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A. Hoorens · F. Gebhard · K. Kraft · N.R. Lemoine
G. Klöppel

Pancreatoblastoma in an adult: its separation from acinar cell carcinoma

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Abstract Pancreatoblastomas are rare tumours, which usually occur in childhood. Here we describe a pancreatoblastoma in a 39-year-old woman. The tumour was located in the tail of the pancreas and consisted of cells forming well-differentiated acinar structures and scattered solid components ("squamoid corpuscles"). Immunocytochemically, the acinar components were positive for pancreatic enzymes and pancreatic stone protein, while the cells of the "squamoid corpuscles" lacked these markers. There was no p53 overexpression nor any mutation at codon 12 of the Ki-*ras* oncogene. The main differential diagnosis of this tumour was acinar cell carcinoma, because both tumours have a number of features in common (scattered solid components, positivity for pancreatic enzymes, lack of p53 overexpression and of Ki-*ras* mutation). Findings which distinguished the pancreatoblastoma and separated it from acinar cell carcinoma were the negativity of the solid components ("squamoid corpuscles") for neuroendocrine markers and their very weak keratin positivity. As the patient is alive and well 30 months after tumour resection, this pancreatoblastoma also differs in biology from the usual acinar cell carcinoma.

Key words Pancreatoblastoma · Acinar cell carcinoma p53 overexpression · Ki-*ras* mutation · Cytokeratins

A. Hoorens (✉) · G. Klöppel
Department of Pathology, Academic Hospital Jette,
Free University of Brussels,
Laarbeeklaan 101, B-1090 Brussels, Belgium

F. Gebhard
Department of Surgery,
Bundeswehrkrankenhauses Ulm, Germany

K. Kraft
Department of Pathology,
Bundeswehrkrankenhauses Ulm, Germany

N.R. Lemoine
Imperial Cancer Research Fund Oncology Unit,
Royal Postgraduate Medical School,
Hammersmith Hospital, London, UK

Introduction

Pancreatoblastoma is an uncommon malignant tumour of the pancreas, occurring in childhood. It is composed of epithelial tissue showing acinar cell differentiation and squamoid cell nests [5, 9, 10]. Occasionally, it also contains some neuroendocrine cells, and rarely a pronounced mesenchymal component [3, 5].

Because of its commonly prevailing acinar cell differentiation, pancreatoblastoma may resemble acinar cell carcinoma [18, 22]. Moreover, in children, acinar cell carcinomas similar in macroscopy and biological behaviour to pancreatoblastomas have been described [18, 19, 20, 32]. Finally, a pancreatoblastoma has been reported in an adult [28]. From all these observations the question arises whether pancreatoblastoma and acinar cell carcinoma are clearly distinct entities or whether they may overlap with each other.

In this report we describe a pancreatoblastoma in an adult and discuss its separation from acinar cell carcinoma.

Materials and methods

A 39-year-old women presented with an upper abdominal mass. Ultrasonography and CT revealed a tumour in the upper abdomen, probably originating from the pancreas. There was no evidence of metastases pre-operatively. At laparotomy a tumour was found in the tail of the pancreas. The tumour was completely removed by distal pancreatectomy. No metastases were observed during laparotomy. Thirty months after surgery the patient is doing well with no evidence of tumour recurrence.

Tissue specimens from the tumour were fixed in 10% formaldehyde and 4 μm thick serial sections were cut from paraffin blocks. Two sections were stained with haematoxylin and eosin and periodic acid-Schiff (PAS) reagent with diastase pretreatment. Immunocytochemical analysis was carried out on serial sections from two blocks using the streptavidin-biotin-peroxidase complex method. The sections were stained with the primary antisera listed in Table 1. The antisera against alpha-amylase, lipase, trypsin and chymotrypsin were generated as previously reported [22]. The histochemical reaction for peroxidase was performed using 3,3 diaminobenzidine-tetrahydrochloride (0.05% w/v) and hydrogen

Table 1 Antisera used (*P* polyclonal, *M* monoclonal)

