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Original Paper

Expression of Tenascin-C in Intraductal Carcinoma of Human Breast: Relationship to Invasion

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Tenascin-C (Tn-C) is an extracellular matrix glycoprotein that appears in areas of epithelial–mesenchymal interaction during fetal development and in neoplasia. The immunohistochemical expression of Tn-C and its relationship to histology, nuclear grade, microinvasion, oestrogen (ER) and progesterone receptors (PR), and to cell proliferation measured by Ki-67 expression were studied in 89 intraductal breast carcinomas (DCIS). Periductal Tn-C was noted in 87% and stromal Tn-C in 25% of the tumours. Stromal expression was associated with moderate to strong periductal expression and microinvasion. Periductal expression was associated with comedo-type, nuclear grade, microinvasion, Ki-67 expression, and lack of PR. The distribution of Tn-C was compared in DCIS and in the intraductal component from another series of small axillary node-negative invasive breast carcinomas ($n = 44$). Tn-C was present in the stroma of pure DCIS in 25% and in the intraductal component of the other series in 82%. Thus, stromal or moderate to strong periductal Tn-C expression in DCIS may relate to early invasion. DCIS with weak periductal or missing Tn-C expression may be a subgroup with benign behaviour. © **1998 Elsevier Science Ltd. All rights reserved.**

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INTRODUCTION

CARCINOMAS ARE composed of two discrete but interdependent compartments, malignant cells and the stroma [1]. The stroma differs from normal connective tissue and resembles the aggregations of mesenchyme observed during morphogenesis [2] or the granulation tissue that forms during wound healing [1]. Tenascin-C (Tn-C) is a large glycoprotein of the extracellular matrix, expressed transiently during fetal development, inflammation, wound healing and neoplasia. It is believed to have active functions in epithelial–mesenchymal interactions. Cell culture studies suggest that it has growth-promoting activity and anti-adhesive functions (for a review see [3]). Tn-C is produced by stromal fibroblasts, and also by epithelial cells of normal and malignant breast tissues, as shown by *in situ* hybridisation techniques [4, 5]. When expressed in normal adult breast tissue, Tn-C is located immunohistochemically as a continuous thin layer around the ducts. In intraductal carcinoma (ductal/carcinomas *in situ*, DCIS), Tn-C appears as broad bands and, in infiltrating ductal carcinomas, extensive immunostaining is noted in the stroma around clusters of carcinoma cells [6]. However, not all breast carcinomas express Tn-C [7, 8]. The reaction is most intense at the invasive edge of the tumour [6]. Tn-C may indicate the site of active cancer spread, since expression of Tn-C in the invasion border of small breast carcinomas is associated with an adverse patient outcome [9, 10].

DCIS is a precursor lesion of breast carcinoma. The epithelial cells are malignant, but growth is limited to the inside of the gland ducts by a basement membrane [11]. The comedo DCIS (which always presents with central necrosis) is known to be associated with occult foci of invasion more often than the other histological subtypes of DCIS [11] and the rate of local recurrences in the comedo type is also higher [12, 13]. Diagnostic classifications of DCIS, based on the nuclear grade and the presence or absence of central necrosis,

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have been proposed [12–14]. The search for treatment criteria for DCIS has started and in 1997 at least three prospective multicentre studies were under way to find out who should be treated with resection only, who needs postoperative radiotherapy and who should undergo mastectomy [15].

In the present study, the immunohistochemical distribution of Tn-C was analysed in tissue from 89 DCIS, with the aim of characterising the staining pattern and intensity of Tn-C and relating the results to subtype, nuclear grade and microinvasion. The level of Ki-67 antigen as a measure of proliferation activity and of oestrogen receptors (ER) and progesterone receptors (PR) had been determined previously in the majority of these tumours and the results are included in the study. The distribution of Tn-C expression was also compared in this group of pure DCIS and in the intraductal component areas of material from another 44 small invasive tumours.

PATIENTS AND METHODS

Patients

133 patients, were operated upon for primary DCIS during 1974–1996 at the Fourth Department of Surgery, Helsinki University Central Hospital (HUCH), Helsinki, Finland. For this study, the pathology reports and specimens of these 133 tumours were reviewed and the most representative sample of each case was selected for Tn-C immunohistochemistry. In 36 cases, either no tumour sample was available or no malignant tissue was left to investigate. A pre-operative core-needle biopsy had been taken of 16 tumours and these cases were excluded to avoid misinterpretation of possible stromal expression of Tn-C related to trauma [16]. The remaining 89 tumours were included in this study.

