CHAPTER 3

Tenascin Expression in Relation to Stromal Tumour Cells in Canine Gastrointestinal Epithelial Tumours

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Published in Journal of Comparative Pathology, 129, 137-146, 2003
Abstract

The expression of tenasin, α-smooth muscle actin (α-SMA), desmin and vimentin was investigated immunohistochemically in the stroma of normal canine stomach, small intestine and colon, and in 30 epithelial tumours of the canine stomach, small intestine and colon by immunohistochemistry. In addition, “co-localisation” of tenasin and α-SMA was investigated by double immunohistochemistry. Tenasin was absent in the normal mucosa of the stomach, but expressed in the normal intestine with a gradual increase from the cryptal glands to the surface epithelium. Tenasin expression was greater in all adenomas and carcinomas. Two different patterns of tenasin expression were observed in carcinomas, irrespective of their site. In well-differentiated tumour regions of both gastric and intestinal tumours, a fibrillary sub-glandular expression was observed; in poorly differentiated tumour regions, however a diffuse network expression pattern was present. Incomplete invasion of the muscularis mucosae was accompanied by thickening and increased tenasin expression. In normal stomach and small intestines, α-SMA and desmin were demonstrated in pericryptal myofibroblasts and smooth muscle cells of the muscle layers. In colonic adenomas and gastric and intestinal carcinomas, α-SMA was demonstrated in all stromal cells surrounding tumour cells. In contrast to α-SMA labelling, desmin labelling was negative in tumour stromal cells (in both gastric and intestinal tumours), except in tumour regions close to the muscularis mucosae. This suggest that myofibroblasts in gastrointestinal tumours originate from pre-existing fibroblasts except in tumour regions close to the muscularis mucosae, where the myofibroblasts seem to originate from smooth muscle cells of the muscularis mucosae. There was a strong co-localisation of tenasin and α-SMA expressing myofibroblasts suggesting that myofibroblasts may be responsible for tenasin secretion.
Introduction

Tenascin is an extracellular matrix glycoprotein that has been shown to have an influence on cell adhesion, growth, proliferation and differentiation (reviewed by Chiquet-Ehrismann et al., 1995). The type of cell producing tenascin in tumours in vivo is not clear. Many researchers have pointed to mesenchymal cells like fibroblasts, smooth muscle cells and glial cells as the main producers of tenascin in vivo (Mackie, 1995) that are thought to be under the influence of cytokines produced by epithelial tumour cells (Jones and Jones, 2000).

Pathological conditions associated with tissue remodelling are characterised by the appearance of stromal cells with ultrastructural features intermediate between those of typical fibroblasts and those of smooth muscle cells; they were first described in granulation tissue and were called myofibroblasts (Gabbiani et al., 1971). Myofibroblasts expressing α-smooth muscle actin (α-SMA) have been considered the most significant single stromal cell type in epithelial tumours of the colon (Sappino et al., 1989). In human mammary (Sappino et al., 1988) and ovarian (Czernobilsky et al., 1989) tumours α-SMA-expressing myofibroblasts have been found in the stroma of the tumours. The origin of myofibroblasts in colonic epithelial tumours is not clear. They may arise from pre-existing fibroblasts of stroma and pericryptal areas, or from smooth muscle cells of the muscularis mucosae and blood vessels. Pre-existing fibroblasts are likely candidates; this opinion is based on the finding of stimulation of stromal fibroblasts in vitro, mediated through various pathways of signalling by cytokines such as TGF-β has been shown to induce α-SMA expression in cultured fibroblasts (Desmoulié et al., 1993; Rønov-Jessen and Petersen, 1993). In addition, fibroblastic cells have been found to express features of muscle differentiation in diverse pathological conditions (Skalli et al., 1989b), in which modulations of the peritumoural extracellular matrix coincide with proliferative characteristics of tumour tissues (Sappino et al., 1988).