Antigen	Antiserum/code	Working dilution	Source
Lipase	P	1:1000	Own
Trypsin	P	1:2000	Own
Chymotrypsin	P	1:10000	Own
Phospholipase A_2	M	1:500	Dr. T. Nevaleinen, Turku, Finland
Alpha-amylase	P	1:6000	Own
Pancreatic stone protein	M	1:2000	Immunotech, Marseille, France
B72.3	M (4HC Sor 26 Anti-TAG72)	1:20	Sorin Biomedica, Brussels, Belgium
Carbohydrate antigen 19.9	M (CA 19.9)	1:200	Dr. H. Kalthoff, Hamburg, Germany
Carcinoembryonic antigen (CEA)	M CEA-BMA-130b CEA-BMA-130c	1:10	Behring, Marburg, Germany
Mucin antigen	M (M1)	1:8000	Dr. E. Solcia, Pavia, Italy
Alpha-fetoprotein	M	diluted solution	Amersham, Buckinghamshire, UK
Alpha-1-antitrypsin	P	1:20	Biogenex, San Ramon, USA
Synaptophysin	P M (SY38)	1:100 1:400	Dr. R. Jahn, New Haven, USA Dr. B. Wiedenmann, Heidelberg, Germany
Chromogranin A	M	diluted solution	Biogenex
Leu 7	M	1:3	Becton Dickinson, Mountain View, USA
Keratins 8, 18	M (CAM 5.2)	1:10	Biogenesis, Bournemouth, USA
Keratins 1, 2, 5, 6, 7, 8, 11, 14, 16, 17, 18	M (KL 1)	1:500	Immunotech
Keratins 8, 14, 15, 16, 18, 19	M (Mak-6 Cocktail)	1:10	Triton Biosciences, Alameda, USA
Keratins 1–19	M (Lu 5)	1:10	Boehringer, Mannheim, Germany
Epithelial Membrane Antigen	M	1:1000	Dako, Glostrup, Denmark
Keratin 7	M (CK 7)	1:100	Biogenex
Keratin 14	M (CK 14)	1:200	Sigma, St. Louis, USA
Keratin 19	M (CK 19)	1:20	Prof. Dr. Ramaekers, Maastricht, The Netherlands
Keratin 20	M (CK 20)	1:20	Dako
Vimentin	M	1:20	Boehringer
p53	P (M1)	1:500	Dr. DP Lane, Dundee, UK

peroxide (0.01% w/v) in phosphate buffered saline-buffer. Controls were performed as appropriate. Expression of the *p53* tumour suppressor gene was analysed using the CM1 polyclonal antibody as previously described [1].

To search for Ki-*ras* mutations at codon 12 the genomic DNA was extracted from a section cut from a paraffin-embedded, formalin-fixed tissue block by the method described by Jackson et al. [15]. Subsequently the method of Mitsudomi et al. [21] was employed, in which mismatched primers are used in the polymerase chain reaction to create restriction fragment length polymorphisms. Appropriate controls were performed as previously described [8].

Pathological findings

The resected body and tail of the pancreas showed a well demarcated tumour measuring 13× 10× 7.5 cm (Fig. 1). The tumour´s cut surface was lobulated, grey-yellow in colour and was focally haemorrhagic. Cysts were absent.

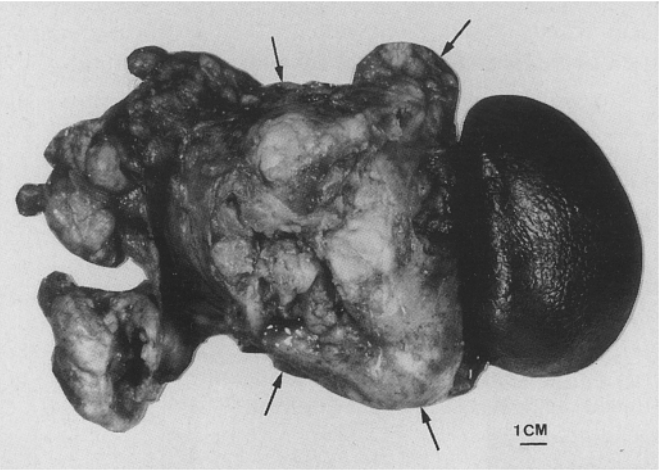

Fig. 1 Resection specimen showing the pancreatic tail containing the tumoral mass (*arrows*) and adherent spleen

