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Surgery

Expression of L-type amino acid transporter 1 in canine and feline intracranial tumors

Shinichi UTSUGI[1,2], Kikumi OGIHARA[3], Yuko NAYA[3], Yuji SUNDEN[4], Yuya NAKAMOTO[5,6], Yoshiharu OKAMOTO[7]*

[1]The United Graduate School of Veterinary Science, Yamaguchi University, Yamaguchi, Japan
[2]Department of Neurology, Saitama Animal Medical Center, Saitama, Japan
[3]Laboratory of Veterinary Pathology, Azabu University, Sagamihara, Kanagawa, Japan
[4]Laboratory of Veterinary Pathology, Joint Department of Veterinary Medicine, Faculty of Agriculture, Tottori University, Tottori, Japan
[5]Neuro Vets Animal Neurology Clinic, Kyoto, Japan
[6]Laboratory of Veterinary Surgery, Department of Graduate School of Life and Environmental Sciences, Osaka Prefecture University, Osaka, Japan
[7]Laboratory of Veterinary Surgery, Joint Department of Veterinary Medicine, Faculty of Agriculture, Tottori University, Tottori, Japan

ABSTRACT. L-type amino acid transporter 1 (LAT1) is upregulated in various malignant tumors in humans. LAT1 expression correlates with the grade of cancer and prognosis. LAT1 is responsible for the supply of many essential amino acids to cancer cells. Inhibition of LAT1 reduces the amino acids that enter the cell and inhibits cancer cell growth. Therefore, novel anticancer drugs targeting LAT1 have attracted much attention in recent years. In this study, to explore the applicability of using LAT1 expression in intracranial tumors as a prognostic factor and therapeutic target, we investigated the expression of LAT1 in surgically resected primary and secondary intracranial tumor tissues from dogs and cats. Immunohistochemical analysis of LAT1 was performed on intracranial tumor tissue from 14 dogs and 3 cats. Primary intracranial tumors were seen in 10 dogs and included meningiomas, histiocytic sarcomas, pituitary tumors, and gliomas, and 9 out of 10 cases were positive for LAT1. Primary intracranial tumors were seen in 2 cats and included meningioma and lymphoma; both cases were positive for LAT1. Secondary intracranial tumors were positive for LAT1 in 3 out of 4 cases in dogs and 1 out of 1 in cats. Since the majority of intracranial tumors in dogs and cats were positive for LAT1, immunostaining for LAT1 is expected to be a prognostic indicator and therapeutic target in the future.

KEYWORDS: canine, feline, intracranial tumor, L-type amino acid transporter 1 expression

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Amino acid transporters play an important role in maintaining cell survival by supplying amino acids to cells, which serve as substrates for protein synthesis and biochemical reactions [2, 14]. The expression of amino acid transporters is upregulated in tumor cells, which require more nutrients than normal cells to maintain cell growth and intracellular metabolism [22, 31]. It has been reported that the amount of amino acids in tumor tissues is about twice as high as that in neighboring normal tissues [6]. The amino acid transporters that are upregulated in tumor cells include LAT1, LAT3, and ASCT2, among which LAT1 is particularly important because it is responsible for the uptake of several essential amino acids and is upregulated in many malignant tumors [1]. LAT1 is a Na^+-independent amino acid transporter that transports amino acids such as valine, leucine, isoleucine, phenylalanine, tryptophan, tyrosine, methionine, and histidine from the extracellular to the intracellular space, and is responsible for supplying amino acids to tissues where cell proliferation and intracellular metabolism are active [15, 25, 31]. LAT1 is upregulated in the fetus and its expression in normal adult tissues is limited to the brain, testis and placenta, but its expression level is thought to be lower than that in tumor tissues [3, 9, 17, 18, 22, 31].

