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The distribution and role of myofibroblasts and CD34-positive stromal cells in normal pancreas and various pancreatic lesions

N. Kuroda[1], M. Toi[1], H. Nakayama[2], E. Miyazaki[1], M. Yamamoto[1], Y. Hayashi[1], M. Hiroi[1] and H. Enzan[1]

[1]Department of Pathology, Kochi Medical School, Kochi, and

[2]Department of Molecular Pathology, Graduate School of Biomedical Science, Hiroshima University, Hiroshima, Japan

Summary. To elucidate the distribution and role of myofibroblasts and CD34-positive stromal cells in various pancreatic lesions, we performed an immunohistochemical study using a streptoavidin-biotin immunoperoxidase technique. We selected 43 pancreatic lesions from 1 biopsied, 22 surgically resected and 12 autopsied specimens: acute pancreatitis (n=3), chronic non-obstructive pancreatitis (n=4), obstructive pancreatitis (n=7), islet cell tumor (n=4), serous cystadenoma (n=7), mucinous cystadenoma (n=6), and invasive ductal carcinoma (n=12). In normal pancreas, myofibroblasts and CD34-positive stromal cells were predominantly present in the peridcutal and periacinar areas, respectively. Both myofibroblasts and CD34-positive cells were observed in the stroma of chronic pancreatitis. In four islet cell tumors, myofibroblasts were present in the stroma of the tumor center, but no CD34-positive stromal cells were identified. Additionally, myofibroblasts and CD34-positive stromal cells were located in the inner layer and the outer layer of the capsule of three islet cell tumors, respectively. In nine of the thirteen cystadenomas, only myofibroblasts were recognized in the cyst wall. In the remaining four cystadenomas, a small number of CD34-positive cells were observed in the cyst wall. In 12 invasive ductal carcinomas, the stroma possessed a lot of myofibroblasts, but there were no or few CD34-positive stromal cells. In conclusion, it seems that the abundant amount of CD34-stromal cells in the main lesions is characteristic of chronic inflammatory lesions. Myofibroblasts and CD34-positive stromal cells may play a role in regulating the tumor growth in the capsule of islet cell tumors of the pancreas.

Key words: Pancreas, Myofibroblasts, CD34

Offprint requests to: Dr. Naoto Kuroda, Department of Pathology, Kochi Medical School, Kohasu, Oko-cho, Nankoku City, Kochi 783-8505 Japan, e-mail: nkuroda@med.kochi-u.ac.jp

Introduction

In the normal pancreas, alpha-smooth muscle actin (ASMA)-positive stromal cells, namely myofibroblasts, and CD34-positive stromal cells are present (Kuroda et al., 1998; Suda, 2000; Barth et al., 2002). However, there are few studies on the distribution of these cells in pancreatic lesions (Suda et al., 1990; Bachem et al., 1998; Izumi et al., 2001; Barth et al., 2002). Therefore, the difference in the distribution and role of these cells in various pancreatic diseases including benign and malignant lesions remains unsolved. In this article, we have studied the distribution of myofibroblasts and CD34-positive stromal cells in normal pancreas and various pancreatic lesions, and discussed the role of these cells.

Materials and methods

Tissue specimens

We selected pancreatic lesions from 1 biopsied and 22 surgically resected specimens from the surgical pathology files of the department of pathology, Kochi Medical School and its affiliated hospitals from 1990 to 2002. Additionally, pancreatic lesions from 12 autopsied specimens performed from 1999 to 2002 were chosen. In total we examined 43 pancreatic lesions obtained from 35 patients (14 men and 21 women, mean age 63.1, range 21-84) in the present study. These lesions are as follows: acute pancreatitis (n=3), chronic non-obstructive pancreatitis (n=4), obstructive pancreatitis (n=7), islet cell tumor (n=4), serous cystadenoma (n=7), mucinous cystadenoma (n=6), and invasive ductal carcinoma (n=12). Clinicopathological data of cases with acute pancreatitis, islet cell tumor and invasive ductal carcinoma are summarized in Tables 1-3, respectively. In 7 cases of the 12 invasive ductal carcinomas, the upstream part, distant from cancer tissue destructing the pancreatic ducts, was used as obstructive

pancreatitis. Additionally, a pancreatic tissue obtained from an autopsy case and 12 cancer-free tissues remotely located from invasive carcinoma were used as a normal control. The paraffin sections were stained with hematoxylin and eosin.

Immunohistochemistry and its interpretation

Using a streptavidin-biotin immunoperoxidase technique as previously described (Naruse et al., 2000; Kuroda et al., 2002), 3-μm sections of each specimen were evaluated for ASMA (1:50 dilution, 1A4, DAKOpatts, Glostrup, Denmark), high molecular weight caldesmon (h-CD) (1:50 dilution, h-CD, DAKOpatts, CA, USA), CD34 (1:20 dilution, MY10, Becton-Dickinson, San Jose, CA, USA) and CD31 (1:20 dilution, JC/70A, DAKOpatts, Glostrup, Denmark). Microwave and pronase treatment was performed only for h-CD and CD31, respectively. Vascular smooth muscle cells and endothelial cells were used as the internal positive controls of immunostaining for ASMA and h-CD, and CD34 and CD31, respectively.

