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Fibrillary inclusions in neoplastic and fetal acinar cells of the pancreas

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Abstract We report a case of pancreatic acinar cell carcinoma which contained a large number of pleomorphic inclusions with fibrillary internal structures and mature zymogen granules. To clarify the significance of fibrillary inclusions in the differentiation of acinar cells of the pancreas, we further investigated fetal pancreases (gestational weeks 16, 17, 19, 20 and 28). We found two types of inclusions: type A, corresponding to fibrillary inclusion of neoplastic acinar cells, was observed only in a 19-week fetus; type B showed a homogeneous density similar to that of zymogen granules. Type B was observed in all the fetuses after the 17th gestational week. Although the type A inclusion might be generated throught a different mechanism than the type B inclusion, the appearance of a large number of fibrillary inclusions in neoplastic acinar cells may represent a transient form of zymogen granule.

Key words Acinar cell carcinoma · Fetal pancreas · Electron microscopy · Fibrillary inclusion · Rough endoplasmic reticulum

Introduction

Acinar cell carcinoma of the pancreas is a rare neoplasm, constituting approximately 1% of pancreatic malignant tumours [2, 3, 16, 20]. It usually consists of cells similar to normal acinar cells with eosinophilic and granular cytoplasm in an acinar structure. However, the tumour can exhibit various patterns (cystic, solid, or trabecular [1, 14, 22]). Using light microscopy alone, it is sometimes difficult to distinguish this tumour from an islet cell tumour, carcinoid, undifferentiated carcinoma, oat cell carcinoma or even ductal carcinoma [16, 25, 26, 29].

Electron microscopy has been an important adjunct in the diagnosis of acinar cell carcinoma. Typical features are abundant rough endoplasmic reticulum (RER) and granules that are easily identified as zymogen granules with a maximum diameter of 600–1200 nm [4, 9, 13, 21, 25]. However, at the ultrastructural level, small granules resembling endocrine granules are occasionally identified in acinar cell carcinoma, which might lead to some confusion in the diagnosis [26, 27, 29]. Some recent reports have demonstrated a unique intracellular structure in neoplastic acinar cells; a pleomorphic, membrane-bound, filament-containing inclusion, which suggests that this inclusion may be helpful in the diagnosis [11, 14, 15, 26, 28].

In this report, we describe a case of acinar cell carcinoma of the pancreas exhibiting this unique inclusion. Furthermore, we investigated fetal pancreases to clarify the significance of this structure in the diagnosis and to determine the relationship between this inclusion and the differentiation of the fetal acinar cell.

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Clinical history

A 59-year-old woman complained of jaundice and epigastric pain for 1 week. No endocrine-related symptoms were noted. Ultrasonography and computed tomography (CT) revealed a neoplastic mass at the pancreatic head. A pancreaticoduodenectomy was performed. No metastases were noted during the operation. The tumour measured 6×4×4 cm. It was a greyish white, solid, and well-demarcated tumour with areas of necrosis; the duodenum showed ulceration due to invasion. The tumour compressed the common bile duct without invasion. However, there was lymphatic and vascular invasion and lymph nodal metastases.

Seven months later, multiple hepatic metastases were detected by CT scan. She died 16 months after surgery. An autopsy was not performed.

Materials and methods

The pancreatic tumour obtained during surgery was fixed in 10% buffered formalin and embedded in paraffin. Sections were cut 3

Table 1 Panel of antibodies used for immunohistochemistry (*PSTI* pancreatic secretory trypsin inhibitor, *NSE* neuron specific enolase, *HCGα* human chorionic gonadotropin α-subunit, *AFP* α-fetoprotein, *CEA* carcinoembryonic antigen)

