Virchows Arch (2002) 440:298–303
DOI 10.1007/s004280100530

ORIGINAL ARTICLE

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CD34+ fibrocytes in invasive ductal carcinoma, ductal carcinoma in situ, and benign breast lesions

Received: 20 February 2001 / Accepted: 21 June 2001 / Published online: 22 November 2001

Abstract The present study was undertaken in order to elucidate the question of whether the distribution of stromal CD34+ fibrocytes and smooth muscle actin (SMA)-reactive myofibroblasts differs between benign and malignant lesions of the breast. We investigated a total of 31 ductal carcinomas and 27 specimens with benign lesions of the breast (ductal hyperplasia, sclerosing adenosis, fibroadenoma, phyllodes tumor) and compared the distribution of CD34+ fibrocytes and SMA-reactive myofibroblasts. The stroma of normal breast tissue contained CD34+ fibrocytes, whereas SMA-reactive myofibroblasts were absent. All benign breast lesions exhibited stromal CD34+ fibrocytes and few lesions (fibroadenomas and phyllodes tumor) showed additional SMA-reactive myofibroblasts. In invasive breast cancer the stroma was devoid of CD34+ fibrocytes but a varying number of stromal SMA-reactive myofibroblasts was detectable. In the setting of the present study the loss of CD34+ fibrocytes was specific for invasive breast cancer and ductal carcinoma in situ, whereas SMA-reactive myofibroblasts were observed in different benign and malignant lesions. These findings may be helpful tools in distinguishing benign breast lesions (e.g., sclerosing adenosis) from invasive breast cancer and in characterizing stromal remodeling associated with invasive cancer.

Keywords Fibrocyte · Myofibroplast · Breast · Ductal carcinoma · DCIS

Introduction

Blood-borne CD34+ fibrocytes derive from myeloid precursors and make up about 0.5% of peripheral blood leukocytes [2]. Experimental studies have shown that blood-borne CD34+ fibrocytes 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. By means of immunohistochemistry, CD34+ fibrocytes have been detected in the skin [14] – in cutaneous [4, 5, 7, 8, 21] and lipomatous tumors [18,20] – and in a multitude of mesenchymal tumors [11, 15, 18, 19]. However, it remains to be clarified to what extent CD34+ fibrocytes observed in various organs are derived from circulating CD34+ fibrocytes. The occurrence of CD34+ fibrocytes in the peritumoral stroma of skin appendage tumors has been considered to be of diagnostic significance in distinguishing basal cell carcinoma from benign appendage tumors, such as trichoepithelioma and its variants [4, 5, 7, 8, 21]. In these studies, the absence of CD34+ fibrocytes favors the diagnosis of basal cell carcinoma, while the presence of CD34+ fibrocytes tends to rule against such a diagnosis. Similar results have been reported in colorectal adenocarcinoma: normal colonic stroma harbors CD34+ fibrocytes, whereas this cell population is absent from the stroma of invasive adenocarcinoma. On the other hand, the tumor-associated desmoplastic stroma was characterized by the presence of SMA-reactive myofibroblasts [13]. To date, reports investigating the occurrence and distribution pattern of CD34+ fibrocytes in benign and malignant breast lesions have – except for one study analyzing the distribution of CD34+ fibrocytes in fibroadenoma and phyllodes tumors [17] – not yet been published.

We therefore analyzed the occurrence of CD34+ fibrocytes and SMA-reactive myofibroblasts in the mammary gland with special respect to malignancy and other specific types of disease processes.

