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Tenascin expression in inflammatory, dysplastic and neoplastic lesions of the human stomach

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Abstract Wie studied the expression of tenascin (Tn) in human stomach. In the normal mucosa of the antrum and body, Tn reaction was only seen in the muscularis mucosae, in the region of the pyloric sphincter and in the duodenum, a Tn-immunoreactive rim was seen underlying surface epithelial cells. Antral gastritis, irrespective of the degree of inflammation, showed a rim-like Tn expression under the surface epithelial cells but no Tn reaction was seen in mild chronic gastritis of the body. In some moderate and severe examples of chronic gastritis a delicate Tn-reactive line was seen to underline the surface epithelium focally and the neck regions of gastric pits. Discontinuous Tn immunoreactivity was sometimes seen beneath hyperplastic epithelium in both parts of the stomach. A Tn-immunoreactive line was seldom seen surrounding glands showing intestinal metaplasia. In both benign and malignant ulcers prominent Tn immunoreaction was seen at the base of ulcers extending deep into the underlying muscularis. Only severely dysplastic lesions displayed Tn in the lamina propria, in close association with capillaries. In early forms of diffuse gastric cancer (DGCA) raggedly increased Tn staining was seen in the lamina propria underlying affected surface epithelial cells. In advanced forms of DGCA consistent Tn expression was seen in the tumour stroma. A distinct Tn reaction was seen surrounding invasive tumour cell nests of intestinal type gastric cancer (IGCA) in the submucosa, whereas in early forms of the tumour enhanced Tn reaction was noted predominantly in the upper part of the lamina propria in the vicinity of dysplastic elements. Notably, while most invading DGCA tumour cell nests showed no Tn in the submucosa and muscle cell layer, invading IGCA islets showed prominent expression of Tn. The most conspicuous Tn enhancement in the stomach is seen in invasive tumours and in ulcers suggesting that Tn is an important stromal component in malignant growth and in lesions undergoing active repair and remodelling.

Key words Tenascin · Stomach · Hyperplasia
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Introduction

Tenascin (Tn) is an extracellular matrix (ECM) glycoprotein with a peculiar molecular structure showing regions homologous to fibronectin type III repeats, fibrinogen and epidermal growth factor [9, 24]. During embryogenesis, the expression pattern of Tn is restricted when compared with other ECM glycoproteins [1, 5]. In adult epithelia. Tn expression is mostly limited to some epithelial-stromal interfaces. However, Tn re-emerges strongly during wound healing [3, 14, 15], proliferative states [21, 28] and in oncogenesis [2], suggesting that it has a role in tissue remodelling.

It has been shown that Tn is a significant stromal component of benign and malignant tumours [6, 12, 16]; however, certain tumours show only slightly enhanced Tn expression in their stroma [12], suggesting that it is not indispensable for malignant growth. Increased Tn content is often seen in association with inflammatory and reparative lesions as well as in hyperplasia [12, 25, 26]. While studying colonic inflammatory and neoplastic lesions, Riedl et al. [19] noticed that in inflammatory lesions Tn expression was only slightly enhanced underneath the affected surface epithelial cells, whereas in colonic cancers invading through the muscularis mucosae marked increase in immunoreactivity was seen. Bearing in mind the association of gastritis with ulcers and tumours of the stomach [23], we thought it interesting to study the expression of Tn in these lesions. Our results

indicate that in the stomach, slight Tn enhancement is associated with superficial inflammation and early cancer whereas marked increase in immunoreactivity is seen in well-established ulcers and in invasive tumours of both diffuse and intestinal types. This suggests that Tn is of importance in infiltrating growth and in repair.

Materials and methods

Paraffin blocks, including both biopsy and surgical material, were retrieved from the files of the Department of Pathology, the Jorvi Hospital, Espoo, Finland, and Rush Medical College, Chicago, Illinois, USA. Samples of normal antrum (n=3), corpus (n=8), pylorus (n=2), mild (n=6), moderate (n=10), severe (n=2) gastritis of antrum, mild (n=3), moderate (n=9), severe (n=2) gastritis of the body bulbitis (n=6), chronic active gastritis with *Helicobacter pylori* infection (n=4) mild (n=4), moderate (n=2), severe (n=3), atrophy of antrum, mild (n=1), moderate (n=1), severe (n=1) atrophy of the body, dysplasias (n=8), ulcers of antrum (n=13), ulcers of corpus (n=7), ulcers of pylorus (n=5), early diffuse gastric cancer (n=6), invasive diffuse gastric cancer (n=2), early intestinal gastric cancer (n=4) and invasive intestinal gastric cancer (n=5) were obtained. Sections were stained by haematoxylin and eosin and diagnosed. The tumour classification described by Lauren [13] was used.