We classified ASMA-positive and h-CD-negative stromal cells as myofibroblasts. Furthermore, CD34-positive and CD31-negative stroma cells were considered as CD34-positive stromal cells which were referred to as fibrocytes in the article by Barth et al. (2002).

Immunoelectron microscopy

A tissue sample of normal pancreas was fixed by immersion in a periodate-lysine-paraformaldehyde solution for 24 hours. Afterwards the material was incubated with a mixed solution of phosphate-buffered saline (PBS) and sucrose and frozen sections were cut into about 20 μm. These tissue sections from tissue samples were incubated with anti-ASMA and anti-CD34 antibodies. Then the above-described immunohistochemical procedures were carried out with the addition of prefixation in 0.5% glutaraldehyde. The thin sections were processed and embedded in epoxy resin. Ultrathin sections stained with uranyl acetate and lead citrate were examined with an electron microscope (JEM100S; JEOL Ltd., Tokyo, Japan).

Results

Distribution of myofibroblasts and CD34-positive stromal cells in normal pancreas

Distribution of myofibroblasts and CD34-positive stromal cells in normal pancreas is summarized in Table 4. Myofibroblasts were predominantly present around the pancreatic ducts, but a few myofibroblasts were also observed around acinar cells (Fig. 1a). CD34-positive stromal cells were chiefly observed around acinar cells (Fig. 1b), but were also present around the pancreatic ducts. Ultrastructurally, myofibroblasts and CD34-positive stromal cells were identified around pancreatic ducts (Fig. 2a,b) and acinar cells (Fig. 2c). In most islets of Langerhans, myofibroblasts and CD34-positive stromal cells were absent. However, these cells were seen focally.

Table 1. Clinical sumamry of cases with acute pancreatitis.

No.	AGE	SEX	HISTOLOGY	CAUSE OF DEATH
1	56	M	necrotic	gastric cancer
2	60	F	necrotic	diffuse alveolar damage
3	76	F	necrotic	hepatocellular carcinoma

Table 2. Clinical summary of cases with islet cell tumor.

No.	AGE	SEX	MAXIMUM SIZE	HORMONAL ACTIVITY	CAPSULE	METASTASIS
1	58	F	1.0 cm	-	+	-
2	45	F	6.7 cm	somatostatin	+	-
3	77	F	1.1 cm	insulin	-	-
4	59	F	4.0 cm	-	+	-

Table 3. Summary of cases with invasive ductal carcinoma.

No.	AGE	SEX	SITE	MAXIMUM SIZE	GRADE	STAGE	THERAPY	OUTCOME	MYOFIBROBLASTS	FIBROSIS
1	57	M	H	1.5 cm	G2	I	operation	AWOD	+++	+
2	50	F	H	2.8 cm	G2	II	operation	?	++	++
3	68	M	B	8.0 cm	G3	IVa	chemotherapy	DOD	+	+++
4	71	F	H	3.0 cm	G2	III	operation	?	+++	+
5	47	M	B	1.5 cm	G1	II	operation	?	+++	+
6	55	M	H	4.5 cm	G2	III	operation	?	+	+++
7	76	M	H	4.0 cm	G2	IVb	none	DOD	+	+++
8	82	M	H	4.0 cm	G2	IVb	none	DOD	+++	+
9	57	M	H	10 cm	G4	IVb	chemotherapy	DOD	+++	+
10	57	M	H	6.2 cm	G2	IVb	chemotherapy	DOD	++	++
11	71	M	H	8.0 cm	G2	IVb	chemotherapy	DOD	+	+++
12	47	M	H	2.7 cm	G1	III	operation	?	+++	+

M: male: F, female; H, head; B, body; AWOD, alive without disease; DOD, died of disease.

Distribution of myofibroblasts and CD34-positive stromal cells in various pancreatic lesions

These overall results are summarized in Table 5. In acute pancreatitis, a small to moderate number of myofibroblasts were identified in the stroma and no or a small number of CD34-positive stromal cells were observed. In chronic inflammatory lesions including non-obstructive, and obstructive pancreatitis, both myofibroblasts and CD34-positive cells were observed in a significant number in the stroma (Fig. 3a,b). In these cases, the degree of fibrosis was mild to moderate. However, the center of fibrosis, containing many mature collagen fibers, seemed to have a smaller number of stromal cells positive for ASMA and CD34 when compared with the peripheral area of fibrosis. In four islet cell tumors, myofibroblasts were present in the intra-tumoral stroma (Fig. 4a), but CD34-positive stromal cells were absent (Fig. 4b). Three out of four islet cell tumors had a fibrous capsule. In the capsule of islet cell tumors, a two-layered structure was observed. Namely, myofibroblasts and CD34-positive stromal cells were located in the inner layer and the outer layer, respectively (Fig. 4c,d). In thirteen cystadenomas including seven serous and six mucinous ones, a small to moderate number of myofibroblasts were identified in the cyst wall (Fig. 5a). However, the cyst wall had no CD34-positive stromal cells in nine cystadenomas (Fig.

