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Expression of ECM-Tenascin in Ethylnitrosourea-Induced Rat Glioma

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Tenascin (TN) is a major extracellular matrix glycoprotein that shows a restricted distribution during fetal organogenesis and is also involved in tumor proliferation and invasion. In this study, the expression of TN and fibronectin (FN) in experimental glioma and the possible role of TN in the proliferation and infiltration of rat glioma were investigated. As a result, the tumor mesenchyme in rat gliomas of various sizes and types was found to be negative for both TN and FN. The frequency of TN-positive vessels was significantly higher in anaplastic glioma than in oligodendroglioma. Furthermore, strong TN immunoreaction was noted in the cytoplasm of anaplastic glioma cells, as was strong TN positivity in the cytoplasm and processes of reactive astrocytes. The distribusion of TN was similar to that of FN, but weak FN positivity was observed only in the cytoplasm of some tumor cells. Thus a positive correlation was observed between the frequency of TN positivity and the proliferation or anaplastic changes and malignancy of rat glioma.

Key words : Tenascin, Extracellular matrix, Rat glioma.

Introduction

Glioma induced by transplacental administration of ethylnitrosourea (ENU) in the rat is a well known experimental model [1]. Previous studies suggest that major extracellular matrices (ECM) such as FN, laminin and collagen [2], various glycosaminoglycans (GAGs) [3] and gangliosides (GM1, GM3, GD3) [4] play different roles in cellular migration, growth and invasion in ENU-induced rat glioma. TN is a major ECM glycoprotein with a unique six-armed macromolecular structure [5-7], and a TN subunit composed of several EGF-like repeats, FN type Ⅲ-like repeats and other components [7, 8]

In early studies, TN showed a restricted distribution during embryogenesis and development [6, 9-16], and strong TN immunoreativity has been observed in various human malignant neoplasms such as gliomas, melanomas, carcinomas and sarcomas [17-20].

In this study, the expression and distribution of TN and FN were investigated in rat glioma using immunohistochemical avidin-biotin peroxidase complex methods, and the author demonstrated that TN was extensively expressed in the vessel wall and in some tumor cells of less differentiated ENU glioma in the rat.

Materials and Methods

Experimental animals

Female Wistar rats weighing about 200 g were housed with males overnight, The day when sperm was confirmed in the vaginal smear was designated as day 0 of gestation. Six pregnant rats received an intravenous injection of 50 mg/kg BW N-ethyl-N-nitrosourea (ENU, Nakarai Chemical Ltd. Kyoto) on day 14 of gestation, and brain tumors were observed in 35 offspring at 9-39 weeks after birth.

Tissue preparation

The dissected brain tissues were fixed in periodate-lysine-paraformaldehyde and embedded in paraffin. To evaluate the effect of the fixative, some of the materials (5 cases) were fixed in 4% paraformaldehyde at 4℃ for 12 h, placed in OCT compound and then quickly frozen in ethanol cooled with dry ice to −80℃. No difference, however, was found either in stainability between the two fixatives or in the immunostaining for TN between frozen sections and paraffin sections.

Immunohistochemistry studies

In this study the avidin-biotin peroxidase complex method was used to demonstrate the distribution of TN and FN in the glioma, and mouse anti-human TN MAb 100EB2 (1 : 50, BIOHIT, Finland), mouse anti-human FN MAb1094 (1 : 100, Transformation Research Inc, USA) and anti-GFAP (glial fibrillary acidic protein) rabbit serum (DAKO Denmark) were used as the primary antibodies in frozen and paraffin sections.

For controls, the primary antibodies were replaced with non-immune mouse IgG, normal rabbit serum and PBS (phosphate buffe red saline). The control staining was negative in all sections. Mouse anti-human TN MAb 100EB2 is known to cross react with rat tissues. There was no difference between the frozen sections and paraffin sections.