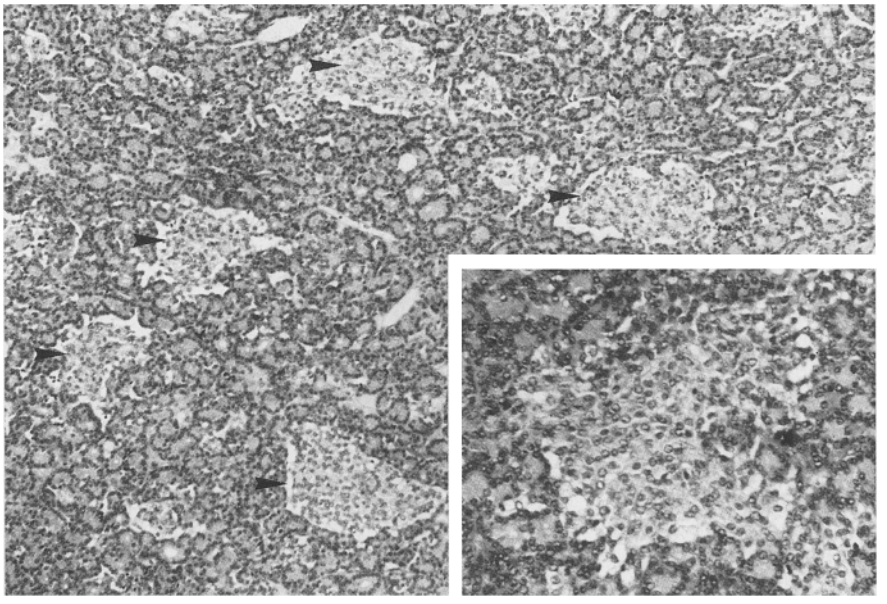

Fig. 2 Microscopy: The majority of the neoplastic cells formed well-differentiated acinar structures, among which scattered squamoid corpuscles were found (*arrowheads*), Haematoxylin and eosin (H&E) × 60. *Inset*: higher magnification of a "squamoid corpuscle", × 120