In humans, LAT1 is upregulated in many tumors, including intracranial tumors, colon cancer, lung cancer, prostate cancer, stomach cancer, breast cancer, and pancreatic cancer, and its expression correlates with the grade of cancer and is a prognostic factor [7, 9, 11, 18, 26, 27, 29]. For example, a study of human renal cell carcinoma patients reported that 92% of cancer tissues expressed LAT1, that patients with higher levels of LAT1 expression had shorter overall survival (OS) and progression-free survival (PFS), and that higher levels of LAT1 expression in tumor tissues were associated with more metastasis and recurrence [5].

*Correspondence to: Okamoto Y: yokamoto@tottori-u.ac.jp, Department of Clinical Medicine, School of Veterinary Medicine, Faculty of Agriculture, Tottori University, 4-101 Koyama-Minami, Tottori 680-8553, Japan

Since pharmacological inhibition of LAT1 can inhibit the growth of cancer cells, LAT1 inhibitors are drawing attention as the new antitumor agents, and novel anticancer agents targeting LAT1, such as JPH203 which has been developed recently [23, 26]. JPH203, a selective LAT1 inhibitor, inhibits tumor cell growth by decreasing the amount of amino acids entering the cell [23]. Therefore, LAT1 is drawing attention as a prognostic biomarker and potential therapeutic molecular target against cancer in human medicine. However, the expression profile of LAT1 has not been well studied in veterinary medicine.

In a previous study by our group analyzing plasma free amino acid (PFAA) profiles in dogs with intracranial tumors, immunohistochemical analysis of LAT1 in anaplastic meningioma tissue was done; although only in two cases, we reported that LAT1 was downregulated in canine meningioma tissue [28]. In this study, we investigated the expression of LAT1 in canine and feline intracranial tumor tissues and its association with malignancy in a larger number of cases for better understanding.

MATERIALS AND METHODS

Animals and tissue sample collection

In this study, histopathological examinations were performed on cases brought to Tottori University Veterinary Medical Center and Animal Hospital in Kyoto Veterinary Hospital between October 2014 and December 2017, which were diagnosed with intracranial tumors by MRI or CT examination, and underwent surgical operation. The study protocol was approved by the Ethics Committee on Animal Trials of the Japan Animal Referral Medical Center (Tokyo, Japan).

Immunohistochemical analysis of LAT1

Immunohistochemical analysis (IHC) of LAT1 was performed using surgically resected intracranial tumor tissue. For immunohistochemical analysis, a rabbit anti-canine LAT polyclonal antibody was used. This antibody was prepared using a synthetic peptide antigen designed based on the C-terminal amino acid sequence of canine LAT1 [19]. Immunohistochemical analysis was performed using the following method: After deparaffinization, the sections were microwaved in 0.01 M citric acid (pH 6.0) for 3 min, heated five times, and washed with 0.01 M phosphate-buffered saline (pH7.4). Endogenous peroxidase was inactivated with methanol containing 0.3% H_2O_2, and the sections were incubated in rabbit anti-dog LAT1 polyclonal antibody at 4°C overnight. Immunostaining was performed using a commercially available kit [EnVision + kit/ HRP (DAB), Dako, Glostrup, Denmark). Subsequently, 3,3′-diaminobenzidine (DAB) H_2O_2 solution was applied to induce a positive color reaction. After the DAB reaction, specimens were washed three to four times with deionized water, and nuclei were stained with hematoxylin for observation.

Histological examination

The tumor tissues were fixed in 10% buffered formalin, embedded in paraffin, sectioned at a thickness of 6–8 µm, and stained with hematoxylin and eosin (HE) for histological examination. Meningiomas were graded into grades I–III based on the WHO classification of meningiomas in humans. For LAT1 staining, the epididymis was used as a positive control in dogs. Although homology with cats could not be confirmed, the staining of capillary endothelial cells in normal areas of the feline cerebrum was determined to be specific in cats. Furthermore, the feline negative control was found to be unstained [21]. Classification and evaluation of LAT1 expression was limited to tumor tissue on the specimen and was done by manual counting. The percentage of LAT1-positive cells among all tumor cells was calculated as the average of 5 fields of view. In cases where the number of tumor cells was less than 200, all tumor cells were counted and calculated. The staining intensity of LAT1 was classified as +, ++, +++, or ++++ based on the following criteria; Percentage of LAT1-positive cells: −, 0% of tumor area; +, <25% of tumor area; ++, 25–50% of tumor area; +++, 50–75% of tumor area; and ++++, 75–100% of tumor area.