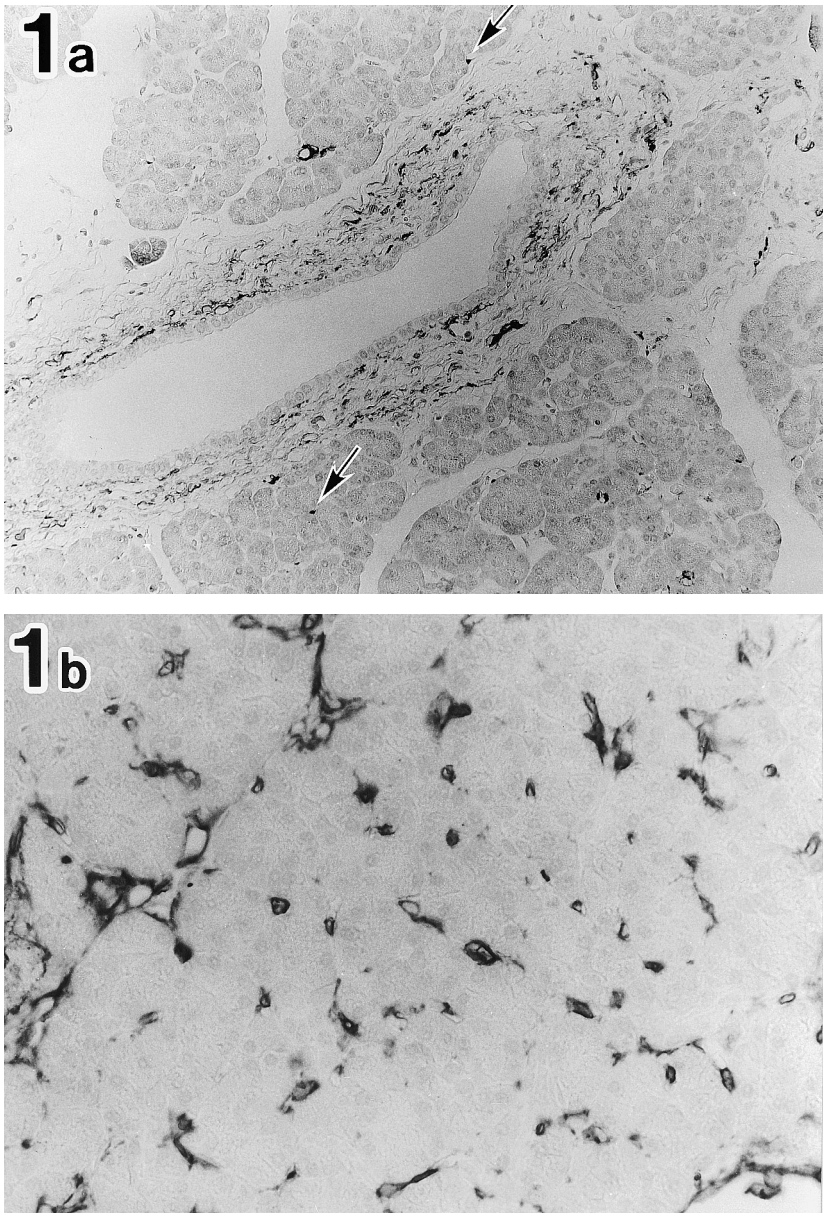
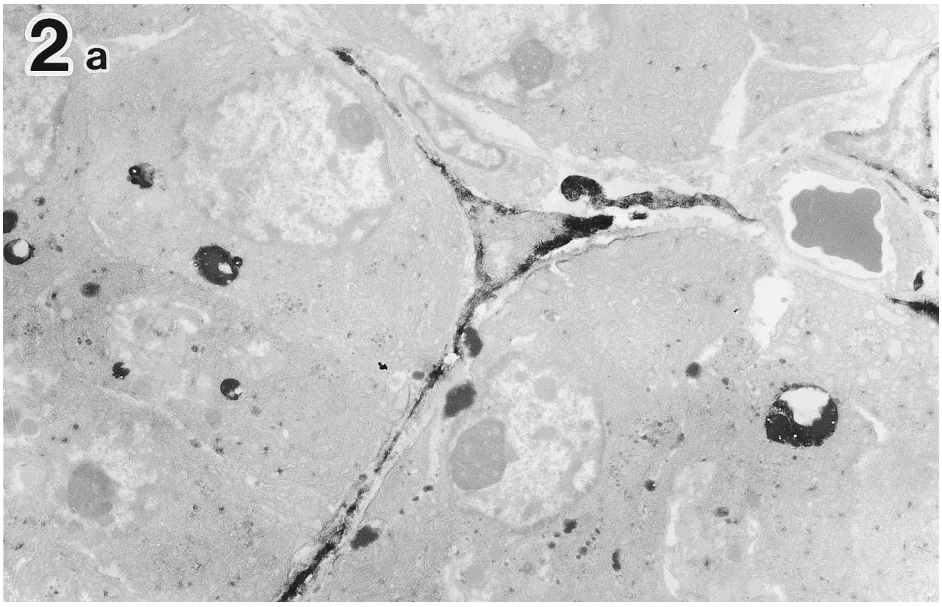
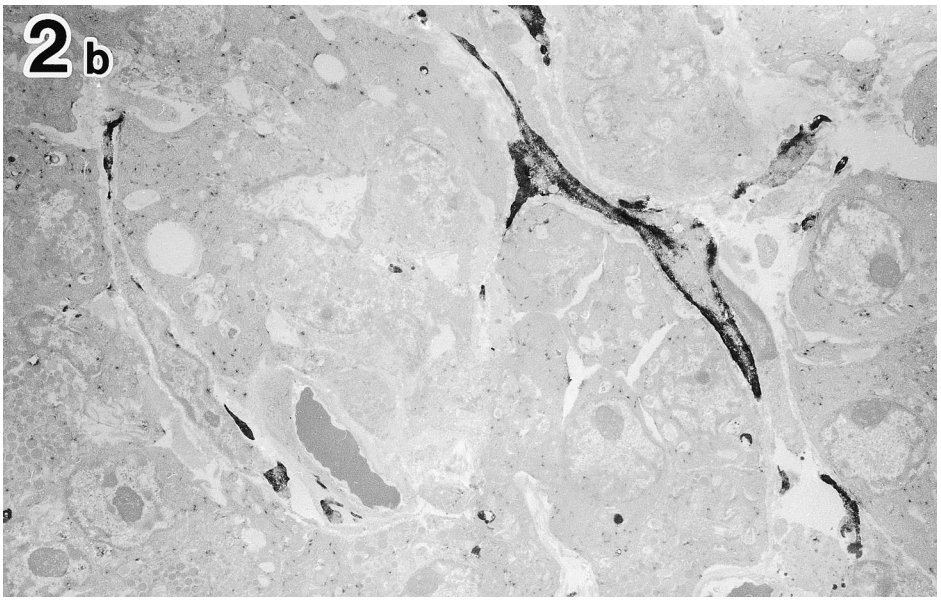


