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ORIGINAL ARTICLE

Hiroshi Nawashiro · Naoki Otani · Youichi Uozumi
Hidetoshi Ooigawa · Terushige Toyooka
Takamoto Suzuki · Hiroshi Katoh · Nobusuke Tsuzuki
Akira Ohnuki · Katsuji Shima · Nariyoshi Shinomiya
Hirotaka Matsuo · Yoshikatsu Kanai

High expression of L-type amino acid transporter 1 in infiltrating glioma cells

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Abstract L-type amino acid transporter 1 (LAT1), a neutral amino acid transport agent, is essential for the transport of large neutral amino acids. LAT1 also corresponds to tumor-associated gene-1 (TA1), an oncofetal antigen that is expressed primarily in fetal tissues and cancer cells such as glioma cells. We have investigated the expression of the transporter in the human primary glioma tissue from 68 patients. Among these patients, we could see the border zone between tumors and normal bain tissues in 10 patients. By WHO criteria, two of the specimens were diagnosed as grade 2, three as grade 3, and five as grade 4 [glioblastoma multiforme (GBM)]. In 9 of 10 cases, we could identify the infiltrating glioma cells associated with stronger immunoreactivity for LAT1. These tumor cells aggregated around the neurons in the border zone and were often found in the perivascular space. In one GBM case, the tumors seemed to develop expansively and separated from the normal brain with a border of arachnoid membrane. The expression of LAT1 was always higher in infiltrating glioma cells than in cells located in the center of the tumor. These findings suggest that LAT1 is one of the molecular targets for glioma therapy.

Key words Glioma · LAT1 · Immunohistochemistry

H. Nawashiro (✉) · N. Otani · Y. Uozumi · H. Ooigawa · T. Toyooka · T. Suzuki · H. Katoh · N. Tsuzuki · A. Ohnuki · K. Shima
Department of Neurosurgery, National Defense Medical College, 3-2 Namiki, Tokorozawa, Saitama 359-8513, Japan
Tel. +81-4-2995-1656; Fax +81-4-2996-5207
e-mail: nawa1957@ndmc.ac.jp

N. Shinomiya
Department of Microbiology, National Defense Medical College, Saitama, Japan

H. Matsuo
1st Department of Physiology, National Defense Medical College, Saitama, Japan

Y. Kanai
Department of Pharmacology and Toxicology, Kyorin University School of Medicine, Tokyo, Japan

Introduction

Most gliomas recur in and around the original tumor bed after surgery. Gliomas infiltrate into surrounding normal brain, making complete surgical removal impossible in almost every case. In cases of glioblastoma multiforme (GBM), several pathology studies have shown that the area of enhancement on magnetic resonance images does not represent the outer tumor border because infiltrating glioma cells can be identified within, and occasionally beyond, a 2- to 3-cm margin. Tumor infiltration often extends into the adjacent cortex or the basal ganglia.

The sodium-independent L-type amino acid transporter 1 (LAT1) has been identified as one of the light chains of the CD98 heterodimer of system L from C6 glioma cells.[1,2] LAT1 also corresponds to tumor-associated gene-1 (TA1), an oncofetal antigen expressed primarily in fetal tissues and cancer cells such as glioma cells.[3] The purpose of the present study was to investigate the expression of LAT1 in glioma cells, especially in those infiltrating the surrounding normal brain.

Materials and methods

Patients and tissues

All 68 patients had primary gliomas of the brain. Patients were treated surgically for the first time between 1990 and 2000 in our hospital. Clinical data were obtained by retrospective chart review. Tumor specimens were obtained by surgical resection in all cases. Formalin-fixed, paraffin-embedded sections were stained with hematoxylin and eosin, and a histological and cytological diagnosis was made. Histological diagnosis and tumor grading were performed according to the grading system established by the World Health Organization (WHO).[4] Ten cases were included in the present study in which the border zone of the tumor could be identified microscopically.

