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CASE REPORT

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Alpha fetoprotein-producing acinar cell carcinoma of the pancreas showing multiple lines of differentiation

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Abstract An alpha fetoprotein (AFP)-producing tumour occurring in the head of the pancreas of a 30-year-old woman is reported. Histological examination revealed a markedly solid proliferation of tumour cells with prominent nucleoli and occasional luminal structures, some of which contained mucinous material stained with mucicarmine and alcian blue. No squamoid corpuscles were recognized. Immunohistochemistry showed intense positivity for lipase trypsin, and AFP basically, and single cells were also positive for carcino-embryonic antigen, CA19-9, synaptophysin and neuron-specific enolase. Pancreatic hormone-positive cells were absent. Electron microscopical examination revealed numerous granules of variable sizes in the tumour cells, which were considered to be zymogen. The tumour is an acinar cell carcinoma with multi-directional differentiation including the ability to produce AFP. Among AFP-positive pancreatic tumours, acinar cell carcinoma and pancreatoblastoma seem to be the most frequent.

Key words Pancreas · Acinar cell carcinoma · Alpha fetoprotein

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Introduction

Pancreatic acinar cell carcinoma (ACC) is regarded as a distinct entity and is thought to be derived from transformed acinar cells [19]. Recently, however, ACCs showing endocrine cells and cells with ductal features in addition to acinar cells have been described [8, 14]. Alpha fetoprotein (AFP)-producing ACCs of the pancreas are rare [8, 14, 20]. We therefore report a case of an ACC with multicellular differentiation and AFP production, and present its morphological, electron microscopical and immunohistological features.

Case report

A 30-year-old woman presented with a 1-month history of epigastric discomfort, which was followed by jaundice. Ultrasonography and computed tomography revealed a relatively well-demarcated mass in the head of the pancreas, which was slightly depressing the vena cava inferior. No metastases were detected in the liver. While most laboratory data were normal, serum AFP level was elevated to 325 ng/ml and indirect bilirubin was 9 mg/dl. A radical pancreatoduodenectomy was performed. The elevated AFP level returned to normal within 2 weeks after operation. The patient received chemotherapy and has been well for 32 months up to the present.

Materials and methods

The resected specimen was fixed in 10% buffered formalin, and embedded in paraffin. Sections were stained with haematoxylin–eosin (HE), periodic acid-Schiff (PAS), alcian blue, mucicarmine, and Grimelius argyrophil reaction. Immunohistochemical analysis was performed by the avidin–viotin–peroxidase complex method on paraffin sections. The antibodies used, source, and dilution were as follows: AFP (Dako Carpinteria, Calif., ×2), lipase (from Prof. G. Klöppel, Brussels, Belgium), trypsin (from Prof. G. Klöppel), amylase (from Prof. G. Klöppel), chymotrypsin (Athens Resarch, Athens, Ga., ×400), alpha-1-antitrypsin (Dako, USA, ×5000), alpha-1-antichymotrypsin (Dako, USA, ×100), pancreatic stone protein (from Prof. G. Klöppel), cytokeratin (Nichirei, Tokyo, Japan, ×10), carcinoembryonic antigen (CEA; Dako, USA, ×50), CA19-9 (CIS, bio International, Jis-sur-Yvette, France,

×50), CA125 (CIS, ×50), epithelial membrane antigen (EMA; Dako, Glostrup, Denmark, ×100), secretory component (Dako, USA, ×100), pancreatic polypeptide (Dako, USA, ×10), somatostatin (Dako, USA ×10), glucagon (Dako, USA ×10), gastrin (Dako, USA, ×10), insulin (Dako, USA, ×10), neuron-specific enolase (NSE; Dako, USA, ×10), chromogranin A (Biogenex, Dublin, Calif., ×20), and synaptophysin (from Prof. G. Klöppel).

For electron microscopy, each specimen was fixed in 1% buffered glutaraldehyde, post-fixed in 4% buffered osmium tetroxide and embedded in Epon 812. Ultrathin sections were contrasted with uranyl acetate and lead citrate and observed using a Hitachi H7000 electron microscope.

Pathological findings

The head of the pancreas contained a 3.5×2.5×4.1 cm grey-white and solid mass showing several small nodules at the periphery. There were several small cysts in the tumour.