RESULTS

Tissue samples were taken from a total of 14 dogs and 3 cats, with a mean age of 11.4 ± 3.4 years (median: 11 years, range: 4–19 years). Of the total sample, 10 animals were male and 7 female (Table 1). In dogs, tumors included five meningiomas (three Grade 1, two Grade 2), three histiocytic sarcomas, and one each of lymphoma, glioblastoma, pituitary adenoma, nasal adenocarcinoma, nasal transitional epithelial carcinoma, and myeloid leukemia. Of these, ten were primary intracranial tumors and four were secondary intracranial tumors. In dogs, primary intracranial tumors originated in the cerebrum in 9 dogs and in the pituitary gland in 1 dog. Secondary intracranial tumors were found in the cerebrum in 2 dogs, in the cerebrum and brainstem in 1 dog, and in the pituitary in 1 dog (Table 1). In cats, tumors include one each of psammomatous meningioma (Grade 1), lymphoma, and squamous cell carcinoma. Of these, two were primary intracranial tumor and one was secondary intracranial tumors. In cats, both primary and secondary intracranial tumors originated in the cerebrum (Table1).

LAT1 was detected in 12 of the 14 dogs by IHC. The two cases that were negative for LAT1 were malignant meningioma and lymphoma (Fig. 1). Of the 5 cases of canine meningioma, 2 were histologically malignant and 3 were benign. Histiocytic sarcoma, glioblastoma and lymphoma were malignant, and pituitary adenoma was benign. Therefore, of the 10 primary intracranial tumors in dogs, 6 were malignant and 4 were benign, 5 of the 6 malignant cases were LAT1 positive, while of the benign cases, all 4 were LAT1 positive (Figs. 2–5). In secondary intracranial tumors, 1 case of nasal adenocarcinoma, 1 case of nasal transitional epithelial carcinoma, and 1 case of myeloid leukemia were positive for LAT1, and negative for lymphoma.

Of the three cat cases, one was a benign meningioma, one was a primary intracranial lymphoma, and one was a metastasis of a squamous cell carcinoma, all of which were positive for LAT1.

Table 1. Characteristics of dogs and cats included in the present study

Breed	Sex	Age (year)	Location of the lesion	Pathological diagnosis	Primary or metastatic	LAT1
Dog	M	11	Olfactory bulb~cerebrum (frontal lobe)	Transitional meningioma (grade 1)	Primary	++++
Dog	M	10	Cerebrum (parietal lobe)	Meningioma (grade 1)	Primary	++++
Dog	F	11	Cerebrum (frontal~temporal lobe)	Meningioma (grade 1)	Primary	++++
Dog	M	12	Cerebrum (frontal lobe)	Atypical meningioma (meningothelial, grade 2)	Primary	++++
Dog	M	19	Cerebrum (frontal lobe)	Meningioma (grade 2)	Primary	-
Dog	F	8	Cerebrum	Histiocytic sarcoma	Primary	++++
Dog	M	10	Cerebrum (temporal lobe)	Histiocytic sarcoma	Primary	++++
Dog	M	10	Cerebrum (frontal lobe)	Histiocytic sarcoma	Primary	+
Dog	M	11	Pituitary gland	Pituitary adenoma	Primary	+
Dog	M	10	Cerebrum	Glioblastoma	Primary	++++
Cat	F	14	Cerebrum	Psammomatous meningioma (grade 1)	Primary	+++
Cat	F	10	Cerebrum	Lymphoma	Primary	++++
Dog	M	13	Olfactory bulb~cerebrum (frontal lobe), brainstem	Intranasal adenocarcinoma	Metastatic	+++
Dog	M	17	Cerebrum (subarachnoid space)	Transitional cell carcinoma of the nasal	Metastatic	+++
Dog	F	8	Cerebrum (chorioid plexus)	Myelocytic leukemia	Metastatic	++++
Dog	F	4	Pituitary gland	Lymphoma	Metastatic	-
Cat	F	15	Cerebrum (temporal lobe)	Squamous cell carcinoma	Metastatic	++

M, male; F, female; LAT1, L-type amino acid transporter 1.

