Histol Histopathol (2011) 26: 297-305

http://www.hh.um.es

Histology and Histopathology

Cellular and Molecular Biology

Appearance and distribution of stromal myofibroblasts and tenascin-C in feline mammary tumors

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Summary. Myofibroblasts and extracellular matrix protein tenascin-C (Tn-C) are known to be implicated in cancer progression in human cancer. In feline mammary tumors that are a suitable model for human breast cancer, however, little is known about stromal myofibroblasts and no information is available on the expression of Tn-C. Feline samples of normal mammary glands and proliferating mammary lesions were routinely processed and serial sections were cut and immunostained with anti-α-smooth muscle actin (α-SMA) or Tn-C antibody. Myofibroblasts were not included in the stroma of 90% (9/10) of normal mammary gland tissues, 92% (12/13) of adenosis, and 63% (5/8) of simple adenomas. On the other hand, all 40 simple carcinomas contained stromal myofibroblasts to a varied extent. Tn-C expression was detected in the stroma of 92% (37/40) of carcinomas, and its global distribution almost coincided with that of myofibroblasts. In addition, Tn-C immunoreactivity was occasionally observed in the basement membrane zone around ducts in some cases of normal mammary glands and benign lesions, but barely observed in the stroma. These results suggest that stromal myofibroblasts may be a major cellular source of Tn-C and be involved in malignant progression of feline mammary tumor.

Key words: Feline, Immunohistochemistry, Mammary tumor, Myofibroblast, Tenascin-C

Introduction

Spontaneous tumors in cats receive attention as models for human cancer biology and translational cancer therapeutics (Vail and MacEwen, 2000). Mammary tumors are the third most common tumor type affecting female cats (Zappulli et al., 2005) and the great majority of them are mammary carcinomas, which are highly infiltrative and metastatic (Misdorp and Weijer, 1980; Zappulli et al., 2005). The histology of feline mammary carcinoma is more similar to human breast cancer than rodents and dogs (Misdorp and Weijer, 1980). Feline mammary carcinoma, because of its biologic similarity to human mammary cancer, are considered to serve as a valuable model for comparative studies and also as an intermediate model between rodent and human for etiology and histogenesis of mammary tumors (Weijer et al., 1972).

Tumor mass consists of not only neoplastic cells but also non-neoplastic stromal cells, such as fibroblasts, vascular component cells, inflammatory cells, and mesenchymal stem cell, extracellular matrix, and extracellular molecules. These stromal components have been recognized as important factors influencing the development of tumors. Fibroblasts are regarded as a key component in all human cancers (Egeblad et al., 2005; Littlepage et al., 2005; Li et al., 2007). Abundant fibroblasts are often observed within invasive carcinomas such a scirrhous cancer of the breast. The reactive fibroblasts within carcinoma tissue, carcinoma-associated fibroblasts (CAFs), are functionally and phenotypically distinct from normal fibroblasts within non-tumorous tissue. The secretion of extracellular matrix molecules, various cytokines and growth factors by the CAFs causes mobility and proliferation of carcinoma cells and promotion of angiogenesis. The CAFs are identified within carcinoma tissue by the expression of α-smooth muscle actin (α-SMA), which indicates their myofibroblastic nature (Powell et al., 1999; Egeblad et al., 2005; Li et al., 2007; McAnulty, 2007; De Wever et al., 2008). Myofibroblasts have also long been observed in feline and canine mammary tumors (Tateyama et al., 1988; Destexhe et al., 1993; Martín de las Mulas et al., 1994, 2004). As we reported recently, in canine simple carcinomas, the appearance of stromal myofibroblasts correlated significantly with histopathological parameters associated with worse

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prognosis, including high histological grade, vascular/lymphatic invasion, and metastasis to the neighboring lymph node (Yoshimura et al., in press).

