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CASE REPORT

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Insulinoma of the pancreas with insular–ductular differentiation in its liver metastasis – indication of a common stem-cell origin of the exocrine and endocrine components

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Abstract We describe an insulinoma of the pancreas in a 56-year-old patient, which showed insular–ductular differentiation in its liver metastasis. Although the primary tumor was uniformly endocrine in nature with insulin production, the metastasis contained two distinct cell types in organoid arrangement. One cell type was insulin-positive and was arranged in islet-like structures; the other was insulin-negative but distinctly pan-cytokeratin and cytokeratin 7 positive and arranged in ducts. In the primary tumor and the metastasis, the tumor cells were surrounded by a desmoplastic stroma. As to the histogenesis of the tumor and its metastasis, we discuss the following possibilities: (1) the tumor cells might derive from a common stem cell that matures into two phenotypically different cell lines, resembling the situation in embryogenesis and (2) one tumor cell type originates from the other by transdifferentiation (metaplasia). We conclude that the parallel occurrence of endocrine and ductal differentiation supports the concept that, under certain conditions, islet cells and ductular cells may also originate from islets and that mixed endocrine/exocrine pancreatic tumors do not necessarily arise from totipotent duct cells but might also have a primary endocrine cell origin.

Introduction

Besides typical pancreatic tumors with ductal, acinar, or endocrine phenotype, mixed exocrine–endocrine tumors of the pancreas have been observed [13, 28]. Cells with endocrine features have been demonstrated in pancreatoblastomas [10] but also in common ductal adenocarcinomas [15, 24] and in acinar cell carcinomas [11, 20]. Conversely, ductular structures have been observed in endocrine pancreatic tumors, and it was concluded that not all endocrine tumors arise within islets but rather originate from pluripotential stem cells or committed endocrine precursor cells of ducts [1].

Only rarely have neoplasms with extensive exocrine and endocrine differentiation been observed [12, 13, 19, 26, 30]. Controversial issues are the existence of hybrid cells with both exocrine and endocrine characteristics [2, 5, 17] and the observations of Pour et al. [22] that probably all pancreatic tumors (exocrine and endocrine) arise from Langerhan's islets. In the present report, a classical insulinoma of the pancreas is described which, in its liver metastasis, exhibited an endocrine insular and an exocrine ductular component. To the best of our knowledge, this situation has not been reported previously.

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Materials and methods

After fixation in 8% buffered formaldehyde solution (pH 7.4), the primary pancreatic tumor and the liver tissue containing the metastasis were processed conventionally and paraffin embedded. Sections, 4-µm thick, were stained with hematoxylin and eosin. In addition, immunohistochemistry was performed using the alkaline phosphatase anti-alkaline phosphatase (APAAP) method. A standard protocol and commercially available reagents were used (Dako, Glostrup, Denmark). For some antibodies, the tissue was protease-digested using 0.1% proteinase XXIV (Sigma, St. Louis, Mo.) for 10 min. The primary antibodies, their source, clones, dilution, and antigen retrieval methods are listed in Table 1. The secondary antibody was a rabbit anti-mouse antibody or a mouse anti-rabbit antibody (Table 1). The peroxidase–antiperoxidase complex was obtained commercially (Dako). Fast red was used as the chromogen and hematoxylin was used as the counterstain.

Clinical history and pathological findings

In a 56-year-old man, a partial pancreatectomy was performed because of a nodule which was clinically regarded as an insulinoma. Upon gross examination, the tumor was a gray–white well-circumscribed firm nodule measuring 12 mm in diameter, surrounded by normal pancreatic tissue. Histologically, the tumor consisted of tumor cells arranged in irregular trabeculae and islands separat-