Histological examination revealed a solid growth of tumour cells with occasional luminal spaces or duct-like structures (Fig. 1, top, left lower corner) invading the surrounding tissue. No acinar arrangement was seen. Fibrovascular stromal septa were present focally. There were no foci of necrosis or haemorrhage. Tumour cells were round, ovoid or polygonal with round nuclei and usually had a prominent nucleolus (Fig. 1). They had granular and slightly eosinophilic cytoplasm, with a high nuclear-cytoplasmic ratio. Mitoses were present in places, but the rate was low. The dilated luminal spaces containing mucinous material stained with PAS, alcian blue, and mucicarmine (Fig. 1, bottom). The tumour cells, which stained with PAS, were resistant to diastase digestion. Argyrophilic reaction with the Grimelius method was negative. There were no papillary structures, calcification, squamoid corpuscles, or mesenchymal tumour elements. Several regional lymph nodes had been invaded by the tumour.

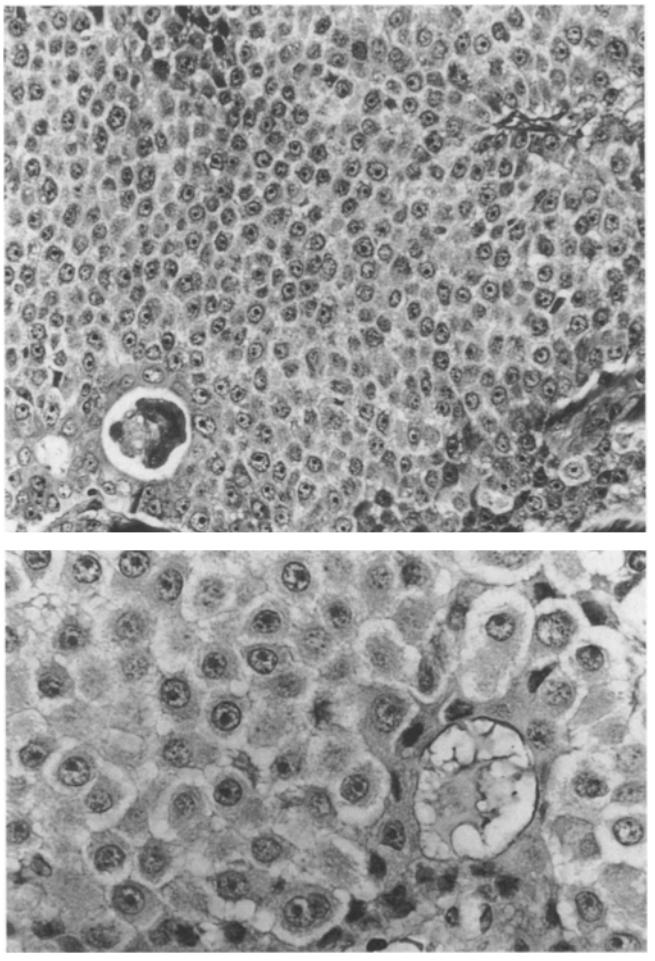

Fig. 1 Histology of acinar cell carcinoma. Note diffusely proliferative round tumour cells with prominent nucleoli and two foci of luminar structure at the *left lower corner* (*top*). HE, ×250. A luminar structure containing mucinous material (*bottom*). Mucicarmine stain, ×400

The immunohistochemical results are listed in Table 1. Most tumour cells were positive for AFP (Fig. 2A), trypsin (Fig. 2B), and lipase (Fig. 2C).

CEA (Fig. 2D) was present in the cytoplasm of single cells or clustered cells. EMA (Fig. 2E) as well as CA19-9 (Fig. 2F) stained the inner surface of lumina and occasionally cells in the solid areas. Synaptophysin and NSE (Fig. 2G) were weakly stained in scattered individual cells.

Electron microscopically, the tumour cells showed moderate numbers of mitochondria, rough endoplasmic reticulum, and numerous osmiophilic granules 200–600 nm in size that were aggregated or dispersed in the cytoplasm (Fig. 3). The granules were considered to be identical to zymogen granules; some had both light and dark areas. Fibrillary structured granules were lacking. No definitive luminal spaces were seen, but occasional villus-like short processes were observed between cellular borders. Small dense core (neuroendocrine) granules were not observed in the examined specimens.