Tenascin-C (Tn-C) is an extracellular matrix protein, which shows markedly increased expression in embryonic and cancerous tissues (Chiquet-Ehrismann and Chiquet, 2003). As has been strongly suggested by histopathological findings in surgical specimens and experimental findings in vitro, Tn-C is a key determinant of the tumor stroma that is involved in initiation of tumorigenesis and progression to metastasis (Orend, 2005; Orend and Chiquest-Ehrismann, 2006). Tn-C, because of its highly restricted expression in adult tissues, is also expected to be a good candidate for tumor targeting therapy (De Santis et al., 2006). In veterinary medicine, Arai, et al. reported that Tn-C expression was observed in canine mammary mixed tumors and Tn-C might play crucial roles in the proliferation of myoepithelial cells and the subsequent development of cartilaginous tissues (Arai et al., 1994). Faustino et al. described that Tn-C was present in all normal, hyperplastic, dysplastic and neoplastic mammary tissues of dogs, with an increased expression in solid and anaplastic carcinomas as well as in areas of initial chondroid metaplasia (Faustino et al., 2002). Our previous study showed that Tn-C immunoreactivity in canine mammary simple carcinomas could be divided into two principal patterns (Yoshimura et al., in press). One was the Tn-C expressed in the basement membrane zone in low-grade simple carcinomas, which was likely produced by neighboring myoepithelial cells and might not be directly involved in cancer invasion and metastasis. The other was the Tn-C expressed in the newly formed stroma of carcinomas, with an increase in high-grade simple carcinomas. The Tn-C was overproduced by stromal myofibroblasts and might be considered to be a variant contributing to cancer invasion and metastasis. Thus, works have been proceeding with Tn-C expression in canine tumors. However, research about Tn-C in feline tumors including mammary carcinomas has not been carried out.

The aims of this study are to detect the appearance and distribution of stromal myofibroblasts and Tn-C expression in feline mammary tissues, and to compare the results with the data described in other species, including humans.

Materials and methods

Tumor specimens

Samples were fixed in 10% neutral-buffered formalin, and embedded in paraffin wax. Serial sections were cut 4 μm. The present study included 10 cases of normal mammary gland, 13 of adenosis, 8 of simple adenoma, and 40 of simple carcinoma (28, tubulo-papillary; 12, solid), which were selected from the biopsy or necropsy files of the Department of Veterinary Pathology, Nippon Veterinary and Life Science University. The diagnostic criteria were based on the WHO classification system for feline mammary tumors (Misdorp et al., 1999). Furthermore, carcinomas were assigned to Grade I, II or III on the basis of the degree of tubule formation, nuclear atypia and mitotic figures, according to the Elston and Ellis grading system for human breast cancer (Elston and Ellis, 1991; Misdorp, 2002).

Enzyme immunohistochemistry

In brief, sections were deparaffinized in xylene, dehydrated, treated for 30 minutes with 0.3% hydrogen peroxide in methanol to quench endogenous peroxidase activity, and incubated in Block Ace (DS Pharma Biomedical Co., Oosaka, Japan) for 30 minutes. For the detection of Tn-C, slides were pretreated with 0.1% trypsin for 15 minutes at 37°C. For α-SMA and p63 sections were immersed in citrate buffer (0.1 M, pH 6.0) and subjected to autoclave (121°C, 10 minutes). Subsequently, all sections were incubated overnight at 4°C with primary antibodies, including mouse monoclonal antibody against Tn-C (clone 4F10TT which recognizes an epitope within the EGF-like sequences that is common to all forms of Tn-C, 1:20, IBL, Takasaki, Japan), α-SMA (clone 1A4, 1:400, DAKO, Glostrup, Denmark), and p63 (clone 4A4, 1 : 200, Neomarkers, Fremont, USA). We used a labeled streptavidin-biotin (LsAB) method and developed the chromogen with immersion of sections in a diaminobenzidine-H_2O_2 substrate. Sections were counterstained in hematoxylin. As positive control for Tn-C, canine mammary simple carcinoma (Yoshimura et al., in press) or vascular smooth muscle within feline mammary tissues was used. As negative controls, sections were incubated with normal mouse Ig (DAKO, Carpinteria, USA) instead of the primary antibodies.

Double-labeled fluorescent immunohistochemistry

Sections were deparaffinized and pretreated with 0.1% trypsin for 15 minutes at 37°C. After incubation for 30 minutes with Block Ace, sections were incubated in the mixture of mouse monoclonal antibody against Tn-C (1:20) and rabbit polyclonal antibody against smooth muscle actin (1:25, Neomarkers, Fremont, USA) for 16 hours at 4°C. After washing, they were incubated in the mixture of Alexa Fluor 488 goat anti-mouse IgG and Alexa Fluor 568 goat anti-rabbit IgG (both, 1:500, Molecular Probes, Eugene, USA) for 30 minutes at room temperature. The slides were mounted using mounting medium containing DAPI (Vector Laboratories, Burlingame, USA) and analyzed by use of a Zeiss Axiovert200M fluorescence microscope (Carl Zeiss Japan, Tokyo, Japan).