The median age of the patients was 52.5 years (range 28–82 years). 39 women (44%) had a breast-conserving operation and 28 of these (72%) received postoperative radiotherapy of 50 Gy to the breast area. No lymph node metastases were found in the 26 women (29%) who had an axillary dissection. The follow-up data were collected in November 1997 from the records of the Fourth Department of Surgery and the Department of Oncology, HUCH and the Finnish Cancer Registry.

Another group of 44 patients with small (1–25 mm) axillary node-negative tumours, comprised of both an invasive area and an intraductal component, has been characterised in detail previously [9].

Tumour samples

The tumours were re-examined and classified for histological subtype and nuclear grade. Nuclear grade was classified as low (1), intermediate (2) and high (3) [17]. The largest dimension of the tumours was obtained from the pathology reports. The mean size was 15 mm (range 2–40 mm). In 26 tumours (29%), the pathologist had not reported the size of the tumour, apparently because of widespread multifocality. The paraffin-embedded tumours were cut at 5 μm for Tn-C immunohistochemistry, and the sections were also stained with haematoxylin–eosin to verify histology.

Steroid receptors and Ki-67 were routinely determined in the laboratory, although in the earlier specimens these determinations were missing. No retrospective staining was carried out. ER was available in 64 (72%), PR in 63 (71%) and Ki-67 antigen in 61 (69%) of the tumours.

Tn-C immunohistochemistry

The monoclonal antibody 143BD7 against Tn-C has been previously characterised [18] and the immunohistochemical detection and evaluation of Tn-C has been described [9]. The extent and intensity of Tn-C expression was scored as −, +, ++ and +++ corresponding to negative, weak, moderate, and strong immunoreactivity. Tn-C staining was scored for periductal and stromal expression.

ER and PR immunohistochemistry

Until October 1995, the steroid receptors were assessed in frozen sections. Frozen sections were used for determinations of ER in 46 tumours and of PR in 45 tumours. Immunohistochemistry was performed using the ERICA and PRICA kits according to the instructions of the manufacturer (Abbott Laboratories, Chicago, Illinois, U.S.A.). The result was scored as weakly positive (+) when 10–40% of cells were stained, as moderately positive (++) when 40–70% were stained, and as strong (+++) when > 70% were stained. The staining intensity was not recorded. After October 1995 the staining was performed on paraffin-embedded sections. The tissue arrived fresh to the laboratory and the tumour specimen was immediately fixed in formaldehyde. Steroid receptors were determined from paraffin sections of 18 tumours using the monoclonal antihuman ER antibody (clone 1D5, Dako, Glostrup, Denmark) and PRICA kit for PR. The staining method for PR has been described previously [19]. ER was detected using the same microwave pretreatment as for PR, but the detection was carried out with a commercial ABC kit (Vectastain Elite, Vector Laboratories, Burlingame, California, U.S.A.). The scoring was the same as in frozen sections.

Ki-67 immunohistochemistry

Ki-67 immunohistochemistry was performed using monoclonal Ki-67 antiserum (Dakopatts) on frozen tissue in 21 tumours before February 1994 (for methods, see [20]) and thereafter, using the polyclonal Ki-67 antibody (Dako) on paraffin-embedded tissue in 40 tumours (for methods, see [21]). The level of immunoreactivity was expressed as the proportion of Ki-67 positive cells. The intensity of the staining was not recorded. The frozen sections were scored as weakly positive (+) when 1–2% of the nuclei were positive, as moderate (++) when 3–10% of the nuclei were positive and as strong (+++) when > 10% of the nuclei were positive. For the paraffin-embedded tumour material, the corresponding proportions of Ki-67-positive nuclei were 5–15% (+), 15–30% (++) and > 30% (+++).

Statistical methods

The statistical significance of differences in periductal and stromal Tn-C distribution between the intraductal component of invasive cancers and pure DCIS was tested with the chi-square test. The chi-square test was also used to test for an association between discrete variables and the Mann–Whitney U test for continuous variables. All tests were two-sided and P values below 0.05 were considered significant.