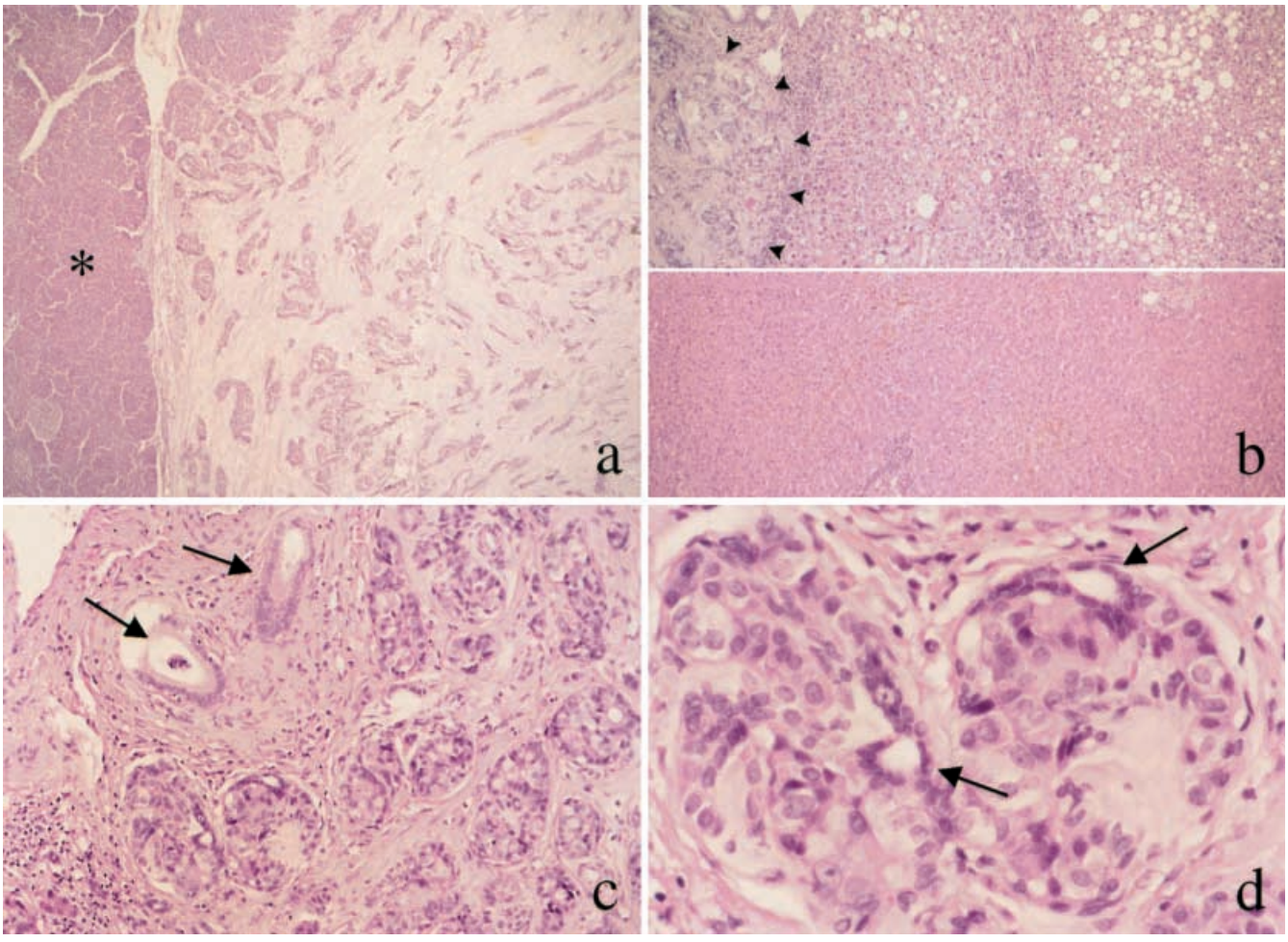

Fig. 1 **a** Pancreatic tissue showing a well-demarcated endocrine tumor adjacent to exocrine tissue (*); hematoxylin and eosin (HE), 20×. **b** *Upper part* insulinoma metastasis (*arrowheads*) adjacent to steatotic liver tissue; HE, 40×. *Lower part* liver tissue distant from the metastasis lacking steatosis; HE, 40×. **c** Liver metastasis in the vicinity of a portal tract showing clusters of tumor cells embedded in fibro-hyalinized stroma. Bile ducts are indicated by *arrows*; HE, 100×. **d** Tumor cells with slightly eosinophilic cytoplasm form islet-like cell clusters associated with small tubules (*arrows*); HE, 630×

Table 1 Antibodies with specification of dilution and antigen retrieval, clones, and sources used. *m* mouse; *ra* rabbit; *Ig* immunoglobulin