Assessment and scoring of myofibroblasts and expression of Tn-C

Myofibroblasts were distinguished immunohisto-chemically from myoepithelial cells by positive staining

for α-SMA and negative staining for myoepithelial marker p63 (Gama et al., 2003; Seixas et al., 2008). Scoring of appearance of α-SMA positive myofibroblasts within tumor stroma was based on a semiquantitative evaluation proposed by Surowiak et al., which was also employed in our previous study of canine mammary tumors (Surowiak et al., 2006; Yoshimura et al., in press). Briefly, the distribution of myofibroblasts was separated into four grades according to the percentage of immunopositive cells: (-) no myofibroblasts, (1+) scanty (<10% of myofibroblasts in stroma), (2+) focally abundant (10-30% of myofibroblasts in stroma), and (3+) globally abundant (>30% of myofibroblasts in stroma). As in our previous study of canine mammary tissues (Yoshimura et al., in press), the extent of Tn-C expression in the stroma or the basement membrane zone was semiquantitatively indicated from (-) to (3+), with (-) indicating no expression of Tn-C, (1+) scanty (<10% Tn-C expression in stroma or basement membrane zone), (2+) focal (10-30% Tn-C expression in stroma or basement membrane zone), (3+) global (>30% Tn-C expression in stroma or basement membrane zone).

Mann-Whitney U-test was used to determine whether there was a statistical significant correlation between samples in the scoring of stromal myofibroblasts or Tn-C expression, and in the histopathological parameters (histological type, histological grade, intravasation, and metastasis to the neighboring lymph node) of carcinomas. All values of p <0.05 were considered significant.

Results

The correlation of the appearance of stromal myofibroblasts or expression of Tn-C between histological types or histopathological parameters of the carcinomas is listed in Tables 1 to 5.

Normal and hyperplastic mammary gland

In 10 cases of normal mammary tissues and 13 cases of adenosis, stromal myofibroblasts were not present (Fig. 1a) with the exception of one case each with scanty or focal myofibroblastic appearance (Table 1). Expression of Tn-C in the stroma was not seen in normal mammary glands (Fig. 1b) or adenosis except one case of normal mammary gland with a scanty Tn-C positive area which coincided with the presence of a few myofibroblasts (Table 2). On the other hand, Tn-C immunoreactivity was occasionally observed in the basement membrane zone around normal ducts and dilated or proliferated ducts (Fig. 1c), but much less frequently around acini (Table 3). In all mammary tissues, Tn-C immunoreactivity was frequently seen at the vascular smooth muscle and adventitia.

Adenoma

In the stroma of 3 (38.5%) of 8 adenomas, not only

Table 1. Appearance of myofibroblasts in feline normal and proliferative mammary tissues.

Histological type	Number of cases	Appearance of myofibroblasts[a]				Significant difference[b]
		(-)	(1+)	(2+)	(3+)	
Normal mammary gland	10	9 (90%)	1 (10%)	0	0	
Adenosis	13	12 (92.3%)	0	1 (7.7%)	0	
Simple adenoma	8	5 (62.5%)	1 (12.5%)	1 (12.5%)	1 (12.5%)	
Carcinoma	40	0	3 (7.5%)	8 (20%)	29 (72.5%)	
Tubulopapillary	28	0	3 (10.7%)	6 (21.4%)	19 (67.9%)	
Solid	12	0	0	2 (16.7%)	10 (83.3%)	

[a]: (-), no myofibroblasts; (1+), scanty; (2+), focally; (3+), globally. [b]: p<0.05, Mann-Whitney U-test

Table 2. Expression of tenascin-C in the stroma of feline normal and proliferative mammary tissues.

Histological type	Number of cases	Expression of tenascin-C in the stroma[a]				Significant difference[b]
		(-)	(1+)	(2+)	(3+)	
Normal mammary gland	10	9 (90%)	1 (10%)	0	0	
Adenosis	13	13 (100%)	0	0	0	
Simple adenoma	8	5 (62.5%)	1 (12.5%)	2 (25%)	0	
Carcinoma	40	3 (7.5%)	1 (2.5%)	6 (15%)	30 (75%)	
Tubulopapillary	28	2 (7.1%)	1 (3.6%)	4 (14.3%)	21 (75%)	
Solid	12	1 (8.3%)	0	2 (16.7%)	9 (75%)	

[a]: (-), no tenascin-C expression; (1+), scanty; (2+), focal; (3+), global. [b]: p < 0.05, Mann-Whitney U-test

myofibroblasts but also Tn-C expression was seen almost simultaneously (Fig. 2a,b, Table 1, 2). The remaining 5 (62.5%) cases did not include myofibroblasts and Tn-C expression in the stroma (Fig. 3a, 3b), although myoepithelial cells investing neoplastic acini were still preserved. In three adenomas, Tn-C-positive basement membrane zone was partially seen around neoplastic ductal structures (Table 3).