RESULTS

Expression of Tn-C in DCIS

Tn-C expression was observed periductally in 77 tumours (87%). It was weak in 23 (26%), moderate in 29 (33%) and strong in 25 tumours (28%). In some samples, Tn-C was not

present around all the affected ducts. In a few cases, periductal staining was also seen around benign hyperplastic ducts. When stromal Tn-C was encountered with microinvasion, it seemed to accompany the invading cells (Figure 1). Typically, the stromal Tn-C was focal, often forming bridges between the ducts of DCIS. In two cases, stromal Tn-C was expressed in areas of benign fibrotic stroma. Stromal expression was absent in 67 tumours (75%), but weak in nine (10%), moderate in nine (10%) and strong in four (5%). For analysis of associations with other factors, the stromal Tn-C-positive groups were combined ($n = 22$).

Expression of ER and PR in DCIS

In the 64 tumours studied, 20 (31%) were ER-negative tumours, four (6%) weakly positive, eight (13%) moderately positive and 32 (50%) strongly positive. For PR ($n = 63$) 32 (51%) were negative, five (8%) were weakly positive, 12 (19%) were moderately positive and 14 (22%) strongly positive. For analysis, the steroid receptors were dichotomised to positive and negative groups.

Expression of Ki-67 in DCIS

Of the 61 tumours studied, 18 (30%) were Ki-67 negative, 24 (39%) weakly positive, 13 (21%) moderately positive and six (10%) strongly positive. For analysis, the Ki-67-positive groups were combined ($n = 42$).

Relationship of Tn-C to other variables

Histological classification and Tn-C staining of the tumours are shown in Table 1. Only two of the tumours (2%) were of low grade, 29 (33%) of intermediate grade and 58 (65%) of high grade. In mixed types, the one with the worse