Accumulation of tenascin has been demonstrated in the stroma of many human mammary adenomas and carcinomas (Koukoulis et al., 1991). In human endometrial tumours, tenascin was identified in periglandular location in cases in which α-SMA was also present (Czernobilsky et al., 1993). Tenascin mRNA been has found to be expressed by myofibroblasts in human colon adenomas and carcinomas (Hanamura et al., 1997). The precise function of myofibroblasts in tumours is not fully understood, but their restricted distribution and association with tenascin point to their function in tissue reorganisation processes in ovarian follicles, intestinal mucosa and tumours (Czernobilsky et al., 1993).

The relationship between tenascin expression and phenotype of stromal cells in human and canine intestinal tumours is not clear. The aims of the present study
were to study (1) the expression pattern of tenascin and α-SMA in normal and neoplastic conditions of the canine stomach, small intestine and colon, (2) the co-localisation of tenascin and α-SMA expressing myofibroblasts in such neoplastic tissues, and (3) the expression of desmin and vimentin in relation to α-SMA in the stroma of tumours, to elucidate the origin of the α-SMA expressing myofibroblasts.

**Materials and methods**

**Case material**
Cases of canine gastrointestinal adenomas (n=11), adenocarcinomas (n=14) and anaplastic carcinomas (n=5) were selected from the files of the Department of Pathology, Faculty of Veterinary Medicine, Utrecht University. Details of the localizations of the various tumours and their numbers are given in table 1. In addition, full thickness samples of normal canine stomach (n=10), small intestine (n=10) and colon (n=10) were obtained. Haematoxylin and eosin-stained sections were made to confirm the original diagnosis made by the pathologists of our department; the tumours were classified according to the WHO classification of tumours of the lower alimentary tract (Head, 1976)

**Table 1. Summary of the number of tumours studied.**

<table>
<thead>
<tr>
<th>Tumour</th>
<th>Stomach</th>
<th>Small intestine</th>
<th>Colon</th>
<th>Rectum</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenoma</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tubular</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Tubulovillous</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mucinous</td>
<td>3</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Tubular</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>Solid/ signet ring</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Anaplastic Carcinoma</td>
<td>3</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td>9</td>
<td>6</td>
<td>7</td>
<td>8</td>
<td>30</td>
</tr>
</tbody>
</table>
Representatives of each tumour and normal tissue were selected for immunohistochemical analysis of tenascin, α-SMA, desmin and vimentin. In each case, 4-μm sections of formalin-fixed, paraffin wax-embedded tissues were mounted on poly-L-lysine coated slides. The slides were de-waxed through xylol and graded alcohols. Sections to be labelled for tenascin were incubated with chondroitinase AC (Sigma, St Louis, MO, USA) at a concentration of 2 units/ml in 0.25M Tris/HCl buffer (pH 8) at 37°C overnight to reduce antigenic masking of tenascin by chondroitin sulphate (Faustino et al., 2002). For tenascin retrieval, antigen was unmasked by treatment for 10 minutes at 37°C with trypsin 0.1% in PBS containing CaCl₂ 0.1% (0.01M, pH 7.4) at a final pH of 7.8. Endogenous peroxidase activity was blocked by H₂O₂ 0.3% in PBS for 30 minutes. After incubation with normal horse serum diluted 1 in 10 for 30 minutes to block background staining, the sections were treated with primary antibodies at room temperature for 60 minutes. The antibodies used were against tenascin (clone TN2; Dako, Glostrup, Denmark) at a dilution of 1 in 50, α-SMA (clone 1A4; Biogenex, CA, USA) at a dilution of 1 in 1200, vimentin (Clone V9; Biogenex) at a dilution of 1 in 150 and desmin (RD 301; Euro Diagnostic B.V., The Netherlands) undiluted. After incubation with horse anti-mouse biotin and avidin-biotin peroxidase complex (Vector, Burlingame, USA), the colour was developed in 3-3’-diaminobenzidine and 0.02% H₂O₂ in Tris buffer pH 7.8. The sections were counter-stained with 10% Mayer’s haematoxylin. The muscularis mucosae was used as internal positive control. For negative controls, the primary antibodies were replaced with normal mouse serum.