Carcinoma

All (100%) 40 carcinomas showed scanty to global distribution of myofibroblasts, and 37 cases (92.5%) of them had focally or globally abundant distribution (10% or more of stromal area) of myofibroblasts (Fig. 4a). The frequency of appearance of stromal myofibroblasts in carcinomas was significantly higher than that in normal mammary glands, adenosis, or adenomas (Table 1). Solid carcinomas had a tendency to contain more stromal myofibroblasts than tubulopapillary ones, but no statistical significance was present between them. The histological grades of 40 carcinomas were as follows: Grade I, 8 (8, tubulopapillary); Grade II, 17 (15, tubulopapillary; 2, solid); and Grade III, 15 (5, tubulopapillary; 10, solid). Stromal myofibroblasts increased in number with enhanced histological grade, but the correlation was not significant statistically (Table 4). Of 40 carcinomas 13 cases (32.5%) had vascular/lymphatic invasion and 11 (27.5%) showed lymph node metastasis. No significant difference was found between the appearance of stromal myofibroblasts and histopathological parameters such as intravasation and metastasis (Table 4).

As for Tn-C expression, 37 (92.5%) of 40 carcinomas exhibited scanty to global Tn-C positive reaction in the stroma and 36 (90%) had focal or global positivity (Table 2). Immunohistochemistry using serial sections revealed that stromal Tn-C expression was mainly restricted to the areas where myofibroblasts were present (Fig. 4b). Furthermore, double-labeled fluorescent immunohistochemistry demonstrated that α-SMA-positive myofibroblasts were distributed in Tn-C-positive extracellular matrix (Fig. 5). However, myofibroblasts alone were occasionally observed without Tn-C expression in the central stromal regions of carcinoma. Necrotic areas were sometimes stained with Tn-C antibody. In addition, in three (7.5%) of 40 carcinomas (2, tubulopapillary; 1, solid), there were occasional Tn-C-positive carcinoma cells (Fig. 6). In a solid carcinoma, a positive reaction for Tn-C was found

Table 3. Expression of tenascin-C in the basement membrane zone of feline normal and proliferative mammary tissues.

Histological type	Number of cases	Expression of tenascin-C in the basement membrane zone[a]				Significant difference[b]
		(-)	(1+)	(2+)	(3+)	
Normal mammary gland	10	6 (60%)	4 (40%)	0	0	
Adenosis	13	7 (53.8%)	6 (46.2%)	0	0	
Simple adenoma	8	5 (62.5%)	2 (25%)	0	1 (12.5%)	
Carcinoma	40	39 (97.5%)	0	1 (2.5%)	0	
Tubulopapillary	28	28 (100%)	0	0	0	
Solid	12	11 (91.7%)	0	1 (8.3%)	0	

[a]: (-), no tenascin-C expression; (1+), scanty; (2+), focal; (3+), global. [b]: $p < 0.05$, Mann-Whitney U-test

Table 4. Correlation of appearance of myofibroblasts with histopathological parameters in feline mammary carcinomas.

Histopathological parameters	Number of cases	Appearance of myofibroblasts[a]			
		(-)	(1+)	(2+)	(3+)
Histological grade					
Grade I	8	0	1 (12.5%)	3 (37.5%)	4 (50.0%)
Grade II	17	0	2 (11.8%)	3 (17.6%)	12 (70.6%)
Grade III	15	0	0	2 (13.3%)	13 (86.7%)
Intravasation					
No	27	0	1 (3.7%)	5 (18.5%)	21 (77.8%)
Yes	13	0	2 (15.4%)	3 (23.1%)	8 (61.5%)
Metastasis					
No	29	0	3 (10.3%)	5 (17.2%)	21 (72.4%)
Yes	11	0	0	3 (27.3%)	8 (72.7%)

[a]: (-), no myofibroblasts; (1+), scanty; (2+), focally; (3+), globally

Table 5. Correlation of expression of tenascin-C in the stroma with histopathological parameters of feline mammary carcinomas.