Table 1. Histopathology and Size of ENU-induced Rat Glioma

	micro	gross	total
oligodendroglioma	31	4	35
anaplastic glioma	0	30	30
total	31	34	65

Table 2. Immunohistochemical Findings of TN, FN and GFAP in the Normal Adult Rat Brain

	TN	FN	GFAP
Neuron perkaryon	−	−	−
neuropile	−	±	−
Glia astrocyte	−	−	++
oligodendrocyte	−	−	−
microglia	−	−	−
Subependymal cell	−	−	−
Ependymal cell	++	++	− or +
Choroid plexus epithel	±	+	−
Meningeal cell	++	++	−
Vessels			
Brain parenchyme			
capillary : endothel	±	−	−
small vessel : endothel	−	−	−
wall	+	+	−
Choroid plexus : capillary	−	−	−
Meningeal vessel			
small vessel : endothel	−	−	−
wall	+	+	−
large vessel : endothel	−	−	−
wall	++	++	−

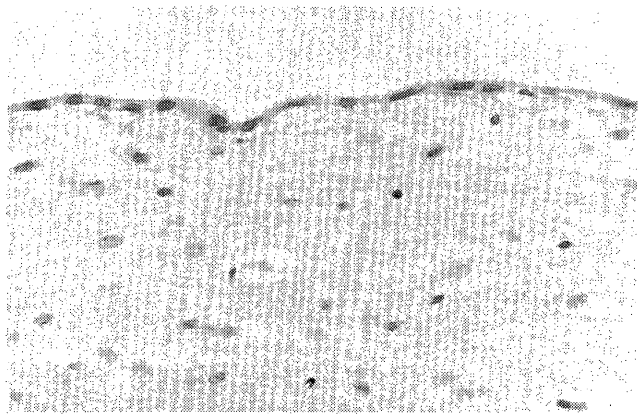
negative : (−), weakly pasitive : (±),
moderately positive : (+), strongly positive : (++).

Classification of ENU-induced rat gliomas (Table 1)

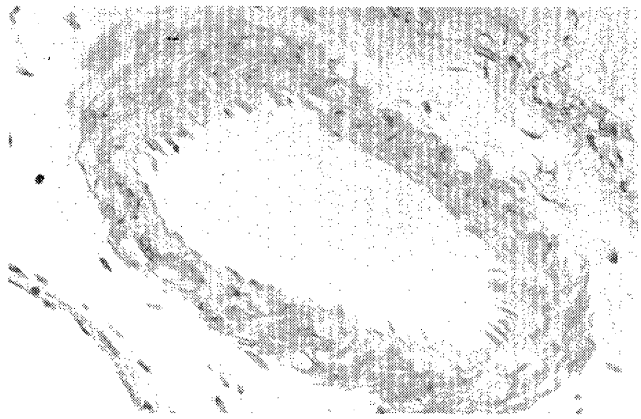
1. microtumor : less than 2 mm in diameter.
2. gross tumor : large than 2 mm in diameter.

Histological types (Table 1)

1. Oligodendroglioma : showing a honeycomb proliferation pattern of small round cells.
2. Anaplastic glioma : showing cellular atypism and polymorphism with necrosis, increased number of tumor vesseles and perivascular "abnormal" cell proliferation.



a



b

Fig. 1 TN immunoreactivity in the normal adult rat brain. Strong TN reactivity is evident in the ependymal cells (a) and medium-sized vessel (b).

Basic type of tumor vessel in ENU-induced rat glioma

Three types of blood vessel were observed in ENU-induced rat glioma [21].

1. Type A : a vessel similar to a normal cerebral small vessel.
2. Type B : a slightly to severely dilated vein-like vessel.
3. Type C : a vessel forming a vascular arcade (vascular proliferation).

Results

1. Normal adult rat brain (Table 2)

The distribution and intensity of TN and FN were similar as shown in Table 2. Normal neuron and glial cells were negative for TN and FN, while ependymal cells were distinctly positive for both antibodies (Fig 1a). In the blood vessels, TN and FN reactivity was distinct in the wall but not in the endothelium (Fing 1b).

GFAP was distinctly positive in astrocytes but weak and slightly variable in the ependymal cells.