Double staining immunohistochemistry

A double immunohistochemical procedure based on a sequential technique (van der Loos, 1999) was performed to establish the co-localisation of tenascin and α-SMA expressing myofibroblasts. The staining procedure started with tenascin primary antibody but without developing the enzymatic activity followed by α-SMA antibody. The sections were treated with chondroitinase AC and tenascin retrieval was performed as explained above. The sections were incubated with normal horse serum 10% for 20 minutes followed by tenascin antibody (Clone TN2; Dako) for 60 minutes and horse anti-mouse biotin (Vector) for 30 minutes diluted as above. For α-SMA assay, the first part of the double staining consisted of blocking the first antibody by incubating with goat anti-mouse immunoglobulin (Dako) at dilution of 1 in 50 for 50 minutes and then incubation with α-SMA (Clone 1A4; Biogenex) at a dilution of 1 in 1200 overnight at 4°C. Incubation with a mixture of goat anti-mouse immunoglobulin alkaline phosphatase (AP; Dako) at a dilution of 1 in 300 and streptavidin horse-radish peroxidase (HRP; Dako) at a dilution of 1 in 50 followed this. The enzymatic activities of AP and HRP
were visualised in blue with 5-bromo-4-chloro-3-indoxyl phosphate and nitro blue tetrazolium chloride (BCIP/NBT; Dako), and red with 3-amino-9-ethylcarbazole (AEC; Dako) respectively. No counterstaining was performed, and slides were mounted with paramount (Dako).

Results

Table 1 contains a summary of the tumours examined. All the adenomas were from the colon or the rectum, whereas the carcinomas involved tumours from stomach and small intestine as well. Table 2 summarises the expression of tenascin and α-SMA in normal gastrointestinal tissues, primary tumours and mesenteric lymph node metastases.

Tenascin expression

Normal tissues. In normal adult stomach tenascin was not expressed in the mucosa

Table 2. Tenascin and α-smooth muscle expression in normal mucosa and tumours and lymph node metastasis

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Tenascin</th>
<th>α-SMA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal stomach</td>
<td>0/10*</td>
<td>10/10</td>
</tr>
<tr>
<td>small intestine</td>
<td>10/10</td>
<td>10/10</td>
</tr>
<tr>
<td>colon</td>
<td>10/10</td>
<td>10/10</td>
</tr>
<tr>
<td>Adenomas</td>
<td>10/11</td>
<td>11/11</td>
</tr>
<tr>
<td>Adenocarcinomas</td>
<td>12/14</td>
<td>14/14</td>
</tr>
<tr>
<td>Anaplastic carcinoma</td>
<td>5/5</td>
<td>5/5</td>
</tr>
<tr>
<td>Metastatic tumour</td>
<td>2/4</td>
<td>4/4</td>
</tr>
</tbody>
</table>

*Numerator, number of positives; denominator, number examined.
even at the epithelial-mesenchymal interface. Tenascin was strongly expressed in the extracellular matrix of the muscularis mucosae and muscularis propria and in the cells themselves. In the submucosa and serosa there was no tenascin expression except in the media of muscular arteries.

In normal small intestine, tenascin was expressed in areas adjacent to the basement membrane. Expression was increased from the crypts to the villi with abundant expression at the top of the villi. Transverse section of the villi showed a thin continuous line at the base of the epithelium representing the basement membrane. Labelling of tenascin in the normal colon was similar to that in the small intestine with an increased expression from the glands to the surface of the mucosa. At the apical portion of the mucosa, a sparse network of tenascin immunolabelling was present (Fig. 1). Granular cytoplasmic labelling was seen in some epithelial cells of the stomach, small intestine and colon. Tenascin was not expressed in the submucosa and serosa except around submucosal (Brunner’s) glands and media of muscular blood vessels. In smooth muscle areas (muscularis mucosae and muscularis propria) tenascin was constantly expressed.