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Materials and methods

We investigated a total of 24 invasive breast carcinomas and qualitatively compared the distribution of CD34+ fibrocytes and SMA-

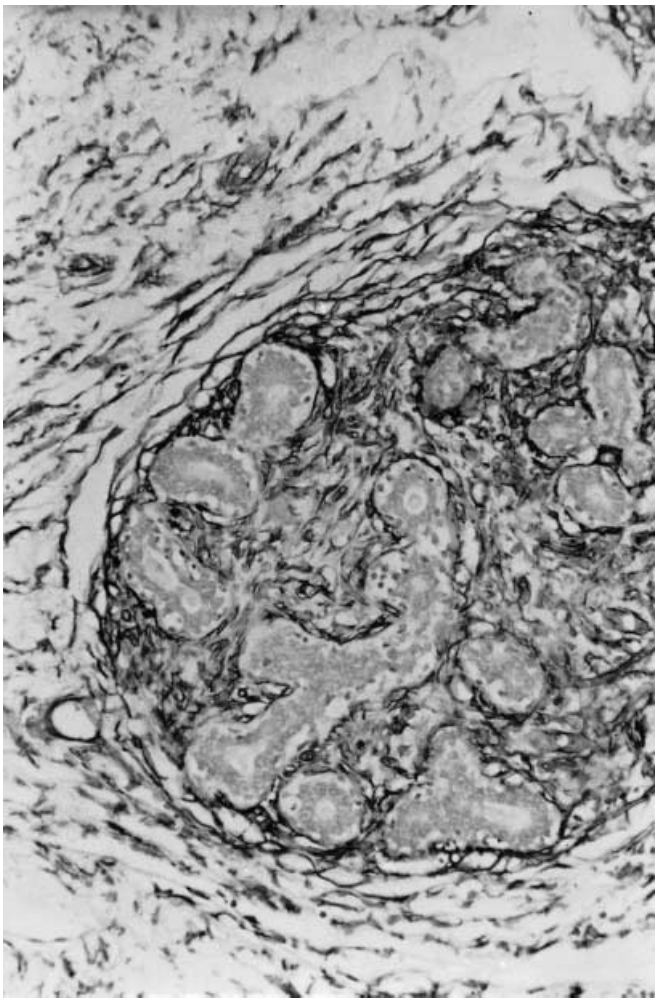

Fig. 1 Normal mammary tissue shows densely packed CD34+ fibrocytes encircling glandular and vascular structures (CD34, ×200 microscopic magnification)

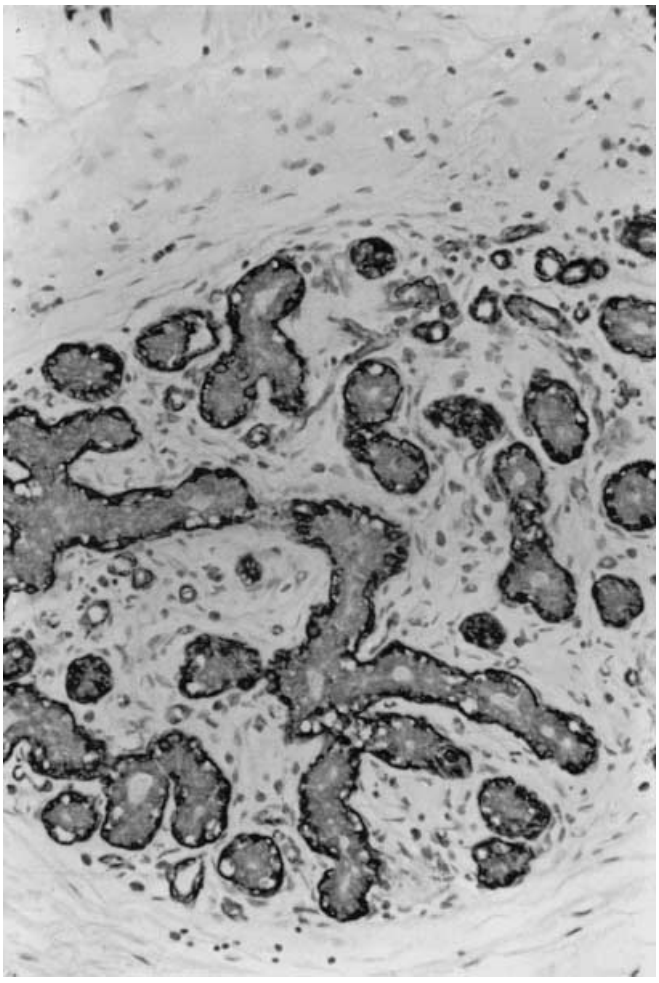

Fig. 2 Smooth muscle actin (SMA) immunohistochemistry decorates acinar myoepithelia whereas the stroma of normal mammary tissue is devoid of SMA-reactive myofibroblasts (SMA, ×200 microscopic magnification)

