Immunohistochemical expression of tenasin in normal stomach tissue, gastric carcinomas and gastric carcinoma in lymph nodes

Y Ikeda1, M Mori2, K Kajiyama1, Y Haraguchi1, O Sasaki4 and K Sugimachi1

1Department of Surgery II, Faculty of Medicine, Kyushu University, Fukuoka, Japan; 2Department of Surgery, Medical Institute of Bioregulation, Kyushu University, Beppu, Japan; 3Department of Gastrointestinal Surgery, Sawara Hospital, Fukuoka, Japan; 4Department of Surgery, Fukuoka Dental College, Fukuoka, Japan.

Summary

The immunohistochemical expression of tenasin was examined in the normal adult mucosa of the stomach, primary tumours and lymph node metastases of gastric cancer patients. In normal gastric tissue tenasin was expressed in the muscularis mucosae, muscularis propria and vessel walls, however it was not expressed in either the mucosal connective tissue or the stromal tissue in the submucosal layer. In gastric cancer, tenasin was expressed in 35 of 85 primary tumours, and in 8 of 25 metastases in lymph nodes. Tenasin was located in the fibrous stroma surrounding foci of cancer. The expression of tenasin in the primary tumour did not correlate with the depth of invasion, lymph node metastasis or prognosis. Tenasin appears during the process of either malignant transformation or tumour progression in gastric cancer, and the positive expression of tenasin may be useful as a stromal marker for the early detection of gastric cancer.

Keywords: gastric cancer; tenasin; lymph node metastasis

Tenasin is a glycoprotein component of the extracellular matrix with a six-armed macromolecular structure of a disulphide-bonded oligomer (Chiquet-Ehrismann et al., 1986), consisting of three isoforms of the molecules with a molecular weight of 190, 200, 230 kDa (Chiquet-Ehrismann et al., 1991). Tenasin is synthesised by fibroblasts and glial cells (Erickson and Bourdon, 1989), and was initially detected as a marker for tendon and muscle morphogenesis in chick (Chiquet and Fambrough, 1984; Chiquet-Ehrismann et al., 1986). Recent studies have demonstrated the appearance of tenasin during fetal development in organs such as the gut (Aufderheide and Ekblom, 1988) and kidney (Aufderheide et al., 1987), as well as in the stromal tissues of benign and malignant tumours (Mackie et al., 1987; Erickson and Lightner, 1988; Erickson and Bourdon, 1989; Vollmer et al., 1990; Natali et al., 1990, 1991; Sakakura et al., 1991; Shoji et al., 1992; Sakai et al., 1993; Soini et al., 1993a,b; Ramkisson et al., 1994). However, in normal adult tissue, tenasin is only slightly expressed or is restricted to a small range of structures. Therefore, tenasin may have an oncofetal potential and thus may play an important role in the mesenchymal cell interaction implicated in the local infiltrative growth and metastasis of human neoplasms.

Since little is known about the molecular interaction of epithelial cells and tenasin during neoplastic transformation, tumour invasion and metastasis in gastric cancer, we studied the expression of tenasin in normal stomach tissue, gastric carcinomas and metastatic gastric carcinoma in lymph nodes using immunohistochemical techniques.

Materials and methods

Tissue specimens

We studied 85 gastric cancer patients who had been surgically treated in the Department of Surgery at Sawara Hospital between 1984 and 1986. The patients' ages ranged from 37 to 83 years (mean 64 years). There were 48 men and 37 women. Of these patients, 31 were diagnosed as having advanced gastric cancer, which is defined as that extending into or beyond the muscle layer, and 54 were diagnosed as having early gastric cancer, which is defined as that confined to the mucosa or submucosa, regardless of the presence or absence of lymph node metastasis. Of the 54 patients with early gastric cancer, 24 showed tumour invasion confined to the mucosa. Tissue specimens were obtained from all 85 primary gastric cancers, and lymph node specimens with metastatic tumour were selected from 25 cases. The metastatic tumours in each lymph node measured more than 5 mm in diameter. Normal tissue specimens were selected from 30 of the resected specimens at sites distant from the carcinoma, avoiding areas affected by histological gastritis or intestinal metaplasia.

