

Tenascin in cerebrospinal fluid is a useful biomarker for the diagnosis of brain tumour

J Yoshida, T Wakabayashi, S Okamoto, S Kimura, K Washizu, K Kiyosawa, K Mokuno

Abstract

Tenascin, an extracellular matrix glycoprotein, has been reported to be expressed predominantly on glioma tissue in the CNS, both in a cell associated and an excreted form. Recently, a highly sensitive sandwich type enzyme immunoassay for quantitative determination of tenascin was developed. In the present study, the amount of tenascin in CSF was measured. An increase of tenascin in CSF (>100 ng/ml) was found in patients with an astrocytic tumour. The concentration was significantly higher (>300 ng/ml) in high grade astrocytoma (anaplastic astrocytoma and glioblastoma) and a further increase (>1000 ng/ml) was found in cases of CSF dissemination of high grade astrocytoma. On the other hand, tenascin concentrations were less than 100 ng/ml in non-astrocytic tumours and non-neoplastic neurological diseases, except meningeal dissemination of tumour cells, meningeal stimulation by infection, and subarachnoid haemorrhage. In cases of treated astrocytomas in remission, tenascin was negligible (<100 ng/ml) in the CSF. The measurement of tenascin in CSF is useful for differential diagnosis of brain tumours and monitoring of astrocytic tumours.

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Tenascin is an extracellular matrix glycoprotein involved in tissue interaction during fetal development and oncogenesis. Chiquet-Ehrismann *et al* reported that tenascin was present in the mesenchyme of the fetal rat mammary gland but not in the adult mammary gland. The protein was, however, predominantly re-expressed in carcinogen induced mammary tumours.¹ This extracellular matrix glycoprotein was discovered independently by several laboratories, each showing a different aspect of its biology, and each giving it a different name: myotendinous antigen,² glioma mesenchymal extracellular matrix antigen,³ J1 glycoprotein,⁴ cytotactin,⁵ hexabrachion,⁶ and tenascin.¹ Bourdor *et al* reported a glioma mesenchymal extracellular matrix antigen defined by monoclonal antibody 81C6, which binds an extracellular matrix antigen shared by glioma and mesenchymal cell lines but not carcinoma, myeloid, or lymphoid cell lines.⁷

Subsequently, Erikson and Taylor showed that this was biochemically identical with tenascin.⁶ Within the CNS the use of peroxidase antiperoxidase immunohistology with 81C6 MCA showed that the antigen was expressed in glioblastomas but not in astrocytomas or in normal adult brain.⁷

In 1991, Herlyn *et al* found that most melanoma cells secreted tenascin in vitro. They also reported that tenascin was present in the serum of patients with melanoma and that the concentration of tenascin in serum was a tumour marker for advanced melanomas because they found lower concentrations in patients with low tumour burden and in normal donors.⁸ Recently, we developed a sandwich type enzyme immunoassay for tenascin⁹ and showed, with this assay system, that not only human melanoma, but also human lung cancer and glioma cells secreted 1-14 µg/ml of tenascin in vitro (data not shown). In the present study, serum and CSF were obtained from patients with various neoplastic and non-neoplastic neurological diseases, and the amount of tenascin was quantitatively determined. It was expected that an increased level of expression of this extracellular matrix glycoprotein would be found in neoplasms compared with other diseases of the CNS, and that the level of expression would be useful in indicating the type of brain tumour.

Patients and methods

SANDWICH TYPE ENZYME IMMUNOASSAY

Details of the methodology of the sandwich type enzyme immunoassay for tenascin has been reported elsewhere.⁹ Briefly, an anti-serum to human tenascin was raised in rabbits. Two antihuman tenascin monoclonal antibodies, 8C9 (RCB1)¹⁰ and 9B2, were supplied by Amano Pharmaceutical Co, Aichi, Japan and F(ab)'2 fragments of both antibodies, prepared from 1gG by pepsin digestion, were used. The RCB1 fragment was immobilised non-covalently on polystyrene beads and the 9B2 fragment was coupled to β-D-galactosidase. Polystyrene beads with antibodies were incubated with various amounts of purified tenascin or samples of serum and CSF (10 µl) in a final volume of 0.51 ml with sodium phosphate buffer for three hours. After washing twice with buffer, the beads were left standing overnight with 1 mU of β-D-galactosidase labelled antibodies (mixed 0.5 mU of 8C9 and 9B2) in 0.2 ml of buffer solution at 4°C. Beads were again washed twice