Fig. 1a. ASMA-positive stromal cells, namely myofibroblasts, are present around pancreatic ducts. A few myofibroblasts (arrows) are also recognized around acinar cells. x 50. **b.** CD34-positive stromal cells are present around acinar cells. x 100





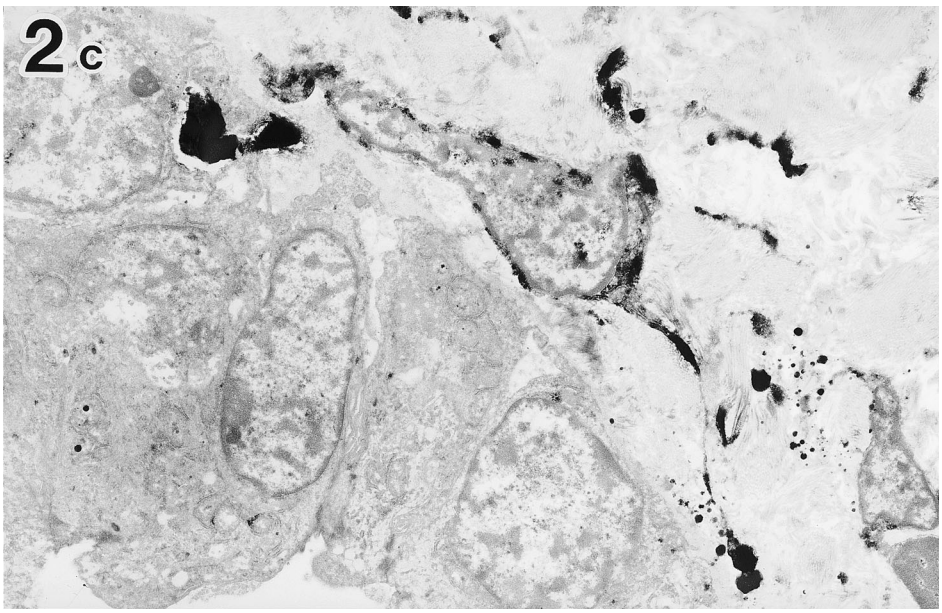


Fig. 2. Immunoelectron microscopic findings of ASMA and CD34 in the periacinar area. **a.** Stellate-shaped cells positive for ASMA, namely myofibroblasts, are recognized in the periacinar area. x 2,000. **b.** CD34-positive stromal cells are identified around acinar cells. x 2,000. **c.** ASMA-positive cells are observed around the pancreatic duct. x 2,000

5b). In the remaining four cystadenomas, a small number of CD34-positive stromal cells were observed in the cyst wall. In 12 invasive ductal carcinomas, the stroma contained many myofibroblasts (Fig. 6a), but there were no or few CD34-positive stromal cells (Fig. 6b). As summarized in Table 3, the number of myofibroblasts in the tumor stroma seemed to be inversely related to the degree of fibrosis. In the area of superficial perineural invasion, some myofibroblasts were observed between carcinoma cells and the bundle of nerve fibers, whereas no myofibroblasts were identified between them in the area of deep perineural invasion (Fig. 6c).

Table 4. Distribution of myofibroblasta and CD34(+)-stromal cells in normal pancreas.