Antibody	Clonality	Dilution	Source
Secretory products			
Trypsin	Polyclonal	1:3,000	Ogawa M[a]
Chymotrypsin	Polyclonal	1:3,000	Ogawa M
Amylase	Polyclonal	1:1,500	Ogawa M
Phospholipase	Polyclonal	1:2,000	Ogawa M
Elastase 1	Polyclonal	1:2,000	Ogawa M
PST1	Polyclonal	1:4,000	Ogawa M
Neuroendocrine markers			
Synaptophysin	Polyclonal	1:200	DAKO
NSE	Monoclonal	1:200	DAKO
Chromogranin	Polyclonal	1:1,500	Immunonuclear Corporation
HCGα	Polyclonal	1:10,000	Okumura H[b]
Glucagen	Polyclonal	1:150,000	Japan Immunoresearch Laboratories
Somatostatin	Polyclonal	1:10,000	DAKO
Insulin	Polyclonal	1:500	Japan Immunresearch Laboratories
Pancreatic peptide	Polyclonal	1:200,000	Chance RE[c]
Tumour marker			
AFP	Polyclonal	1:500	DAKO
CEA	Monoclonal	1:5,000	Mochida Pharmacy
Other marker			
Alpha-1-antitrypsin	Polyclonal	1:2,000	DAKO

[a] Second Department of Surgery, Osaka University, Osaka, Japan
[b] Teikoku Hormone Manufacturing Company
[c] Eli Lilly and Company, Ind., USA

μm thick and stained with haematoxylin and eosin (H & E), alcian blue and periodic acid-schiff (PAS), or were subjected to immunohistochemical studies.

Fetal pancreas was obtained from five fetuses aged 16, 17, 19, 20 and 28 gestational weeks. All were previously involved in a study of the development of fetal pancreatic secretory proteins [5, 6].

Immunohistochemical studies were performed on formalin-fixed, paraffin-embedded tissue of the pancreatic tumour of the present case, using the avidin-biotin-peroxidase complex method as reported previously [5, 6]. The primary antibodies used are shown in Table 1.

For ultrastructural examination, fresh tissue from the pancreatic tumour and fetal pancreatic tissue were placed in 2.5% glutaraldehyde, postfixed in 1% osmium tetroxide, and embedded in epoxy resin. Ultrathin sections were stained with uranyl acetate and lead citrate, and examined under an electron microscopy (H 7000, Hitachi, Japan). We examined at least 500 cells of each fetal pancreas.

Immuno-electron microscopical examination was applied to the tumour by the pre-embedding method using antibodies for cytokeratin, trypsin, chymotrypsin and elastase 1 antibody, as reported previously [7, 8].

Results

Light microscopic examination of the tumour showed an acinar pattern, with solid growth in some areas. The neoplastic cells showed uniform large, round nuclei with prominent nucleoli, and were uniformly eosinophilic with finely granular cytoplasm (Fig. 1). The neoplastic cells were frequently in mitosis. Immunohistochemical studies showed that almost all the cells were positive for trypsin, chymotrypsin, alpha-1-antitrypsin, pancreatic secretory trypsin inhibitor and elastase 1, but negative for amylase, lipase, tumour markers and all the neuroendocrine markers. Some neoplastic cells had a perinuclear eosinophilic hyaline area. The hyaline area in the cytoplasm was negative with alcian blue and PAS stains and all immunohistchemical stains used.

