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ORIGINAL ARTICLE

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CD34+ fibrocytes in neoplastic and inflammatory pancreatic lesions

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Abstract Besides its function as a matrix-producing cell, the CD34+ fibrocyte has been reported to be an antigen-presenting cell capable of priming naive T cells in situ. Therefore, it has been claimed that the CD34+ fibrocyte may play an important role in host response to tissue damage. The objective of the present study was to analyze the presence and distribution of CD34+ fibrocytes and smooth muscle actin (SMA) reactive myofibroblasts in relation to the underlying pancreatic disease. We investigated a total of 12 pancreatic adenocarcinomas, 7 endocrine tumors of the pancreas, and 8 cases of chronic pancreatitis; in 11 cases, normal pancreatic tissue was available. The stroma of normal pancreatic tissue harbored diffusely scattered CD34+ fibrocytes. Chronic pancreatitis was characterized by an increased number of stromal CD34+ fibrocytes paralleled by a gain of SMA reactive myofibroblasts which were not observed in the normal pancreatic stroma. The stroma of pancreatic ductal adenocarcinomas and endocrine tumors was devoid of CD34+ fibrocytes or showed at least a focal loss of this cell type, whereas SMA reactive myofibroblasts were detected in both endocrine tumors and adenocarcinomas. We conclude that detection of CD34+ fibrocytes may constitute an adjunctive tool in distinguishing chronic pancreatitis from ductal adenocarcinoma since the absence of this cell population strongly favors a neoplastic process. Moreover, CD34+ fibrocytes and myofibroblasts appear to be involved in stromal remodeling associated with chronic pancreatitis and ductal adenocarcinoma.

Keywords Fibrocytes · Myofibroblast · Pancreas · Pancreatitis · Adenocarcinoma · Endocrine tumor

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Introduction

CD34+ fibrocytes have been detected in normal skin [16], normal breast tissue [4, 20, 24], and in various cutaneous [5, 6, 9, 10, 23] and mesenchymal tumors [13, 17, 19, 20, 21, 22]. To date, the origin of this cell population remains enigmatic, but experimental studies support the assumption that CD34+ fibrocytes derive from myeloid precursors [2]. They can be found in the peripheral blood and invade sites of tissue damage and are capable of connective tissue matrix synthesis [2]. Besides its function as a matrix-producing cell, the CD34+ fibrocyte is a potent antigen-presenting cell capable of priming naive T cells in situ [3]. Therefore, it has been claimed that the CD34+ fibrocyte may play an important role in host response to tissue damage of whatever cause and in specific immune-mediated host defense against invading tumor cells [8].

The occurrence of CD34+ fibrocytes in the peritumoral stroma of skin tumors has been considered to be of diagnostic significance in distinguishing basal cell carcinoma from benign skin appendage tumors, such as trichoepithelioma and its variants [5, 6, 9, 10, 23]. In these studies, the absence of CD34+ fibrocytes in the tumor-associated stroma favors the diagnosis of basal cell carcinoma and vice versa. Similar results have been reported in colon cancer: normal colonic stroma harbors CD34+ fibrocytes, whereas this cell population is absent from the stroma of invasive colon cancer. However, the tumor-associated desmoplastic stroma is characterized by the presence of smooth muscle actin (SMA) reactive myofibroblasts [15]. Recently, a solitary fibrous tumor of the pancreas with CD34+ fibrocytes was described [13], but the pertinent literature yields no information concerning the occurrence and distribution of CD34+ fibrocytes in the pancreas and benign and malignant lesions of the pancreatic parenchyma.

The present study was undertaken to investigate this topic with special reference to pancreatitis and ductal pancreatic carcinoma since the differential diagnosis of these lesions is of clinical significance and may cause significant problems to the surgical pathologist.