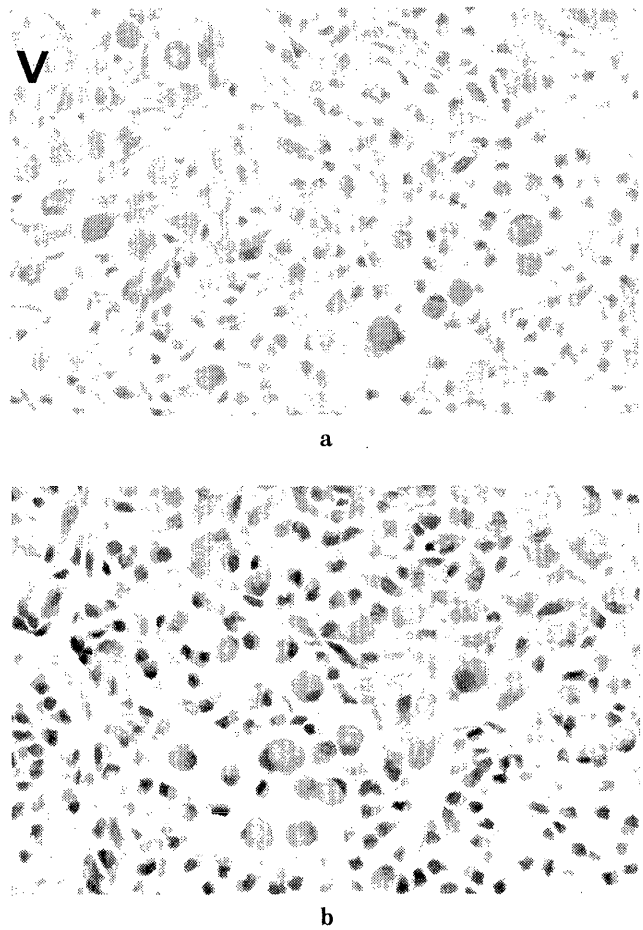


Fig. 2 TN and FN immunoreactivity in perivascular abnormal cell proliferation of anaplastic glioma.

(a) A moderate or strong TN reaction is observed in proliferating cells surrounding the vessels (v) and in some tumor cells.
(b) A weak FN reaction is ovserved in some tumor cells.

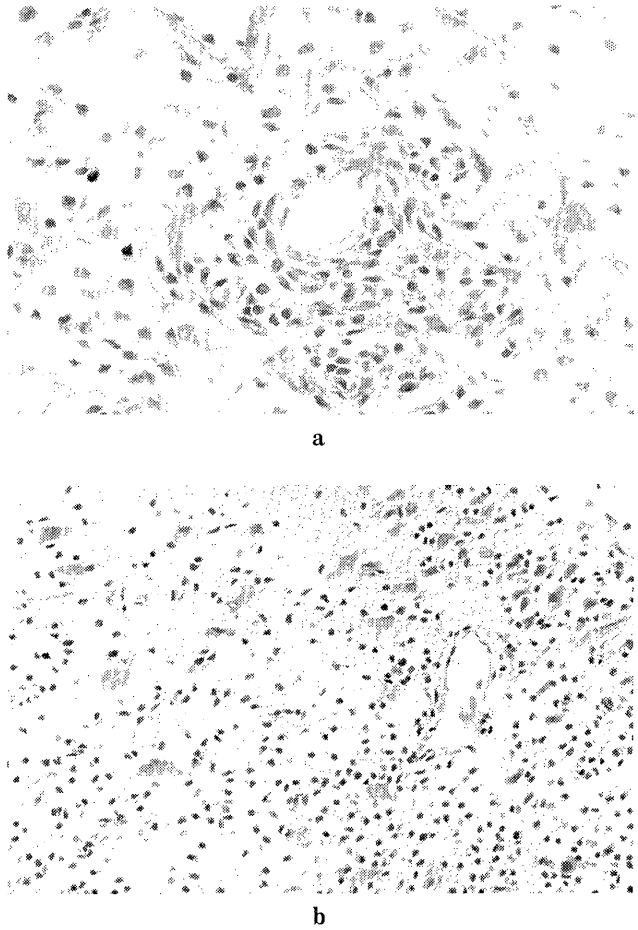


Fig. 3 (a) TN immunoreactivity in anaplastic glioma. A strong TN reaction is observed in some tumor cells and reactive astrocytes.
(b) FN immunoreactivity in a microtumor of oligodendroglioma. Only reactive astroytes show a positive reaction in the circumference of the tumor.