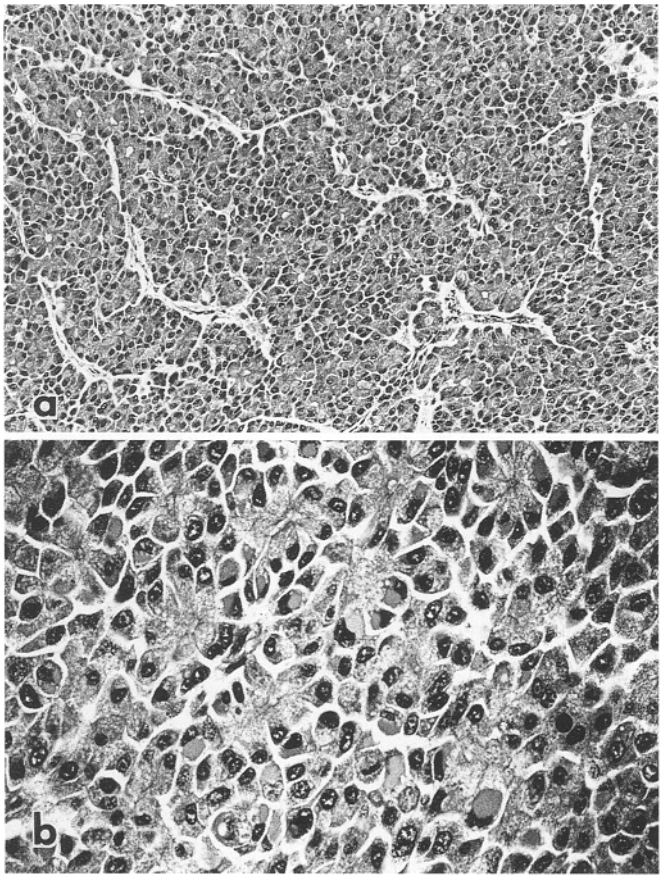

Fig. 1 **a** Neoplastic cells exhibit an acinar pattern and show granular eosinophilic cytoplasm typical of acinar cell carcinoma. Uniform tumour cells with large nuclei and prominent nucleoli growing in an acinar pattern. Haematoxylin and eosin stain, ×40. **b** Some neoplastic cells have an eosinophilic hyaline area beside the nuclei in the cytoplasm. Haematoxylin and eosin stain, ×200

