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ORIGINAL ARTICLE

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Mixed acinar–endocrine carcinoma of the pancreas. A clinicopathological study and comparison with acinar-cell carcinoma

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Abstract We compared the clinicopathological features of acinar-cell carcinomas (ACCs) with those of mixed acinar–endocrine carcinomas (MAECs). Specimens from 37 patients with ACC and 6 patients with MAEC were examined histologically and immunohistochemically. The mean age of ACC and MAEC patients was similar (61.3 years versus 58.4 years), but the sex ratio differed (ACC, 29 males and 8 females; MAEC, 2 males and 4 females). The size of the tumor was large in both cases (ACC, 13.8 cm in diameter; MAEC, 8.2 cm). Immunohistochemically, more than half of the tumor cells in all tumors, whether ACC or MAEC, stained for trypsin. In 20 of the 37 ACCs (54%), scattered endocrine cells (SECs) were found, which stained positively for synaptophysin (SYN) and/or chromogranin A (CGA). Interestingly, there was also a difference in the sex ratio between ACC patients without SECs (16 males and 1 female) and ACC patients with SECs (13 males and 7 females). In MAECs, the cells staining for SYN were more common than those staining for CGA and made up more than one-third of the neoplastic-cell population. In all but one case (in which the endocrine component was arranged in islet-like cell clusters), the endocrine cells were intimately mixed with trypsin-positive tumor cells. The endocrine cells only rarely expressed one of the known pancreatic or gastrointestinal hormones. Both ACCs and MAECs had a high proliferation rate and lacked p53 overexpression or progesterone and estrogen receptors. This study revealed that ACCS and MAECs share most clinicopathological features and, therefore, may form a single tumor entity, though they differ in the number of endocrine cells. The frequent identification of endocrine cells in these tumors suggests the existence of a pluripotent cell of origin that is capable of differentiating into acinar and endocrine cells.

Keywords Pancreas · Acinar-cell carcinoma · Mixed acinar–endocrine carcinoma · Immunohistology

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Introduction

Acinar-cell carcinoma (ACC) constitutes a phenotypically and genotypically different entity from ductal adenocarcinomas and endocrine tumors [1, 2, 4, 11, 14], but it is well known that one-third of the tumors may express neuroendocrine markers [2, 4]. Though the endocrine component is usually limited to scattered cells, occasionally the endocrine cells constitute a significant proportion of the tumor tissue [5, 6, 7]. If they exceed 30% of the tumor, the neoplasm is called mixed acinar–endocrine carcinoma (MAEC). Because of its histological and biological similarity to ACC, MAEC is considered a variant of ACC [15], but it is so rare that we do not yet know whether it may have some features that are distinct from those of the usual ACCs. Another question is whether MAECs show a special hormonal expression profile. We therefore evaluated the clinicopathological features of a series of ACCs and contrasted them with six cases of MAEC.

Materials and methods

The cases were retrieved from the surgical files and the consultation files of the Department of Pathology of the University of Kiel, Germany. Tumor specimens from 43 patients, including 37 with ACC and 6 with MAEC, were examined. Of these patients, 18 had been investigated in a previous study [2]. All tumors but one occurred in the pancreas. This tumor was a MAEC that was localized in the stomach wall and probably originated from heterotopic pancreas tissue. The clinicopathological features that were studied included patient age and sex, tumor size and histological growth pattern (acinar, solid or glandular). Clinical information was ob-

tained from the patients' records, but follow-up information was not available. The tumor tissue was fixed in 10% formalin and routinely processed for paraffin embedding. The sections were stained with hematoxylin and eosin (H&E) and periodic acid-Schiff for microscopic study.