Table 1 Epidemiologic data and type of benign breast lesions investigated

Number of patients	27
Age range (years)	22–78
Type of lesion	
Sclerosing adenosis	12
Fibroadenoma	7
Phyllodes tumor	1
Microglandular adenosis	1
Ductal hyperplasia	9
Tubular adenoma	1

Table 2 Epidemiologic data of patients with breast carcinoma

Number of patients	31
Age range (years)	34–79
Type of carcinoma	
Invasive ductal	24
Intraductal carcinoma[a]	7
Lesions associated with invasive carcinomas	
Sclerosing adenosis	2
Intraductal carcinoma	14
Ductal hyperplasia	7

[a] Pure intraductal carcinoma not associated with invasive ductal carcinoma

reactive myofibroblasts between stromal areas located within the tumor to areas of tumor free mammary tissue surrounding the carcinoma. Epidemiological data of patients with invasive carcinomas investigated and data concerning tumor properties are summarized in Table 1. In order to be sure that in patients with invasive carcinoma the distribution and number of CD34+ fibrocytes and myofibroblasts in the surrounding tumor-free tissue was unaffected by the fact that an invasive carcinoma was remotely located in the same or contralateral breast, we investigated biopsy specimens of mammary tissue of 27 patients with benign breast lesions. The epidemiological data of these patients are depicted in Table 2. The analysis of stromal alterations associated with intraductal carcinoma (DCIS) was based on specimens of seven patients showing pure DCIS without associated invasive ductal carcinoma (Table 1). In brief, after resection the tissues were fixed in a 4% formaldehyde solution and representative tissue blocks were selected. Tissues were embedded in paraffin, cut, and stained H&E and PAS for routine purposes.