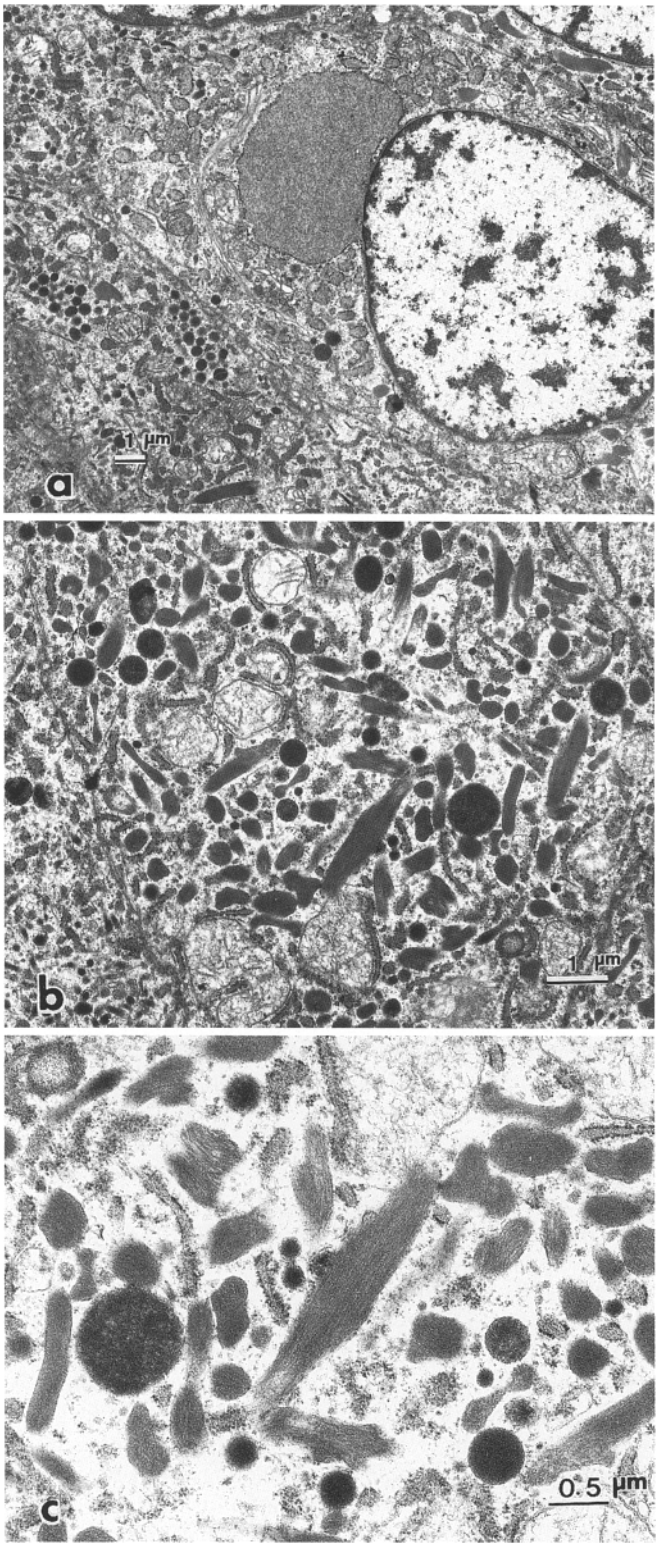

Fig. 2a–c Electron microscopy of acinar cell carcinoma. Neoplastic acinar cells contain abundant Golgi apparatus and mitochondria. An abundance of 200–1,000 nm, uniform-density zymogen-like granules can be seen. **a** Some rough endoplasmic reticulum is widely dilated, resembling nuclei, and contain medium-density material. ×5,000. **b** Almost all of the neoplastic acinar cells exhibit a large number of pleomorphic inclusions with a fibrillary internal structure. ×10,000. **c** The fibrillary inclusions are arranged in a bundle, and often cross each other. All of the inclusions are surrounded by a limiting membrane. ×20,000

Table 2 Frequencies of pleomorphic inclusions and zymogen granules in the acinar cell carcinoma and fetal pancreas (– absent, + small number, ++ moderate number, +++ large number)

	Pleomorphic inclusion		Zymogen granule
	Type A	Type B	
Acinar cell carcinoma	+++	–	+++
Fetus 16 weeks	–	–	+
17 weeks	–	+	+
19 weeks	+	+	++
20 weeks	–	++	++
28 weeks	–	++	+++

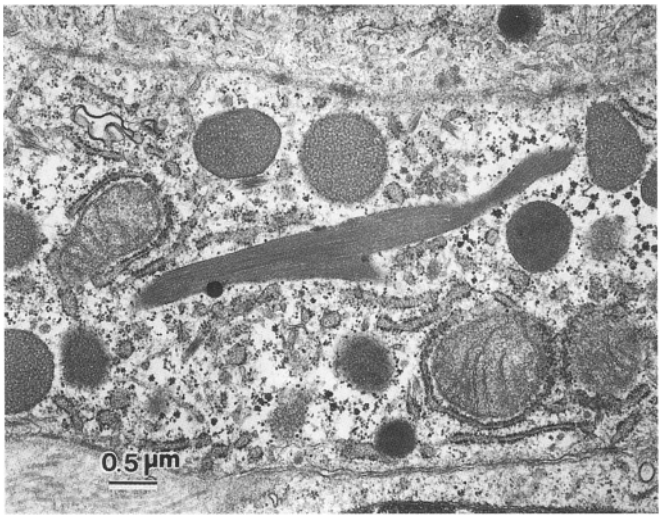

Fig. 3 Fetal pancreas at 19th week of gestation. Fibrillary inclusions resembling those found in neoplastic acinar cells are seen. These inclusions also show a fibrillary internal structure and are surrounded by a limiting membrane. However, the number of inclusions per cell is less than that in the neoplastic acinar cells. ×18,000

Electron microscopy of neoplastic acinar cells showed that tumour cells had good polarity and contained hyperchromatic nuclei (Fig. 2a). Many zymogen-like granules were observed: membrane-bound granules, with a fairly uniform density, ranging in size from 200 to1000 nm. Golgi apparatus and mitochondria were well developed. These features are typical of acinar cell carcinoma. Vesiculated rough endoplasmic reticulum (RER) was observed; some widely dilated to the size of nuclei, containing medium-density material. The eosinophilic hyaline area in the cytoplasm on light microscopy corresponded to these dilated RER.

In addition, almost all the neoplastic acinar cells exhibited a large number of pleomorphic inclusions (Fig. 2b, c; Table 2). The inclusions varied in size and shape, and all showed a fibrillary internal structure. The fibrillary structures were arranged in a bundle and often crossed each other. The long axis of the inclusion varied from 200 to 3000 nm and the short axis varied from 250 to 600 nm. All the inclusions were surrounded by a limiting membrane. Immuno-electron microscopy revealed that the inclusions were negative for trypsin, chymotryp-