Department of
Neurosurgery, Nagoya
University School of
Medicine, Nagoya,
Japan

J Yoshida
T Wakabayashi
S Okamoto

Department of
Research and
Development, Amano
Pharmaceutical
Company, Aichi,
Japan

S Kimura
K Washizu

Department of
Neurology, Nagoya
University School of
Medicine, Nagoya,
Japan
K Kiyosawa
K Mokuno

Correspondence to:
Dr Jun Yoshida,
Department of
Neurosurgery, Nagoya
University School of
Medicine, 65 Tsurumai-
cho, Showa-ku, Nagoya,
466 Japan.

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Table 1 Concentrations of tenascin in CSF (ng/ml)

Disease	n	Negligible (<100)	Low (100-300)	Medium (300-1000)	High (>1000)
Non-neoplastic disease	57	54	3§	0	0
Pituitary adenoma	10	10	0	0	0
Craniopharyngioma	2	2	0	0	0
Neurinoma	2	2	0	0	0
Meningioma	6	5	1‡	0	0
Germ cell tumour	4	4	0	0	0
Pineoblastoma	1	1	0	0	0
CNS lymphoma	1	0	1	0	0
Oligodendroglioma	1	1	0	0	0
Ependymoma	1	1	0	0	0
Medulloblastoma	2	1	0	1*	0
Astrocytoma	5	1†	4	0	0
Anaplastic astrocytoma	5	1†	0	3	1*
Glioblastoma	9	2†	3	2	2*
Brainstem glioma	6	3†	1	2	0
Metastatic brain tumour	3	1	1	1	0

*Associated with CSF dissemination.

†Postoperative state after radiotherapy or chemotherapy.

‡Meningeal sarcoma.

§Meningitis, subarachnoid haemorrhage, normal pressure hydrocephalus.

with chilled buffer and the galactosidase activity bound was measured after incubation at 37°C for 30 minutes with 0.15 mmol 4-methylumbelliferyl β-D-galactoside (Sigma St Louis, MO, USA) as a substrate.

SAMPLES

Serum samples were obtained from two healthy donors and 10 patients with brain tumours. Samples of CSF were taken from patients with various neurological diseases by spinal tap or puncture of an Ommaya reservoir connected to the lateral ventricle. Fifty eight patients had brain tumours. (table 1) Samples of CSF from four patients were taken serially and the concentrations of tenascin were compared with the tumour progression and regression. Fifty seven patients had non-neoplastic neurological disease (table 2). Brain tumour cystic fluid was also obtained from eight patients at surgery or by puncture of an Ommaya reservoir connected to the cyst.

Results

STANDARD CURVE

Figure 1 shows a standard curve for our sandwich type enzyme immunoassay of tenascin. The minimum detectable amount of tenascin was 0.1 ng per assay tube or 10 ng/ml. A linear dose response of the absorbance v tenascin added was obtained at between 0.1 to 30 ng per assay tube. The results for diluted CSF samples were parallel with the standard curve.

CEREBROSPINAL FLUID

Table 1 shows the concentrations of tenascin in CSF obtained from patients with various neurological diseases. For non-neoplastic disease, 54 patients (94.8%) had tenascin concentrations of less than 100 ng/ml and most were less than 50 ng/ml. Only three patients (5.2%) had concentrations above 100 ng/ml: post-subarachnoid haemorrhage (186 ng/ml), meningitis (188 ng/ml), and normal pressure hydrocephalus (136 ng/ml). For neoplastic disease, we divided primary brain tumours into three subgroups; non-astrocytic (n = 30), low grade astrocytoma (n = 5), and anaplastic

Table 2 Numbers of patients with non-neoplastic diseases

Non-neoplastic disease	n
Cerebral infarction	9
Intracerebral haemorrhage	1
Subarachnoid haemorrhage	3
Meningitis or encephalitis	9
Cervical or lumbar spondylosis	9
Multiple sclerosis	4
Spinocerebellar degeneration	6
Amyotrophic lateral sclerosis	1
Parkinson's disease	1
Huntington's disease	1
Bulbospinal muscular atrophy	1
Neuropathy	3
Dementia	1
Involuntary movement	1
Normal pressure hydrocephalus	1
Aneurysm	2
Radiation necrosis	1
Arachnoid cyst	1
Tuberculosis	1
Head injury	1