Table 1 Results (++ 20–50% positive, + 10–20% positive, (+) individual cell positive, –negative)

Antisera	Result
Alpha-fetoprotein	++
Lipase	++
Trypsin	++
Amylase	–
Chymotrypsin	(+)
Alpha-1-antitrypsin	+
Alpha-1-antichymotrypsin	+
Pancreatic stone paotein	(+)
Cytokeratin	+
Carcinoembryonic antigen	(+)
CA19-9	(+)
Epithelial membrane antigen	(+)
CA125	–
Secretory component	–
Pancreatic polypeptide	–
Somatostatin	–
Glucagon	–
Gastrin	–
Insulin	–
Neuron specific enolase	(+)
Chromogranin A	–
Synaptophysin	(+)

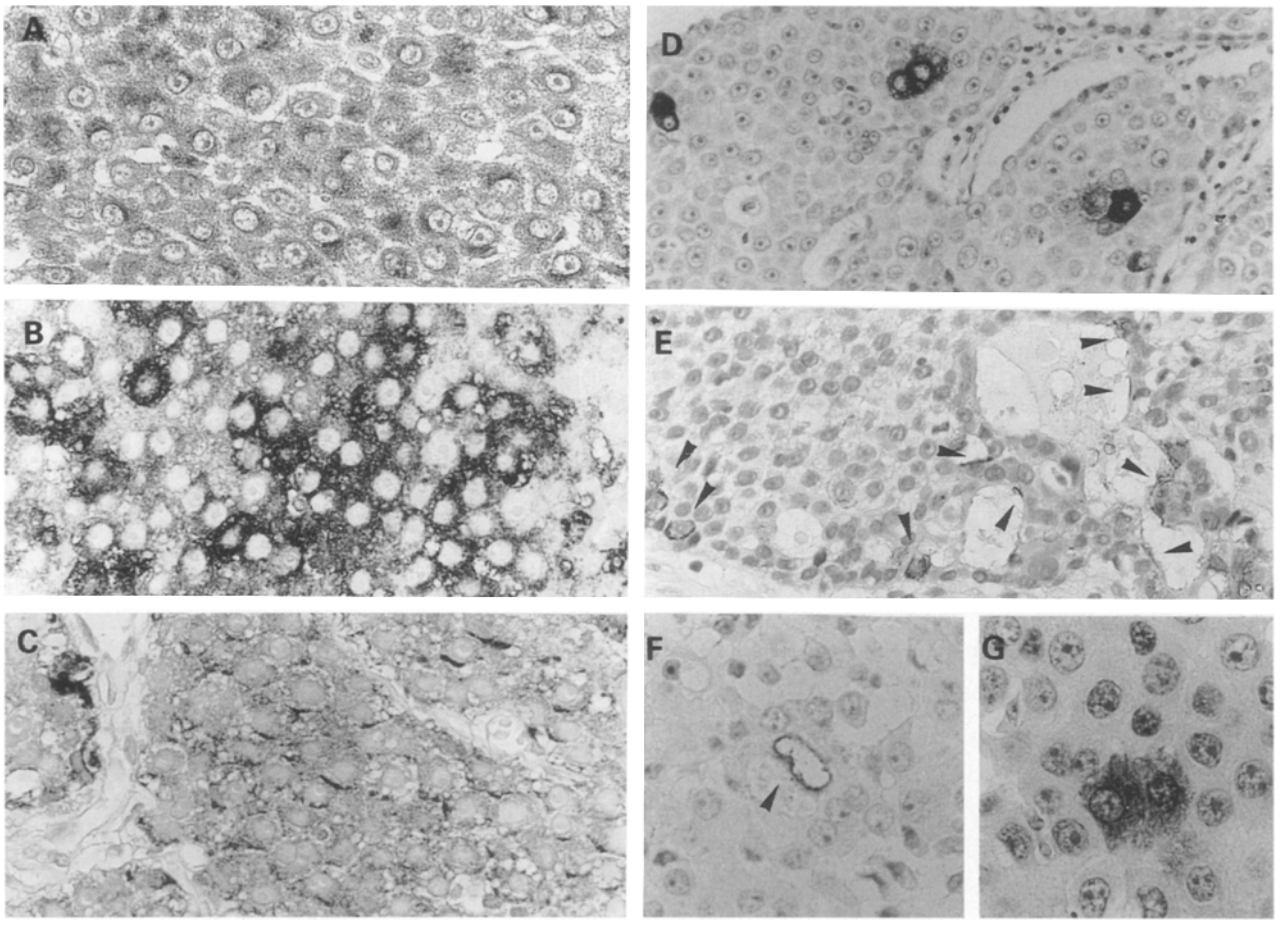

Fig. 2A–G Immunohistochemistry. **A** Alpha fetoprotein. ×375. **B** Trypsin. ×400. **C** Lipase. ×400. **D** Carcino-embryonic antigen. ×250. **E** Epithelial membrane antigen. ×200. **F** CA19-9. ×175. **G** Neuron-specific enolase. ×400. **A–C** Diffuse staining; **D–G** Scattered positive cells. Inner surface of a lumen is stained with CA19-9 (*arrowhead*). EMA is stained at the luminal surface partially and scattered individual cells (*arrowheads*)

Discussion

The diagnosis of ACC for the pancreatic tumour presented here was established on the basis of the immunohistochemical and electron microscopical results. The tumour cells were found to produce pancreatic enzymes such as trypsin and lipase and contain zymogen granules, and apart from these ACC-typical features the tumour showed AFP-positivity. ACCs have been reported to account for 1% of exocrine pancreatic malignant tumours [2, 18]. The incidence of pancreatic tumours with elevated AFP level ranges widely from 6% [4] to 24% [17] clinically, but AFP-producing ACCs confirmed immunohistochemically are few, comprising only 4.5 [8] and 