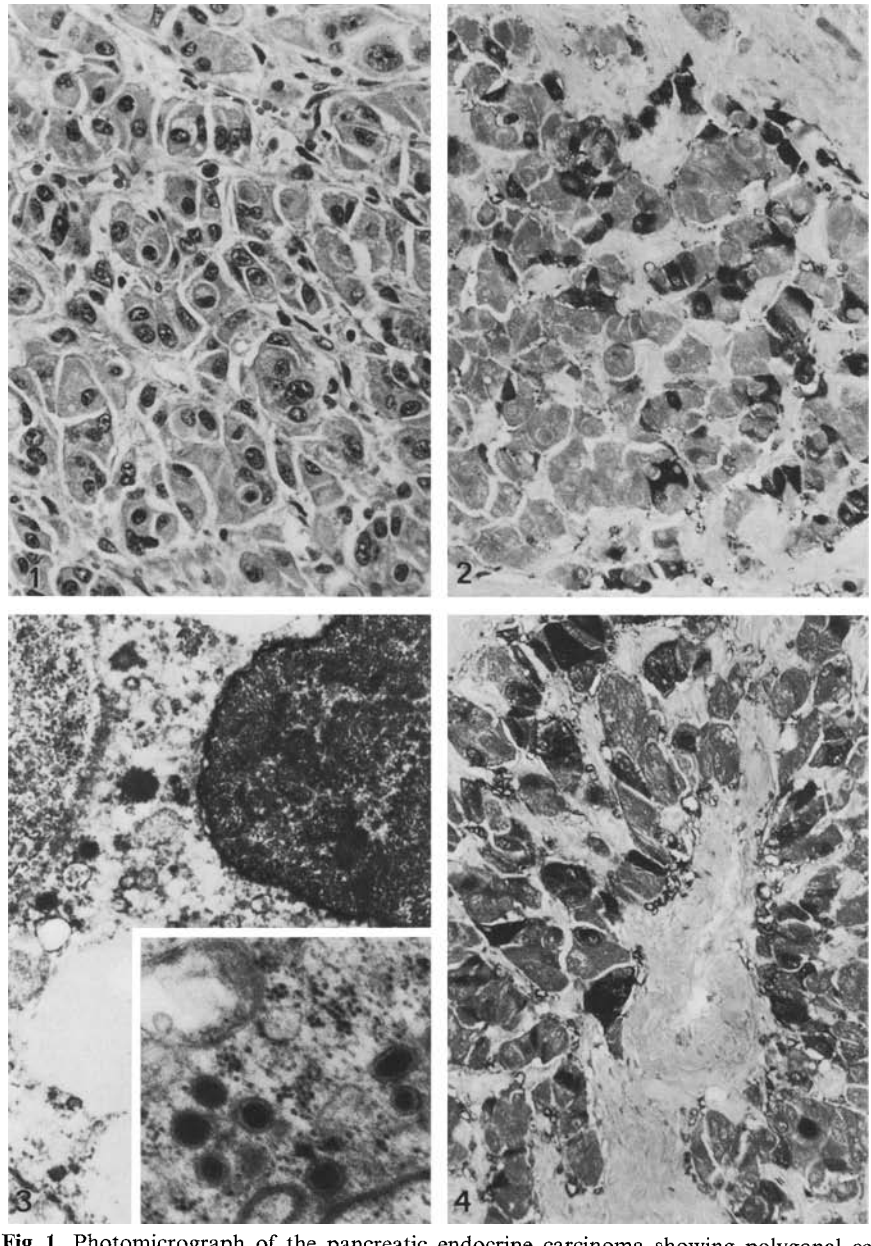

Fig. 1. Photomicrograph of the pancreatic endocrine carcinoma showing polygonal cells in solid pattern. (H&E, final magnification ×250)

Fig. 2. Positive immunostaining of tumour cells for neuronspecific enolase in a liver metastasis. (DAB-immunohistochemistry, final magnification ×175)

Fig. 3. Electron micrograph of a tumour cell showing neurosecretory granules at the cell membrane. Inset: Higher magnification of another group of secretory granules. (final magnification ×10,500, inset ×21,000)

Fig. 4. Several tumour cells positive for PTH in the primary tumour. (DAB-immunohistochemistry, final magnification ×175)

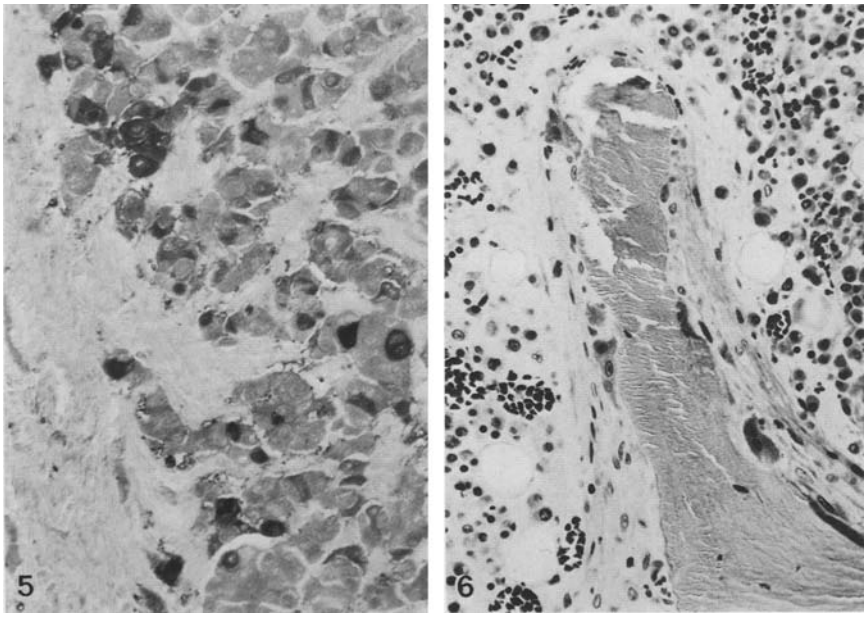

Fig. 5. Focal positivity for PTH in the tumour cells in a liver metastasis. (DAB-immunohistochemistry, final magnification ×175)