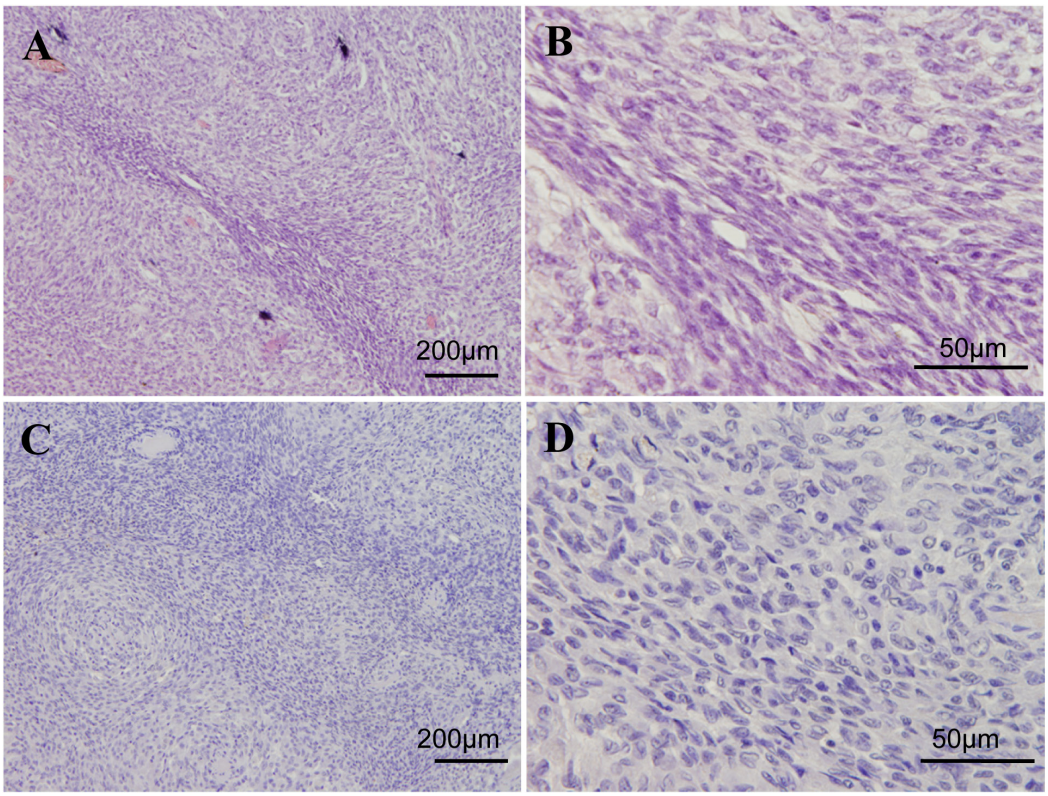

Fig. 1. Histologic sections from L-type amino acid transporter 1 (LAT1)-negative (−) grade 2 meningioma specimens of the dog. Hematoxylin and eosin stained (**A**, **B**), and LAT1 immunostained (**C**, **D**).