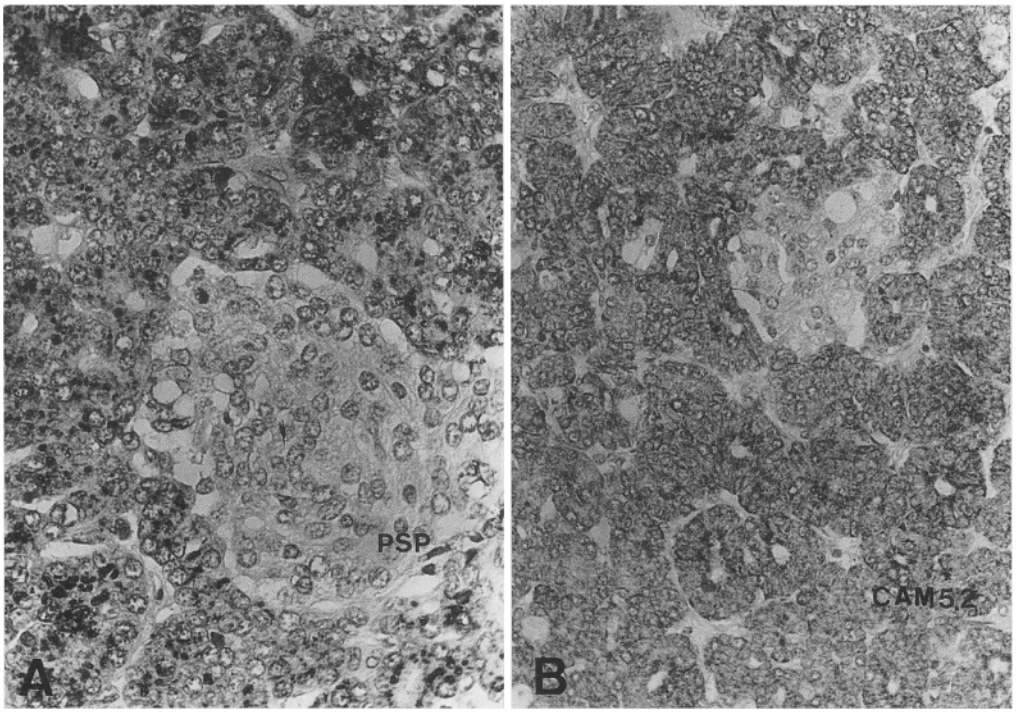

Fig. 3 **A** The tumour cells forming the acinar structures were positive for pancreatic stone protein (PSP), while the cells of the "squamoid corpuscles" were negative for this marker. × 600. **B** The cytokeratins 8 and 18 recognised by the CAM5.2 antibody were strongly expressed in the acinar structures, but not in the cells forming the "squamoid corpuscles". × 250

Histological examination revealed a cellular tumour with scanty stroma. The neoplastic cells formed large lobules which were separated by thin and often incomplete fibrous bands. They grew in well-differentiated acinar patterns which blended with scattered solid cell nests ("squamoid corpuscles"; Fig. 2). The acinar structures were composed of columnar or cuboidal cells with faintly granular eosinophilic cytoplasm and slightly irregular round to oval nuclei. The nuclei were situated at the basal pole of the cells and often contained a prominent nucleolus. The apical pole of the cells showed PAS-positive, diastase resistant, intracytoplasmatic fine granules. The mitotic rate was rather high, ranging between 1 and 15 mitoses per ten high power fields (HPF; mean 8 mitoses per ten HPF). The "squamoid corpuscles" contained polygonal cells, which were slightly larger than the cells forming the acinar structures. Their cytoplasm was faintly eosinophilic to clear. There was no keratinization. The nuclei were oval, slightly irregular in size, lacked prominent nucleoli and showed no mitoses.

Immunocytochemically, the tumour cells forming the acinar structures stained strongly for lipase, trypsin, chymotrypsin, phospholipase A_2 and pancreatic stone protein, while the cells of the "squamoid corpuscles"