Antigens	Antibodies	Clone (source)	Dilution	Antigen retrieval
Pan-cytokeratin	m-a-CK	Clone MNF 116 (Dako, Glostrup, Denmark)	1:50	Protease
Cytokeratin 7	m-a-h-CK7	M 7019 (Dako)	1:100	Protease
Insulin	m-a-h Insulin	Subclass IgG1, kappa (Novo Biolabs, Bagsvaerd, Denmark)	1:500	Protease
Pancreatic polypeptide	ra-a-h PP	Polyclonal (Dako)	1:3000	–
Gastrin	ra-a-h Gastrin	Polyclonal (Dako)	1:50	Protease
Glucagon	m-a-h Glu	Subclass IgG1, kappa (Novo Biolabs)	1:50	–
Chromogranin	m-a-h CGA	Clone LK2 H10 (Boehringer Mannheim, Germany)	1:50	–
Serotonin	m-a-Synth serotonin	5HAT-H209 (Dako)	1:500	Protease

ed by dense fibro-hyalinized stroma (Fig. 1a). The tumor cells were round to polygonal with finely granular eosinophilic cytoplasm. The nuclei were slightly polymorphic. Mitotic figures were rare. Insulin production was detected immunohistochemically. Vascular invasion was not detected. Five peripancreatic lymph nodes were removed; they were free of metastasis.

A single liver metastasis was diagnosed by means of computed tomography 10 years later and resected. There was no indication of a recurrent tumor in the pancreas at the time of operation. The metastasis showed subcapsular localization in segment five of the liver. It was a gray–white well-circumscribed nodule measuring 2 mm in diameter and arose within a portal tract. It consisted of