Table 1 Epidemiological data of patients with chronic pancreatitis

Case no.	Age (years)	Sex	Presumed cause of pancreatitis
CP1	47	Male	Alcoholism
CP2	58	Female	Unknown
CP3	59	Male	Obstructive, pancreatic acinar cell carcinoma
CP4	61	Male	Obstructive, main pancreatic duct stenosis*
CP5	77	Male	Obstructive
CP6	76	Male	Obstructive, carcinoma of the ampulla of Vater
CP7	74	Female	Obstructive, main pancreatic duct stenosis*
CP8	76	Male	Obstructive, choledocholithiasis

* Main pancreatic duct stenosis proven by means of endoscopic retrograde pancreatography

Table 2 Data of patients with ductal adenocarcinoma

Case no.	Age (years)	Sex	Grading	TNM classification*
DA1	45	Male	G1	pT3 pN0 M0
DA2	56	Female	G2	pT3 pN0 M0
DA3	85	Female	G2	pT3 pN0 M0
DA4	68	Male	G2	pT3 pN0 M0
DA5	70	Male	G2	pT3 pN0 M0
DA6	62	Male	G2	pT3 pN0 M0
DA7	33	Male	G3	pT3 pN0 M0
DA8	69	Male	G2	pT3 pN0 M0
DA9	62	Male	G2	pT4 pN1b M0
DA10	54	Female	G2	pT3 pN1a M0
DA11	67	Male	G2	pT3 pN1a M0
DA12	76	Female	G2	pT2 pN1b M0

* UICC, 4th edn, 1997

Table 3 Data of patients with pancreatic endocrine tumors. *d.n.a.* data not available

Case no.	Age (years)	Sex	Functional type	Size (cm)	Metastasis	Follow-up (years)
ET1	48	Male	Insulinoma	4.0	–	7
ET2	86	Female	Insulinoma	1.2	–	6
ET3	68	Female	Insulinoma	3.5	–	6
ET4	57	Male	Gastrinoma	4.0	–	6
ET5	50	Male	Insulinoma	1.6	–	4
ET6	40	Male	Insulinoma	d.n.a	–	4
ET7	50	Female	Glucagonoma	4.5	+ (Liver)	0.5

Materials and methods

The present study comprises 20 patients who underwent classic Whipple resection or a modified pylorus sparing Whipple procedure. In 12 cases, a ductal adenocarcinoma of the pancreas was diagnosed; in 8 cases the diagnosis of chronic pancreatitis was rendered. The epidemiological data of patients with chronic pancreatitis and ductal adenocarcinoma are summarized in Table 1 and Table 2, respectively. Histologically, the cases of chronic pancreatitis, mostly obstructive in origin, were characterized by stromal fibrosis associated with a mild to moderately interstitial lymphocytic infiltration. Abscess formation or diffuse polymorphonuclear infiltration were not observed; in one case small necroses were found. The presumed cause of chronic pancreatitis is listed in Table 1. Cases of hereditary or auto-immune pancreatitis were not included in the present study.

In 11 of 12 cases with ductal adenocarcinoma, tumor-free pancreatic tissue remotely located from infiltrating carcinoma and devoid of features characteristic of chronic pancreatitis was available and served as normal control.

We additionally investigated a total of seven functionally active differentiated pancreatic endocrine tumors, six of which were treated by enucleation (Table 3). In the remaining case (ET7), a resection of the pancreatic tail was performed. One of the seven patients with an endocrine tumor (ET1) suffered from multiple endocrine neoplasia (MEN) I syndrome, whereas the remaining cases were sporadic endocrine tumors.

After resection or enucleation, the tissues were fixed in a 4% formaldehyde solution and representative tissue blocks were selected. Tissues were embedded in paraffin, cut, and stained with hematoxylin and eosin and Periodic acid–Schiff base for routine purposes.

Immunohistochemistry

Immunohistochemistry was performed using a standard avidin–biotin complex (ABC)-peroxidase method using 3,3'-diaminobenzidine (DAB) as chromogen. CD34 antigen was detected using a monoclonal antibody (QBEND10, Immunotech, Marseille, France) without any tissue pretreatment or dilution of the antibody. SMA was detected using a monoclonal antibody (ASM-1, Progen, Heidelberg, Germany; dilution 1:200) after tissue pretreatment with 0.1% trypsin for 15 min at 37°C.

The number and distribution of stromal $CD34^+$ fibrocytes and SMA reactive myofibroblasts was assessed semi-quantitatively strictly considering that endothelia are also positive for CD34, and SMA reactive cells occur in vessel walls and in periductal myofibroblasts of the pancreas [7].