For immunohistochemistry paraffin sections were cut at 6 μm and kept in an oven overnight at 37° C. Sections were then deparaffinized and treated with pepsin (0.01 M pepsin in 0.01 M HCl at 37° C). After pepsin treatment, sections were incubated in 3% hydrogen peroxide to block endogenous peroxidase activity; they were then incubated with normal rabbit serum and subsequently with the mAb 143DB7 to Tn [25]. Sections were treated then with rabbit anti-mouse immunoglobulins, and then with avidin-biotin peroxidase complex (Dako, Glostrup, Denmark). The colour reaction was developed with 3-amino-9-ethylcarbazole (Sigma, St. Louis, Mo., USA). Finally, sections were counterstained with Mayer's haematoxylin. Negative controls concerning each part of stomach included in the study were used to reveal nonspecific binding.

Results

Both biopsy and surgical specimens from two laboratories were used in the study. No differences in the reactions with the mAb 143BD7 between different types of specimens were noted. The results described below show the major findings without any marked intersample variability.

Normal antrum and body of stomach

In the normal gastric antrum and body Tn immunoreaction was only seen in the muscularis mucosae (Figs. 1, 2). Vessels in the submucosa and adventitia stained mostly for Tn. Both circular and longitudinal smooth muscle layers showed a diffuse but delicate Tn reaction. Immunoreaction for Tn was also seen in perineural sheaths.

Normal pyloric sphincter region/duodenum

Tn staining was seen underneath the surface epithelial cells of the pylorus and duodenum (Fig. 3) as a continuous delicate line; occasionally, extensive Tn reactions were seen in the lamina propria. Muscularis mucosae and muscle layers were consistently Tn immunoreactive. Septa amongst Brunner glands stained for Tn.

Gastritis

In pure chronic antral gastritis ranging from mild to severe forms, Tn staining was sometimes noted surrounding the round cell infiltrates in the lamina propria just above the muscularis mucosae; irrespective of the severity of the inflammation, a rim-like reaction was often seen underneath the epithelium. Gastritis associated with ulcerations or carcinomas (of both intestinal and diffuse type) were often accompanied with increased Tn staining in the lamina propria surrounding the ulcers. Gastritides associated with hyperplastic epithelium showed a rim-like Tn immunoreaction in the basement membrane region.

In mild chronic gastritis of the body of the stomach, Tn immunoreaction retained the normal pattern. In moderate and severe chronic gastritis, a delicate and discontinuous Tn reaction was focally seen under the surface epithelium (Fig. 4) and lining the neck and sometimes the base of the gastric pits. Tn-immunoreactive rims seen surrounding gastric glands in the lamina propria were occasionally seen. In these cases, inflammatory cells were often seen amongst glandular cells. In gastritis associated with carcinomas a diffuse Tn reaction was often noted in the lamina propria flanking the affected mucosa.

Chronic active gastritis associated with *H. pylori* infection showed often a clear Tn immunoreactive rim underneath the surface epithelial cells, often in close association with capillaries. Some diffuse enhancement was sometimes seen in the lamina propria also.

Fig. 1 Normal lamina propria of antrum. No tenascin (Tn) immunoreaction is seen. × 150

Fig. 2 Body of stomach lacking Tn immunoreactivity in the lamina propria. × 150

Fig. 3 In duodenum a Tn-immunoreactive line is seen underlying the surface epithelial cells. × 150

Fig. 4 In chronic gastritis Tn appears as a delicate line underneath the slightly hyperplastic surface epithelium. × 150