Histopathological parameters	Number of cases	Expression of tenascin-C[a]			
		(-)	(1+)	(2+)	(3+)
Histological grade					
Grade I	8	0	1 (12.5%)	1 (12.5%)	6 (75%)
Grade II	17	2 (11.8%)	0	3 (17.6%)	12 (70.6%)
Grade III	15	1 (6.7%)	0	2 (13.3%)	12 (80%)
Intravasation					
No	27	1 (3.7%)	1 (3.7%)	4 (14.8%)	21 (77.8%)
Yes	13	2 (15.4%)	0	2 (15.4%)	9 (69.2%)
Metastasis					
No	29	2 (6.9%)	1 (3.4%)	4 (13.8%)	22 (75.9%)
Yes	11	1 (9.1%)	0	2 (18.2%)	8 (72.7%)

[a]: (-), no tenascin-C expression; (1+), scanty; (2+), focal; (3+), global

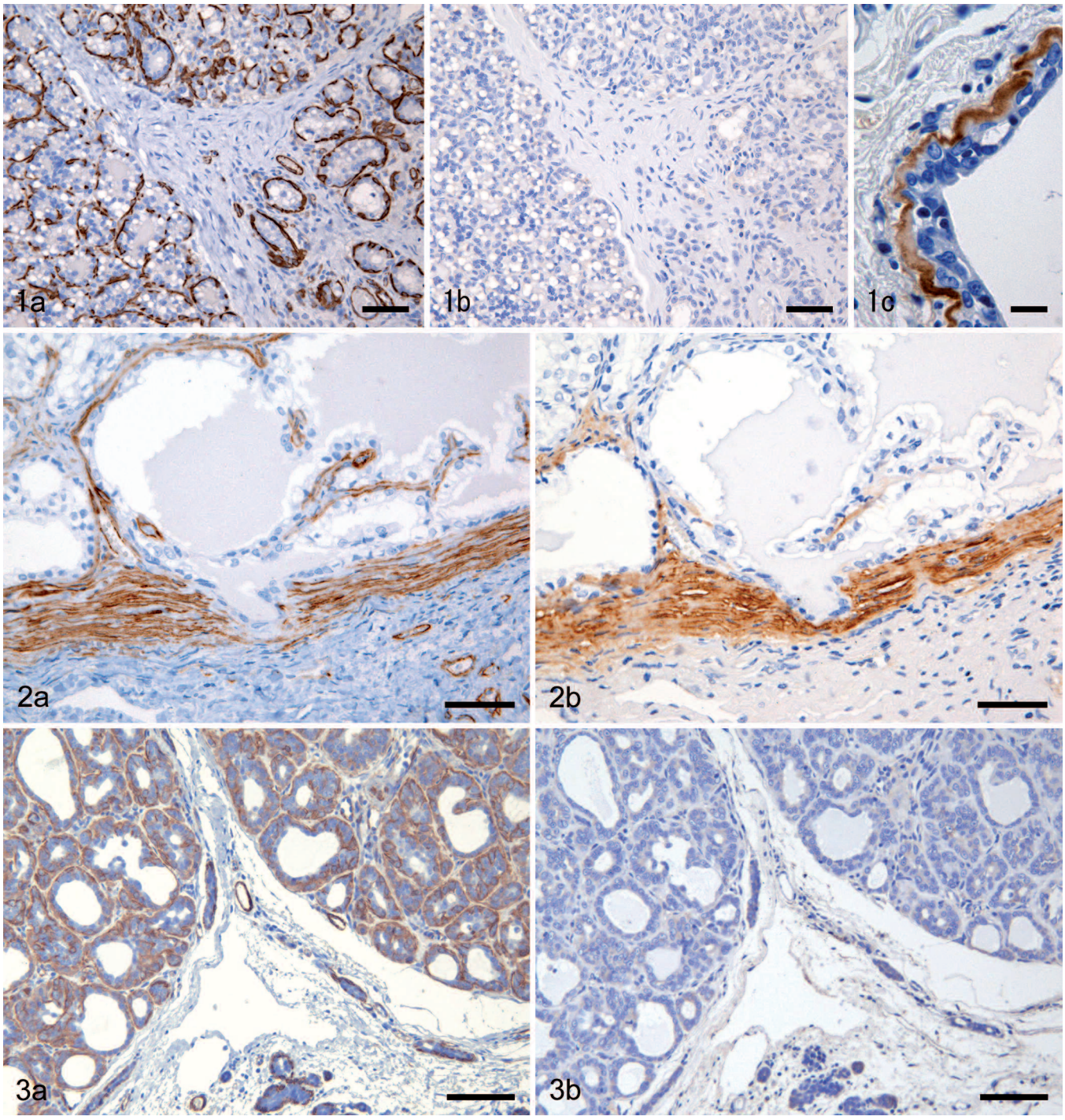

Fig. 1. Normal mammary gland. **a.** Immunohistochemical staining with α-smooth muscle actin (α-SMA). Although myoepithelial cells and vascular walls are stained positive, stromal fibroblasts are not positive. **b.** Serial section of Fig. 1a. Immunohistochemical staining for Tenascin-C (Tn-C). Tn-C immunoreactivity is not seen in the stroma. **c.** An interlobular duct. Immunohistochemical staining for Tn-C. The basement membrane zone is positive for Tn-C. Scale bars: a, b, 50 μm; c, 15 μm.