**Adenomas.**

In 10 adenomas, tenascin was expressed when stroma was present (Fig. 2). Expression

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**Fig. 1.** Normal colon immunolabelled for tenascin. Sparse network-like immunoreaction of tenascin at the apical portion of the mucosa. Avidin-biotin peroxidase complex (ABC), haematoxylin counter-stain. x 400

**Fig. 2.** Colorectal adenoma immunolabelled for tenascin. Increased tenascin immunoreactivity in the tumour stroma and cytoplasmic granular staining in tumour cells. ABC, haematoxylin counter-stain. x 200
of tenascin at the tip of adenomatous growth was intense and greatly exceeded that in normal mucosa. Granular staining of tenascin in the cytoplasm of tumour cells was seen in four cases (Fig. 2). There was no relation between cytoplasmic labelling of tenascin and the intensity of extracellular expression.

Alteration in the thickness of muscularis mucosae and tenascin expression was seen in eight of the tumours where the adenoma was near or in contact with the muscularis mucosae. In these cases the thickness of muscularis mucosae changed from a regular line into an outgrowing mass filling the base of the tumour with filamentous pattern of tenascin-positive stroma projecting into the adenomatous growth. In cases where the tumour was growing from the top of the normal mucosa, there was increased expression of tenascin at the junction of tumour and normal mucosa, suggesting that the tumour was founded on this tenascin positive base. The submucosa, muscularis propria and serosa showed the same pattern as the normal tissue.

Adenocarcinomas.

Samples of 14 adenocarcinomas were examined. Twelve cases had tumour proliferation in the mucosa and tenascin was expressed when stroma was present. In two cases the stroma was completely negative for tenascin but there was granular cytoplasmic labelling of tumour cells (Fig. 3). Cytoplasmic granular staining of tenascin was seen in three other adenocarcinomas.

Eight cases had invasion into the muscularis mucosae. In these cases two different patterns were observed. When incomplete invasion was present the muscularis mucosae was thickened with intense expression of tenascin and tenascin positive stromal projections completely surrounding invading tumour cells, as in the adenomas. When complete invasion was present, there was a break in the muscularis mucosae by tumour cell-containing lymph vessels; some tenascin expression was found around these vessels (Fig. 4), but deeper in the submucosa tenascin expression around these vessels was frequently absent. In 12 cases there was invasion into the submucosa,
muscularis propria and serosa. The expression pattern of tenascin seen in these regions was significantly different, even within the same tumour, depending on the degree of differentiation and site of invasion. In general two patterns were observed. In well-differentiated tumour regions with gland or cyst formation, the immunoreactivity, which was fibrillary, was subglandular or located in the basement membrane (Fig. 5). In poorly differentiated tumour regions, a more diffuse, net-like expression was present (Fig. 6). In the tumour stroma, tenascin expression was less in the submucosa than in the muscularis propria and serosa. In the submucosa, labelling varied according to the depth of invasion. Stroma of tumour regions near muscularis mucosae was always positive for tenascin and the stroma seemed to be connected to the muscularis mucosae. Tumour regions deep in the submucosa were always negative.

Anaplastic carcinomas.

The same pattern of tenascin expression was found as in poorly differentiated regions of adenocarcinomas. In all the cases there was invasion into the submucosa, but tenascin was expressed in only two cases (of small intestinal origin),
although there was abundant stroma (desmoplasia) in these tumours. Lymph node metastasis. Four cases with mesenteric lymph node metastasis were examined. In all the four cases tenascin was expressed in the primary tumour. In two cases tenascin was expressed in the metastasis.