Histological examination and immunohistochemical procedures

All resected specimens were fixed in 10% formalin and routinely processed for paraffin embedding. In this study 1–3 tissue blocks were selected in each case to include the largest diameter of the tumours in both primary and metastatic lesions. Five-micron-thick sections made from each block were stained with haematoxylin and eosin. The gastric carcinomas were classified into two types with regard to the degree of glandular formation: differentiated type (intestinal, expanding and well-differentiated type, characterised by origin from intestinal metaplasia) and undifferentiated type (diffuse, infiltrated and poorly differentiated type, characterised by origin from proper gastric gland) (Lauren, 1965; Nakamura et al., 1968; Ming, 1977; Sugano et al., 1982). All pathological diagnoses and classifications were based on the TNM classification of the stomach, as confirmed by the International Union Against Cancer (Hermanek and Sobin, 1987).

Five micron sections were deparaffinised and washed in phosphate-buffered saline (PBS). After treatment with 3% hydrogen peroxide, the sections were incubated at 4°C overnight with monoclonal antibody to tenasin (DB7 1:200, Biohit Helsinki, Finland). The sections were treated with anti-mouse IgG–biotin complex (Vector Laboratories, CA, USA) followed by avidin–peroxidase complex and then were stained with 3, 3'-diaminobenzidine (DAB) solution with 0.15% hydrogen peroxide. All sections were briefly counterstained with Mayer's haematoxylin. For negative controls, sections were incubated with non-immune rat serum (1:1000 dilution) instead of the primary antibody. Distinct staining for tenasin in normal tissue and stromal tissue in the

Correspondence: Y Ikeda, Department of Surgery II, Faculty of Medicine, Kyushu University, 3-1-1, Maidashi, Higashi-ku, Fukuoka, 812, Japan

Received 21 September 1994; revised 6 March 1995; accepted 7 March 1995
tumours was scored as positive (+). Cases with absent tenascin staining in the normal tissue or the stromal tissue in the tumours were scored as negative (-). Staining of tenascin in the muscularis mucosae, muscularis propria or vessel walls was not regarded as positive.

Results

Table I summarises the expression of tenascin in the normal tissue of the stomach, primary tumours and lymph node metastases in the gastric cancer patients. In the normal tissue, the muscularis mucosae, muscularis propria and vessel walls showed positive expression of tenascin. However, tenascin was not expressed in the mucosa or the submucosal connective tissue. In gastric cancer, tenascin was expressed in 41% (35/85) of the primary tumours and in 32% (8/25) of the metastatic tumours in the lymph nodes. Tenascin was located mainly in the fibrous stroma surrounding the malignant cells or tubules (Figure 1). Tenascin was also seen in vessel walls and any normal gastric smooth muscle present in the section.

Table I The expression of tenascin in the normal mucosa, primary tumours and metastatic tumours of lymph nodes

|                  | Negative (%) | Positive (%) |
|------------------|--------------|--------------|
| Normal mucosa    | 30 (100)     | 0            |
| Primary tumour   | 50 (59)      | 35 (41)      |
| Metastatic tumour| 17 (68)      | 8 (32)       |

Figure 1 The immunohistochemical expression of tenascin in gastric cancer. Positive expression of tenascin in the stroma between malignant glands (a, b).

Table II summarises the expression of tenascin according to the clinicopathological factors. Tenascin was expressed in 46% (16/35) of the differentiated carcinomas and 38% (19/50) of undifferentiated carcinomas. The positive expression of tenascin did not depend on the degree of tumour differentiation. Tumours of undifferentiated type usually showed a rich fibrous stroma, however the intensity of tenascin staining was stronger in the differentiated tumours. When the expression of tenascin was compared between the patients with tumour invasion within and beyond the submucosal layer, no statistical difference was observed. Furthermore, in the 54 patients with early gastric cancer, a positive expression of tenascin was found in 45.8% (11/24) of patients with intramucosal invasion and 40.0% (12/30) of patients with submucosal invasion, there being no statistical difference. The expression of tenascin regarding the patient's sex, tumour location and lymph node status was studied, but no statistical differences were observed when the expression of tenascin was compared in tumours separated on the basis of these clinicopathological factors.