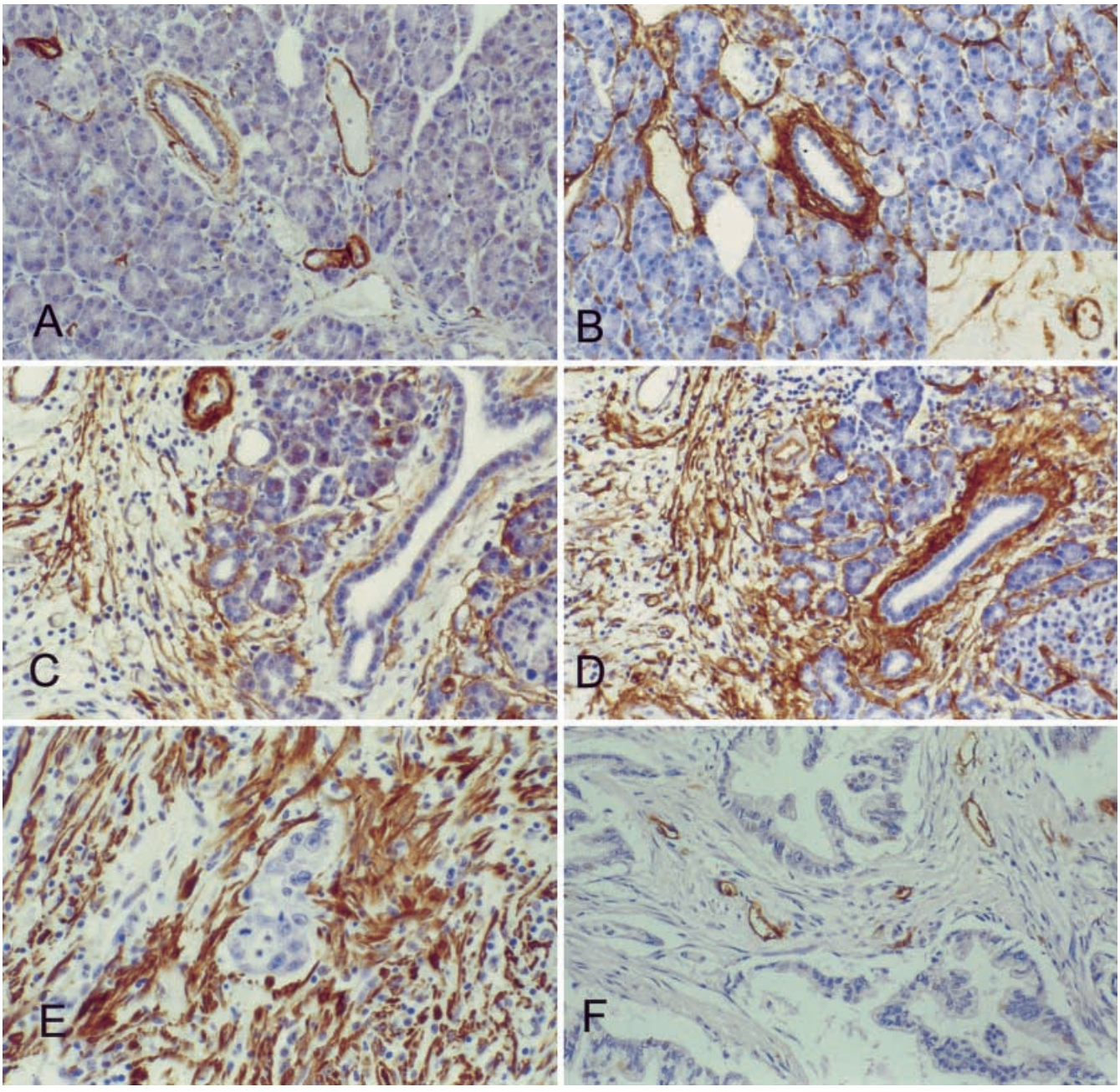

Fig. 1 Normal pancreatic parenchyma harbors few smooth muscle actin (SMA) reactive myofibroblasts surrounding intralobular ducts. SMA is also seen in the walls of arterioles and venules (**a**). $CD34^+$ fibrocytes are characterized by long dendrite-like projections (**b**, *inset*) and encircle acinar, ductal, and vascular structures of the normal pancreas (**b**). In chronic pancreatitis, the numbers of SMA reactive myofibroblasts (**c**) and $CD34^+$ fibrocytes (**d**) appear to be increased. The stroma of ductal adenocarcinoma shows densely packed SMA reactive myofibroblasts (**e**) whereas $CD34^+$ fibrocytes (**f**) are lacking