Immunohistochemistry

Depending on the amount of material available, immunostaining was performed with several or all of the following antibodies: anti-trypsin monoclonal antibody (Ventrex Laboratories, Portland, OR, USA; 1:500), anti-chromogranin A (CGA) monoclonal antibody (Ventana Medical Systems, Tucson, AZ, USA; 1:50), anti-synaptophysin (SYN) polyclonal antibody (Ventana; 1:50), anti-insulin monoclonal antibody (BioGenex, San Ramon, CA, USA; 1:40), anti-glucagon polyclonal antibody (BioGenex; 1:60), anti-somatostatin polyclonal antibody (Dako Cytomation, Glostrup, Denmark; 1:200), anti-pancreatic polypeptide (PP) polyclonal antibody (Dako Cytomation; 1:10,000), serotonin (Dako Cytomation; 1:20), anti-gastrin (Paesel, Frankfurt, Germany; 1:3000), anti-progesterone receptor (PgR) monoclonal antibody (Ventana; 1:50), anti-estrogen receptor (ER) monoclonal antibody (Ventana, 1:50), anti-Ki-S5 (which recognizes a formalin-resistant epitope of the Ki-67 proliferation antigen) [10] monoclonal antibody (Department of Hemapathology, University of Kiel, Germany) and anti-p53 monoclonal antibody (Oncogene Research Products, Boston, MA, USA; 1:20). The immunostaining was carried out using the avidin-biotin-peroxidase complex method. The tissue samples were subjected to pressure cooker treatment for 3.5 min prior to SYN, Ki-S5 and p53 immunostaining. The positivity of CGA and SYN immunostaining was interpreted on a three-point scale: negative, scattered (less than 30% of the neoplastic cell population was immunoreactive) and diffuse (greater than 30% of the neoplastic cell population). Tumors with scattered CGA or SYN positivity were designated as ACCs with scattered endocrine cells (SECs), and those with diffuse positivity were designated as MAECs. For PgR, ER and p53, we considered strong nuclear labeling in >30% of neoplastic cells as the cutoff for positivity. The Ki-S5 labeling index (LI) was calculated as percentage of positive cells in at least 1000 tumor cells and was scored as high when the Ki-S5 LI was higher than 5%.

All statistical evaluations were carried out using a χ^2 test (2×2 contingency table). Statistical significance was tested at a probability level of 0.05.

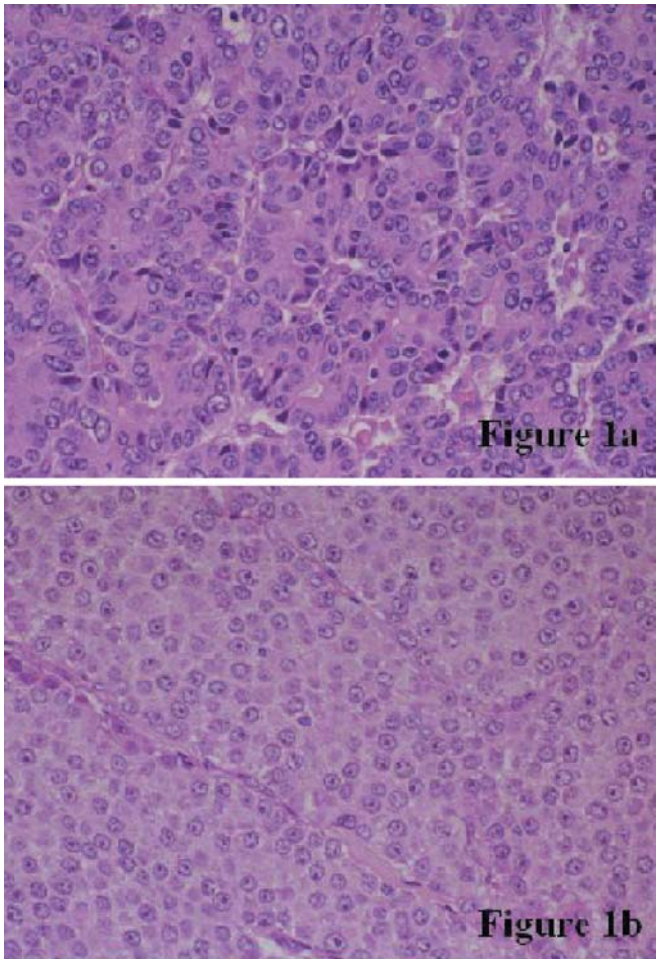

Fig. 1 The main histological patterns found in acinar cell carcinomas and mixed acinar–endocrine carcinomas: acinar pattern (**a**) and solid pattern (**b**). Hematoxylin and eosin, ×250