	PERIACINAR AREA	PERIDUCTAL AREA	WITHIN ISLET OF LANGERHANS
Myofibroblasts	+	++	-~+-
CD34-positive stromal cells	++	+	-~+-

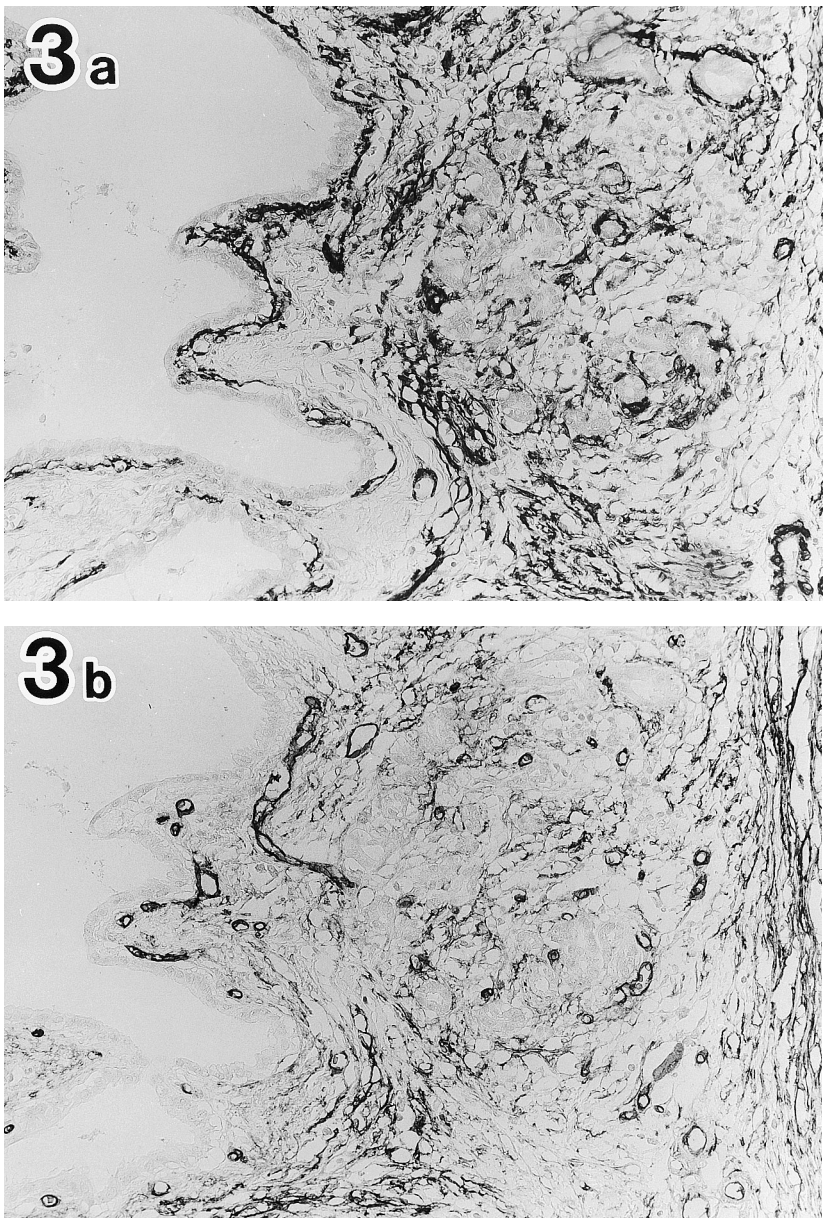


Fig. 3. Immunohistochemical findings of ASMA and CD34 in obstructive pancreatitis. **a.** Myofibroblasts are abundant in the stroma. **b.** CD34-positive cells are also seen in the stroma. x 50

Discussion

In the present study, we have confirmed that myofibroblasts were observed in the stroma of various pancreatic lesions. Among them, myofibroblasts were relatively rich in the stroma of invasive ductal carcinoma, and chronic non-obstructive and obstructive pancreatitis. We suggest that stellate-shaped cells,

Table 5. Intensity of distributin of stromal cells in various pancreaaticl lesions.

	MYOFIBROBLASTS	CD34-POSITIVE STROMAL CELLS
Acute pancreatitis (n=3)	+~++	-~+
Chronic non-obstructive pancreatitis (n=4)	++~+++	++~+++
Obstructive pancreatitis (n=7)	+++	+++
Islet cell tumor (n=4)		
tumor center (4/4)	++	-
capsule, outer layer (3/4)	-	++
capsule, inner layer (3/4)	++	-
Serous cystadenoma (n=7)	+~++	-~+
Mucinous cystadenoma (n=6)	+~++	-~+
Invasive ductal carcinoma (n=12)	+~+++	-~+-