Results

Normal pancreas

The intra- and extralobular lobular stroma of normal pancreatic tissue was devoid of SMA reactive myofibroblasts except for a few myofibroblasts concentrically surrounding the intralobular ducts (Table 4). SMA staining was also observed in the smooth musculature of muscularized vessels (Fig. 1a). CD34 immunostaining of normal pancreatic tissue disclosed evenly scattered $CD34^+$ fibrocytes encircling the intralobular ducts and acini (Fig. 1b). The $CD34^+$ fibrocytes showed small elongated centrally located nuclei and long slender dendrite-like cytoplasmatic projections intervening with those of neighboring $CD34^+$ fibrocytes forming a loose reticular meshwork. Morphologically $CD34^+$ fibrocytes were clearly distinguishable from CD34-positive endothelial cells (Fig. 1b, inset). The distribution of $CD34^+$ fibrocytes and SMA reactive myofibroblasts showed no significant variation within the examined population (Table 5).

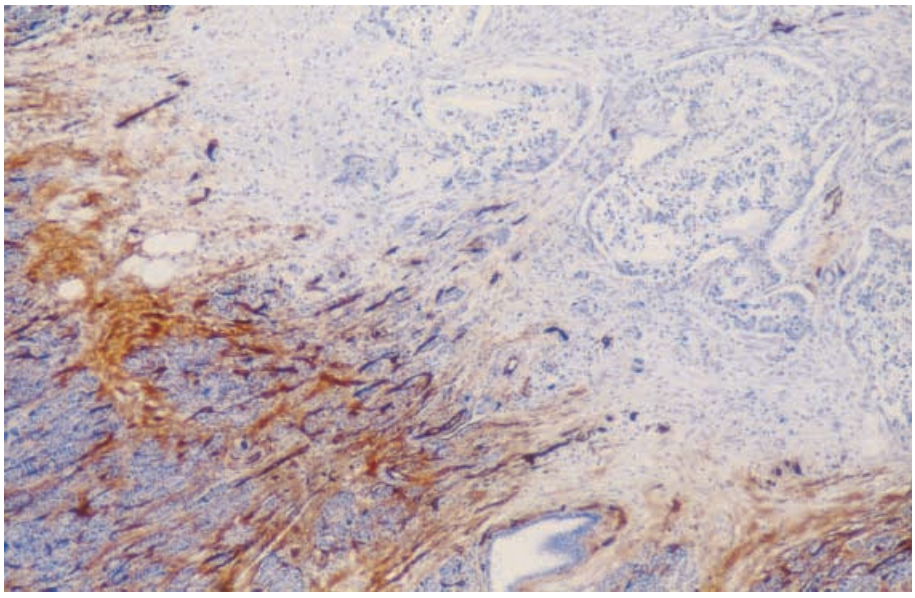

Fig. 2 The interface between tumor-free pancreatic parenchyma (*left* and *lower side*) and ductal adenocarcinoma (*upper right*) is characterized by an abrupt loss of $CD34^+$ fibrocytes. Note positive CD34 staining in intratumoral capillaries which can be well distinguished from the stromal staining due to densely packed $CD34^+$ fibrocytes even at low magnification

Table 4 Distribution of smooth muscle actin (SMA) reactive myofibroblasts in the pancreatic stroma. *0* no SMA reactive myofibroblasts detectable, *0*–+ focal accumulations of SMA reactive myofibroblasts, + diffusely scattered SMA reactive myofibroblasts

Staining score	Normal (*n*=11)	Chronic pancreatitis (*n*=8)	Endocrine tumor (*n*=7)	Adenocarcinoma (*n*=12)
0	11	0	1	0
0–+	0	4	6	0
+	0	4	0	12