**α-smooth Muscle Actin, Desmin and Vimentin Expression**

Normal tissue. α-SMA was expressed in different muscular layers of the stomach, small intestine and colon, and in the media of muscular vessels and pericytes. Pericryptal fibroblasts at the bases of the crypts were also positive for α-SMA. The stromal cells in the submucosa and serosa were completely negative. Desmin showed the same pattern of expression as α-SMA. All stromal cells were positive for vimentin.

**Adenomas.**

In nine adenomas a persistent sheath of α-SMA positive cells was found bordering the epithelia, and labelling was also observed in foci of stromal cells within the tumour. In contrasts, however, none of the adenomas examined expressed desmin in the tumour stroma, except in areas close to the muscularis mucosae. In such areas, the desmin-positive tumour stromal cells seemed to be connected to the muscularis mucosae.

**Adenocarcinoma.**

The 14 cases of adenocarcinomas examined showed no evidence of α-SMA in the pericryptal fibroblasts in the mucosa. However, foci of stromal cells representing desmoplastic reaction were positive for α-SMA. At the point of muscularis mucosae invasion the intensity of α-SMA expression was increased. In the submucosa stromal cells of the tumour were positive when close to the muscularis mucosae, but as with tenascin labelling, less strongly when in the submucosa. The stroma of tumour nests in the muscularis propria and serosa was positive for α-SMA (Fig. 7).

When the tumour was present in the mucosa, desmin was not expressed

![Fig. 7. Colon adenocarcinoma with invasion into the serosa immunolabelled for α-SMA. Tumour stromal cells are positive for α-SMA. ABC, haematoxylin counter-stain. x 200](image)
even in regions where α-SMA was positive. Stroma of tumour nests in the muscularis propria and serosa were negative for desmin. Interestingly, tumour regions in the submucosa close to the muscularis mucosa showed intensely desmin-positive stromal cells, while stromal cells deep in the submucosa were negative for desmin, even when they were positive for α-SMA.

Carcinomas.
The same pattern of α-SMA actin and desmin expression was seen as in adenocarcinomas. In three cases (two gastric and one small intestine), α-SMA negative stromal cells surrounded individual carcinoma cells.

Co-localisation of and α-Smooth Muscle Actin
This was evaluated by comparing serial sections labelled for tenasin and α-SMA and then confirmed by double labelling.

Normal tissue.
In normal tissue tenasin was expressed mainly in conjunction with α-SMA expressing cells. Thus the muscularis mucosae and muscularis propria, which were strongly positive for α-SMA actin and appeared to be the major sites for tenasin expression. This area served as our internal positive control for the double labelling. In the gastric mucosa, there was no tenasin expression, but α-SMA was positive in cells surrounding glands. In the mucosa of small intestines and colon, there was co-localisation of tenasin and α-SMA at the tips of the villi or surface epithelium. Double labelling showed that isolated stromal cells (representing smooth muscle cells) in the lamina propria co-expressed tenasin and α-SMA. However, pericryptal myofibroblasts surrounding glands close to muscularis mucosae were strongly positive for α-SMA but negative for tenasin.

Tumours.
In the mucosa there was almost 100% co-localisation of tenasin and α-SMA. In three cases with desmoplasia, the stromal cells were positive for α-SMA, but negative for tenasin. In all cases tenasin was always expressed in conjunction with α-SMA positive cells. Within the same tumours there were regions where tenasin was not expressed, but α-SMA was expressed.

Tenasin was strongly expressed in conjunction with α-SMA in the muscularis mucosae at the base of adenomas or areas of incomplete invasion in adenocarcinomas and anaplastic carcinomas. Stroma projecting from muscularis mucosae into the tumour was positive for both tenasin and α-SMA.