Immunohistochemistry for human LAT1 in gliomas

LAT1 expression was determined by immunohistochemical staining with a polyclonal rabbit anti-human LAT1 antibody. Oligopeptides corresponding to amino acid residues 497–507 of human LAT1 (CQKLMQVVPQET) and amino acid residues 516–529 of the human heavy chain of 4F2 cell surface antigen (4F2hc) (EPHEGLLLRFPYAAC) were synthesized. The N-terminal cysteine residues were introduced for conjugation with keyhole limpet hemocyamine. Anti-peptide polyclonal antibodies were generated as described elsewhere.[1,5]

Immunohistochemical staining was performed on paraffin sections using an avidin-biotinyl peroxidase complex method. Briefly, deparaffinized, rehydrated sections were treated with 0.6% hydrogen peroxide in methanol for 30 min to block endogenous peroxidase activity. After rinsing in 0.05 M Tris-buffered saline containing 0.1% Tween-20, the sections were incubated with anti-LAT1 antiserum (1:250) or anti-4F2hc antiserum (1:500) overnight at 4°C. Thereafter, they were incubated with Envision (+) rabbit peroxidase (Dako, Carpinteria, CA, USA) for 30 min. The peroxidase reaction was performed using 0.02% 3,3′-diaminobenzidine tetrahydrochloride and 0.01% hydrogen peroxide in 0.05 M Tris-HCl buffer pH 7.4. Finally, nuclear counterstaining was performed with Mayer's hematoxylin. To verify the specificity of immunoreactions by absorption experiments, we treated the tissue sections with primary antibodies in the presence of antigen peptides (200 μg/ml).

Results

By WHO criteria[4], two of the specimens were diagnosed as grade 2, three as grade 3, and five as grade 4 (GBM). In 9 of 10 cases, we could identify the infiltrating glioma cells associated with stronger immunoreactivity for LAT1. These tumor cells aggregated around the neurons at the border zone (Fig. 1) and were often found in the perivascular space (Fig. 2). In one GBM case, the tumor seemed to develop expansively and was separated from the normal brain with a border of arachnoid membrane (Fig. 3). The expression of LAT1 was always higher in infiltrating glioma cells (Fig. 1, 2) than in glioma cells located at the center of the tumor (Fig. 4).

Discussion

The transport of large neutral amino acids with branched or aromatic side chains are mediated by amino acid transport system L.[6] System L is a Na^+-independent neutral amino acid transport agency and is thought to be a major route for providing cells with branched or aromatic amino acids such as leucine, isoleucine, valine, phenylalanine, tyrosine, tryptophan, histidine, and methionine.[6] It is speculated that

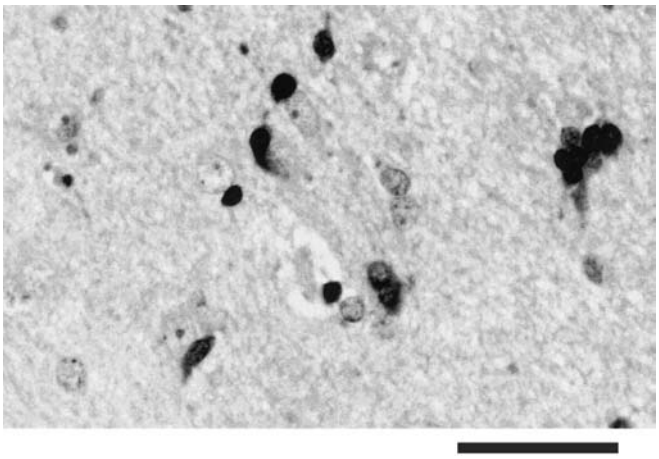

Fig. 1. Glioblastoma cells with strong staining for L-type amino acid transporter 1 (LAT1) showing perineuronal satellitosis. *Bar* 20 μm

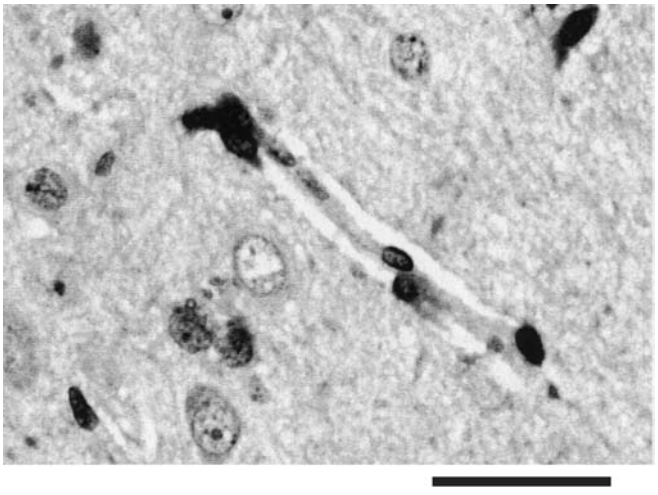

Fig. 2. Glioblastoma cells with strong staining for LAT1 showing extension into the perivascular space. *Bar* 20 μm