DISCUSSION

This is the first report investigating the expression of LAT1 in primary and secondary intracranial tumors in dogs and cats. In this study, LAT1 was highly expressed in intracranial tumor tissues regardless of the primary or secondary site, suggesting that increased expression of LAT1 is associated with tumor progression and metastasis. An especially high rate of LAT1 expression was observed

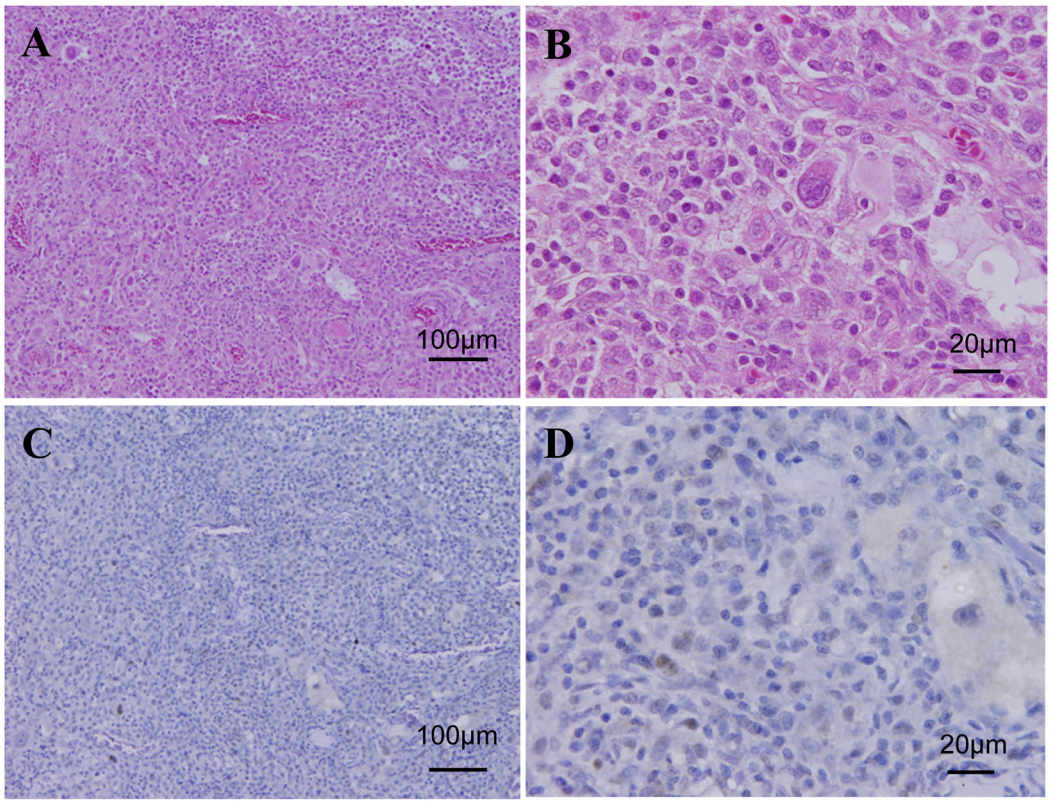

Fig. 2. Histologic sections from L-type amino acid transporter 1 (LAT1) positive (+) dogs with histiocytic sarcoma. Hematoxylin and eosin stained (**A**, **B**), and LAT1 immunostained (**C**, **D**).