The expression of tenascin in primary tumours was compared with that in metastatic tumours in lymph nodes in 25 cases (Table III). Of ten cases with tenascin-positive expression in the primary tumour, five cases also showed positive expression in the metastatic tumour in lymph nodes; however, the five other cases showed negative expression in the metastatic tumour in lymph nodes. Three further cases with positive expression of tenascin in the lymph node metastases did not show positive expression in the primary tumours.

The survival curves are shown with respect to the expression of tenascin in Figure 2. The 5 year survival rates were 61% with a positive expression of tenascin and 72% with a negative expression of tenascin. No statistical difference in survival was observed.

Discussion

The present study demonstrates that tenascin was expressed in the stromal tissue of gastric cancer, but not in the normal mucosa or submucosa. It has been reported that tenascin
appears in the stromal tissues of various human neoplasms, such as breast cancer (Mackie et al., 1987; Shoji et al., 1992), colon cancer (Sakai et al., 1993), lung cancer (Soimi et al., 1993a), malignant bone marrow disease (Soimi et al., 1993b) and malignant melanoma (Natali et al., 1990). Tenasin has been isolated from cultured fibroblasts and cultured medium (Oike et al., 1990), and tenasin synthesis in fibroblasts is also induced by tumour growth factor beta (TGF-β) (Pearson et al., 1988; Erickson and Bourdon, 1989; Chiquet-Ehrismann, 1990). Therefore, it is thought that tenasin in tumour tissue is synthesised by stromal fibroblasts, which are induced by the tumour to produce TGF-β. Tenasin contains epidermal growth factor-like repeats (Jones et al., 1988), and it has been suggested that tenasin also has growth-promoting properties. Furthermore, the adherent growth of the human colon carcinoma cell line HT-29 can also be inhibited by a tenasin-containing substrate (Probstmeier et al., 1990), supporting the theory of a major fibronectin-antagonising role of tenasin (Chiquet-Ehrismann et al., 1988). Therefore, an increased amount of tenasin in the surrounding extracellular matrix is considered to play an important role in the process of neoplastic transformation, tumour invasion and metastasis. In this study, tenasin was expressed in the stromal tissue of gastric cancer but not in normal tissue, however the expression of tenasin did not correlate with the depth of tumour invasion, lymph node metastasis or the prognosis. These results indicate that, in gastric cancer, the appearance of tenasin is involved in some way in malignant transformation and tumour progression, although the positive expression of tenasin does not predict either the metastatic or aggressive potential of the gastric cancer. It has been reported that, in colon cancer, tenasin is more highly expressed in well-differentiated tumours than in poorly differentiated tumours (Sakai et al., 1993) and, while the expression of tenasin in gastric cancer also shows the same tendency, the difference is not statistically significant.

The existence of positive expression of tenasin in normal gland tissues remains controversial. In the mammary glands, tenasin has been reported to be prominent in malignant disease, but it is rare in benign mammary lesions or normal tissue (Mackie et al., 1987). In contrast, Howedy et al. (1990) concluded that tenasin is not a transient extracellular matrix component restricted to development and transformation but may be viewed as a consistent, albeit variably distributed, component of the normal and pathological peripartal stromal regions. In colonic tissue, tenasin has been described in the basement membrane of the mucosal epithelium, muscularis mucosae and the muscularis propria of normal adult colon (Oike et al., 1990; Riedl et al., 1992). And Sakai et al. (1993) reported the distinct localisation of tenasin in the stroma of tubular adenomas as well as in the superficial layer of well-differentiated adenocarcinomas; they also reported an absence of tenasin in normal mucosa. These discrepancies may be explained by the use of different kinds of monoclonal antibodies and by differing sensitivity of the antibody depending on tissue preparation, e.g. frozen sections of paraffin-embedded sections (Sakai et al., 1993). In contrast to the controversy surrounding the expression of tenasin in normal tissue, the expression of tenasin appears more intense in the stromal tissue of human neoplasms than in normal tissue. With regard to the stomach, only a few studies have been carried out (Natali et al., 1991; Ramkisson et al., 1994). In the present study tenasin was expressed in the muscularis mucosae, the muscularis propria and the vessel walls of the stomach, but not in the mucosa or submucosal connective tissue, which is consistent with the findings of Natali et al. (1991) or Ramkisson et al. (1994).