Table 5 Distribution of $CD34^+$ fibrocytes in the pancreatic stroma. *0* no $CD34^+$ fibrocytes detectable, *0*–+ focal accumulations of $CD34^+$ fibrocytes, + diffusely scattered $CD34^+$ fibrocytes, ++ diffusely scattered and focally crowded $CD34^+$ fibrocytes

Staining score	Normal (*n*=11)	Chronic pancreatitis (*n*=8)	Endocrine tumor (*n*=7)	Adenocarcinoma (*n*=12)
0	0	0	7	8
0–+	0	0	0	4
+	11	2	0	0
++	0	6	0	0

Chronic pancreatitis

In all cases of chronic pancreatitis, SMA reactive myofibroblasts occurred in the intra- and extralobular parenchyma which, under normal conditions, was devoid of this cell type (Fig. 1c). The distribution of SMA reactive myofibroblasts showed slight variations ranging from focal accumulations to diffusely scattered cells which virtually occupied the whole pancreatic stroma (Table 4). The occurrence of SMA reactive myofibroblasts in chronic pancreatitis was accompanied by an increased number of $CD34^+$ fibrocytes. In most cases investigated (6 of 8), chronic pancreatitis was characterized by an increased number of $CD34^+$ fibrocytes which were predominantly located in areas of diffuse or nodular stromal fibrosis around intralobular ducts and acini (Fig. 1d). Additionally, $CD34^+$ fibrocytes appeared to be increased in number in areas infiltrated by lymphocytes. In areas with predominant stromal edema, $CD34^+$ fibrocytes were less densely arranged, but no areas with complete loss of $CD34^+$ fibrocytes were seen in chronic pancreatitis. In two cases, the distribution of $CD34^+$ fibrocytes was similar to that observed in normal pancreatic parenchyma (Table 5).

Ductal adenocarcinomas

In all cases of ductal adenocarcinoma investigated, SMA reactive myofibroblasts formed thick sheets surrounding the infiltrating tumor cells (Fig. 1e, Table 4). The stroma associated with invasive ductal adenocarcinoma was free of $CD34^+$ fibrocytes in eight cases (Fig. 1f). In the tumor-free pancreatic parenchyma located at the infiltrating border of carcinoma, the $CD34^+$ fibrocytes were increased in number and more densely packed; the transition from tumor-free tissue to invasive

carcinoma was characterized by an abrupt loss of the stromal CD34+ fibrocytes (Fig. 2). Four cases disclosed a subtotal loss of CD34+ fibrocytes with small foci of preserved mophologically normal appearing CD34+ fibrocytes (Table 5).

Pancreatic endocrine tumors

In six of seven endocrine tumors examined, few scattered stromal cells exhibiting a weak immunohistochemical expression of SMA were found (Table 4). CD34+ fibrocytes were completely absent from the stroma of all endocrine tumors investigated, whereas the endothelium of intratumoral vessels showed a normal endothelial expression of CD34 (Table 5).

Discussion

The present study was undertaken in order to analyze the presence and distribution of CD34+ fibrocytes and SMA reactive myofibroblasts in relation to the underlying pancreatic disease. We found that stromal remodeling associated with chronic pancreatitis was characterized by an increased number of stromal CD34+ fibrocytes paralleled by a gain of SMA reactive myofibroblasts which were absent from the normal pancreatic stroma. In contrast, the stroma associated with pancreatic ductal adenocarcinoma and endocrine tumors was devoid of CD34+ fibrocytes in most cases investigated, whereas SMA reactive myofibroblasts were detected in a quantity comparable to that found in chronic pancreatitis.

