Virchows Archiv A Pathol Anat (1992) 421:53–56

Virchows Archiv A
Pathological Anatomy and Histopathology

Immunohistochemical staining patterns of tenascin in invasive breast carcinomas

T. Shoji [1], T. Kamiya [2], A. Tsubura [1], T. Hatano [2], T. Sakakura [3], M. Yamamoto [2], and S. Morii [1]

Departments of [1] Pathology and [2] Surgery, Kansai Medical University, Moriguchi, Osaka 570, Japan
[3] Riken, Tsukuba Life Science Center, The Institute of Physical and Chemical Research, Tsukuba, Ibaragi 305, Japan

Received December 24, 1991 / Received after revision February 3, 1992 / Accepted February 4, 1992

Summary. Eighty-two cases of primary invasive breast carcinoma and adjacent "normal" mammary glands were examined immunohistochemically for tenascin expression and distribution. Formalin-fixed tissues pretreated with actinase were processed by the avidin-biotin complex method using anti-human tenascin monoclonal antibody (RBC1). In normal mammary glands, tenascin was distributed around the ducts and ductules but not around the acini. In carcinomas, a high incidence of tenascin-positive cases (>67%) was seen with various histological appearances, with the exception of lobular carcinoma where a low incidence was found (25%). Although intense staining was seen around cancerous foci when compared with normal mammary glands, tenascin was often expressed at cancer-mesenchymal junctions with dense fibrotic stroma, but not at junctions with active inflammatory change and a loose fibrotic stroma. Tenascin expression is not an all-or-none marker for mammary malignancy and the staining pattern suggests either a role in stimulating cancer cells or a host defence mechanism accompanied by a desmoplastic response to them.

Key words: Tenascin – Breast carcinoma – Immunohistochemistry

Introduction

Tenascin, a major extracellular matrix glycoprotein, was originally found in myotendinous junctions and has been reported to be present abundantly in embryonic tissues (Chiquet-Ehrismann et al. 1986; Inaguma et al. 1988; Erickson and Bourdon 1989). It has been reported in active phases of organogenesis and in neoplastic mesenchyme (Sakakura et al. 1991). In the mammary gland, tenascin was initially observed selectively in the condensing mesenchyme near budding epithelial cells of developing mammary glands (Chiquet-Ehrismann et al. 1986) and also in the stroma of malignant tumours (Mackie et al. 1987). It was thus initially considered to be a stromal marker for mammary malignancy. Later, tenascin was noted in normal as well as physiological and pathological hyperplastic adult breast tissues (Ferguson et al. 1990; Howeedy et al. 1990). Several monoclonal antibodies (mAbs) to tenascin have been developed (Oike et al. 1990) and an immunoperoxidase method using mAb RCB1 yielded satisfactory staining for tenascin in pronase-treated formalin-fixed paraffin-embedded tissues in our hands. The specific distribution and possible function of tenascin in invasive breast carcinomas and in normal mammary glands is discussed from a histological viewpoint.

Correspondence to: T. Shoji

Materials and methods

Eighty-two Japanese women with primary invasive breast carcinoma, resected surgically at Kansai Medical University hospital, were investigated. Tissues were fixed in 10% formalin and embedded in paraffin. Several samples were also fixed in methacarn (methanol:chloroform:acetic acid; 6:3:1). Sections were cut to 4 μm in thickness and stained with haematoxylin and eosin (H&E); other serial sections were subjected to the immunohistochemical study. Tumours were classified from the H&E-stained sections using conventional histological criteria. Immunoperoxidase staining was performed as previously described (Tsubura et al. 1988) with slight modification. In brief, following deparaffinization, sections were treated with ethanol containing 0.03% hydrogen peroxide for 20 min and digested with actinase (Kaken Chemical, Tokyo), 0.1 mg, in 1 ml of 0.05 M phosphate buffered saline (pH 7.2) at 37° C for 10 min to enhance antigenic exposure. After digestion, sections were rinsed with 0.05 M TRIS-buffered saline (pH 7.2) and pre-incubated in 5% non-immune goat serum for 20 min, incubated overnight at 4° C with the primary antibody at a dilution of 1:1000. The primary antibody (RCB1), purified against tenascin from human fibroblast, has been described in detail previously (Oike et al. 1990). The immunoglobulin subclass is IgG_{2a}. An avidin-biotin complex staining kit (Vectastain kit; Vector, Burlingame, Calif., USA) was used according to the manufacturer's instructions. Peroxidase activity was visualized with diaminobenzidine (Wako Pure Chemical, Osaka, Japan), and sections were weakly