Fig. 2. Adenoma. **a.** Immunohistochemical staining with α-SMA. The marginal area is enriched with α-SMA-positive myofibroblasts. **b.** Serial section of Fig. 2a. Immunohistochemical staining for Tn-C. Tn-C-positive matrix coincides with the area abundant in myofibroblasts shown in Fig. 2a. Scale bars: 50 μm.

Fig. 3. Adenoma. **a.** Immunohistochemical staining with α-SMA. Myoepithelial cells and vascular walls are stained. However, no myofibroblasts are present in the stroma. **b.** Serial section of Fig. 3a. Immunohistochemical staining with Tn-C. Tn-C immunoreactivity is not observed. Scale bars: 80 μm.

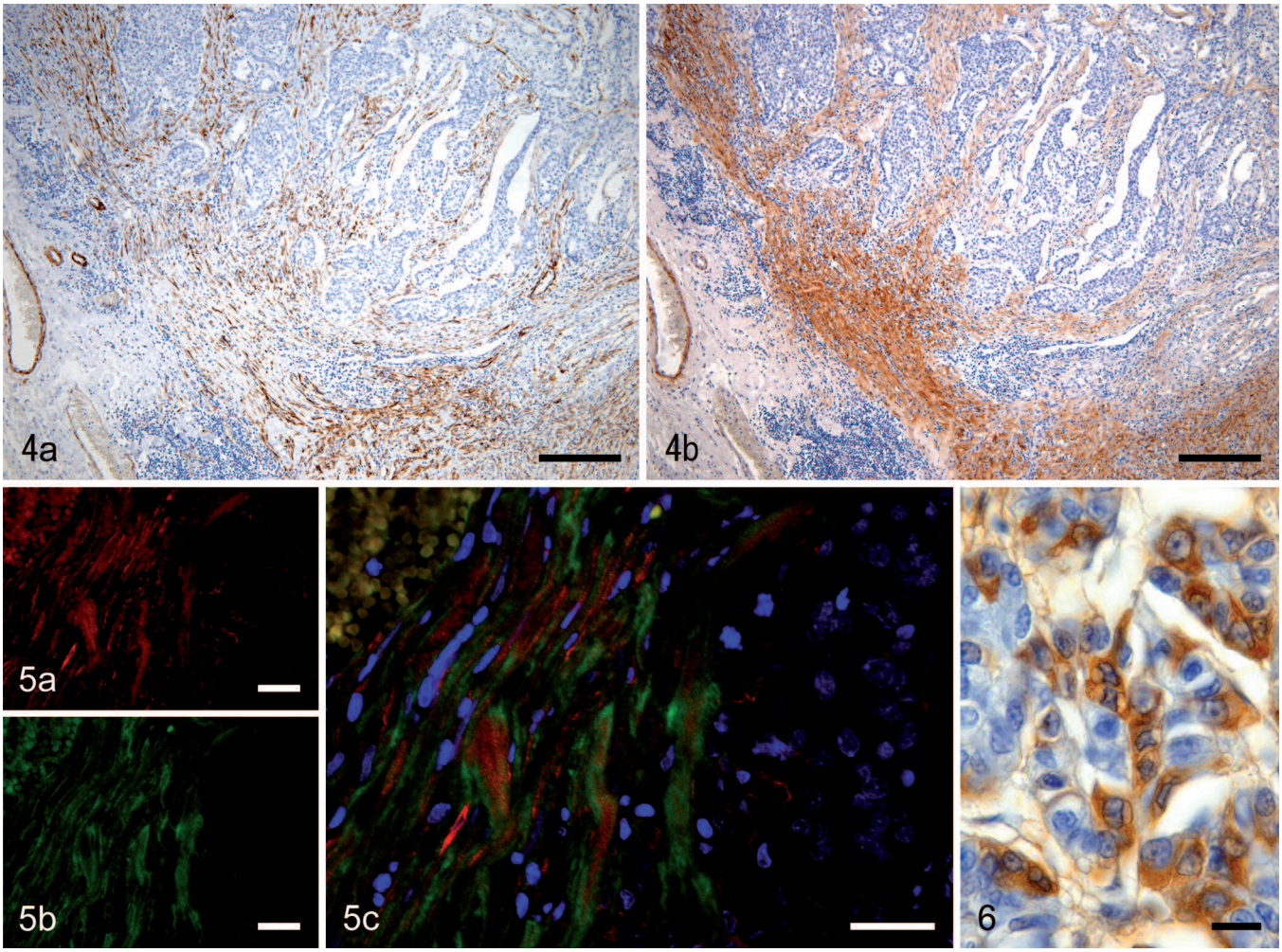

Fig. 4. Carcinoma (solid type). **a.** Immunohistochemical staining with α-smooth muscle actin (α-SMA). There are abundant α-SMA-positive myofibroblasts in the peripheral stroma. **b.** Serial section of Fig. 4a. Immunohistochemical staining with Tenascin-C (Tn-C). Consistent with the distribution of myofibroblasts in Fig. 4a., Tn-C immunoreactivity is observed in the stroma. Scale bars: 240 μm.

Fig. 5. Carcinoma (tubulopapillary type). Double-labeled fluorescent immunohistochemical staining using an anti-smooth muscle actin antibody (Red signal) and an anti-Tn-C antibody (Green signal). Nuclei are colored blue with DAPI. **a.** Red signal indicates the presence of myofibroblasts. **b.** Green signal shows the presence of Tn-C. **c.** Merge. Red signals closely surrounded by green signals are noted. Right one-third of the photograph consists of carcinoma cells with no stromal myofibroblasts and the left two-thirds consist of both types of cells. Scale bars: 25 μm.

Fig. 6. Carcinoma (tubulopapillary type). Immunohistochemical staining with Tn-C. Some carcinoma cells express Tn-C. Scale bar: 15 μm.

in the basement membrane zone adjacent to myoepithelial cells surrounding solid growths of cancer cells. The frequency of Tn-C expression in the stroma was significantly greater in carcinomas than in normal mammary glands, adenosis, or adenomas (Table 2). On the other hand, Tn-C expression in the basement membrane zone was detected less frequently in carcinomas than in normal mammary glands, adenosis, or adenomas with a statistical significance (Table 3). No significant difference was found between the expression of Tn-C in the stroma and histopathological parameters such as histological grades, intravasation and metastasis (Table 5).

Discussion