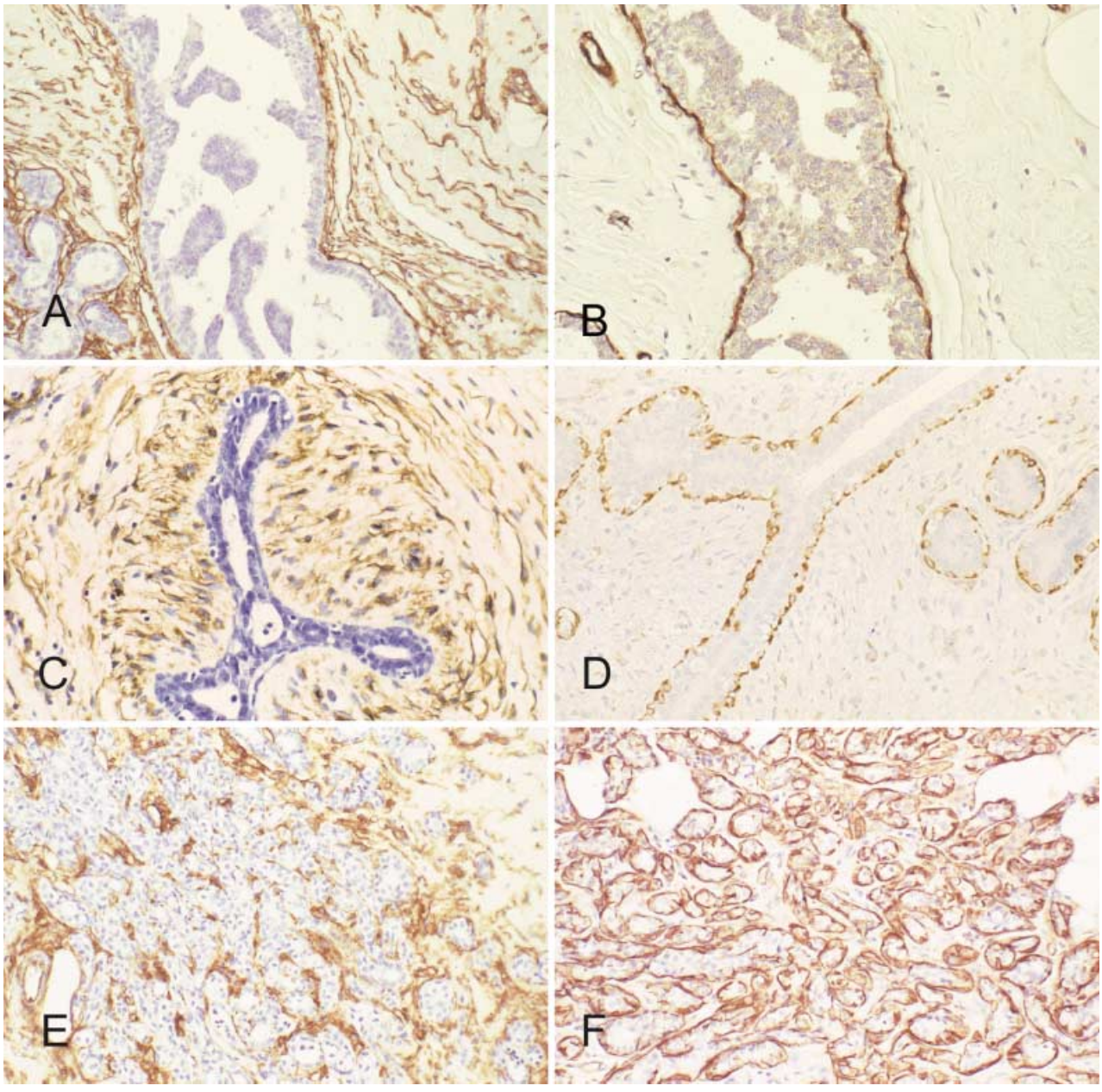

Fig. 3 Ducts with epithelial hyperplasia are surrounded by a normal appearing population of CD34+ fibrocytes (**A**), and smooth muscle actin (SMA) stains myoepithelial cells and smooth muscle of muscularized vessels (**B**). The stroma of fibroadenoma contains numerous CD34+ fibrocytes (**C**), SMA-reactive myofibroblasts are absent. SMA-reactive myoepithelia cover the basement membrane of compressed ducts (**D**). In sclerosing adenosis the stroma contains densely packed CD34+ fibrocytes (**E**), the stroma is free of SMA-reactive myofibroblasts. The small acinar structures are lined by SMA-reactive myoepithelia (**F**). (**A–D**, ×200 microscopic magnification: **E,F** ×100 microscopic magnification)

Immunohistochemistry

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

Results

In normal mammary tissue muscularized blood vessels, glandular ducts, and acini were surrounded by a dense concentric network of CD34+ fibrocytes. These cells showed slender elongated dendrite-like processes featuring a bipolar arrangement; the nuclei were small and inconspicuous. With increasing distance from the aforementioned glandular and vascular structures the density of CD34+ fibrocytes decreased (Fig. 1). SMA was detected in the wall of muscularized vessels and in myoepithelia lining the ductal and acinar basement membranes, whereas SMA-reactive myofibroblasts were not detected