In summary, tenasin appears during the process of either malignant transformation or tumour progression in gastric cancer, while the positive expression of tenasin in gastric cancer is not necessarily considered to indicate clinically malignant potential such as lymph node metastasis or prognosis.

References

AUFERHEIDE E, CHIQUET-EHRISMANN R AND EKBLOM P. (1987). Epithelial–mesenchymal interaction in the developing kidney lead to expression of tenasin in the mesenchyme. J. Cell Biol., 105, 599–608.

AUFDERHEIDE E AND EKBLOM P. (1988). Tenasin during gut development: appearance in the mesenchyme, shift in molecular forms, and dependence on epithelial–mesenchymal interactions. J. Cell Biol., 107, 2341–2349.

CHIQUET M AND FAMBROUGH DM. (1984). Chick myotendinous antigen (M). A monoclonal antibody as a marker for tendon and muscle morphogenesis. J. Cell Biol., 98, 1926–1936.

CHIQUET-EHRISMANN R. (1990). What distinguishes tenasin from fibronectin? FASEB J., 4, 2598–2604.

CHIQUET-EHRISMANN R, MACKIE EJ, PEARSON CA AND SAKAKURA T. (1986). Tenasin: an extracellular matrix protein involved in tissue interactions during fetal development and oncogenesis. Cell, 47, 131–139.

CHIQUET-EHRISMANN R, KALLA P, PEARSON CA, BECK K AND CHIQUET M. (1988). Tenasin interferes with fibronectin action. Cell, 53, 383–390.

CHIQUET-EHRISMANN R, Matsuoka Y, Hofer U, Spring I, Bernasconi C AND CHIQUET M. (1991). Tenasin variants: differential binding to fibronectin and distinct distribution in cell cultures and tissues. Cell Regul., 2, 927–938.

ERICKSON HP AND LIGHTNER VA. (1988). Hexabrachion protein (tenasin, cytokeratin, brachionectin) in connective tissue, embryonic brain and tissues. Adv. Cell Biol., 2, 55–90.

ERICKSON HP AND BOURDON MA. (1989). Tenasin: an extracellular matrix protein prominent in specialized embryonic tissues and tumours. Ann. Rev. Cell Biol., 5, 71–92.

HERMANEK P AND SOBIN LH. (1987). TNM Classification of Malignant Tumours, 4th edn. Springer and the International Union Against Cancer: New York.

HOWEYDE AA, VIRMANEN I, LAITINEN L, GOUQLD NS, KOUKULIS GK AND GOULD VE. (1990). Differential distribution of tenasin in the normal, hyperplastic and neoplastic breast. Lab. Invest., 63, 798–806.

JONES FS, BURGOON MP, HOFFMAN S, CROSSIN KL, CUNNINGHAM BA AND EDELMAN GM. (1988). A cDNA clone for cytosin contains sequence similar to epidermal growth factor-like repeats and segments of fibronectin and fibrogen. Proc. Natl Acad. Sci. USA, 85, 2186–2190.

LAUREN P. (1965). The two histological main types of gastric carcinoma: diffuse and so-called intestinal-type carcinoma: an attempt at a histo-clinical classification. Acta Pathol. Microbiol. Scand., 64, 31–49.
Expression of tenascin in gastric cancer

Y Ikeda et al

MACKIE EJ, CHIQUET-EHRISMANN R, PEARSON CA, INAGUMA Y, TAYA K, KAWARADA Y AND SAKAKURA T. (1987). Tenascin is a stromal marker for epithelial malignancy in the mammary gland. Proc. Natl Acad. Sci. USA, 84, 4621–4625.