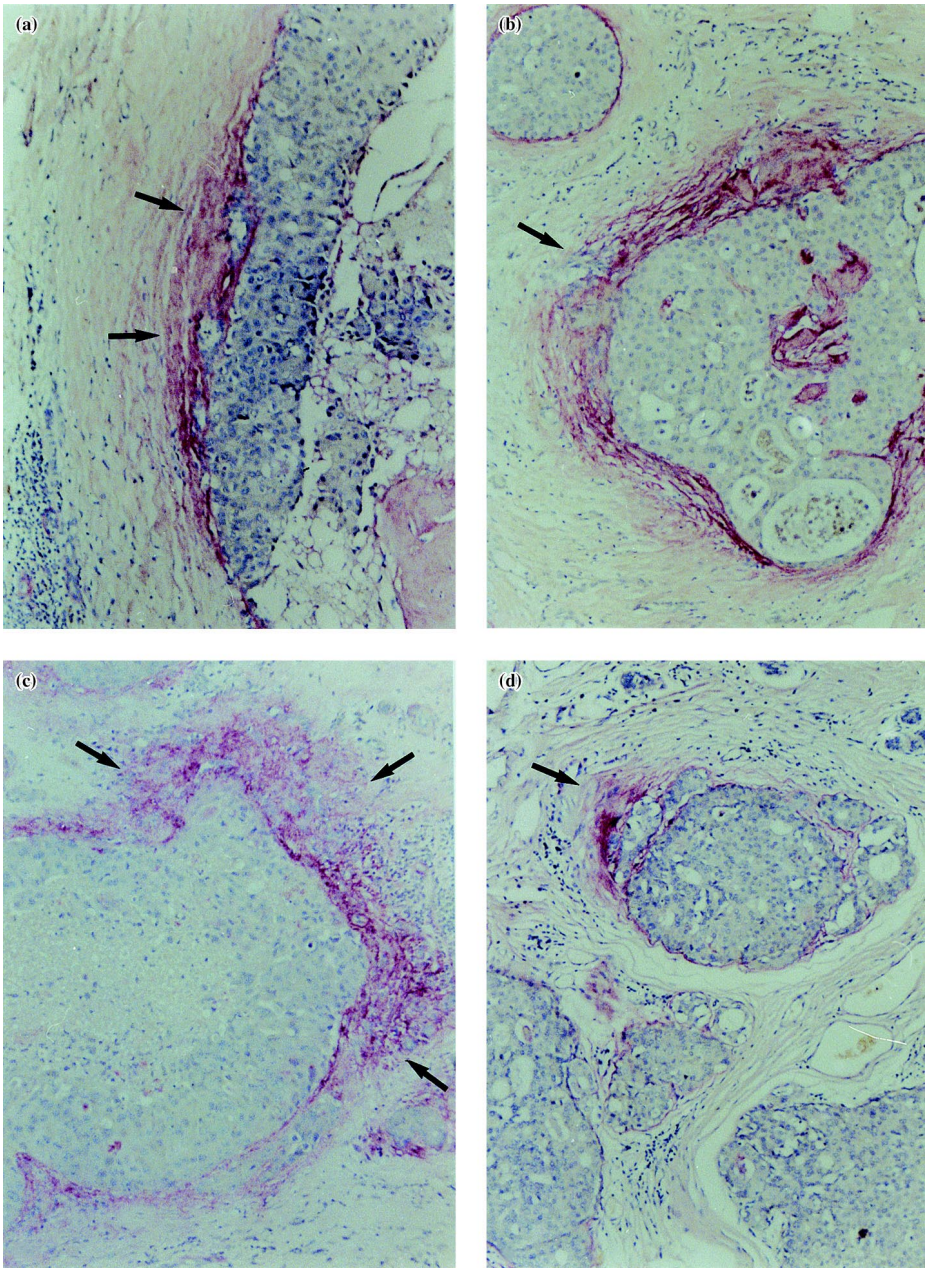

Figure 1. Periductal tenascin-C expression in four tumours of intraductal carcinomas (a–d). Note focally enhanced expression in microinvasive foci (arrows in a–d). (magnification×125).

Table 1. *Distribution of periductal and stromal tenascin-C (Tn-C) immunoreactivity in intraductal carcinoma (DCIS) and subtypes*

	Periductal				Stromal			
	– *n* (%)	+ *n* (%)	++ *n* (%)	+++ *n* (%)	– *n* (%)	+ *n* (%)	++ *n* (%)	+++ *n* (%)
All DCIS ($n=89$)	12 (13)	23 (26)	29 (33)	25 (28)	67 (75)	9 (10)	9 (10)	4 (5)
Comedo ($n=47$)	1 (2)	12 (26)	15 (32)	19 (40)	35 (74)	7 (15)	4 (9)	1 (2)
Non-comedo ($n=42$)	11 (26)	11 (26)	14 (33)	6 (14)	32 (76)	2 (5)	5 (12)	3 (7)
Cribriform ($n=12$)	2 (17)	1 (8)	6 (50)	3 (25)	6 (50)	2 (17)	3 (25	1 (8)
Micropapillary ($n=4$)	2 (50)	1 (25)	1 (25)	0	4 (100)	0	0	0
Not specified ($n=26$)	7 (27)	9 (35)	7 (27)	3 (12)	22 (84)	0	2 (8)	2 (8)

Table 2. *Associations between periductal tenascin-C (Tn-C) expression and other variables of intraductal carcinoma (DCIS)*

	Periductal Tn-C expression				
	– *n* (%)	+ *n* (%)	++ *n* (%)	+++ *n* (%)	Chi-square *P* value
All ($n=89$)	12 (13)	23 (26)	29 (33)	25 (28)	
Nuclear grade 1 ($n=2$)	0	1 (50)	1 (50)	0	
Nuclear grade 2 ($n=29$)	8 (28)	10 (34)	5 (17)	6 (21)	
Nuclear grade 3 ($n=58$)	4 (7)	12 (21)	23 (40)	19 (33)	0.04
Comedo ($n=47$)	1 (2)	12 (26)	15 (32)	19 (40)	
Non-comedo ($n=42$)	11 (26)	11 (26)	14 (34)	6 (14)	0.002
No microinvasion ($n=71$)	12 (17)	21 (30)	21 (30)	17 (24)	
Microinvasion ($n=18$)	0	2 (11)	8 (44)	8 (44)	0.04
ER- ($n=20$)	1 (5)	2 (10)	9 (45)	8 (40)	
ER+ ($n=44$)	8 (18)	12 (27)	14 (32)	10 (23)	0.13
PR- ($n=32$)	1 (3)	5 (16)	12 (37)	14 (44)	
PR+ ($n=31$)	8 (26)	8 (26)	11 (35)	4 (13)	0.008
Ki-67+ ($n=43$)	2 (5)	9 (21)	15 (35)	17 (39)	
Ki-67- ($n=18$)	5 (28)	3 (17)	7 (39)	3 (17)	0.04

ER, Oestrogen receptor; PR, Progesterone receptor.

known prognosis was chosen. Comedo or cribriform tumours were more frequently Tn-C-positive than other types (Table 1). Cribriform tumours were often positive for ER (8/9) and PR (6/9), and they expressed Tn-C periductally in 10/12 tumours (in 9/12 moderate to strong staining) and stromally in 6/12 tumours (Table 1).