Results

ACC versus MAEC

Clinicopathological findings

The age of 35 patients with ACC ranged from 35 years to 79 years, with a mean of 61.3 years. There were two exceptions (a 13-year-old girl and a 19-year-old boy). The age of 5 patients with MAEC ranged from 49 years to 65 years (mean 58.4 years). The one exception was a 16-year-old girl. The patients with ACC included 29 males and 8 females, while among the patients with MAEC, females (4 patients) outnumbered males (2 patients). The mean tumor size of both ACCs and MAECs was large (13.8 cm and 8.2 cm in diameter). They showed no preferential localization in the pancreas. Histologically, both tumor types exhibited a nodular pattern at low power, with the nodules often separated by fibrous cords. In ACCs, the tumor cells showed an acinar growth pattern in 5 cases (Fig. 1a), a solid pattern in 10 cases (Fig. 1b) and a mixed pattern in 22 cases (acinar-glandular in 3 cases, acinar-solid in 17, acinar-glandular-solid in 2). MAECs showed a solid pattern in 2 cases and a mixed acinar-solid pattern in 3. There was one case with scattered cell clusters the size of islets composed of cells with pale cytoplasm (Fig. 2a) (Table 1).

Immunohistochemical findings

All tumors, whether ACC or MAEC, showed scattered to diffuse staining for trypsin. The reactivity was more intense than that of the endocrine markers SYN or CGA. Among 37 ACCs, SECs with SYN and occasional CGA positivity were found in 16 (43%) and 11 cases (30%), respectively. Of the 6 MAECs, 3 showed positivity for both SYN and CGA and 3 only for SYN. In the tumors with SYN and CGA positivity, there were more SYN-positive than CGA-positive cells. CGA and SYN positivity was found mainly in tumor cells growing in a solid pattern reminiscent of low-grade endocrine tumors. In 1

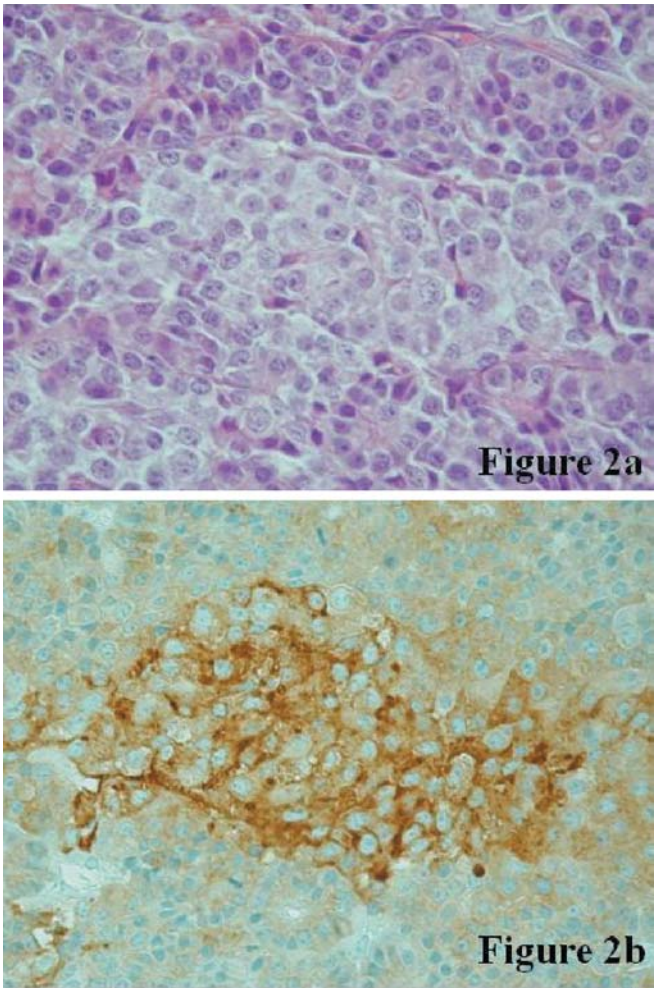

Fig. 2 Histological features of a mixed acinar–endocrine carcinoma showing a cluster of tumor cells with pale cytoplasm resembling an islet (**a**). The cell clusters stain with endocrine markers (synaptophysin) but not acinar markers (**b**). Hematoxylin and eosin, ×300 (**a**) and immunostaining for synaptophysin, ×250 (**b**)

of 6 MAEC, approximately 5% of the tumor cells stained for serotonin, and a few individual cells stained for somatostatin and glucagon. None of the MAECs stained for insulin, PP or gastrin. The endocrine cells were intimately mixed with trypsin-positive tumor cells in all but one tumor. In this tumor, clusters of cells with pale cytoplasm that had been recognized in the H&E sections were found to stain for endocrine markers (Fig. 2b) but not for trypsin. In the other tumors, there was a mixture of endocrine and acinar cells (Fig. 3). PgR and ER positivity was not found in either type of tumor cell. Of 21 ACCs, 14 showed high proliferative activity, as did 2 of 2 MAECs. p53 expression was detected in only 2 of 20 ACCs and in neither of the 2 MAECs investigated. Statistically, there was a significant difference between ACC and MAEC cases in the sex ratio but not in the other factors (Table 1).