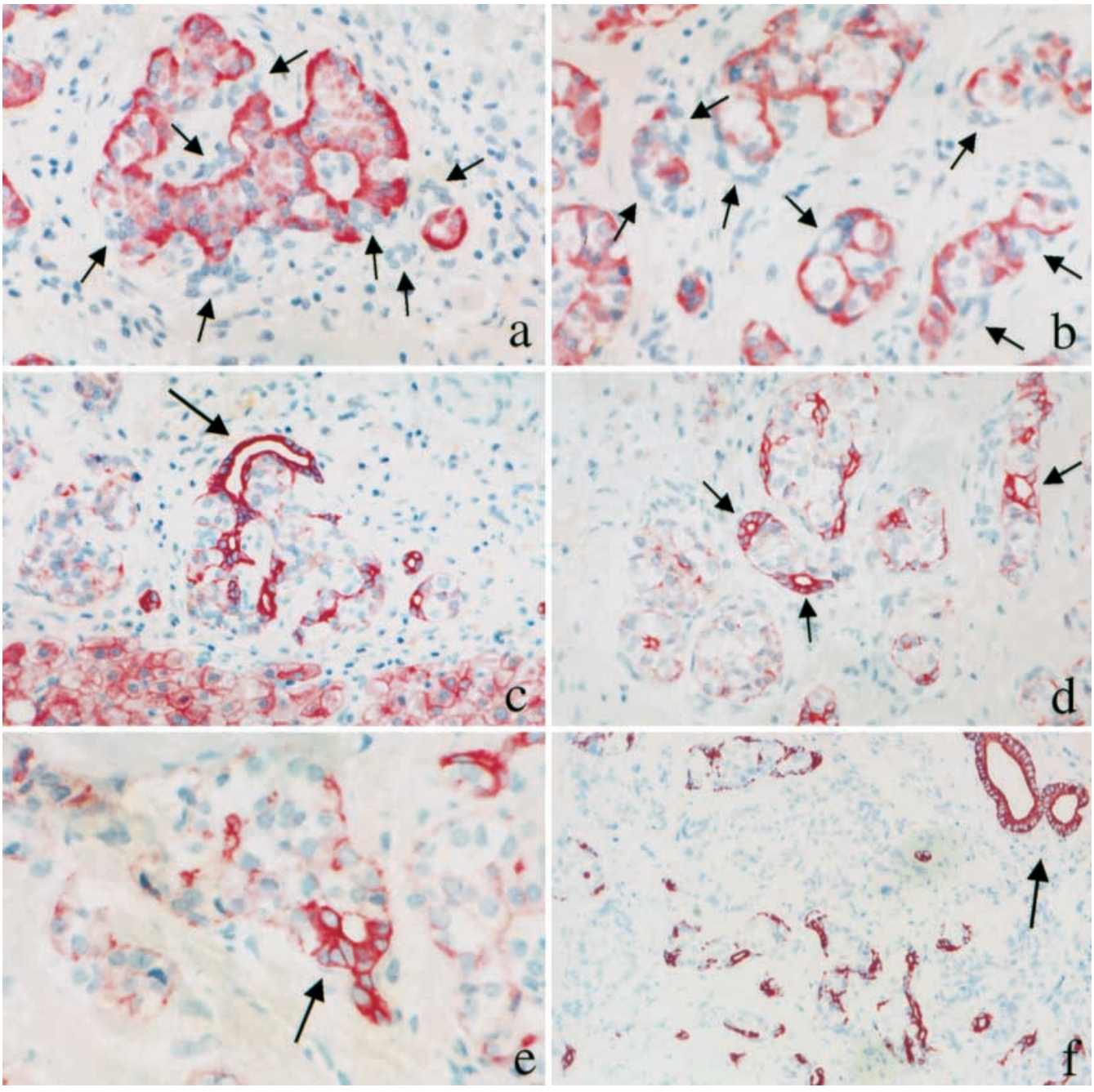

Fig. 2 Insulinoma metastasis in the liver. **a** Immunostaining for insulin demonstrates insulin-positive tumor cells, which are in direct contact with small insulin-negative tubules (*arrows*); 400×. **b** The same change shown at a higher magnification; 630×. **c–e** Immunostaining for pan-cytokeratin shows a positive reaction in tubular structures within the metastasis and in hepatocytes at the *bottom of* **c**; 100× and 630×. **f** Immunostaining for cytokeratin 7 labels ductular structures within the metastasis and an intrahepatic bile duct (*arrow*); 40×

clusters of tumor cells embedded in fibro-hyalinized stroma (Fig. 1c). Two different tumor cell populations could be discriminated in conventional hematoxylin-eosin histology. The first cell population had uniform tumor cells with slightly eosinophilic cytoplasm. The round to oval-shaped nuclei showed fine chromatin and no or only small nucleoli. These tumor cells formed spherical solid nests resembling small islets (Fig. 1d). A second cell population was in direct contact with these cells. They formed small tubules, 20–40 µm in diameter and contained smaller more hyperchromatic nuclei. The cytoplasm was more eosinophilic, and the shape of the cells was mostly cuboidal or flat (Fig. 1d). In immunohistochemistry, the latter cells were strongly positive for pan-cytokeratin (Fig. 2c–e) and cytokeratin 7 (Fig. 2f) and negative for insulin, whereas the former were negative for cytokeratin 7 but only weakly positive for pan-cytokeratin and strongly positive for insulin (Fig. 2a,b) and chromogranin. Pancreatic polypeptide, gastrin, glu-

cagon, and serotonin were undetectable in both tumor cell types. No mitotic figures were observed in either cell population. Bile ducts in the same portal tract and in the surrounding liver tissue showed a positive reaction for both pan-cytokeratin and cytokeratin 7 (Fig. 2f upper left). Hepatocytes reacted with the pan-cytokeratin antibody (Fig. 2c, bottom). In the portal tracts in the vicinity of the tumor, a mild nonspecific lympho-histiocytic infiltrate with some admixed eosinophils was recognized. The hepatocytes surrounding the metastasis showed pronounced steatosis (Fig. 1b, upper part), increased cytoplasmic glycogen content, and glycogen nuclei (characteristic of a localized effect of insulin produced by the endocrine tumor cells). The patient is still alive without tumor recurrence 8 years after the removal of the metastasis.

Discussion

Primary pancreatic carcinomas containing both endocrine and exocrine (ductal or acinar) components are rare and only few cases have been described in the literature [12, 13, 19, 26, 27, 28, 30, 31]. Nonomura et al. [19] reported a pancreatic tumor consisting of neoplastic cells with endocrine morphology and insulin expression intermingled with ducts which were negative for insulin. Occasionally, direct transitions between ductal- and islet cell-type cells were observed. Similarly, Schron and Mendelsohn [26] observed a poorly differentiated adenocarcinoma with glandular structures and signet ring cells which, in several areas of the primary tumor and in its lymph-node metastases, resumed the histologic appearance of an islet cell tumor reacting with antibodies to somatostatin. Moreover, Klimstra et al. [12] reported five cases of pancreatic carcinomas with mixed acinar–endocrine phenotype, one with segregated areas consisting of acinar and endocrine cells and the others with a morphologically uniform cell population where the different cell types, i.e., acinar and endocrine cells, could only be discriminated immunohistochemically. These authors also emphasized the rarity of ductular–insular carcinomas with either distinct or intermingled ductal and endocrine components.