It has been shown that CD34+ fibrocytes invade areas of tissue damage and take part in matrix repair via collagen synthesis [2]. In the present study, the presumed role of CD34+ fibrocytes in tissue repair was underlined by the fact that the number of stromal CD34+ fibrocytes was found to be increased together with that of SMA reactive myofibroblasts in chronic pancreatitis. Intra- and interlobular proliferation of myofibroblasts mediate fibrogenesis in chronic pancreatitis irrespective of the initiating event. In focal pancreatitis, myofibroblast proliferation plays an important role in the development of duct stenosis and, thus, in further aggravation of the disease process [7]. It remains to be clarified whether SMA reactive myofibroblasts and CD34+ fibrocytes are related in origin. SMA reactive myofibroblasts were also detected in adenocarcinomas and in endocrine tumors, whereas CD34+ fibrocytes disappeared at least focally from the stroma of adenocarcinomas and endocrine tumors. The phenomenon of CD34+ fibrocyte loss is not site specific and has meanwhile been described in the stroma of various invasive carcinomas such as colon cancer [15], basal cell carcinoma of the skin [5, 6, 9, 10, 23], and invasive ductal breast cancer [4]. It is widely accepted that stromal matrix degradation and subsequent stromal invasion are crucial steps in the process of local tumor growth and systemic tumor spread [1, 11, 12, 14, 18].

With the CD34+ fibrocytes being capable of collagen I and III synthesis and therefore taking part in tissue repair, these cells play an important role in maintaining stromal integrity and inhibition of tumor cell migration [2]. Besides its function as a matrix-producing cell, the CD34+ fibrocytes have been shown to express HLA class-II molecules and to act as an antigen-presenting cell [3] indicating that CD34+ fibrocytes might be involved in specific host immune responses to tumor antigens. Therefore, it is of special interest to elucidate what causes CD34+ fibrocytes to disappear from the stroma of pancreatic adenocarcinomas and endocrine tumors. Cells of human endocrine pulmonary tumors are capable of inducing apoptosis in dendritic cells and blocking the differentiation of CD34+ myeloid precursors into mature dendritic cells [8]. This mechanism might explain our finding that the stroma of pancreatic endocrine tumors lacks CD34+ fibrocytes. However, this remains to be clarified in further studies together with the question whether the same is true for adenocarcinomas.

While the diagnosis of pancreatic endocrine tumors is straightforward and can be well substantiated using immunohistochemistry, problems occur when chronic pancreatitis has to be distinguished from pancreatic ductal adenocarcinoma. According to the data of the present study, detection of CD34+ fibrocytes in pancreatic lesions with suspected adenocarcinoma renders this differential diagnosis less probable and favors a benign lesion such as chronic pancreatitis. However, the absence of CD34+ fibrocytes from the stroma of a questionable pancreatic lesion renders the diagnosis of ductal adenocarcinoma much more likely than that of chronic pancreatitis. Generally, negative immunostaining is regarded to be less valuable, but small vessels and capillaries provide an internal control of high reliability when CD34 immunostains are performed. However, due to the relatively low number of cases investigated, the diagnostic impact of CD34+ fibrocytes in distinguishing chronic pancreatitis from adenocarcinoma could not be assessed precisely. Nevertheless, regarding the experiences of the present study, this approach appears to be promising and therefore merits further investigation.

References

1. Brummer O, Athar S, Riethdorf L, Loning T, Herbst H (1999) Matrix-metalloproteinases 1, 2 and 3 and their tissue inhibitors 1 and 2 in benign and malignant breast lesions: an in situ hybridization study. Virchows Arch 435:566–573
2. Bucala R, Spiegel LA, Chesney J, Hogan M, Cerami A (1994) Circulating fibrocytes define a new leukocyte subpopulation that mediates tissue repair. Mol Med 1:71–81
3. Chesney J, Bacher M, Bender A, Bucala R (1997) The peripheral blood fibrocyte is a potent antigen-presenting cell capable of priming naive T cells in situ. Proc Natl Acad Sci USA 94:6307–6312
4. Ebrahimsade S, Barth PJ, Ramaswamy A, Moll R (2001) CD34+ fibroblasts in benign and malignant breast lesions. Cancer Res Clin Oncol 127[Suppl 1]:S41
5. Humphreys TR, Monteiro MR, Murphy GF (2000) Mast cells and dendritic cells in basal cell carcinoma stroma. Dermatol Surg 26:200–203