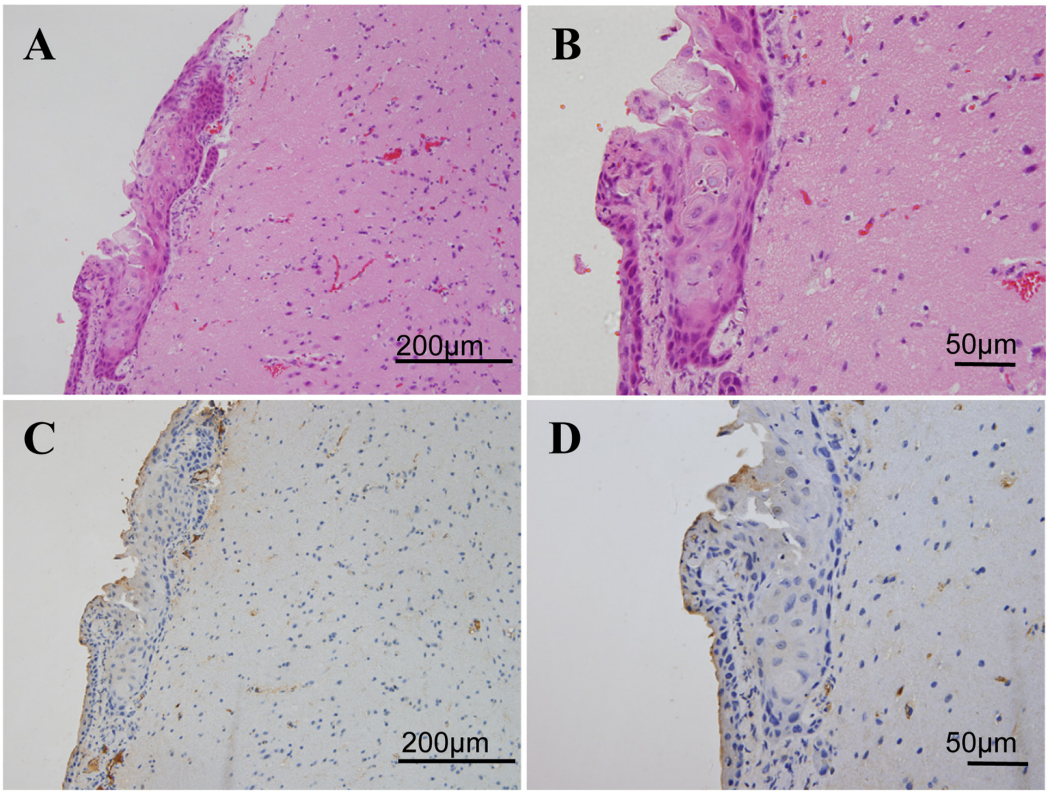

Fig. 3. Histologic sections from L-type amino acid transporter 1 (LAT1) positive (++) squamous cell carcinoma metastasized to the right temporal lobe specimens of the dog. Hematoxylin and eosin stained (**A**, **B**), and LAT1 immunostained (**C**, **D**).