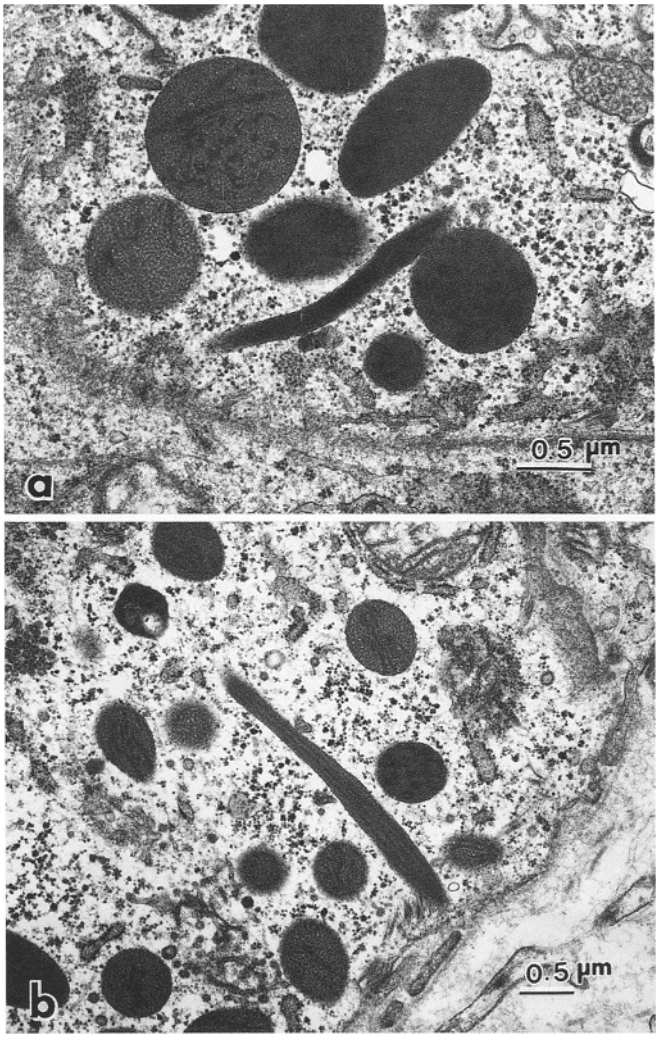

Fig. 4a, b Fetal pancreas at 20th wek of gestation. **a** Pleomorphic inclusions with a limiting membrane similar to the fibrillary inclusion in the neoplastic acinar cell are seen, but their internal structure has almost the same electron density as zymogen granules. ×24,000. **b** Few fibrillary structures can be seen in the internal structure of the inclusion. ×18,000

sin and elastase 1, while zymogen granules were densely positive. The inclusions and zymogen granules were negative for cytokeratin.

Zymogen granules were found in all the fetal pancreatic tissues electron-microscopically (Figs. 3, 4). There were few granules in acinar cells at the 16th and 17th weeks of gestation, but their numbers increased with time (in the 19th, 20th and 28th gestational weeks). Two types of inclusions were found in fetal acinar cells (types A and B). Type A inclusions resembled the fibrillary inclusion in neoplastic acinar cells, and were only observed in the fetal pancreas at the 19th week of gestation (Fig. 3). Although the number of type A inclusion per cell was fewer than that of the neoplastic acinar cells, the inclusions showed a similar fibrillary internal structure and were surrounded by a limiting membrane. The size range of type A inclusion was similar to that of the inclusion in acinar cell carcinoma.

Type B inclusions were found in the fetal pancreas at the 17th, 19th, 20th and 28th gestational weeks (Fig. 4). They varied in size and shape and had a limiting membrane. Their internal structure had almost the same electron density as zymogen granules and occasionally had a few fibrillary structures (Fig. 4b) or none (Fig. 4a). Type B inclusions were not observed in acinar cell carcinoma. The size ranges of the type A and type B inclusions were almost the same. Dilated RER was not seen in the fetal pancreases.

Discussion

A unique intracytoplasmic inclusion was first reported in a retroperitoneal tumour which was throught to be an acinar cell carcinoma in 1990 [28]. In a response to that report, Henderson stated that any ultrastructural finding should ideally fulfill three criteria to be useful in tumour diagnosis: the structure should be sufficiently distinctive in appearance that it can be readily recognized; the structure should be specific for, or characteristic of, a particular pattern of cell differentiation, either alone or in combination with other features; preferably, the structure should occur with sufficient frequency in a particular cell type that it is useful in at least a considerable proportion of cases [12].