ACC without SECs versus ACC with SECs

The mean age of 17 patients with ACC without SECs was 64.3 years, while that of 18 patients (excepting two pediatric patients) with ACC with SECs was 58.0 years. The patients with ACC without SECs included 16 males and 1 female, while there were 13 males and 7 females with ACC with SECs. There was a significant difference in the sex ratio between ACC without SECs and ACC with SECs. None of the pathological and biological factors investigated revealed a significant difference between the two tumors, as shown in Table 1.

Discussion

Our study revealed that the two groups of ACCs, i.e., those with or without SECs and those with a MAEC population share most clinicopathological features and may, therefore, be of similar origin. In addition, we found that MAECs, even when they contain many endocrine cells, only rarely express one of the known pancreatic or gastrointestinal hormones.

Table 1 Clinicopathological findings in 37 acinar-cell carcinomas and 6 mixed acinar–endocrine carcinomas. *NS* not significant

	Acinar cell carcinoma	Mixed acinar-endocrine carcinoma	*P* value	Acinar cell carcinoma		*P* value
				Endocrine component		
				Absent	Present	
No. of patients	37	6		17	20	
Mean age (years)	61.3*	58.4**	NS	64.3	58.0	NS
Sex (male:female)	29:8	2:4	0.03	16:1	13:7	0.04
Tumor size (cm)	13.8 (*n*=18)	8.2 (*n*=2)	NS	15.0 (*n*=6)	13.2 (*n*=12)	NS
Acinar pattern	27/37	4/6	NS	14/17	13/20	NS
Solid pattern	29/37	6/6	NS	14/17	15/20	NS
PgR positivity	0/19	0/2	NS	0/7	0/12	NS
ER positivity	0/19	0/2	NS	0/7	0/12	NS
Ki-S5 >5%	14/21	2/2	NS	4/8	10/13	NS
p53 positivity	2/20	0/2	NS	0/7	2/13	NS

* Excluding a 13-year-old girl and a 19-year-old boy
** Excluding a 16-year-old girl