astrocytoma or glioblastoma (n = 14). The concentrations of tenascin were statistically different between the groups. (fig 2) In the non-astrocytic group, tenascin in CSF was less than 100 ng/ml except for three tumours (10%) each with CSF dissemination. More than 100 ng/ml of tenascin was detected in 15 cases (79.0%) in the astrocytic tumour group. In three metastatic tumours, the level was 249.7 ng/ml in meningeal carcinomatosis of gastric cancer, 771.7 ng/ml in metastatic parietal tumour of lung cancer, but only 45.5 ng/ml in an extradural skull base undifferentiated carcinoma. We classified the concentration of tenascin in CSF into four groups; negligible if less than 100 ng/ml, low if 100-300 ng/ml, medium when 300-1000 ng/ml, and high for more than 1000 ng/ml (table 1). In low grade astrocytoma, 80% of the tumours expressed low concentrations of tenascin and, none of them had medium or high concentrations, whereas more than 50% of high grade astrocytomas (anaplastic astrocytoma or glioblastoma) expressed medium or high concentrations. Furthermore, all patients with high expression of tenascin showed CSF

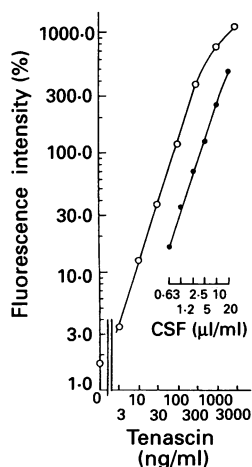


Figure 1 Standard curve and CSF dilution curve for sandwich enzyme immunoassay of tenascin. Each point represents the mean of a duplicate assay.

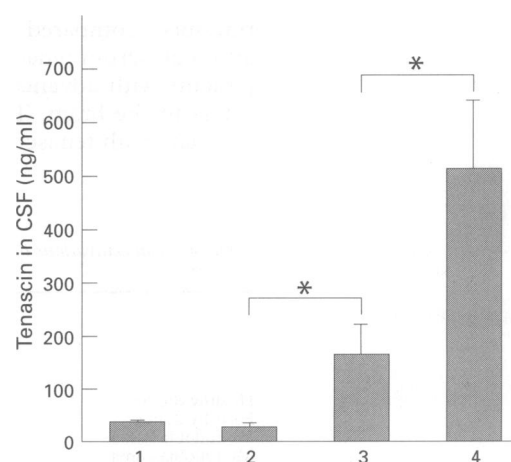


Figure 2 Concentrations of tenascin in CSF obtained from patients with neoplastic and non-neoplastic CNS diseases. (1) Non-neoplastic disease (36.32 (SD 4.36), n = 57); (2) non-astrocytic brain tumour (30.35 (6.66), n = 30); (3) low grade astrocytoma (166.36 (52.53), n = 5), (4) anaplastic astrocytoma or glioblastoma (506.94 (125.75), n = 14). *p < 0.05; Welch's t test.

Table 3 Concentrations of tenascin in cystic fluid of brain tumours

Tumour type	Tenascin (ng/ml)
Cystic meningioma	263.9
Haemangioblastoma	457.8
Astrocytoma (optic glioma)	1010.3
Metastatic lung cancer	2152.0
Metastatic lung cancer	32172.5
Craniopharyngioma	20860.5
Craniopharyngioma	22496.5
Glioblastoma	45667.4

dissemination of their high grade astrocytoma. For this group of patients, negligible tenascin expression was only found in the CSF of post-operative patients who had received some radiotherapy or chemotherapy.

Samples of CSF were also serially taken from four patients with a primary brain tumour. In an embryonal carcinoma of the suprasellar region and a pineoblastoma, negligible tenascin (9.4 and 48.2 ng/ml) increased to low-expression (220.7 and 234.9 ng/ml) with CSF dissemination of the tumour cells. In a patient with malignant lymphoma, a low level of tenascin (132.7 ng/ml) became negligible (57.6 ng/ml) in accordance with regression of the tumour after chemotherapy. In a patient in whom an astrocytoma was totally removed, tenascin in CSF was negligible (28.9 ng/ml) and continued to be negligible (37.8 ng/ml) after a seizure, at which time CT confirmed continued remission of the tumour.

CYSTIC FLUID

Table 3 summarises the tenascin concentrations in cystic fluid from brain tumours. It was low in a meningioma and a haemangioblastoma, and high in an astrocytoma, metastatic tumours, and a glioblastoma. In craniopharyngiomas, tenascin in tumour cysts was very high (>20 000 ng/ml), but was less than 100 ng/ml in CSF.