2. ENU-induced rat glioma

(1) TN (Table 3, 4, 5)

TN immunoreaction was negative and weak or considerably variable in the cells of microtumors and gross tumors of oligodendroglioma, respectively. In anaplastic glioma, however, a strongly positive TN staining was observed in the cytoplasm of some tumor cells (Fig 2a). In the areas of perivascular abnormal cell proliferation, a moderately positive reaction was noted (Fig 2a). The cytoplasm and processes of reactive astrocytes showed strong positivity in both oligodendroglioma and anaplastic glioma (Fig 3a).

As shown in Table 3, type B and C vessels increased with the growth of tumor and anaplastic transformation. The immunoreactivity to TN in the vessels of tumor tissue is summarized in Tables 4 and 5. The intensity of TN tended to be weaker in the microtumor than in the gross tumor.

Table 3. Frequency (%) by Type of Blood Vessel and Tumor Size in ENU-Induced Rat Glioma

Type of Vessel	Type A	Type B	Type C
oligodendroglioma			
microtumor	80.9	14.4	4.8
gross tumor	64.3	23.0	12.7
anaplastic glioma			
microtumor	/	/	/
gross tumor	41.6	37.6	20.8

In the microtumor of oligodendroglioma, approximately half of all type A, B and C vessels showed immunoreactivity in the endothelium. In the gross tumor, the TN positivity was elevated to about 80% in type A and B, and nearly 90% in type C vessels. TN was expressed in the

Table 4. Intensity of TN and FN Immunostaining in ENU-induced Rat Glioma

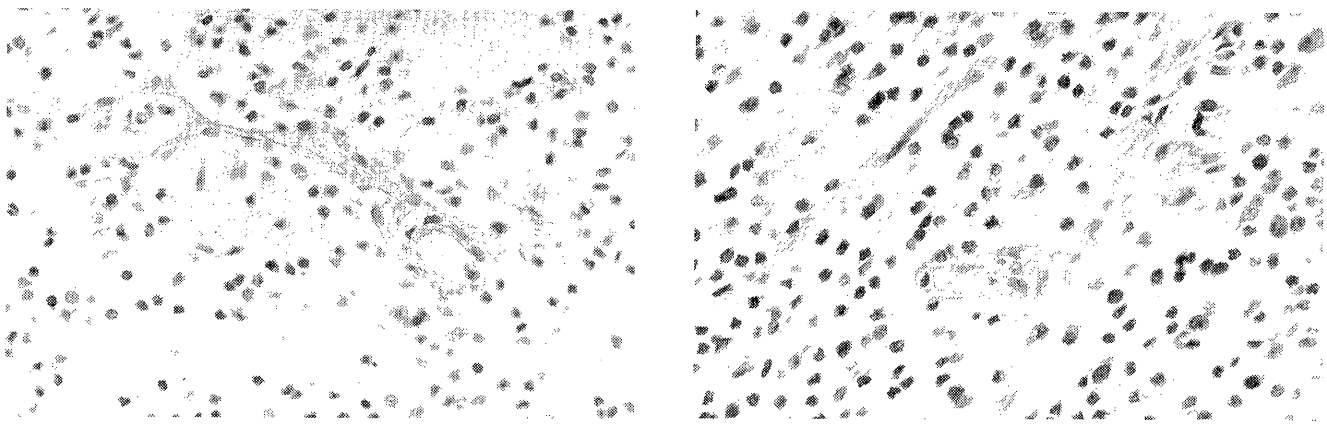
	Tenascin			Fibronectin		
	Oligo		Anapl	Oligo		Anapl
	Micro	Gross	Gross	Micro	Gross	Gross
Cells						
Tumor cell	−	−～±	−～+	−	−	−～±
React astro	+	++	++	+	++	++
Perivasc. prolif	/	+	++	/	±	±
Vessels						
Type A : endothel	+	+	+	±	±	+
Type B : endothel	+	+	+	±	±	+
circumf	±	+	+	±	±	+
Type C : endothel	±	+	+	±	+	+
circumf	±	+	+	±	+	+

Oligo ; oligodendroglioma, Anapl ; anaplastic glioma,
Micro ; microtumor, Gross ; gross tumor.
React astro ; reactive astrocyte, ECM ; extracellular matrix,
Perivasc. prolif ; perivascular abnormal cell proliferation,
endothel ; endothelium, circumf ; circumference of vessels,
− ; negative, ± ; weakly positive,
+ ; moderately positive, ++ ; strongly positive.