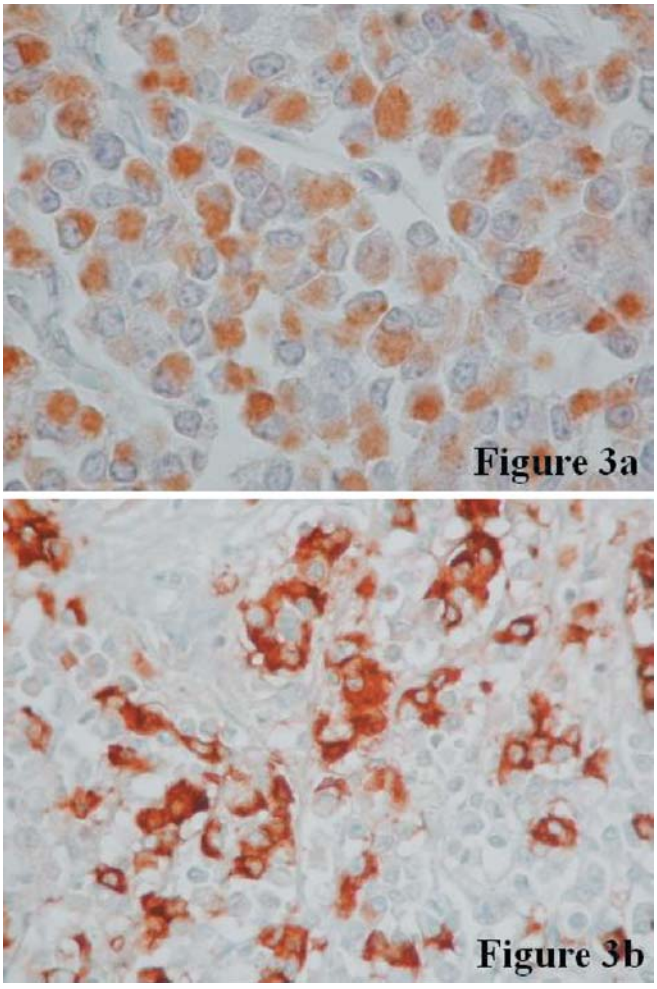

Fig. 3 Trypsin and chromogranin A-positive cells are intimately mixed in mixed acinar–endocrine carcinomas. Immunostaining for trypsin (**a**) and chromogranin A (**b**), ×400

It appears that the only difference between ACCs and MAECs is the number of endocrine cells. Most of the features, such as histological differentiation, tumor size and location and nuclear p53 expression, are similar. The only exception is the gender, since the ACCs in our series showed the well-known male preponderance of this tumor group, whereas MAECs were more common in women than men. Considering the small number of MAECs, this difference could be incidental. However, we noted that there was also a female predominance (three women and two men) among the five MAECs reported by Klimstra et al. [5], and there was again a similar sex distribution in our ACCs with SECs compared with those without SECs. The reason why MAECs occur predominantly in females is unclear. Among pancreatic tumors, the tumors with a strong female predominance include solid-pseudopapillary tumors, mucinous cystic neoplasms and serous microcystic adenomas [8]. It has been hypothesized that they might derive from genital ridge/ovarian anlage-related cells, which become attached to the pancreatic tissue during early embryogenesis [9]. Since MAECs, however, show a typical pancreatic acinar phenotype, they seem to have no relationship with the female genital tract. Moreover, MAECs did not show expression of PgR or ER, which might have an influence on the growth and prognosis of female-predominant tumors [16]. A clinicopathological study of a larger number of MAECs is necessary to clarify the reason for the female predominance.

The presence of SYN- and, to a minor degree, also CGA-expressing cells in MAECs and ACCs with SECs indicates that the cells giving rise to these tumors are pluripotent and may differentiate towards acinar and endocrine cells. The endocrine cells, however, seem to remain at a low differentiation level, since most of these tumors do not stain with antisera against the common gastroenteropancreatic hormones. There was only one MAEC in which a small number of cells expressed serotonin, somatostatin and glucagon. Among Klimstra's five MAECs, there were two tumors that contained a few hormone-positive cells staining for somatostatin, glucagon, PP, gastrin or vasoactive intestinal polypeptide. This scarcity of hormone expression in MAECs demonstrates that either these tumors produce immature hormone products or the hormone production in the tumor cells is generally so low that it escapes immunocytochemical detection. The fact that usually somatostatin, but never insulin, was among the hormones identified thus far in ACCs and MAECs might indicate that the neoplastic endocrine cells are comparable in their genetic equipment to the endocrine cells that occur early in the embryological development of the endocrine pancreas [13]. It is also of interest in this connection that the endocrine cells fail to express the receptor for progesterone, which normal islet cells and cells in pancreatic endocrine tumors constantly show [12]. Finally, MAECs, though equipped with many endocrine cells, differ from the typical well-differentiated neuroendocrine tumors of the pancreas in their much higher proliferation rate. This difference is also reflected in their poorer prognosis, which is identical to that of ACCs with or without SECs.

The endocrine cells that occur in ACCs with SECs and in MAECs might derive from a common progenitor cell with a potential for dual acinar–endocrine differentiation. A second possibility leading to endocrine cells in ACCs is a non-dysjunctional mitosis in which dual differentiation arises initially and concurrently from a single precursor cell [3]. MAECs with segregated areas showing only endocrine marker positivity may follow the latter pathway of differentiation. However, as we had only one MAEC that showed this pattern, it seems that this type is the exception, and a complete mixture is the usual case. The factors driving the pronounced endocrine differentiation that characterizes MAECs are not yet known.

In summary, we found no distinct differences between ACCs and MAECs, except for the sex ratio. This suggests that they may form a single tumor entity, though they differ in the number of endocrine cells, and that they have in common a progenitor cell that is able to give rise to acinar and endocrine cells. In the case of the endocrine

cells, it seems that these cells still have a precursor cell status, since most of them lack definite hormone production, as is usually seen in endocrine tumors of the pancreas.

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