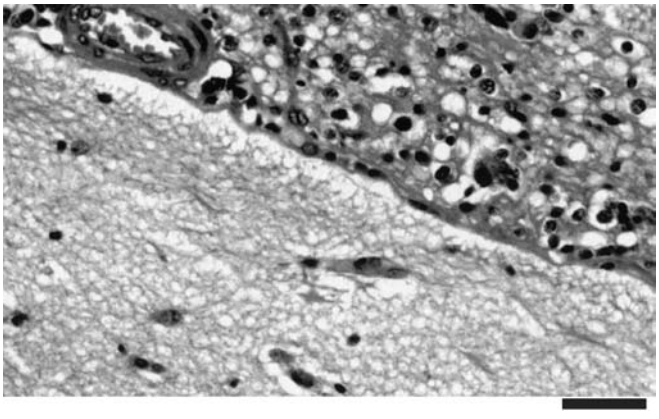

Fig. 3. Glioblastoma seems to develop expansively and is separated from the normal brain with a border of arachnoid membrane. *Bar* 20 μm

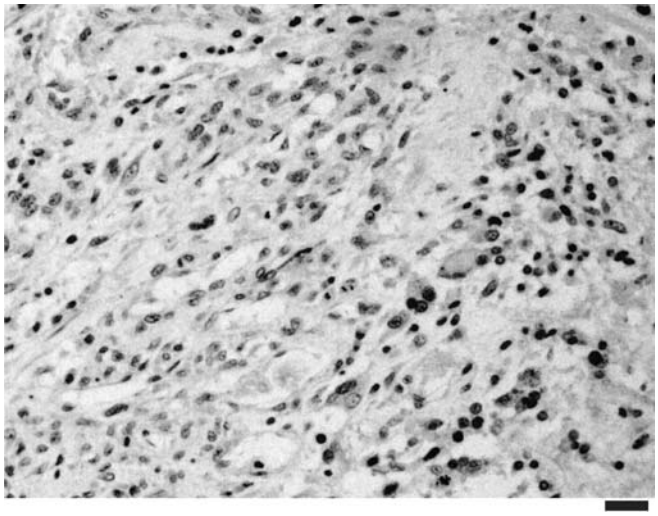

Fig. 4. Glioblastoma with diffuse weak immunoreactivity for LAT1 in the center of the tumor. *Bar* 10 μm

LAT1 expression is up-regulated to provide cells with essential amino acids for high levels of protein synthesis associated with cell activation and to support rapid growth or continuous proliferation. Higher expression of LAT1 in the invading glioma cells might reflect the higher metabolic demands in these cells. Neurons surrounded by the glioma cells often appear to be shrunken and dark. These findings suggest that there could be some metabolic competition between neurons and invading glioma cells.

A significant correlation of iodine-123–methyltyrosine (IMT) uptake in gliomas and the expression of the proliferation marker Ki-67 has been reported.[7] Recent studies also demonstrated significantly longer survival times in patients with cerebral gliomas with low amino acid uptake than with gliomas with high amino acid uptake.[8] The results of this study support the hypothesis that the uptake of radiolabeled acids such as IMT is dependent on the proliferative activity of human gliomas. It is noteworthy that in cultured human glioma cells membrane transport of IMT is dominated by LAT1.[9,10]

Glioma cells infiltrating the surrounding normal brain could cause local recurrence. However, leptomeningeal tissue may be a barrier against brain tumor cell invasion.

Conclusions

High expression of LAT1 at the invading edge, especially at secondary structures such as extension into perivascular spaces or perineuronal satellitosis, may be a good molecular target for glioma therapy.

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