MING, S-C. (1977). Gastric carcinoma. A pathobiological classification. Cancer, 39, 2475–2485.

NAKAMURA K, SUGANO H AND TAKAGI K. (1968). Carcinoma of the stomach in incipient phase: its histogenesis and histological appearances. Gann, 59, 251–258.

NATALI PG, NICOTRA MR, BARTOLAZZI A, MOTTOLESE M, COSCIA N, BIGOTTI A AND ZARDI L. (1990). Expression and production of tenascin in benign and malignant lesions of melanocyte lineage. Int. J. Cancer, 46, 586–590.

NATALI PG, NICOTRA MR, BIGOTTI A, BOTTI C, CASTELLANI P, RISSO AM AND ZARDI L. (1991). Comparative analysis of the expression of the extracellular matrix protein tenascin in normal human fetal, adult and tumour tissues. Int. J. Cancer, 47, 811–816.

OIKE Y, HIRAIWA H, KAWAKATSU H, NIHIKAI M, OKINAKA T, SUZUKI T, OKADA A, YATANI R AND SAKAKURA T. (1990). Isolation and characterization of human fibroblast tenascin. An extracellular matrix glycoprotein of interest for developmental studies. Int. J. Dev. Biol., 34, 309–317.

PEARSON CA, PEARSON D, SHIBAHARA S, HOFSTEEENGE J AND CHIQUET-EHRISMANN R. (1988). Tenascin: cDNA cloning and induction by TGF-beta. EMBO J., 7, 2977–2981.

PROBSTMEIER R, MARTINI R AND SCHACHTER M. (1990). Expression of J1 tenascin in the crypt-villus unit of adult mouse small intestine: implications for its role in epithelial cell shedding. Development, 109, 313–321.

RAMKISsoon DY, DEL BUONO R, FILIPE MI, BUK S, HALL AP AND PIGNATELLI M. (1994). Integrins and their extracellular matrix ligands in gastric cancer. Int. J. Oncol., 5, 689–695.

RIEDEL SE, FAISSNER A, SCHLAG P, VON HERBAY A, KORETZ K AND MOLLER P. (1992). Altered content and distribution of tenascin in colitis, colon adenoma, and colorectal carcinoma. Gastroenterology, 103, 400–406.

SAKAI T, KAWAKATSU H, HIROTA N, YOKOYAMA T, SAKAKURA T AND SAITO M. (1993). Specific expression of tenascin in human colonic neoplasms. Br. J. Cancer, 67, 1058–1064.

SAKAKURA T, ISHIHARA A AND YATANI R. (1991). Tenascin in mammary gland development: from embryogenesis to carcinogenesis. In: Regulatory Mechanism in Breast Cancer, Lipman M and Dickson R. (eds) pp. 383–400. Kluwer: Boston.

SHOJI T, KAMIYA T, TSUBURA A, HATANO T, SAKAKURA T, YAMAMOTO M AND MORII S. (1992). Immunohistochemical staining patterns of tenascin in invasive breast carcinomas. Virch. Arch. A Pathol. Anat., 421, 53–56.

SOINI Y, PAAKKO P, NUORVA K, KAMEL D, LINNALA A, VIRTANEN I AND LEHTO V-P. (1993a). Tenascin immunoreactivity in lung tumors. Am. J. Clin. Pathol., 100, 145–150.

SOINI Y, KAMEL D, APAJA-SARKKINEN M, VIRTANEN I, LEHTO V-P. (1993b). Tenascin immunoreactivity in normal and pathological bone marrow. J. Clin. Pathol., 46, 218–221.

SUGANO H, NAKAMURA K, KATÔ Y. (1982). Pathological studies of human gastric cancer. Acta Pathol. Jpn., 32 (Suppl. 2), 329–347.

VOLLMER G, SIEGAL GP, CHIQUET-EHRISMANN R, LIGHTNER VA, ARNOLDT H AND KNAPPEN R. (1990). Tenascin expression in the human endometrium and in endometrial adenocarcinomas. Lab. Invest., 62, 725–730.