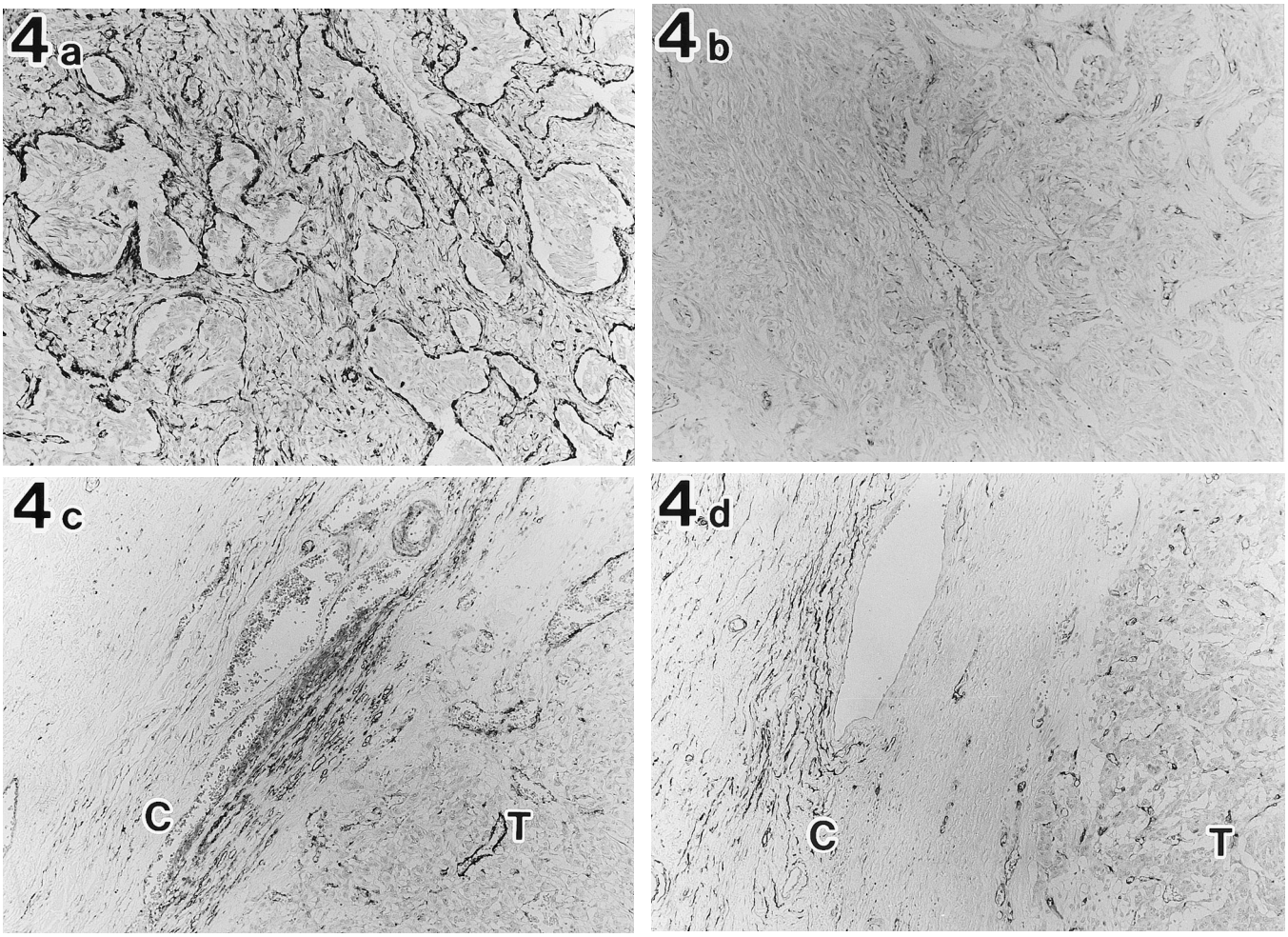


Fig. 4. Immunohistochemical findings of ASMA and CD34 in islet cell tumors. **a.** Myofibroblasts are present in the stroma of the tumor center. x 25. **b.** CD34-positive stromal cells are absent in the stroma of the tumor center. x 25. **c.** In the capsule, myofibroblasts are located in the inner layer. x 25. **d.** In the capsule, CD34-positive stromal cells are present in the outer layer. x 25. (T, tumor cells; C, capsule).

located around normal pancreatic ducts, are activated by cytokines such as transforming growth factor-ß (TGF-ß) or platelet-derived growth factor (PDGF) and acquire the nature of myofibroblastic cells and proliferate (Apte et al, 1999). Additionally, myofibroblasts around acinar cells, which was confirmed by immunoelectron microscopy in the present study, may play a role in intralobular and interlobular fibrosis. These cells may be referred to as activated pancreatic stellate cells (PSCs) in other articles (Apte et al., 1998, 1999; Bachem et al., 1998). In cases of chronic non-obstructive pancreatitis or obstructive pancreatitis in the present study, the number of myofibroblasts in the center of the fibrosis tended to be smaller than that in the peripheral area of fibrosis. Additionally, the number of myofibroblasts seemed to have an inverse correlation with the degree of fibrosis in invasive ductal carcinoma. These results suggest that myofibroblasts tend to disappear with the progression of fibrosis.

There are some reports on immunohistochemical studies of stromal cells in the capsule of tumors of various anatomic sites (Ooi et al., 1997; Nakayama et al. 1999, 2002). Nakayama et al. (1999) reported a two-layered structure in the capsule of major salivary gland pleomorphic adenomas. On the other hand, they found h-CD-positive stromal cells in the capsule of thyroid follicular tumors and tumor-like lesions. In this study, we found that the capsule of islet cell tumors was