The inclusion in neoplastic acinar cells does have a distinctive ultrastructure, as illustrated in the present case and in other studies. Klimstra et al. identified these structures in several cases of acinar cell carcinoma and in one case of a mixed acinar-endocrine carcinoma of the pancreas [14, 15]. Subsequent reports have described six cases of acinar cell carcinoma and one case of malignant mixed exocrine-endocrine tumour [11, 26].

Some previous studies on fetal pancreatic acinar cells have found elongated inclusions with a fibrillary internal structure from the 12th–20th weeks of gestation [17, 18]. Klimstra et al. suggested that the inclusions of acinar cell carcinoma may represent primitive zymogen granules due to their resemblance to fetal zymogen granules. In the present study, we found two types of inclusion. The type B inclusion was pleomorphic, but showed the same density as that of a zymogen granule. Although the type A inclusion was similar in shape to type B and the size ranges of both types were almost the same, it contained fibrillary structures. There is a possibility that technical factors contribute to the ultrastructural difference between type A and type B inclusions. However, every specimen was processed in the same fashion, and preservation of ultrastructure was excellent in all cases. The morphological resemblance between type A and type B inclusions may indicate that both inclusions are derived from zymogen granules but the temporal profiles of these inclusions were different in development: type B were observed from the 17th to the 28th gestational week, while type A appeared only transiently at the 19th gestational week. Furthermore, in neoplastic acinar cells, only the type A inclusion was observed with zymogen

granules. The two types of inclusions may be processed differently in acinar cells, and the type A inclusion may be a transient form of zymogen granule which is found only in a certain stage of fetal acinar cell differentiation. Thus, these facts confirm that fibrillary inclusions meet Henderson's criteria and may be useful for the diagnosis of acinar cell carcinoma.

In immuno-electron microscopy, there was no immunoreaction against trypsin, chymotrypsin, elastase 1 or cytokeratin. This result supports our hypothesis that type A inclusions may be a transient form of zymogen granule.

There are two different views on the possible derivation of the inclusions. Klimstra et al. revealed immunoreaction against chymotrypsin in the inclusion [15] and concluded the inclusion was derived from the zymogen granule. Their different results may be due to the different immuno-electron method used; we used the preembedding method which is more sensitive in detecting antigens than the postembedding technique applied by Klimstra et al. Furthermore, their immunogold particles were very scanty on the inclusion, and the difference of the immunoreactivity between the inclusion and the background of their Figure was difficult to see.

Pasquinelli et al. suggested that the inclusions are aggregates of intermediate filaments, because they showed immunoreactivity against cytokeratin and vimentin by immunogold labelling [23]. Although their electron microscopic Figure of the low power view showed that the inclusions were almost surrounded by a limiting membrane, high power view and immunolabelled Figures showed that the inclusion lacked a limiting membrane and its fibrillary structures were dispersed. We think that the appearance of the inclusion-like structure which they showed is different from the fibrillary inclusion reported here and previously [11, 14, 15, 26].

Some reports have suggested that the inclusion is similar to the cytoplasmic inclusions which are seen in Paneth cells of the bowel in cases of acrodermatitis enteropathica or experimentally induced zinc deficiency [19, 24]. However, the fibrillary-like structure of the inclusions did not form a bundle. This inclusion is thus also different from the fibrillary inclusion described.

The widely dilated RER was recognized as a homogeneous hyaline area on light microscopy. Such a finding has not been previously reported in acinar cell carcinoma of the pancreas. Ghadially [10] has suggested three possible explanations for this phenomenon: a rate of synthesis of secretory products in excess of that which can be handled by the transport mechanism; a defect in the transport system, such as a mechanical or enzymatic abnormality in the RER; the production of an abnormal secretory product with which the normal transport mechanism is unable to cope. Although we have no evidence to answer this question, this phenomenon may indicate a close relationship between the fibrillary inclusion and an abnormal transport mechanism, since a large number of fibrillary inclusions and dilated RER were found only in neoplastic acinar cells.

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