6% [14] in two recent ACC series . Markedly elevated serum AFP levels were detected in five tumours in other published series [13, 20, 23]. A sixth pancreatic tumour was thought to be a ductal adenocarcinoma, but the illustrations suggest that it was actually an acinar cell carcinoma [28].

AFP is an oncofetal protein that is produced in the liver and yolk sac and in the fetal gastrointestinal tract [6]. It is thus likely that tumours originating from these tissues may show AFP production. AFP-producing tumours of the stomach can histologically mimic hepatocellular carcinoma and have therefore been named hepatoid adenocarcinoma [11]. Among pancreatic malignancies, AFP has been demonstrated in pancreatoblastomas [1, 22] and ACCs [8, 12, 14, 20], but rarely in other tumours [10]. Experimentally Rao et al. [27] induced the appearance of hepatocyte-like cells (pancreatic hepatocytes) in the rat pancreas. In the human pancreas and its neoplasms, however, such hepatocyte-like cells have yet not been described. AFP-producing cancers do not always derive from hepatoid differentiated cells of the foregut, as pointed out by Ooi et al. [24], who found AFP-positive gastric carcinomas resembling yolk sac tumours. Because different patterns of glycosylated chains in AFP have recently been identified, differential reactivity of AFP affinity to concanavalin A (Con A) and lens culinalis agglutinin (LCA) have been frequently used to elucidate the causative organ or nature. AFP produced by primary hepatocellular carcinoma binds strongly to Con A, whereas AFP of yolk sac tumour usually (50–70%) fails to react with Con A. We did not study the affinity of