Fig. 6. Vertebral bone with signs of activated metabolism: Stimulation of osteoclastic bone resorption and osteoid formation. (Masson-Goldner, final magnification ×175)

peroxidase anti-peroxidase complex were used as the second and third layer, respectively. The immunecomplexes were visualized with diaminobenzidine. Controls performed by omitting the first antibody or by replacing the second antibody with normal rabbit serum remained negative. Immunostaining was positive in about 70% of the tumour cells expressing the typical irregular distribution pattern as often found in tumours with paraneoplastic hormone production (Figs. 4 and 5). Immunostaining for other secretory products (α-1-antitrypsin, gastrin, human pancreatic polypeptide, somatostatin, glucagon, serotonin, α-human chorionic gonadotropin, chymotrypsin, α-1-fetoprotein, α-amylase, and calcitonin) was negative.

In agreement with the finding of an ectopic overproduction of PTH, the histology of undecalcified, metacrylate-embedded bone tissue showed fibroosteoclasia with numerous activated osteoclasts, loss of mineralized bone structure, and enlarged areas of osteoid (Fig. 6). This condition is often observed in primary hyperparathyroidism and represents increased bone turnover due to elevated PTH levels.

Discussion

70% of all patients with hypercalcaemia suffer from malignant tumours. The tumour-induced hypercalcaemia may be due to multiple metastases with destruction of bone. This mechanism is now attributed to the presence of factors with local osteolytic activity, released by the cells of the metastases (Delling 1981; 1982). Among these, the prostaglandins (Besarab and Caro 1978) and so-called "osteoclast activating factors" of unknown chemical structure (Heitz and Staub 1981) have been examined most widely. Alterna-

tively, the stimulation of osteoclast activity may be due to humoral factors produced by the tumour or by its distant metastases outside the bone. Hormonal factors increasing bone resorption in this way have been identified to be paraneoplastically secreted PTH (Riggs et al. 1971; Arps et al. 1984a; Buckle et al. 1970) or to be derivatives of vitamin D-like sterols (Gordan et al. 1966).

Unequivocal demonstration of ectopic PTH secretion is difficult biochemically because elevation of blood PTH levels results in identical serological changes regardless of its source. A paraneoplastic PTH syndrome can be presumed to be present when one or more of the following criteria are met:

(1) hypercalcaemia (Riggs et al. 1971) and/or elevated iPTH levels in the patient's serum (Besarab and Caro 1978; Buckle et al. 1970) in combination with arterial-venous gradient of iPTH across the tumour (Besarab and Caro 1978; Rees 1975; Dietel 1982);

(2) PTH reactivity of tumour cells in immunohistochemistry (Tashjian et al. 1964; Buckle et al. 1970; Rees 1975; Samaan et al. 1976; Arps and Dietel 1984) and characteristic bone lesions in about 50% of the cases;

(3) presence of PTH in cell free extracts from the tumour tissue (Palmieri et al. 1974; Arps et al. 1984a; Arps and Dietel 1984) and/or in vitro production of PTH by the tumour in tissue or cell culture (Greenberg et al. 1973; Dietel 1982; Arps et al. 1984a; 1984b).

In the case presented a pancreatic endocrine carcinoma had been diagnosed by conventional histology. The diagnosis was confirmed by immunohistological detection of neuron specific enolase and electrone microscopical demonstration of secretory granules. The patient died in deteriorated hypercalcaemia. At autopsy, the lack of bone metastases and the demonstration of four macroscopically and histologically normal parathyroid glands prompted the search for paraneoplastic PTH production by the tumour. This was substantiated by immunohistochemistry performed on tissues from the primary and a liver metastasis showing a large number of positive cells. Thus, two of the criteria for the demonstration of a paraneoplastic PTH syndrome are met in the case investigated.

The histogenetic origin of the tumour from endocrine tissue was demonstrated by positive immunostaining for neuron specific enolase (Klöppel et al. 1983; Marangos 1985). The endocrine nature was additionally demonstrated by electrone microscopical visualization of endocrine secretory granules. Supporting evidence for ectopic PTH production by the pancreatic endocrine carcinoma was provided by bone histology. The morphological analysis of osseous tissue disclosed distinct fibroosteoclasis as found in cases of hyperparathyroidism. For the relatively late onset of clinically overt disturbances of calcium homeostasis the following explanation is feasible:

Relatively slow tumour growth in combination with recurrent therapeutic reduction of tumour mass (surgery, embolization) resulted in a history of six years of cancer. This may be a hint for the fact that during a long time only few vital tumour cells had been present. Of these only a small percentage was able to produce PTH, a fact that leeds to a further reduction

of the amount of PTH secreted. Thus, increase of Ca^{2+} and PTH in the serum led to clinically overt pseudohyperparathyroidism only during the last seven month. Clinical interpretation of the hypercalcaemia to be based on skeletal metastases prevented the evaluation of serum PTH levels.

The case presented serves as an example of the usefulness of immunohistochemistry in disclosing tumour-associated hypercalcaemia as paraneoplastic. With the use of this immunological method paraneoplastic hypercalcaemia can be distinguished from parathyroid disease or other causes of hypercalcaemia. Immunohistochemistry of hormonal factors may become a routinely used diagnostic tool in cases of suspected paraneoplastic syndromes.

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