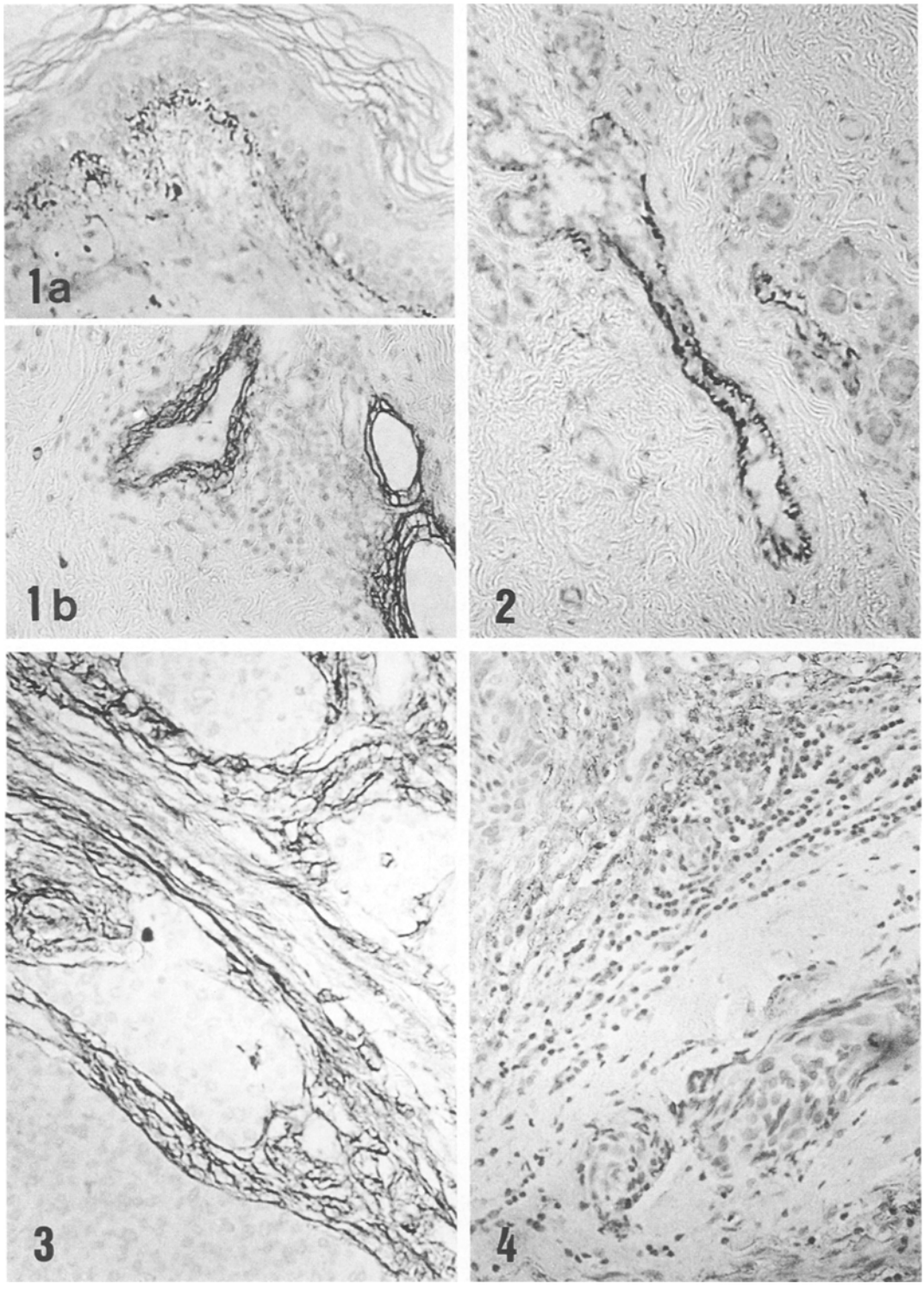

Fig. 1. a. Normal skin overlying mammary gland. Narrow band like staining can be seen in papillary dermal matrix. **b** Normal blood vessels intermingled in the breast tissue. Staining was noted in the intimal, medial, and adventitial layers. Tenascin, × 200

Fig. 2. Normal mammary gland. A fairly thick well-defined band can be seen in sub-basement membrane zone of the duct and ductule but not around the acini. Tenascin, × 200