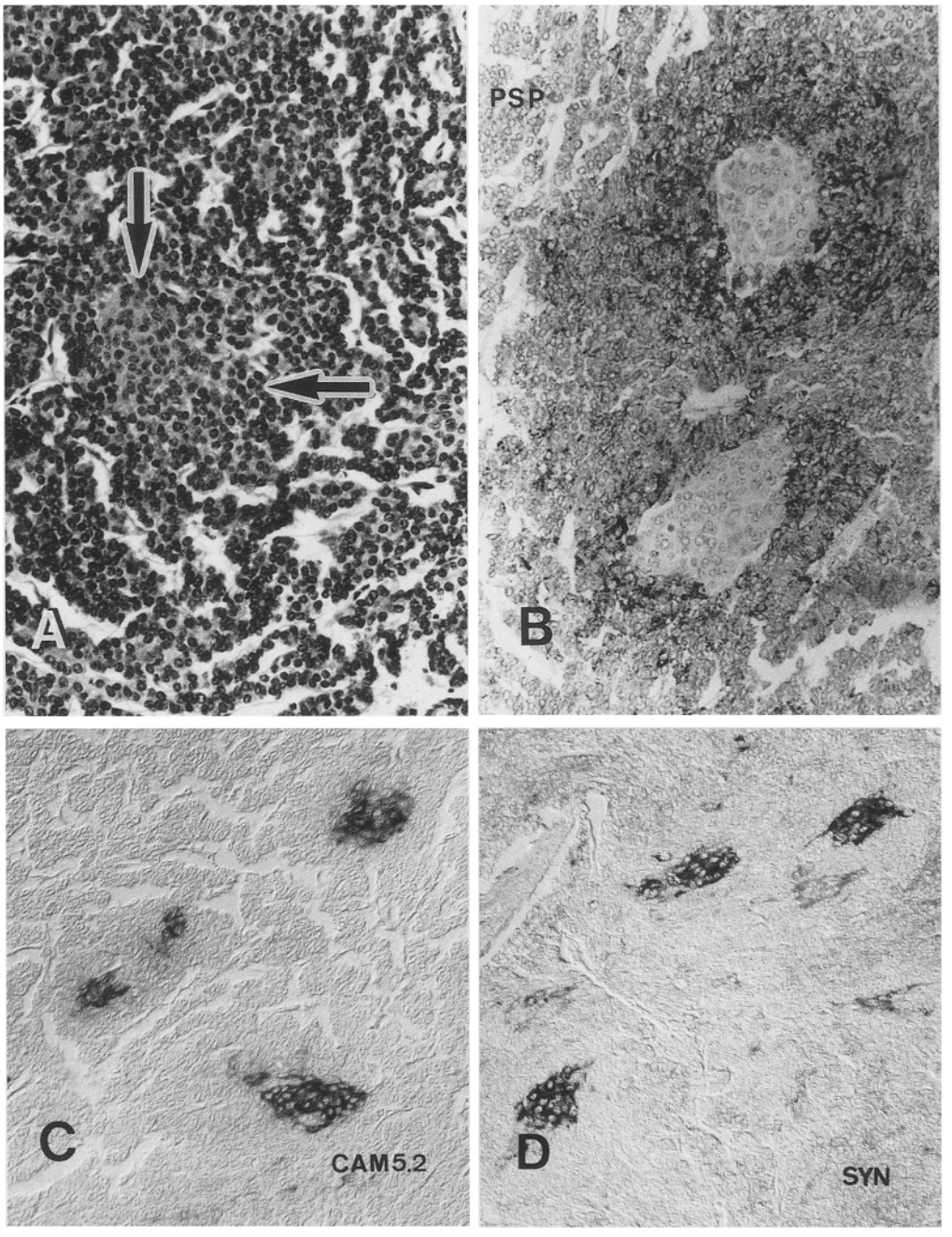

Fig. 4 **A** Microscopy: acinar cell carcinoma with a mixed acinar-solid pattern, the solid cell nests strongly resemble "squamoid corpuscles" in pancreatoblastoma (*arrow*), H&E × 120. **B** In contrast to what we usually find in acinar cell carcinomas with a mixed acinar-solid pattern, the solid cell nests in this peculiar acinar cell carcinoma case were negative for PSP, × 120. **C** The solid cell nests in this peculiar acinar cell carcinoma case showed however, in contrast to what we found in our pancreatoblastoma case, a stong positivity for the epithelial marker CAM5.2, × 120. **D** In addition, the solid cell nests in this acinar cell carcinoma were positive for the neuroendocrine marker synaptophysin, × 120

lacked these markers (Fig. 3A). All tumour cells were negative for alpha-amylase. The cytokeratins 8 and 18 recognized by the CAM 5.2 antibody were strongly expressed in the acinar structures, but not in the cells forming the "squamoid corpuscles" (Fig. 3B). The "squamoid corpuscles" were also negative for the broad spectrum cytokeratin markers KL1, Lu 5 and epithelial membrane antigen (EMA) and antibodies against CK7, CK14, CK19 and CK20, but weakly positive for the broad spectrum epithelial marker MAK-6 which recognizes cytokeratins 8, 14, 15, 16, 18 and 19. The cells forming the acinar structures were also negative for KL1 and CK7, but expressed a weak positivity for CK19, a finding in contrast to normal pancreatic acinar cells which are negative for both CK7 and CK19. The antiserum against alpha-1-antitrypsin gave in most tumour cells a diffuse coarsely granular positivity. All tumour cells failed to stain for vimentin, alpha-fetoprotein and the neuroendocrine markers synaptophysin, chromogranin A and Leu 7. A series of antisera known as duct cell markers (CA19.9, B72.3, carcinoembryonic antigen and the mucin antigen M1) gave also negative results. CM1 antibody revealed no overaccumulation of the p53 protein, and no mutation at codon 12 of the Ki-*ras* oncogene was detected.