In feline mammary tissues, stromal myofibroblasts were almost absent in normal glands and benign lesions, but they increased markedly in number in malignant lesions. A combined histochemical and ultrastructural study revealed the participation of myofibroblasts in all 17 cases of feline mammary carcinoma (Tateyama et al., 1988). In feline mammary carcinomas there were no significant differences between the appearance of

stromal myofibroblasts and histopathological parameters such as histological grades, intravasation or metastasis, unlike the results obtained in human and canine mammary carcinomas (Yazhou et al., 2004; Surowiak et al., 2006, 2007; Yoshimura et al., in press). However, it is clear that the rate of appearance of stromal myofibroblasts in feline mammary carcinomas is higher than that in mammary carcinomas of other animal origin. For instance, the field percentage of myofibroblasts occupying 10% or more in the stroma was detected in 92.5% of feline mammary carcinomas, which was higher than 44.4% of human breast ductal carcinomas *in situ*, 56.9% of human breast invasive ductal carcinomas (Yazhou et al., 2004), or 54.8% of canine mammary simple carcinomas (Yoshimura et al., in press). In addition, another study (Martín de las Mulas et al., 2004) indicated that the appearance rate of stromal myofibroblasts in mammary carcinomas was higher in feline cases (100%; 32/32) than in canine (73%; 11/15) or human cases (89%; 25/28). Thus, a high rate of myofibroblastic appearance implies that myofibroblast may be involved in a malignant behavior of this tumor. In general, feline mammary carcinomas are known to be more aggressive than their counterparts in dogs and have a highly invasive potential even though they exhibit neither intravasation nor metastasis.

We and other authors have described the following 4 different immunolabeling patterns of Tn-C in canine mammary tissues (Arai et al., 1994; Faustino et al., 2002; Yoshimura et al., in press): 1) Tn-C expressed in the reactive stroma containing myofibroblasts in high-grade simple carcinomas; 2) Tn-C expressed in the basement membrane zone in normal glands, benign legions, and low-grade simple carcinomas, which is seemingly produced by neighboring myoepithelial cells; 3) Tn-C expressed in the cytoplasm of carcinoma cells themselves; and 4) Tn-C expressed in the areas of proliferated myoepithelial cells and chondroid metaplasia in complex or mixed tumors. In this feline study, the former 3 patterns were recognized. Tn-C has a number of splice variants generated by alternative splicing of fibronectin-like types repeats (Tsunoda et al., 2003). The different localizations of Tn-C observed in this study and other studies on mammary tumors might represent splice variants and play different roles.