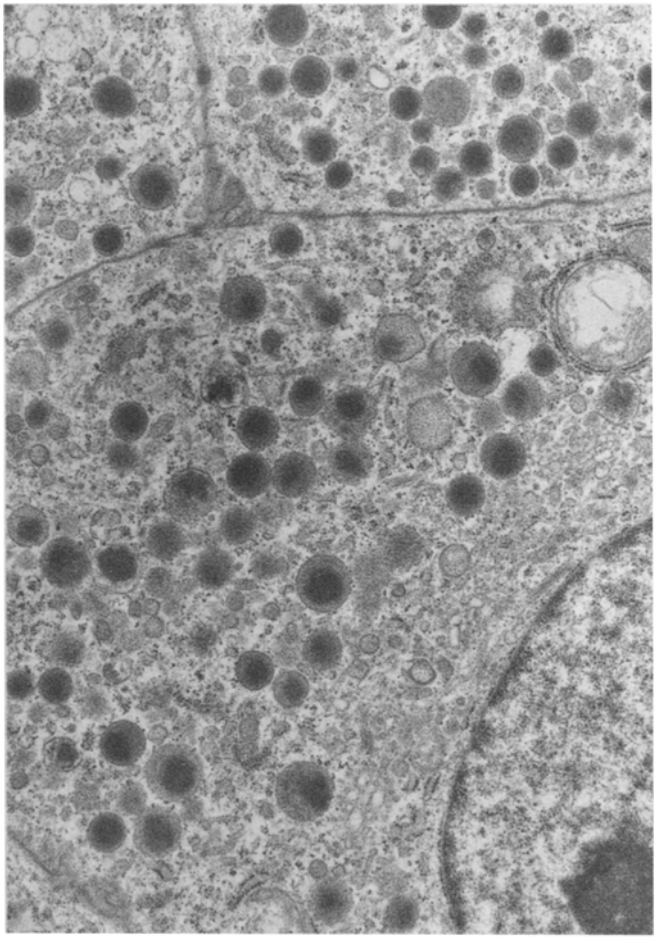

Fig. 3 Electron microscopy. Obvious zymogen granules measured 200–600 nm. Note short villus-like processes. ×12145

AFP for Con A or LCA in this case, and there are only two reports on the AFP affinity of pancreatic ACC in the literature. Itoh et al. [12] demonstrated the binding of serum AFP from an ACC to Con A and LCA, while Nojima et al. [20] reported the opposite.

Although the present tumour exhibited acinar cells predominantly, some tumour cells also displayed neuroendocrine and ductal features. Ductal differentiation in ACCs has been reported by several investigators [3, 8, 14, 29, 32]. In two recently published series, CEA and CA19-9, which are well known markers of pancreatic ductal adenocarcinomas [7, 19], were found to be positive in 18% [14] and 41% [8] of ACCs, respectively. Ductal differentiation within ACCs, sometimes considered as mucous metaplasia of tumour cells [5], probably reflects the capacities of ACCs for differentiating into several directions, including ductally [8].

Exocrine and endocrine cells of the pancreas are known to develop from a common endodermal epithelium [16], and human tumours may imitate this intimate relationship between ducts and islet cells during the early developmental stage [26]. Wiedenman et al. [33] demonstrated immunohistochemically and electron microscopically coexpression of zymogen granules and neuroendocrine vesicles in the cells of a rat acinar carcinoma [5], which seems to be amphicrine in origin. Scattered neuroendocrine cells are encountered in approximately 40% of human ACCs [8, 14], but the unequivocal immunocytochemical demonstration of exocrine (pancreatic enzymes) and neuroendocrine markers in the same tumour cells is infrequent [8, 15]. Based on electron microscopical examination, Ulich et al. [31] described neuroendocrine and zymogen granules within the same tumour cells. However, ultrastructurally zymogen and endocrine granules may be difficult to distinguish in neoplasms. The zymogen granules of many ACCs, including the present case, are often smaller than zymogen granules of normal acinar cells. Recent ultrastructural studies have disclosed irregularly shaped fibrillary granules in the ACC cells of the pancreas [14, 15, 30], which appeared to be specific for acinar differentiation. These fibrillary granules, however, were not observed in the present case. Nonomura et al. [21] reported two pancreatic tumours composed of three elements, duct, acinar and islet cells.

Recently, Klimstra et al. [15] described ACCs in which more than 25% of the tumour cells exhibited endocrine features. For these tumours they proposed the term "mixed acinar-endocrine carcinoma". The current case (as well as most other ACCs published so far) does not represent a mixed tumour but rather belongs to the "pure" ACC type, containing only a minor component of non-acinar cells.

Pancreatoblastoma, which occurs predominantly in children, is mainly composed of acinar cells. This tumour has to be included in the differential diagnosis of ACC, as it secretes AFP and endocrine and ductal differentiation are found in half the cases [1, 22]. Because of these similarities, differential diagnosis between ACCs and pancreatoblastomas may be very difficult in the absence of squamoid corpuscles, which are a hallmark [14]. In the present case, squamoid corpuscles were not detected. Nevertheless, it can be speculated that AFP-producing ACCs may be pancreatoblastomas that have lost their squamoid corpuscles. Therefore, the relationship between ACC with multi-directional differentiation and AFP production and pancreatoblastoma should be investigated in more detail.

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