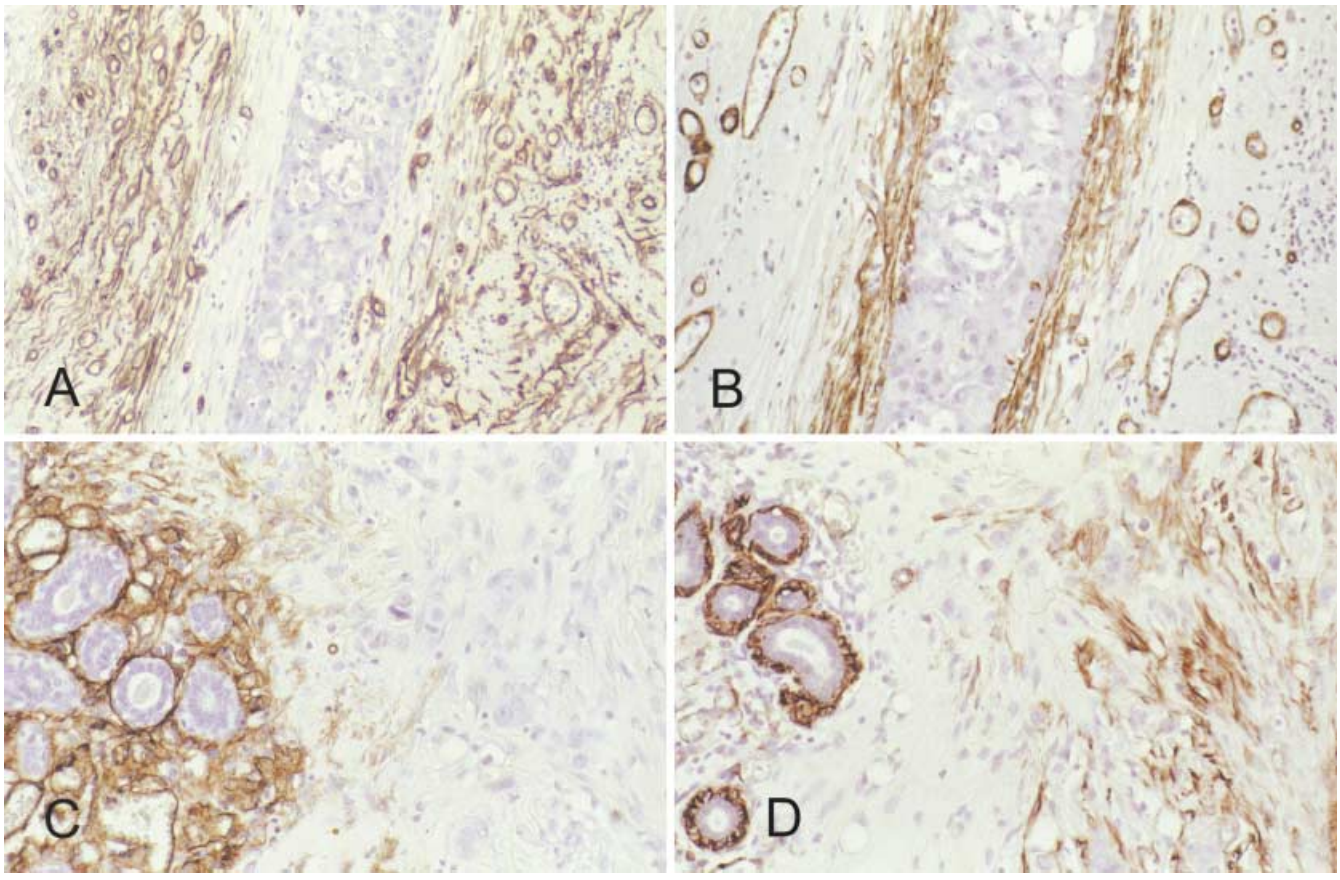

Fig. 4 The stroma surrounding the intraductal carcinoma (DCIS) lacks $CD34^+$ fibrocytes (note CD34 positive endothelial cells in capillaries adjacent to DCIS); areas located more remotely from DCIS show a normal distribution pattern of $CD34^+$ fibrocytes (**A**). DCIS is encircled by a thick layer of smooth muscle actin (SMA)-reactive myofibroblasts (**B**). The stroma of invasive carcinoma is free of $CD34^+$ fibrocytes in the adjacent tumor-free tissue and the CD34+ fibrocytes appear somewhat condensed and closely packed (**C:** *right*, invasive carcinoma; *left*, tumor-free tissue). SMA-reactive myofibroblasts are visible in the stroma of invasive carcinoma (**D:** *right*, invasive carcinoma; *left*, tumor-free mammary tissue). ×200 microscopic magnification