Table 5. The Frequency of TN Positivity in Rat Brain Tumor Vessels

Histologic diagnosis	No. of positive vessels		
	Type A	Type B	Type c
oligodendroglioma			
Micro tumor	44/84 (52.4%)	8/15 (53.3%)	2/ 5 (40%)
Gross tumor	63/81 (77.8%)	23/29 (79.3%)	14/16 (87.5%)
	(P＊<0.01)	NS	NS
anaplastic glioma			
Gross tumor	68/72 (94.4%)	64/65 (98.4%)	36/36 (100%)
	(P＊＊<0.001)	(P＊＊<0.001)	(P＊＊<0.001)
	(P＊＊＊<0.01)	(P＊＊＊<0.01)	NS

P＊, vs microtumor. P＊＊, vs microtumor of oligodendroglioma.
P＊＊＊, vs gross tumor of oligodendroglioma. NS, not significant.
/ : vessels with positive TN/vessels observed.



a b

Fig. 4 TN and FN immunoreactivity in a type-A vessel in a gross tumor of anaplastic glioma. Both TN (a) and FN (b) are positive in the endothelium of capillary or small-size vessels.

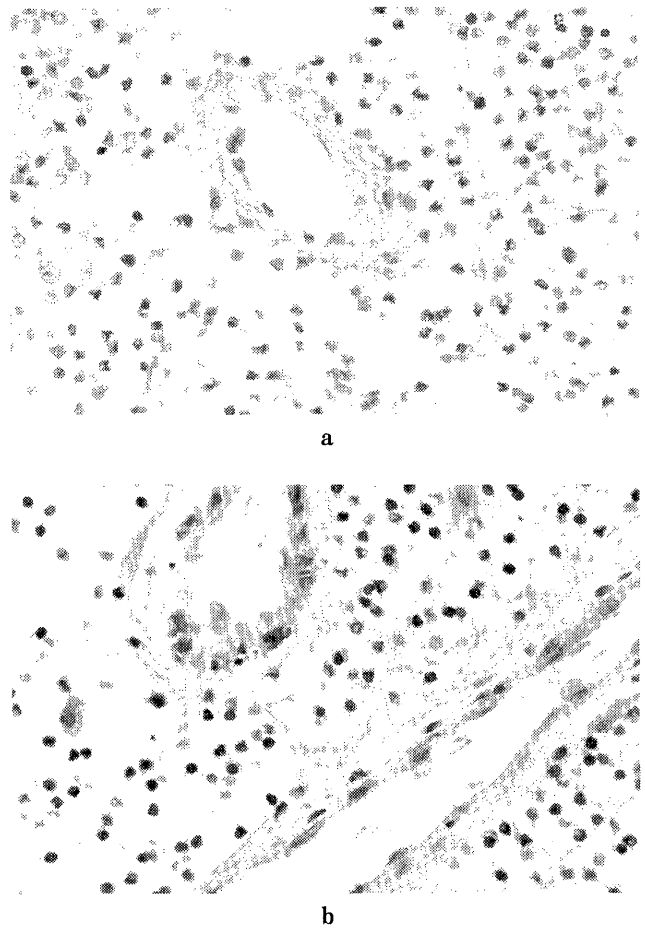


Fig. 5 TN and FN immunoreactivity in a type-B vessel in a gross tumor of oligodendroglioma.

A moderate TN (a) and weak FN (b) positivity are observed in the endothelium and circumference of the vessels.