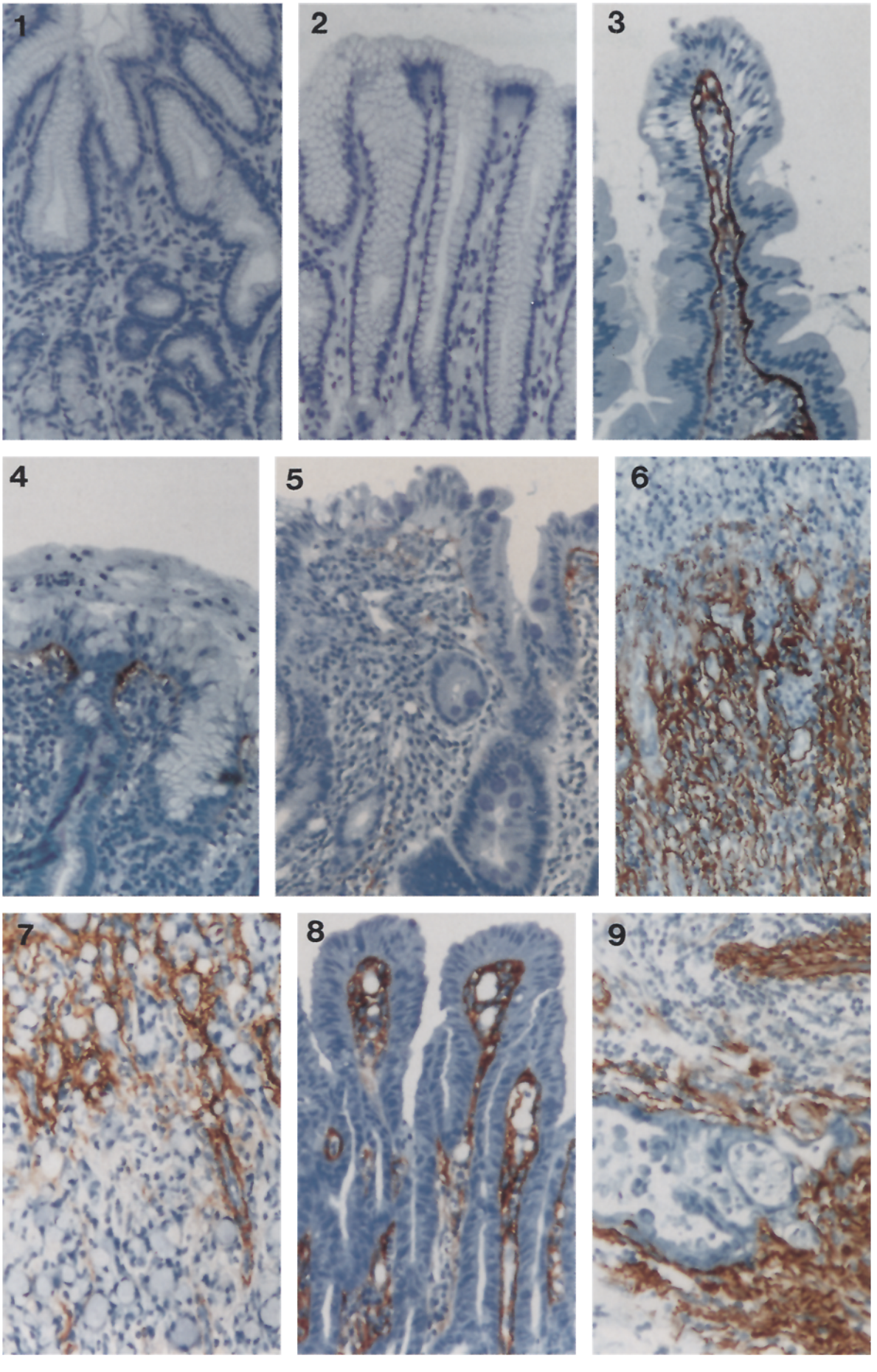
Fig. 5 Intestinal metaplasia showing Tn immunoreactivity underlying the surface epithelial cells but not gastric glands. × 150

Fig. 6. Massive Tn reaction is seen in the ulcer base. ×150

Fig. 7 Early form of diffuse gastric cancer showing markedly enhanced Tn reaction in the upper part of lamina propria containing many vessels; however, in the lower part of the lamina propria showing lots of signet ring cells, minimal reaction is seen. × 150

Fig. 8 Early form of intestinal type of gastric cancer revealing clearly enhanced Tn staining in the highly vascular lamina propria. × 150

Fig. 9 Advanced form of intestinal type of cancer showing submucosal Tn enhancement in the invading front of the tumour. × 150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Bulbitis

In bulbitis slight Tn increase was often seen in the lamina propria. A prominent Tn immunoreaction was sometimes seen surrounding small capillaries in the lamina propria. The most distinct increase in Tn content was seen in association with pyloric and antral ulcers.