in the stroma of normal breast tissue (Fig. 2). The stroma harboring ducts with epithelial hyperplasia showed a similar distribution of $CD34^+$ fibrocytes (Fig. 3 A) and SMA-reactive myoepithelia (Fig. 3B).

In fibroadenomas $CD34^+$ fibrocytes were scattered uniformly throughout the stroma and did not show the predilection for periglandular and perivascular areas as observed in the normal mammary gland (Fig. 3C). Fibroadenomas with loose, myxoid stroma showed an additional population of SMA-reactive myofibroblasts which were not observed in more sclerotic fibroadenomas (Fig. 3D). In one phyllodes tumor investigated the stroma harbored $CD34^+$ fibrocytes and SMA-reactive myofibroblasts in a fashion similar to that observed in fibroadenomas with loose, myxoid stroma. A similar stromal composition with SMA-reactive myofibroblasts and coexisting $CD34^+$ fibrocytes was observed in the tubular adenoma investigated.

Table 3 $CD34^+$ fibrocytes and smooth muscle actin (*SMA*)-reactive myofibroblasts in the lesions investigated

Type of lesion	$CD34^+$ fibrocytes	SMA-reactive myofibroblasts
Ductal hyperplasia	16/16	0/16
Fibroadenoma	7/7	3/7
Phyllodes tumor	1/1	1/1
Tubular adenoma	1/1	1/1
Sclerosing adenosis	14/14	1/14
Microglandular adenosis	1/1	0/1
DCIS [a]	0/7	5/7
Invasive ductal carcinoma	0/24	20/24

[a] Pure intraductal carcinoma (*DCIS*) not associated with invasive ductal carcinoma

In sclerosing adenosis, the stroma showed abundant densely packed $CD34^+$ fibrocytes (Fig. 3E), and the lobules showed a population of SMA-reactive myoepithelia (Fig. 3F), but SMA-reactive stromal myofibroblasts were not observed except in one case. In this case, an adenosis tumor, stromal SMA-reactive myofibroblasts were detected additionally to the $CD34^+$ fibrocytes (Table 3).

In contrast to normal mammary tissue and ductal hyperplasia, the number of $CD34^+$ fibrocytes was reduced in the stroma surrounding ducts harboring carcinoma in situ, although capillaries with CD34 reactive endothelia were still detectable adjacent to these ducts (Fig. 4 A). On the other hand, ducts with DCIS were encircled by a rim of densely packed SMA-reactive myofibroblasts

which were not detected in the normal breast tissue or in ductal hyperplasia (Fig. 4B).

In all cases investigated, the stroma of invasive carcinomas showed a complete loss of $CD34^+$ fibrocytes, whereas the surrounding mammary tumor-free tissue disclosed a normal distribution of this cell population except for areas which were located at the margin of invasive carcinoma (Fig. 4C). In these regions the $CD34^+$ fibrocytes appeared to be more densely packed than in tumor-free tissue remotely located from the margin of invasive cancer. The endothelium of vessels located in the stroma of invasive carcinoma and those located in tumor-free mammary tissue showed a similar degree and extent of CD34 immunostaining. Twenty of 24 invasive ductal carcinomas disclosed SMA-reactive myofibroblasts forming focal accumulations of various extent (Fig. 4D) (Table 3).