Tn-C was expressed in the stroma of almost all carcinomas in varying degrees, whereas it was hardly noted in normal mammary glands and benign lesions. The appearance and distribution of stromal Tn-C immunoreactivity often coincided with those of myofibroblasts in tumors. Although an increase in myofibroblasts was not necessarily accompanied by Tn-C expression, this might suggest a functional immaturity or senescence of myofibroblasts. Thus, myofibroblasts are considered to be a major cellular source of Tn-C in feline mammary tumors. Tn-C production by myofibroblasts in cancer stroma has been demonstrated in several human cancers and canine gastrointestinal carcinomas (Czernobilsky et al., 1993; Hanamura et al., 1997; Tuxhorn et al., 2002; Mukaratirwa et al., 2003). Recently, we have also obtained similar results in canine mammary carcinomas (Yoshimura et al., in press). However, the frequency and amount of stromal Tn-C expression seem to differ between canine and feline mammary carcinomas. Tn-C expression occupying 10% or more of cancer stroma was detected in 90% of feline mammary carcinomas examined, which exceeded 64.3% in canine mammary simple carcinomas (Yoshimura et al., in press). As discussed above on stromal myofibroblasts, the abundance of stromal Tn-C in feline mammary carcinomas may reflect their aggressive behavior. Earlier studies in human cancer have generated the theory that tissue-derived fibroblasts and bone marrow-derived mesenchymal stem cells in cancer stroma are converted into myofibroblasts by physical stimuli due to tumor growth or by a cytokine, such as TGF-ß secreted by cancer cells themselves, and then the stromal myofibroblasts contribute to cancer development by secreting factors including Tn-C (Egeblad et al., 2005; Li et al., 2007; De Wever et al., 2008).

Tn-C expressed in the basement membrane zone was occasionally observed in normal mammary glands and benign lesions, but very seldom in carcinomas. In feline mammary tumors, like canine ones (Yoshimura et al., in press), Tn-C immunoreactivity in the basement membrane zone was confined to the areas adjacent to the resting myoepithelium. Therefore, Tn-C expressed in the basement membrane zone is considered to be secreted by myoepithelial cells. Since feline mammary carcinomas generally lack the resting myoepithelium, such a Tn-C immunoreactivity pattern seems to be rarely or never recognized, unlike canine mammary simple carcinomas (Yoshimura et al., in press). In this respect, feline mammary carcinoma is similar to human breast cancer and may be more suited for a human breast cancer model rather than canine mammary carcinoma. A high expression of Tn-C has been proposed in stem cell niche of some organs (Orend, 2005). Since mammary ducts are known as a site in which mammary stem/progenitor cells reside (Villadsen et al., 2007), the fact that expression of Tn-C in normal mammary glands was confined mainly to the basement membrane zone surrounding ducts suggests that Tn-C might be associated with the maintenance of mammary stem/progenitor cells.

In human breast cancer, it has been claimed that Tn-C expression in cancer cells themselves appears to indicate unfavorable patient prognosis (Yoshida et al., 1995; Ishihara et al., 1995). We expected that in more feline cases carcinoma cells would express Tn-C, since feline mammary carcinomas are generally highly aggressive. However, 7.5% as the frequency in feline mammary carcinomas with Tn-C-positive carcinoma cells was compatible with 7.1% in canine mammary simple carcinomas (Yoshimura et al., in press). In order to elucidate the significance of Tn-C expression in mammary carcinoma cells in cats, it will be necessary to accumulate more data on similar cases.

In conclusion, we demonstrated that most feline

mammary carcinomas contained abundant stromal myofibroblasts and Tn-C-positive extracellular matrix. This evidence makes us think that the role of the stromal microenvironment may be of importance in invasiveness of these tumors. Therefore, we confirm that feline mammary carcinoma is a good research subject to clarify the mechanism of cancer-stromal interaction. Furthermore, because the distribution of myofibroblasts and Tn-C expression in feline mammary carcinomas are largely similar to those in high-grade human breast cancer, it is possible that feline mammary carcinoma could serve as an animal model for cancer therapies targeting the stroma.

Acknowledgements. The authors wish to thank Mr. Rei Nakahira for figure editing.

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Accepted September 21, 2010