The age of the patient was not related to Tn-C expression nor was the size of the tumour (data not shown). The tumours with a known size and the mainly multifocal tumours with no size definition were also compared and no difference was found in Tn-C distribution (data not shown).

The DCIS tumours that expressed (moderate to strong) periductal Tn-C were associated with microinvasion ($P=0.04$), comedo type ($P=0.002$), high nuclear grade ($P=0.04$), lack of PR ($P=0.008$) and positive Ki-67 expression ($P=0.04$) (Table 2). They tended to be ER-negative, but this association did not reach statistical significance. Stromal Tn-C expression was associated with periductal Tn-C expression ($P=0.05$) and microinvasion ($P=0.005$). A non-significant association with positive Ki-67-immunoreactivity was also noted ($P=0.07$; Table 3).

Microinvasion was considered to be present if it was mentioned in the original pathology report on paraffin samples or if it was noted in the sections made for this study. There was microinvasion in 18/89 tumours (20%). It was associated with periductal Tn-C expression ($P=0.04$), stromal Tn-C

Table 3. *Association between tenascin-C (Tn-C) expression in the stroma and other variables of intraductal carcinoma (DCIS)*

	Tn-C positive in stroma *n* (%)	Chi-square *P* value
All ($n=89$)	26 (29)	–
Tn-C periductal- ($n=12$)	1 (8)	
Tn-C periductal+ ($n=23$)	2 (9)	
Tn-C periductal++ ($n=29$)	8 (28)	
Tn-C periductal+++ ($n=25$)	11 (44)	0.02
Nuclear grade 1 ($n=2$)	1 (50)	
Nuclear grade 2 ($n=29$)	6 (21)	
Nuclear grade 3 ($n=58$)	15 (26)	0.6
Comedo ($n=47$)	12 (26)	
Non-comedo ($n=42$)	10 (24)	0.9
Microinvasion ($n=18$)	9 (50)	
No microinvasion ($n=71$)	13 (18)	0.005
ER- ($n=20$)	4 (20)	
ER+ ($n=44$)	13 (30)	0.4
PR- ($n=32$)	9 (28)	
PR+ ($n=31$)	8 (26)	0.8
Ki-67+ ($n=43$)	15 (35)	
Ki-67 - ($n=18$)	2 (11)	0.07

ER, Oestrogen receptor; PR, Progesterone receptor.

Table 4. Distribution of periductal and stromal expression of tenascin-C (Tn-C) in purely intraductal carcinomas (DCIS) (n = 89) *and in the intraductal component of small invasive ductal carcinomas* (n = 44)

	Pure DCIS (%)	Intraductal component of invasive ductal carcinoma* (%)	*P* value
Tn-C periductal			
–	12 (13)	2 (5)	
+	23 (26)	6 (14)	
++	29 (33)	20 (45)	
+++	25 (28)	16 (36)	0.1
Tn-C stromal			
–	67 (75)	8 (18)	
+	9 (10)	11 (25)	
++	9 (10)	13 (30)	
+++	4 (5)	12 (27)	< 0.0001

*Detailed in [9].

expression ($P = 0.005$), comedo type ($P = 0.02$) and a Ki-67-positive immunoreaction ($P = 0.02$), but not with nuclear grade (data not shown).

Follow-up of the DCIS patients

None of the DCIS patients had metastases. The median follow-up for the local status of the patients treated with breast-conserving surgery was 2 years (range 5 months–9 years). Four local recurrences occurred 1.5–5.5 years, median 3.3 years, after the operation. All recurrences were DCIS and no invasive growth was encountered. In 2 patients the resection margin had not been tumour-free and in 1 patient the tumour was multifocal with an uncertain resection margin, the smallest distance from DCIS being 3 mm. 1 patient had not received postoperative radiotherapy and there was no reference to the resection margin in the pathology report. None of the histopathological variables were related to these local recurrences, but because of non-standardised surgical treatment, the series was not suitable for evaluation of local recurrence.