Discussion

$CD34^+$ fibrocytes are a small population of blood-borne dendritic cells capable of antigen-presentation and T-cell priming which have been considered to play a significant role in specific instances of immune response and tissue repair [2, 3]. The distribution of $CD34^+$ fibrocytes has been claimed to be a valuable tool in the differential diagnosis of benign and malignant tumors of the skin appendages [4, 5, 7, 8, 21]. The present study is the first to correlate the presence and distribution of $CD34^+$ fibrocytes with the malignancy of primary breast lesions. The data reported here indicate a strong negative association between the presence of $CD34^+$ fibrocytes and the malignancy of a ductal breast lesion in that invasive ductal carcinomas disclose a complete loss of stromal $CD34^+$ fibrocytes. Moreover, distinct lesions such as sclerosing adenosis that may cause severe differential diagnostic confusion concerning malignancy show a preserved population of $CD34^+$ fibrocytes. Therefore, assessment of $CD34^+$ fibrocytes might play an important role whenever this problem arises. We additionally investigated the occurrence of SMA-reactive stromal myofibroblasts in relation to the type of underlying breast disease. In invasive breast cancer the desmoplastic stroma showed focal accumulations of SMA-reactive myofibroblasts and a complete loss of $CD34^+$ fibrocytes in all cases investigated. Therefore, the data reported in the present study indicate that the loss of $CD34^+$ fibrocytes is a sensitive and specific marker of stromal changes associated with invasive breast cancer. In contrast, the accumulation of SMA-reactive myofibroblasts is less specific and can be observed in benign as well as in malignant lesions of the breast. Moreover, the stroma surrounding ductal carcinoma in situ was also characterized by a loss of $CD34^+$ fibrocytes. Therefore the detection of a loss of $CD34^+$ fibrocytes may be a valuable tool in the detection of DCIS.

The loss of $CD34^+$ fibrocytes is not restricted to invasive breast cancer since similar findings have been reported in basal cell carcinoma of the skin [4, 5, 7, 8, 21] and colorectal adenocarcinoma [13].

Stromal remodeling is an important feature of invasive breast cancer and has predominantly been investigated for tenascin [12, 16] and fibronectins [9]. Breast cancer cells have been shown to be capable of matrix-metalloproteinase synthesis which initiates stromal alterations in invasive cancer [1, 10]. However, this approach might fail to completely explain the periductal loss of $CD34^+$ fibrocytes in DCIS. Neuroendocrine pulmonary tumor cells are capable of inducing apoptosis in dendritic stromal cells which are closely related to the $CD34^+$ fibrocytes by a not yet precisely defined soluble factor [6]. We speculate that in parallel to this finding apoptosis of $CD34^+$ fibrocytes might be initiated by a soluble factor secreted by DCIS cells.

Taking into account that $CD34^+$ fibrocytes are capable of collagen I and collagen III synthesis, it seems to be likely that this cell type may be involved in the process of stromal remodeling in breast cancer [2]. However, it remains unclear whether the phenotypical alterations of the stroma in invasive breast cancer can be explained by alterations of one single type of stromal cell characterized by downregulation of CD34 and upregulation of SMA in this cell type or by a loss of $CD34^+$ fibrocytes and subsequent repopulation of the stroma by SMA-reactive myofibroblasts. The latter process would require an increased proliferation of stromal cells which up to now has not been documented in breast cancer. For explaining the data of the present study, it seems probable that $CD34^+$ fibrocytes are replaced by alternatively differentiated and less immunocompetent mesenchymal cells. This process is probably related to matrix metalloproteinase-induced stromal damage and subsequent stromal remodeling. Interestingly, sites of tissue damage have been shown to be invaded by blood-borne $CD34^+$ fibrocytes [2]. In invasive breast cancer this mechanism seems to fail, leading to a loss of the $CD34^+$ fibrocytes. Bearing in mind that $CD34^+$ fibrocytes may mediate specific immunologic reactions related to antigen-presentation and T-cell priming [3], the loss of this cell population might play an important role in that part of the host response directed against invasive cancer cells that is mediated by infiltrating T-cells [22].

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