SERUM

Serum concentrations of tenascin were 733 to 4853 ng/ml (table 4). They were not significantly increased in patients with primary brain tumours compared with healthy donors, although serum tenascin was relatively high in patients with advanced carcinoma and metastases to the brain. Tenascin in CSF did not correlate with tenascin in serum.

Table 4 Concentrations of tenascin in patients with brain tumours

	Tenascin	
	Serum (ng/ml)	CSF (ng/ml)
Healthy donor	1136	—
Healthy donor	1657	—
Medulloblastoma	1563	—
Medulloblastoma	1672	—
Astrocytoma	2016	130
Anaplastic astrocytoma	988	522
Anaplastic astrocytoma	733	652
Brainstem glioma	1108	576
Diffuse gliomatosis	796	—
Glioblastoma	1434	—
Metastatic gall bladder carcinoma	3035	—
Metastatic lung cancer	4853	—

Discussion

Human tenascin has two major isoforms, with molecular weights of 320 kDa and 220–230 kDa.^{11,12} Ventimiglia *et al* found no difference in electrophoretic mobility of glioma specific isoforms and those of the normal brain.¹² They also indicated that both 320 kDa and 220 kDa isoforms were expressed not only in glioma cells but also in normal adult brain other than the cerebellum. Despite the presence of tenascin in the normal human brain, 8IC6 anti-tenascin monoclonal antibody did not show significant specific binding to normal brain in either immunohistochemical studies of normal human brain sections or in *in vivo* localisation studies with a radiolabelled 8IC6 monoclonal antibody in patients with brain tumours. These researchers considered that this was due to differences in the quantitative expression of tenascin between normal and malignant tissues.

It has also been reported that tenascin is expressed in a secreted form, and the amount of secreted tenascin has been measured by various quantitative assay systems. A tenascin concentration of 5–10 µg/ml was detected in culture of human glioma cells by Erikson and Lightner,¹³ 3–18 µg/ml by Ventimiglia *et al*¹² and 1–14 µg/ml in our study. In the present study, we determined the amount of tenascin in serum, intratumoural cystic fluids, and CSF in patients with glioma and compared these concentration with those from patients with non-gliomatous brain tumours and non-neoplastic diseases. Our highly sensitive sandwich type enzyme immunoassay has already been described by Washizu *et al*.⁹ The assay system has a minimum detectable sensitivity of 0.1 ng per assay tube or 10 ng/ml and no cross reactivities with laminin, vitronectin, hEGF, fibrinogen, and fibronectin. Our present results indicate that the serum concentration of tenascin is not useful for the diagnosis of brain tumours except metastases. The quantitative determination of tenascin in CSF proved useful, however, for the diagnosis and monitoring of gliomas. A negligible expression of tenascin was found in patients with non-neoplastic disease or non-gliomatous tumours. The expression of tenascin seen in patients with gliomas depended on the grade of the tumour and the progress of the disease. A tenascin concentration of greater than 100 ng/ml suggests an astrocytic tumour, low grade if values are 100–300 ng/ml and high grade if they are 300–1000 ng/ml. A tenascin concentration greater than 1000 ng/ml suggests CSF dissemination of a high grade glioma. Interestingly, negligible concentrations of tenascin were found in patients in whom high grade tumours had been treated with surgery, radiation, or chemotherapy, and who were in clinical remission.

High concentrations of tenascin were found in cyst fluid from an astrocytic tumour as well as a craniopharyngioma. The CSF concentration in the patient with the astrocytic tumour was high whereas that in the patient with the craniopharyngioma was low, however, possibly due to the presence of the craniopharyngioma

tumour capsule. Pseudopositive tenascin concentrations (>100 ng/ml) were found in conditions of meningeal irritation such as meningitis and subarachnoid haemorrhage. It is possible that some tenascin may be secreted from reactive fibroblasts, astrocytes, or endothelial cells.

In a previous study, we reported that a radiomunoassay of glioma associated antigen (G-22) in CSF was useful for the diagnosis and monitoring of human gliomas.¹⁴ This assay is, however, cumbersome and fails to differentiate between high grade glioma and medulloblastoma. The new method reported here is easy and practical for differentiation of brain tumours, and monitoring of the disease state in treated patients.

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