Tumour regions in the submucosa, muscularis propria and serosa
showed a significant difference in the co-localisation of tenascin and α-SMA, depending on the location and degree of differentiation. In the submucosa, tumour regions close to the muscularis mucosae co-expressed tenascin and α-SMA, and those deeper were faintly positive for α-SMA actin and negative for tenascin. In well-differentiated tumour regions co-localisation was more evident (Fig. 8). Tumour regions in the muscularis propria and serosa showed strong co-localisation in eight cases, but there was variation within the same tumour. In some regions of the same tumour the stroma expressed both tenascin and α-SMA, but in some regions stromal cells were positive for α-SMA and negative for tenascin (Fig. 8). In no case was tenascin expressed in absence of α-SMA-positive cells.

**Fig. 8. Gastric adenocarcinoma double immunolabelled for tenascin and α-SMA. Co-localisation of tenascin and α-SMA in stroma surrounding well-differentiated tumour regions. Note some areas are positive for α-SMA, but tenascin is not expressed. AEC and NBT/BCIP, no counter-stain. x 200 (refer to colour picture page 156)**

**Discussion**

There is increasing evidence that tumour stromal components play a role in tumourigenesis by modulating growth and differentiation. Increased tenascin expression has been documented in many human tumours (Koukoulis et al., 1991; Natali et al., 1991) and in dog mammary tumours (Faustino et al., 2002), but no reports are available on tenascin expression in other canine tumours. Few studies on the type of stromal cells present in human gastrointestinal tumours have been published (Ohtani and Sasano, 1983; Sappino et al., 1989), and there are no published studies on the origin and type of cells responsible for tenascin secretion in both human and canine gastrointestinal tumours.

The present study showed that in the normal canine gastric mucosa tenascin expression was absent, whereas in the small intestine and colon tenascin was expressed at the epithelial-mesenchymal interface with a gradual increase from the crypts to the mucosal surface. The observations in the stomach are in line with the reported tenascin expression pattern in human stomach (Tiitta et al., 1994; Ikeda et al.,...
The observations in the small intestine and those in the colon are in agreement with the reported tenascin expression pattern in human small intestine and colon (Reidl et al., 1992; Gulubova and Vlaykoya, 2001) and murine intestine (Thor et al., 1987). It appears paradoxical that tenascin immunoreactivity was not present at the epithelial-mesenchymal interface in the normal stomach where the epithelium is also undergoing rapid renewal, yet it was present in the small intestine and colon. The differences in tenascin expression may be explained by the different functions of epithelial cells or the difference in the rate of migration and turnover of surface epithelium as suggested by Tiitta et al., (1994). Another reason for such a differential distribution of tenascin in the stomach may be that epithelial cells of the stomach are not able to produce cytokines like TGF-β that stimulate tenascin synthesis by stromal cells.

The presence of tenascin granular cytoplasmic immunoreactivity in epithelial cells in both normal and tumour cells suggest that epithelial cells synthesise tenascin. Similar results have been found in canine mammary tumours (Faustino et al., 2002) and human epithelial tumours, including those of colorectal origin (Gulubova & Vlaykoya, 2001). In vitro experiments have shown that tenascin can be synthesised and secreted by epithelial cells (Kawakastu et al., 1992; Latijnhouwers et al., 1997). The increased tenascin expression in adenomas suggests two important functions of tenascin: proliferation and anti-adhesion. Under normal conditions the steadily dividing cells of the lower parts of the crypts of the intestine are situated on a tenascin positive basement membrane. In adenomas there is increased proliferation and turnover of cells; therefore tenascin expression is expected to increase. A remarkable finding in our study was the thickening of and increased tenascin expression in the muscularis mucosae seen in adenomas in contact with the muscularis mucosae and in carcinomas invading the muscularis mucosae. This suggests that tumour cells are stimulating tenascin synthesis by stromal cells. Lymph vessels which contained tumour cells, adjoining the muscularis mucosae, were surrounded by thin bands of tenascin (Fig.4) whereas around similar vessels deeper in the submucosa this tenascin pattern was absent in many cases; this suggests that after invasion of the muscularis mucosae, tenascin is no longer necessary. A similar process has been demonstrated in wound healing where tenascin is increased during the active process of wound healing and lost in old scars (Mackie et al., 1988).