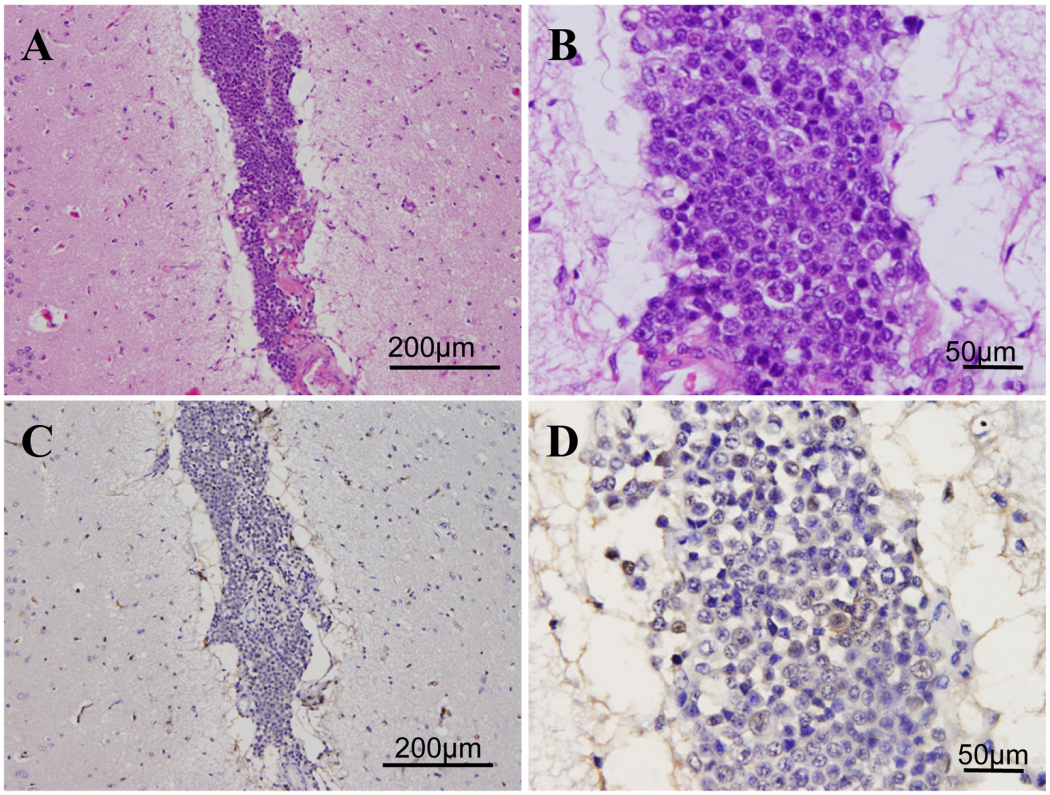

Fig. 4. Histologic sections from L-type amino acid transporter 1 (LAT1) positive (+++) dogs with adenocarcinoma in the nasal cavity and local extension to the cerebrum and metastasis to the brainstem. Hematoxylin and eosin stained (**A**, **B**), and LAT1 immunostained (**C**, **D**).

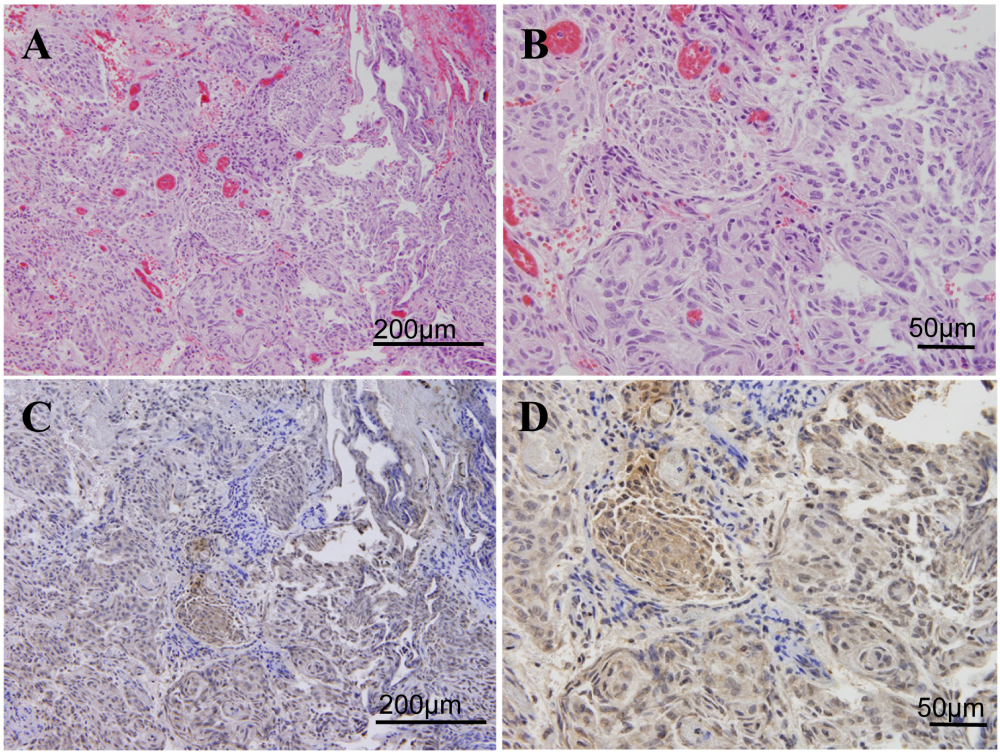

Fig. 5. Histologic sections from L-type amino acid transporter 1 (LAT1) positive (++++) grade 2 meningioma specimens of the dog. Hematoxylin and eosin stained (**A**, **B**), and LAT1 immunostained (**C**, **D**).

in canine meningiomas and histiocytic sarcomas, suggesting that an increased expression of LAT1 plays an important role in the progression of these tumors.