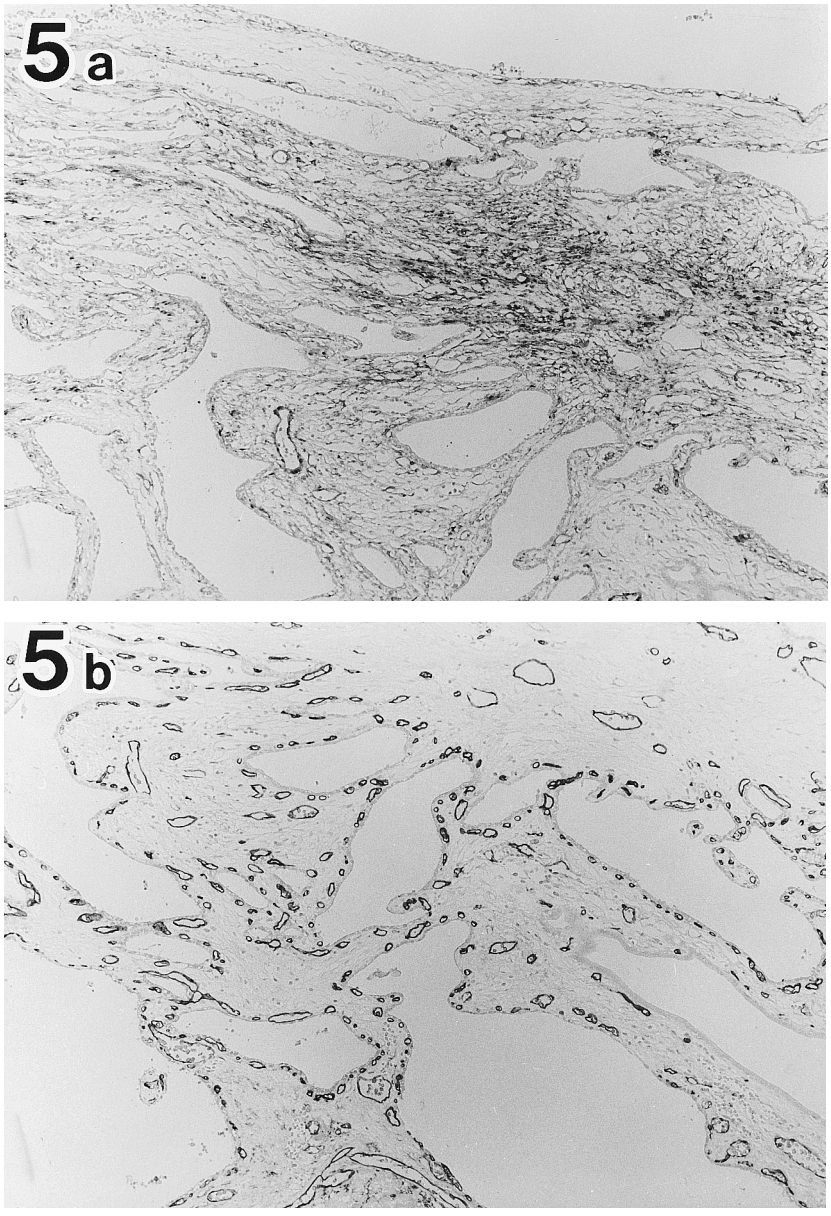
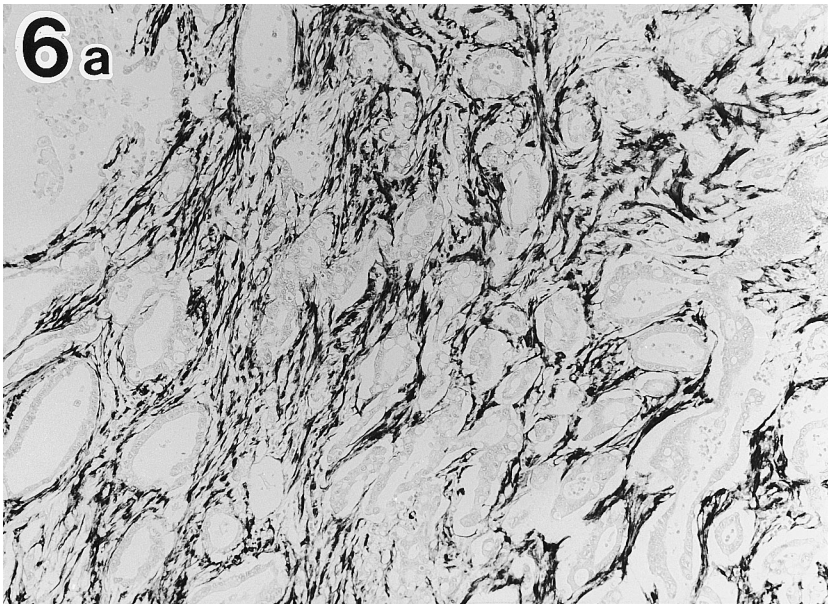
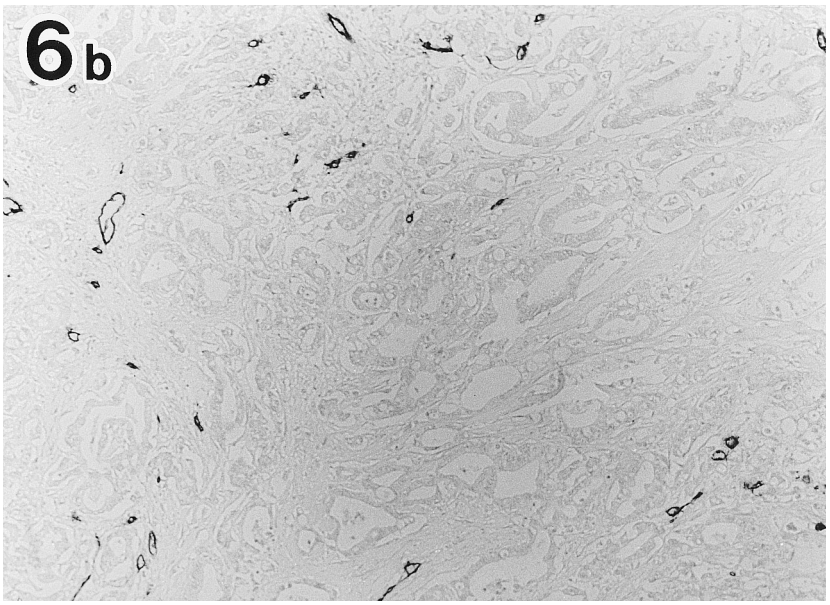