Intestinal and gastric metaplasias

In severe mucosal atrophy a Tn-immunoreactive rim was rarely seen, surrounding gastric glands affected by intestinal metaplasia. However, surface epithelium displaying intestinal metaplasia showed a delicate Tn-immunoreactive line in the basement membrane region (Fig. 5). Some increase in Tn content of atrophic mucosae was seen around ulceration in the antrum and in the corpus. Some cases of bulbitis displaying gastric metaplasia showed a slightly enhanced Tn immunoreaction in the lamina propria beneath the surface epithelial cells.

Gastric and pyloric ulcers

Both gastric (Fig. 6) and pyloric ulcers showed a notably increased Tn content ranging from the superficial ulcer base to the deep muscle layers. No difference in Tn expression was seen between benign and malignant ulcers. Tn enhancement was strongest in areas of florid granulation tissue but a marked immunoreaction persisted in fibrous granulation tissue and extended deeply into the muscle layers. At the margins of ulcers, the altered mucosa and the lamina propria showed clearly increased Tn reactions.

Dysplasias

In mild and moderate dysplasia only focal Tn enhancement in the lamina propria was seen. In severe dysplasias (not shown) a clear Tn reaction was seen in association with capillaries and also diffusely in the lamina propria underneath the folded surface epithelium.

Diffuse gastric cancer

In early forms of diffuse gastric cancer (DGCA, tumour limited to mucosa) enhanced Tn immunoreaction was seen in the upper portion of the lamina propria around capillaries, and glands that were being destroyed. Areas of the lamina propria, displaying a significant amount of signet ring cells but not capillaries and glands, showed no significant stromal Tn reaction (Fig. 7). Neoplastic ulceration associated with carcinomas showed similar immunoreactivity to benign ulcers. In advanced forms of DGCA, delicate Tn reactive lines were seen around capillaries and small vessels in the upper parts of the lamina propria and diffusely in the deep lamina propria. Tumour cell islets of DGCA located just under the muscularis mucosae in the submucosa showed no Tn immunoreaction; however, in the muscle layers and in the adventitia, Tn reaction was sometimes seen in close association with tumour cell nests and lines. Nevertheless, single signet ring cells were seen between muscle bundles, lacking Tn immunoreaction in the surrounding stroma.

Intestinal gastric cancer

In early forms of intestinal type of cancer, Tn immunoreaction was seen beneath the affected folded surface epithelium either as ragged bands extending into the lamina propria or diffusely in the lamina propria, especially in association with capillaries. Tn reactions were limited to the vicinity of transformed glands and no Tn enhancement was seen in the deep lamina propria (Fig. 8). In some samples, a Tn-containing rim was noted surrounding cystically dilated glands. In advanced cancers, the tumour stroma showed markedly increased Tn content in the submucosa and muscle layers. Tumour cell aggregates surrounded by a Tn-immunopositive rim were sometimes noted in the muscle of the muscularis mucosae (Fig. 9) in areas of normal mucosa flanking in the carcinoma.

Lymph nodes

Normal lymph nodes showed a reticular Tn immunoreaction in the cortical regions. In hyperplastic nodes a distinct Tn reaction was also seen around vessels. In nodes containing metastatic carcinoma, strong Tn-reactive rims were consistently noted around neoplastic aggregates.

Discussion

Our results show that in the normal adult gastric mucosa, Tn immunoreaction was seen only in the muscularis mucosae, whereas in the duodenum Tn was seen to underline the surface epithelial cells as a delicate line. Our findings in the stomach parallel data of Sakai et al. [20], who did not note any immunoreaction at the epithelial stromal interface of the normal colonic mucosa. However, Riedl et al. [19] detected a discontinuous Tn pattern in the lower part of the crypts that developed into a broadened continuous line underlining the mucosal surface epithelial cells. Since Tn expression was often seen to be associated with epithelial proliferation [21, 25, 28], its presence in the "germinative" part of the colonic epithelium appears predictable. Therefore, it appears paradoxical that Tn immunoreactivity was not seen at the epithelial-stromal interface in the normal stomach where there are proliferative zones in which the epithelium is known to undergo constant and rapid renewal [4]. In the duodenum, Tn-immunoreactive bands were noted be-

neath the surface epithelial cells. Such a differential distribution in stomach and in bowel may reflect the different functions of these epithelia and the active migratory functions of the colonic cryptal cells. In the small intestine it has been suggested that Tn is involved in the process of epithelial cell shedding [18].