Two different patterns of tenascin expression were found in adenocarcinomas: the fibrillary expression pattern around glands and cysts of well-differentiated tumours and the network-like expression pattern in poorly differentiated tumours, as has been described in human tumours (Broll et al., 1995; Gulubova & Vlaykoya, 2001). In human colon cancer tenascin is expressed more in well-differentiated tumours than in poorly differentiated tumours (Sakai et al., 1993; Gulubova & Vlaykoya, 2001). Tenascin expression in canine tumours showed the same
tendency. The absence of tenascin in some regions of adenocarcinomas and carcinomas demonstrates that tenascin is not indispensable for invasion. The presence of tenascin in both adenomas and carcinomas in our study suggests that tenascin cannot be used as a stromal marker for malignancy as claimed in human mammary tumours (Howeedy *et al.*, 1990).

α-SMA expression in the normal gastric and intestinal mucosa has been described in cells around crypts (pericryptal myofibroblasts), pericytes and smooth muscle fibres of the muscularis mucosae and muscularis propria (Sappino *et al.*, 1989; Skalli *et al.*, 1989a). Our results in canine tissues showed the same reaction pattern. Theoretically myofibroblasts in gastrointestinal tumours can arise from phenotypic modulation of preexisting fibroblasts, pericytes and smooth muscle cells of the muscularis mucosae and muscularis propria. The strategy frequently applied to determine the origin of myofibroblasts is to examine the phenotype using cytoskeletal proteins α-SMA, vimentin, and desmin. It has been proposed that myofibroblasts in human breast carcinomas originate from preexisting fibroblasts and not from smooth muscle cells, because the cells expressed vimentin and not desmin (Schürch *et al.*, 1984). In our study a persistent sheath of α-SMA positive cells was found, bordering epithelial cells in all adenomas regardless of their histological type. These cells did not express desmin except in areas near the muscularis mucosae. This is in contrast to findings in human colon adenomas, where α-SMA was only expressed in stromal cells of villous adenomas and not in tubular adenomas (Sappino *et al.*, 1989). In carcinomas, stromal cells representing desmoplasia were positive for vimentin and α-SMA and negative for desmin. Desmin was only positive in areas near the muscularis mucosae. Based on these results we propose that myofibroblasts in the stroma of canine gastrointestinal tumours are most likely to originate from pre-existing fibroblasts and not smooth muscle cells except in tumour regions near the muscularis mucosae.

The co-localization of tenascin and myofibroblasts can be explained by two possible different mechanisms. First, tenascin can act as a mediator in the epithelial mesenchymal interaction resulting in the stimulation of stromal cells to express α-SMA filaments as suggested in the process of human intestinal development (Beaulieu *et al.*, 1993). Second, the regulation of tenascin synthesis and transformation of fibroblasts to myofibroblasts is influenced by the same cytokines produced by the tumour cells. A variety of tumour cells secrete cytokines that confer a transformed phenotype of fibroblasts to myofibroblasts (Desmoulière *et al.*, 1993, Rønnov-Jessen and Petersen, 1993). Cytokines of particular interests are TGF-β and GM-CSF. When TGF-β and GM-CSF are administered subcutaneously in rats, they induce formation of granulation tissue in which α-SMA expressing myofibroblasts are present (Rubbia-Brandt *et al.*, 1991; Desmoulière *et al.*, 1993). The assumption that cytokines produced by tumour
cells induce transformation of fibroblasts to myofibroblasts and tenascin synthesis need to be confirmed by evaluation of cytokine production at the cellular level in tumours where myofibroblasts and tenascin are abundant, and similarly by use of combinations of growth factors in in vitro studies.

Reference