Discussion

All but one of the reported cases of pancreatoblastoma have occurred in children. The age at diagnosis in these children ranged from birth to 8 years [2, 3, 4, 5, 6, 10, 11, 12, 13, 14, 23, 24, 25, 26, 29, 30]. The only exception was a 37-year-old man who developed a metastasizing pancreatoblastoma [28]. Our patient is a 39-year-old woman who has remained disease free for now more than $2^1/_2$ years.

The diagnosis of pancreatoblastoma in our patient was established on the basis of the histological pattern which was characterized by the combination of well-formed acinar structures with scattered squamoid cell nests. The cells of the latter structures are thought to be epithelial in nature, and some authors described signs of keratinization in these "squamoid corpuscles" [5, 13]. In our case we found neither keratinization nor positivity for a number of epithelial markers, such as CAM 5.2, KL1, Lu 5, CK7, CK14, CK19, CK20 and EMA. However, there was a weak positivity for the broad spectrum epithelial marker MAK-6, recognizing the keratins 8, 14, 15, 16, 18 and 19. As the "squamoid corpuscles" were negative for the mesenchymal marker vimentin and also showed no neuroendocrine or acinar features, we concluded that these tumour components are of epithelial origin but without any indication of differentiation towards one of the pancreatic cell types [17]. This may suggest that they represent a pool of undifferentiated cells within the pancreatoblastomas. Alternatively, the cells may represent a compartment of terminally differentiated epithelial cells that share no relationship with any of the known pancreatic cell types.

The main differential diagnosis of the tumour was acinar cell carcinoma, because of the positivity of the acinar cell complexes for pancreatic enzymes including pancreatic stone protein [8, 18, 22]. In addition, there was neither p53 overexpression nor a mutated Ki-*ras* oncogene at codon 12, findings which also characterise the distinction of acinar cell carcinoma from most other malignant tumours of the exocrine pancreas [8]. However, acinar cell carcinomas usually lack the distinct solid cell nest, called "squamoid corpuscles", that are typical for pancreatoblastomas.

In our series of acinar cell carcinomas [8] there was one exception from this rule. This acinar cell carcinoma contained conspicuous solid cell nests negative for acinar cell markers (Fig. 4A, B) and resembling "squamoid corpuscles". However, as these structures were strongly positive for keratin (Fig. 4C) and neuroendocrine markers (Fig. 4D), they could easily be distinguished from "squamoid corpuscles". Neuroendocrine cells have also been described in some pancreatoblastomas, but were never identified in "squamoid corpuscles" [4, 13, 30].

Pancreatoblastoma in infants appear to have a good prognosis if adequately treated [3, 4, 10, 13, 14, 29]. Whether this is also true for the pancreatoblastomas occurring in adults is unclear. Palosaari's patient [28] developed metastasis 15 months after tumour resection, while our patient has been disease free for more than 2 years. In this context it is of interest that acinar cell tumours have also been described in infants [7, 16, 18, 19, 20, 27, 31, 32], but with a natural history different from that of acinar cell carcinomas in adults. In 4 of the 11 cases so far reported in infants and children less than 15 years of age (mean age 8.5 years) there was no evidence of tumour recurrence 1, 1.5, 8, and 11 years respectively after resection of the tumour [18, 19, 32]. Thus in children, acinar cell tumours, if completely resected, seem to behave like pancreatoblastomas and differ from the corresponding tumours in adults [8, 18]. The possibility has therefore to be considered that the acinar cell tumours described in children might have been pancreatoblastomas in which the number of "squamoid corpuscles" was extremely low.

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