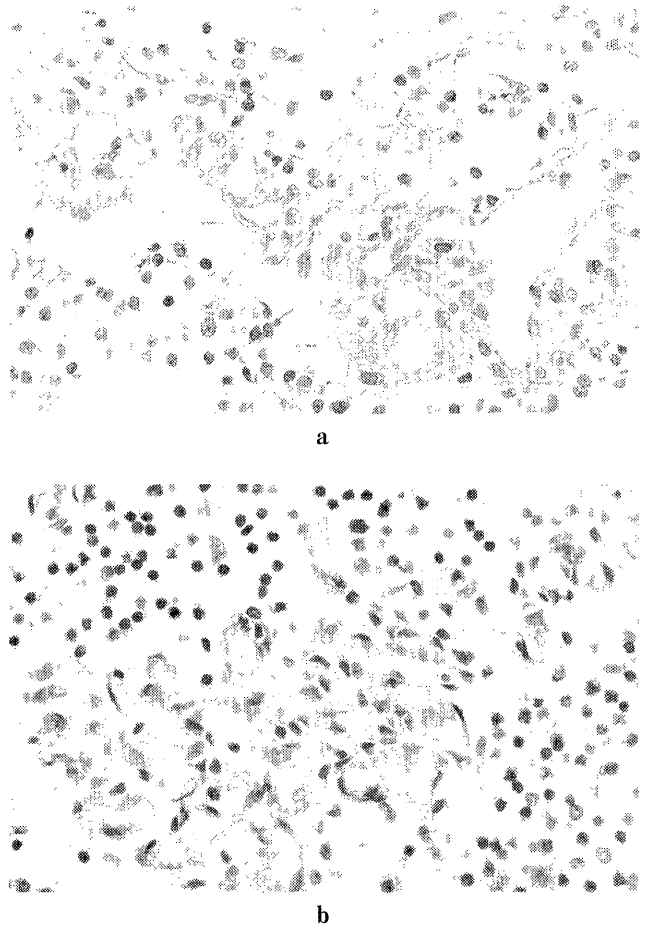


Fig. 6 TN and FN immunoreactivity in a type-C vessel in a gross tumor of anaplastic glioma.

The endothelium and circumference of the vessels are moderately positive for both TN (a) and FN (b).

endothelium and the circumference of vessels. In anaplastic glioma, TN was expressed in almost all vessels of tumors (Fig 4a, 5a, and 6a). Thus the frequency of TN positive vessels was significantly higher in anaplastic glioma than in oligodendroglioma.

(2) FN (Table 3, 4)

In every size and histological type of glioma, the distribution and intensity of FN were very similar to those of TN. In anaplastic glioma, weak FN positivity was observed only in the cytoplasm of some tumor cells (Fig 2b), but the cytoplasm and processes of reactive astrocytes showed strong FN positivity (Fig 3b).

In the tumor vessels the positivity of FN was very similar to that of TN, although the intensity was somewhat weaker in FN (Fig 4b, 5b and 6b).

(3) GFAP

The processes of reactive astrocytes in rat glioma showed distinct staining for GFAP. However, no positive finding was detected in the tumor cells.

Discussion

In this study the distribution of TN and FN was analyzed in the normal rat brain and ENU-induced rat glioma. Rat glioma is composed of less differentiated oligodendroglial cells and anaplastic glial cells [1] and is characterized histologically by perivascular abnormal cell proliferation [4]. In the present study, a strong TN but weak FN immunoreaction were observed in the cytoplasm of some of the less differentiated tumor cells and perivascular proliferating cells. High grade human astro-

cytoma showed strong TN staining of the neoplastic cells and their processes [22]. The results of these studies suggest that TN is produced by immature glial cells and glioma cells [12, 22, 23].

The intensity of TN and FN reactions showed some differences among the vessels in rat glioma. TN expression in the blood vessels was stronger in anaplastic glioma than in oligodendroglioma, showing a positive correlation between the frequency of TN positivity and the malignancy of rat glioma ($P<0.01$). These findings suggest that TN is involved in vascular proliferation and that it promotes the invasion and proliferation of glioma cells [17, 22, 23]. On the contrary, it has also been suggested that TN inhibits the adhesion of tumor cells to the vessel wall. This phenomenon, which may be related to the difficulty of metastasis by the vascular route, may strengthen the role of the blood-brain barrier (BBB) and thus prevent metastasis of the tumor. TN may therefore not only promote perivascular tumor cell proliferation but also diminish tumor cell adhesion in the surroundings and the resulting invasion of glioma.

In conclusion, TN may regulate proliferation and invasion through both the promoting and inhibiting actions of cell adhesion.

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