Fig. 5. Immunohistochemical findings of ASMA and CD34 in serous cystadenoma. **a.** Myofibroblasts are identified in the cyst wall. x 25. **b.** CD34 only reacts with capillary endothelial cells, but there are no CD34-positive stromal cells in the cyst wall. x 25





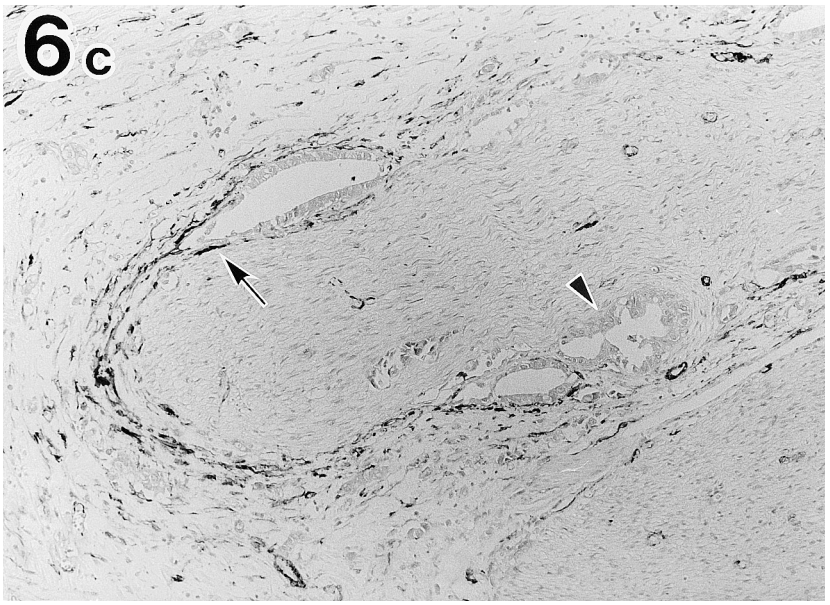


Fig. 6. Immunohistochemical findings of ASMA **(a, c)** and CD34 (**b)** invasive ductal carcinoma. **a.** Myofibroblasts are abundant in the stroma. x 25. **b.** There are no CD34-positive stromal cells in the tumor stroma. x 25. **c.** Between carcinoma cells and the nerve bundles, myofibroblasts are present in the area of superficial perineural invasion (arrow), but absent in the area of deep perineural invasion (arrowhead). x 25

composed of two cell layers. ASMA-positive and CD34-positive stromal cells were located in the inner and outer layers of the capsule, respectively. However, we found no h-CD-positive stromal cells in the capsule of islet cell tumors. Therefore, the two-layered structure in the capsule of islet cell tumors resembles that of salivary gland tumors. Accordingly, myofibroblasts and CD34-positive stromal cells may play a role in tumor growth regulation in the capsule of islet cell tumors of the pancreas.

On the other hand, a significant number of CD34-positive stromal cells were identified only in the stroma of inflammatory lesions except for the capsule of islet cell tumors. In other words, it seems that the abundant number of CD34-positive stromal cells in the main lesions is characteristic of non-neoplastic lesions except for acute pancreatitis. In cases with cystadenomas, the number of CD34-positive stromal cells was nil or small. Fukushima and Mukai (1997) reported that ovarian-type stroma of pancreatic mucinous cystic tumors expressed ASMA but not CD34. Our study shows that the stroma of mucinous cystadenoma phenotypically resembles that of serous cystadenoma, irrespective of the presence of the ovarian stroma. In contrast, CD34-positive stromal cells were absent in malignant lesions of the present study. Nakayama et al. (2000) reported that CD34-positive stromal cells were not present in the tumor stroma and peritumoral inflammatory tissue of the colon cancer, but present in normal colorectal tissue. The lack of CD34-positive stromal cells in the stroma of pancreatic carcinoma resembles that of colorectal carcinoma. It is possible that the lack of CD34-positive stromal cells in the stroma of pancreatic cancer may be associated with the peculiar fibrotic process caused by the invasion of carcinoma cells. Further examinations will be required to clarify whether myofibroblasts and CD34-positive stromal cells in various pancreatic lesions share the same origin or not.

Finally, as described by Barth et al.(2002), we speculate that the detection of the significant number of CD34-positive cells may be helpful in distinguishing invasive ductal carcinoma from chronic inflammatory lesions. Additionally, myofibroblasts and CD34-positive stromal cells may play a role in regulating the tumor growth in the capsule of islet cell tumors of the pancreas.

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