Tn-C in pure DCIS and in the intraductal component of invasive carcinomas

There was no significant difference in periductal Tn-C distribution in the pure DCIS group and the 44 small, invasive ductal carcinomas with an intraductal component [9] (Table 4). Stromal Tn-C expression was present in 25% of the pure DCIS and in 82% of the intraductal components ($P < 0.0001$; Table 4).

DISCUSSION

In this study of 89 DCIS tumours, an increased periductal expression of Tn-C was correlated with microinvasion and with comedo type. Comedo type is known to be associated with a higher risk of both invasion [11] and recurrence [12, 13]. Tn-C was also associated with lack of PR expression and with cell proliferation, measured by the Ki-67 antigen. Using *in situ* hybridisation, it has recently been shown that Tn-C is produced by the carcinoma cells of early invasive nests of DCIS and that these mRNA-positive cells are particularly frequent at the margins of the carcinoma cell nests [4, 5]. This is consistent with our observations of a connection between Tn-C and early invasion.

Tn-C expression in morphogenesis and in carcinogenesis have marked resemblances. Tn-C accumulates specifically in the dense mesenchyme around epithelial organ primordia [22] and in invasive nests of breast carcinoma [5, 6]. The source of Tn-C at these sites is predominantly or entirely the epithelial cells themselves [4, 5, 23]. After differentiation, this Tn-C production is downregulated [22–24]. An analogous downregulation may take place in infiltrating breast carcinomas, where the expression at the invading periphery is often intense, whereas the central parts of the tumour express less Tn-C [4, 6]. This downregulation of Tn-C expression within fully evolved advanced carcinomas may explain why Tn-C-negative tumours have a worse prognosis in breast cancer [25] and colorectal cancer [26]. The origin of the stromal Tn-C in infiltrating carcinomas may be different, produced by fibroblasts and related to desmoplasia, as shown in the scirrhotic type of breast carcinoma [5]. This type of tumour represents 'the wound that does not heal' [1] and Tn-C may reflect and participate in a process resembling wound healing or inflammation related to host-response and different from cancerous invasion. It seems as if carcinoma cells produce Tn-C at the invasion front in a manner similar to actively growing epithelia during fetal development.

The distribution of Tn-C in other benign, premalignant and malignant epithelial tissues resembles that of the breast. In colon adenomas, Tn-C expression in the basal lamina is increased as compared with the normal mucosa and in invasive adenocarcinomas Tn-C is found in the basal lamina and also in the stroma [27]. Analogous changes in expression have been reported in the prostate [28], the endometrium [29], the cervix uteri and the vulva [18], the urinary bladder [30], in salivary gland tumours [31], lung tumours [32], and premalignant oral lesions and squamous cell carcinoma [33]. It seems possible that measurement of Tn-C may be of clinical use and aid the identification of early stages of invasion in any carcinoma. Future studies are needed to show whether Tn-C can be used to help decisions on the extent of surgery and the need for radiotherapy and adjuvant medication in early carcinomas.

In vitro Tn-C participates in the control of cell proliferation and migration. According to both *in situ* hybridisation and immunohistochemical studies, Tn-C is produced by breast carcinoma cells at the site of early invasion and may, thus, have an active function in cancer invasion and metastasis. The expression of Tn-C in the invasion border of small infiltrating breast carcinomas predicts both local recurrence after breast-conserving surgery [10] and distant metastasis [9]. In the present study, moderate to strong periductal expression of Tn-C in DCIS was associated with comedo type, microinvasion, high nuclear grade, high Ki-67 expression and lack of PR. Expression in the stroma was associated with moderate to strong periductal staining and microinvasion. Enhanced periductal Tn-C expression may be a sign that DCIS is likely to progress into invasive breast cancer and it may aid in finding microinvasion. The stromal expression of Tn-C was considerably higher in the intraductal component of early infiltrating breast carcinomas than in pure DCIS. We suggest that Tn-C is related to invasion and it may be of value when measuring disease character and selecting treatments for carcinomas.

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