6. Illueca C, Monteagudo C, Revert A, Llombart-Bosch A (1998) Diagnostic value of CD34 immunostaining in desmoplastic trichilemmoma. J Cutan Pathol 25:435–439
7. Izumi M, Suda K, Torii A, Inamada E (2001) Pancreatic ductal myofibroblasts. Proliferative patterns in various pathologic situations. Virchows Arch 438:442–450
8. Katsenelson NS, Shurin GV, Bykosvkaia SN, Shogan J, Shurin MR (2001) Human small cell lung carcinoma and carcinoid tumor regulate dendritic cell maturation and function. Mod Pathol 14:40–45
9. Kirchmann TT, Prieto VG, Smoller BR (1994) CD34 staining pattern distinguishes basal cell carcinoma from trichoepithelioma. Arch Dermatol 130:589–592
10. Kirchmann TT, Prieto VG, Smoller BR (1995) Use of CD34 in assessing the relationship between stroma and tumor in desmoplastic keratinocytic neoplasms. J Cutan Pathol 22:422–426
11. Koukoulis GK, Howeedy AA, Korhonen M, Virtanen I, Gould VE (1993) Distribution of tenascin, cellular fibronectins and integrins in the normal, hyperplastic and neoplastic breast. J Submicrosc Cytol Pathol 25:285–295
12. Lebeau A, Nerlich AG, Sauer U, Lichtinghagen R, Löhrs U (1999) Tissue distribution of major matrix metalloproteinases and their transcripts in human breast carcinomas. Anticancer Res 19:4257–4264
13. Lüttges J, Mentzel T, Hubner G, Klöppel G (1999) Solitary fibrous tumour of the pancreas: a new member of the small group of mesenchymal pancreatic tumours. Virchows Arch 435:37–42
14. Moch H, Torhorst J, Dürmüller U, Feichter GE, Sauter G, Gudat F (1993) Comparative analysis of the expression of tenascin and established prognostic markers in human breast cancer. Pathol Res Pract 189:510–514
15. Nakayama H, Enzan H, Miyazaki E, Kuroda N, Naruse K, Hiroi M (2000) Differential expression of CD34 in normal colorectal tissue, peritumoral inflammatory tissue, and tumour stroma. J Clin Pathol 53:626–629
16. Narvaez D, Kanitakis J, Faure M, Claudy A (1996) Immunohistochemical study of CD34-positive dendritic cells of human dermis. Am J Dermatopathol 18:283–288
17. Seidal T, Edvardsson H (1999) Expression of c-kit (CD117) and Ki67 provides information about the possible cell of origin and clinical course of gastrointestinal stromal tumours. Histopathology 34:416–424
18. Shoji T, Kamiya T, Tsubura A, Hamada Y, Hatano T, Hioki K, Morii S (1993) Tenascin staining positivity and the survival of patients with invasive breast carcinoma. J Surg Res 55:295–297
19. Silverman JS, Tamsen A (1996) Mammary fibroadenoma and some phyllodes tumour stroma are composed of CD34+ fibroblasts and factor XIIIa+ dendrophages. Histopathology 29:411–419
20. Silverman JS, Tamsen A (1997) Fibrohistiocytic differentiation in subcutaneous fatty tumors. Study of spindle cell, pleomorphic, myxoid, and atypical lipoma and dedifferentiated liposarcoma cases composed in part of CD34+ fibroblasts and FXIIIa+ histiocytes. J Cutan Pathol 24:484–493
21. Sircar K, Hewlett BR, Huizinga JD, Chorneyko K, Berezin I, Riddell RH (1999) Interstitial cells of Cajal as precursors of gastrointestinal stromal tumors. Am J Surg Pathol 23:377–389
22. Suster S, Fisher C (1997) Immunoreactivity for the human hematopoietic progenitor cell antigen (CD34) in lipomatous tumors. Am J Surg Pathol 21:195–200
23. Swanson PE, Fitzpatrick MM, Ritter JH, Glusac EJ, Wick MR (1998) Immunohistologic differential diagnosis of basal cell carcinoma, squamous cell carcinoma, and trichoepithelioma in small cutaneous biopsy specimens. J Cutan Pathol 25:153–159
24. Yamazaki K, Eyden BP (1995) Ultrastructural and immunohistochemical observations on intralobular fibroblasts of human breast, with observations on the CD34 antigen. J Submicrosc Cytol Pathol 27:309–323