In previous studies [7, 12, 25, 26], Tn enhancement was often associated with inflammation but general increase in Tn pattern was not found in chronic gastritis lesions of various degrees either in the antrum or in the body. However, in both parts of the stomach, focal rim-like Tn immunoreactivity was sometimes seen underlining surface epithelial cells in association with gastritis, especially when associated with *Helicobacter* infection. In line with this finding are previous results showing that condylomatous infection was associated with marked stromal enhancement [25]. However, in gastritis the appearance of Tn may suggest increased renewal or necrosis of epithelium. Notably, enhancement of Tn was always seen at ulcer margins showing intense inflammation, consisting of both acute and chronic inflammatory cell compnents, in the lamina propria and sometimes underlying hyperplastic epithelial cells. Our previous data on bladder neoplasms and inflammatory conditions suggest that increased Tn reactivity is associated with active inflammatory infiltrates and myofibroblastic proliferation [26]. In addition, inflammatory cells are able to produce tumour growth factor beta [10, 27] which enhances Tn production [17]. Our present results further support the idea of Tn being a marker for the active phase of inflammation by showing the virtual absence of Tn immunoreactivity in atrophic gastritis which is generally regarded as a burn-out stage in the evolution of gastritis. However, it has been shown that in atrophic gastritis and in the mucosa adjacent to deep ulces there is a marked increase in epithelial cell proliferation when compared with normal mucosa [20], suggesting that in gastritis Tn expression may not reflect enhanced proliferation as is often seen in other tissues [21, 25, 28] unless associated with significant stromal remodelling.

Tn has been reported to emerge during wound healing process and disappear when the healing process is complete [3, 14, 15]. We noted that in gastric and in pyloric/duodenal ulcers, Tn appeared during the process of mucosal regeneration. Our studies contrast with the findings of Chuong and Chen [3] who noted only mildly increased Tn immunoreactivity in experimental wounds of the mouse stomach wall. We found an intense Tn reaction extending from the ulcer base and continuing deeply into the underlying muscle layers. Although clinically ulcers in the stomach and duodenum have a dissimilar background [11], they showed similar Tn patterns.

In mild and moderate gastric dysplasia, Tn enhancement was not seen, but in severe changes prominent Tn immunoreaction was seen in close association with capillaries in the stromal septa. These findings contradict our previous findings in cervical lesions [25] where we found that irrespective of the degree of dysplasia, some Tn enhancement was seen. This difference may be due to variable classification of preneoplastic changes or to different epithelial responses of cervix and stomach. It is also of interest that early types of intestinal type of cancer show similar Tn immunoreaction to that seen in severely dysplastic lesions. This finding supports the notions that metaplasia and dysplasia are associated in the development of intestinal types of cancers [29]. While studying colonic adenomas, Riedl et al. [19] showed that Tn reaction was enhanced in the tumour stroma, supporting our findings [25, 26] and those of others [8] that increase in Tn content is associated with premalignant changes.

Enhancement of Tn expression was seen in early forms of cancers of both diffuse and intestinal type. In early cancers, Tn immunoreaction was seen in association with surface epithelial cells and gastric glands as a ragged band or diffusely in the lamina propria. In advanced tumours of both DGCA and IGCA, Tn immunoreactivity in the stroma was markedly increased. These findings are in keeping with previous data on invasive tumours [16, 22, 26]. Metastatic islets and cell lines of DGCA and IGCA differed in their immunoreactions for Tn. Most invading nests of IGCA showed Tn reaction in the surrounding stroma, but this did not occur in DGCA which showed only focal Tn expression in the vicinity of invading cell lines clearly, not all aggressive tumours show enhancement of their stromal Tn content [12].

In the stomach, Tn enhancement was seen both in benign and malignant lesions. The most prominent increase in Tn content was seen in association with ulcers and malignant lesions, suggesting that Tn is involved in the process of benign and malignant remodelling of tissues.

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