Fig. 3. Breast carcinoma. Intense stainings can be seen in condensing stroma interwoven with clusters of unstained cancer cells. Tenascin, × 200

Fig. 4. Breast carcinoma. No staining of tenascin can be detected in loose cancer-mesenchymal junctions containing many active mesenchymal cells and sparse fibrotic stroma. Tenascin, × 200

Table 1. Tenascin staining in invasive breast carcinomas

Histology	Positive cases/ cases examined	Positivity (%)
Invasive ductal	33/47	70%
Scirrhous	23/28	82%
Mucinous	2/3	67%
Invasive lobular	1/4	25%
Total	59/82	72%

counterstained with Gill's haematoxylin. In the preliminary experiment, actinase-untreated formalin-fixed tissues showed no tenascin staining, but those of methacarn-fixed tissue expressed distinct tenascin staining. In formalin-fixed tissues, after actinase digestion, tenascin immunoreactivity was observed comparable to that in methacarn-fixed tissues (Anbanzhaghan et al. 1990). The staining pattern did not differ significantly with methacarn- or formalin-fixed actinase-treated tissues, and was similar to those reported using cryostat sections. The positive staining of the sub-basement membrane zone of the epidermis and/or vessel walls served as a built-in positive control. For negative controls, sections were incubated with non-immune rat serum (1:1000 dilution) instead of the primary antibody. Distinct staining for tenascin in stroma near some cancer foci was scored positive, while negative cases showed no tenascin staining anywhere in tumour foci.

Results

Tenascin staining was generally observed under the epidermis overlying the breast (Fig. 1 a), and was constantly seen in blood vessel walls (Fig. 1 b). The entire papillary dermis adjacent to basement membrane showed positive staining, and constant staining was noted in vessels ranging from small arterioles and venules to muscular arteries and large veins. In normal mammary ductal trees, tenascin staining was seen in sub-basement membrane zones of the ducts and ductules as a thick homogeneous band, but not in those of acini (Fig. 2). Intralobular and interlobular stroma were completely negative.

In carcinomas, when compared with normal mammary glands, most tissues examined revealed a striking increase in tenascin staining in the mesenchyme closely surrounding the cancer cell nests. Heterogeneous staining was seen in different areas of invasive ductal carcinomas. Tenascin was intensely stained where mesenchymal cells were sparse with a dense fibrotic stroma (Fig. 3). Condensing stroma interwoven with cancer foci manifested fibrils of tenascin surrounding big clusters and/or small cords of cancer cells. In contrast, no staining was seen in loose cancer-stromal junctions showing active inflammatory change and containing very few fibrotic stroma (Fig. 4). In poorly differentiated areas of carcinoma, tenascin was more heterogeneously distributed from area to area, but the pattern of tenascin staining and stromal localization were unrelated to the degree of tumour differentiation. The pattern of distribution was generally the same regardless of histological types of carcinoma. Epithelial cells did not react to tenascin. The incidence of tenascin-positive cases was 59 among the 82 cases examined (72%); most of the carcinomas expressed tenascin. Tenascin positivity was low in invasive lobular carcinomas (1/4; 25%) (Table 1) but small numbers were examined.

Discussion

Human tenascin was isolated from cultured fibroblasts or from cultured media, and a cell line which produces tenascin-specific antibodies has been established (Oike et al. 1990). In the present study, one of the mAbs, RCB1, was selected for immunohistochemical observations after pronase treatment, essential for hidden antigen unmasking (Tsubura et al. 1988, 1991) and necessary in conventionally processed material (Vollmer et al. 1990). In our present experiment, pre-treatment with 0.01% actinase for 10 min was adequate for tenascin expression comparable to that in methacarn-fixed tissues (Anbanzhaghan et al. 1990) and gives great advantages for immunohistochemical analysis of tenascin, using surgical materials.