We feel that our case is remarkable for two reasons: (1) The primary tumor was a classical insulinoma with solid/trabecular morphology, whereas the liver metastasis clearly revealed a mixed endocrine–exocrine pattern with organoid-like arrangement of the endocrine and exocrine elements, the endocrine cells being recognized by insulin production and secretion and the exocrine cells on the basis of their ductal arrangement, lack of insulin expression and pronounced pan-cytokeratin and cytokeratin 7 positivity. Cytokeratin 7 is a characteristic marker for ductal cells of the hepato-pancreatic ductal tree [3]. (2) Despite the occurrence of a liver metastasis 10 years after surgical removal of the primary pancreatic tumor, the patient is still alive and apparently tumor-free 8 years after resection of the liver metastasis. The possibility that the nodule in the liver resembled ectopic pancreatic tissue in a portal tract is highly unlikely since, in a study of 1000 consecutive autopsy livers, only 4.1% contained pancreatic tissue, all without Langerhan's islets [29].

The occurrence of mixed tumors with endocrine and exocrine differentiation raise the question of the relationship of endocrine and exocrine elements in the pancreas. It is now accepted that both are derived from the endoderm and arise from a primitive precursor (stem) cell capable of differentiation in several directions, finally leading to the complex cellular composition of the mature pancreas, (for review see Peters et al. [21]). Depending on the potency of the precursor cell at the time of neoplastic transformation, the resulting tumor may either show pure endocrine, pure exocrine, or a spectrum of endocrine and exocrine differentiation [12]. However, even in the normal adult pancreas, cells with morphologic characteristics of exocrine, ductal, and acinar cells have been detected [5].

A close relationship of pancreatic endocrine cells to pancreatic ducts has been demonstrated by studies in humans and in experimental animals. Nesidioblastosis in children is a pathological condition, which is characterized by persistent hyperinsulinemic hypoglycemia and the occurrence of hypertrophic insulin cells, which may show a close association with pancreatic ductules [25]. However, the occurrence of ductulo–insular complexes is not restricted to children but is also found in adults and in the course of a variety of conditions, including the Zollinger–Ellison and watery-diarrhea syndromes and after sulfonylurea medication [4, 6, 7, 8, 9, 16, 18].

Moreover, also in the non-neoplastic pancreas, connections between islets and the ductal system have been observed even beyond the embryonic period. Particularly, the presence of ducts within islets is suggestive of transformation of islet cells from duct epithelium [33]. Islets seem to be able to regenerate from ducts after injury (Weichselbaum, 1909; Laguesse, 1895 and 1910 cited in [33]), suggesting that duct epithelium may retain a certain degree of totipotency. Consequently, islet cell tumors could arise as a reaction of the duct epithelium to a carcinogenic stimulus. However, the islets of the adult pancreas, once differentiated out of the duct, seem to grow by proliferation on their own.

In our case, a small liver metastasis with clear-cut endocrine and exocrine morphology arose from a classical and at least light microscopically uniform insulin-producing endocrine pancreatic tumor. We have to assume, therefore, that endocrine cells, at least at a certain stage of differentiation in the neoplastic process, are also able to convert into exocrine ductal elements. The ability of islet cells to transform into duct cells has already been suggested by Weichselbaum and Kyrle [32]. Moreover, Yuan et al. [34] observed "transdifferentiation" of human islets to exocrine pancreatic cells if cultured on a collagen matrix. These authors observed cystic transformation of islets in culture with the appearance of primitive ductal epithelium. At 10 days of culture, insulin protein

and messenger (m)RNA were absent from islet cells, whereas all cells expressed the duct cell marker cytokeratin 19. Moreover, transformation of islet cells into duct cells was also observed during experimental pancreatic carcinogenesis in Syrian golden hamsters. In these animals treated with N-nitrosobis (2-oxopropyl)-amine, ductules arose within islets, which progressively developed malignant cellular features upon continuation of carcinogen treatment [23]. Intrainsular ductules were also observed in pancreatic tissue of patients with chronic pancreatitis and pancreatic cancer. Moreover, evidence exists that at least some human pancreatic cancers develop within islets [22].

These experimental results, together with our observations, support the concept that pancreatic endocrine cells at a certain stage of differentiation are able to produce a progeny with exocrine and endocrine features. This suggests that exocrine pancreatic regeneration and duct formation may, under certain conditions, also start from the islets and that mixed endocrine/exocrine pancreatic tumors do not necessarily arise from totipotent duct cells but might also have a primary endocrine cell origin. In consideration of the evolution of islet cells from ducts reported in the literature, it could also indicate that islet regeneration potentially proceeds through a ductular intermediate phase.

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