In mammary embryogenesis, two different mesenchymes can be distinguished: dense mammary mesenchyme and mammary fat pad precursors (Sakakura 1987; Sakakura et al. 1991). The former produces fibronectin and tenascin, and the latter basal lamina. The tenascin staining seen in the present study differed completely from the type IV collagen staining (Tsubura et al. 1988). In mammary oncogenesis, tenascin has been reported to be prominent in many species, but it is rare in benign mammary lesions (Chiquet-Ehrismann et al. 1986; Mackie et al. 1987; Inaguma et al. 1988). It has therefore been thought to be a mesenchymal marker for mammary epithelial malignancy. In contrast with earlier reports and in agreement with Howeedy et al. (1990) and Ferguson et al. (1990), we found tenascin to be abundant in carcinomas but it was also expressed in normal resting mammary glands, precluding its use as an all-or-none marker of mammary epithelial malignancy. It is clear, however, theat tenascin staining was both more intense and more widely distributed in the carcinomas. With respect to histology, though the numbers of cases examined was small, there was a lower incidence of tenascin-positive cases in invasive lobular carcinomas compared with ductal carcinomas.

Transformed epithelial cells appear to induce fibroblasts in the underlying connective tissue to synthesize tenascin. Such tenascin may not only promote cancer cell proliferation but may also diminish adhesiveness and result in invasion and metastasis. Tenascin is a large, complex molecule on which two contrary signals co-exist: an anti-adhesive effect and a strong cell binding capacity. This co-existence of apparently paradoxical functions may be responsible for the variable effects of tenascin (Spring et al. 1989). Of interest in the present study was the fact that prominent staining of tenascin was found in dense cancer-mesenchymal junctions, and little or no staining was observed in the loose cancer-mesenchymal junctions. Tenascin may stimulate tumour growth, but it might also play a role in inhibiting malignancy by the formation of densely hyaline tissue.

References

Anbazhaghan R, Sakakura T, Gusterson BA (1990) The distribution of immuno-reactive tenascin in the epithelial-mesenchymal junctional areas of benign and malignant squamous epithelia. Virchows Arch [B] 59:59–63

Chiquet-Ehrismann R, Mackie EJ, Pearson CA, Sakakura T (1986) Tenascin: an extracellular matrix protein involved in tissue interactions during fetal development and oncogenesis. Cell 47:131–139

Erickson HP, Bourdon MA (1989) Tenascin: an extracellular matrix protein prominent in specialized embryonic tissues and tumors. Annu Rev Cell Biol 71–92

Ferguson JE, Schor AM, Howell A, Ferguson MWJ (1990) Tenascin distribution in the normal human breast is altered during the menstrual cycle and in carcinoma. Differentiation 42:199–207

Howeedy AA, Virtanen I, Laitinen L, Gould NS, Koukoulis GK, Gould VE (1990) Differential distribution of tenascin in the normal, hyperplastic, and neoplastic breast. Lab Invest 63:798–806

Inaguma Y, Kusakabe M, Mackie EJ, Pearson CA, Chiquet-Ehrismann R, Sakakura T (1988) Epithelial induction of stromal tenascin in the mouse mammary gland: from embryogenesis to carcinogenesis. Dev Biol 128:245–255

Mackie EJ, Chiquet-Ehrismann R, Pearson CA, Inaguma Y, Taya K, Kawarada Y, Sakakura T (1987) Tenascin is a stromal marker for epithelial malignancy in the mammary gland. Proc Natl Acad Sci USA 84:4621–4625

Oike Y, Hiraiwa H, Kawakatsu H, Nishikai M, Okinaka T, Suzuki T, Okada A, Yatani R, 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

Sakakura T (1987) Mammary embryogenesis. In: Naville MC, Daniel CW (eds) The mammary gland, development, regulation and function. Plenum Press, New York, pp 17–66

Sakakura T, Ishihara A, Yatani R (1991) Tenascin in mammary gland development: from embryogenesis to carcinogenesis. In: Lippman M, Dickson R (eds) Regulatory mechanism in breast cancer. Kluwer, Boston, pp 383–400

Spring J, Beck K, Chiquet-Ehrismann R (1989) Two contrary functions of tenascin: dissection of the active sites by recombinant tenascin fragments. Cell 59:325–334

Tsubura A, Shikata N, Inui T, Morii S, Hatano T, Oikawa T, Matsuzawa A (1988) Immunohistochemical localization of myoepithelial cells and basement membrane in normal, benign and malignant breast lesions. Virchows Arch [A] 413:133–139

Tsubura A, Okada H, Sasaki M, Dairkee SH, Morii S (1991) Immunohistochemical demonstration of keratins 8 and 14 in benign tumors of the skin appendage. Virchows Arch [A] 418:503–507

Vollmer G, Siegal GP, Chiquet-Ehrismann R, Lightner VA, Arnholdt H, Knuppen R (1990) Tenascin expression in the human endometrium and in endometrial adenocarcinomas. Lab Invest 62:725–730