In the present study, LAT1 expression was upregulated at a high frequency in both secondary and primary intracranial tumors. In a previous study on human metastatic intracranial tumors, LAT1 was reported to be upregulated in almost all brain metastatic tissues (98.5%), which is considerably higher than the frequency of upregulation in primary tumors [24]. In a study comparing primary tumors and their pulmonary metastases in humans, the positive rate of LAT1 expression in colorectal cancer, breast cancer, head and neck cancer, genital cancer, and soft tissue sarcoma were 40%, 24%, 56%, 41%, and 45%, respectively in primary tumors. In contrast, in metastases, the rates were 65%, 45%, 84%, 67%, and 73%, respectively, indicating that LAT1 is more frequently upregulated in metastases [10]. In this study, as in the above report on neoplasms in humans, LAT1 upregulation was found in as high as 80.0% of secondary intracranial tumors, although the rate of LAT1 positivity in the primary tumor is unknown. Kaira *et al.* reported that the expression levels of LAT1 and CD98 are markedly increased in metastases compared to primary tumors [10]. In addition, in a study of metastatic tumors in the liver using a rat model, the tumor size in the LAT1-positive and CD98-positive group were significantly larger than in the LAT1-negative and CD98-negative group [22]. This suggests that LAT1 along with CD98 promotes tumor growth. Therefore, inhibition of LAT1 function is expected to be a potential therapeutic target for many types of cancer.

Many previous studies have reported positive expression of LAT1 in mammary gland tumors, hepatocellular carcinoma, and malignant melanoma in dogs [3, 4, 20]. To the best of our knowledge, there are no reports on LAT1 expression in neoplastic diseases in cats, and our study is the first to report on LAT1 expression in cats. Tumor cells in canine hepatocellular carcinoma expressed 28 times more LAT1 than normal hepatocytes [20]. A study of canine mammary gland tumors, both benign and malignant, reported a 20-fold increase in LAT1 expression compared to normal mammary gland tissue [3]. Furthermore, it has been reported that LAT1 expression is upregulated in mammary tumors with vascular invasion compared to those without invasion [3]. It has been reported that LAT1 expression levels in malignant melanoma were significantly higher than in normal tissue [4]. Furthermore, malignant melanomas with distant metastases had higher LAT1 expression than those without distant metastases [4]. In human gliomas, the level of LAT1 expression increases with higher grade of malignancy [16]. These findings suggest that LAT1 is upregulated in both benign and malignant tumors, with higher upregulation in malignant tumors.

In the canine meningiomas in this study, three were benign and two were malignant, and LAT1 was positive in all benign cases and one malignant case. In a previous study which analyzed PFAA profiles in dogs with intracranial tumors by the authors, two cases of malignant meningiomas were negative for LAT1 [28]. When combined with the results of this study, only one out of four malignant cases was positive for LAT1, and all three benign cases were positive for LAT1. In canine meningiomas, the rate of LAT1 positivity was higher in benign cases, which is different from previous reports of LAT1. The reason for this contrasting results is that LAT1 is expressed in normal brain tissue in dogs, which may be down-regulated in meningiomas, or LAT1 may reflect not only the malignancy of the tumor but also its proliferative potential. Another reason may be the small number of cases. Therefore, if the correlation between LAT1 overexpression and malignancy is clarified through an aggregate analysis of all published cases in the future, it is expected that it may be used as a prognostic indicator.

Since LAT1 plays an important role in the proliferation and progression of cancer cells, it has been suggested as a potential diagnostic marker and therapeutic target for cancer in humans, and several drugs targeting LAT1 have been developed in recent years [13]. In human medicine, it has been reported that pharmacological inhibition or genetic cleavage of LAT1 inhibits the transport of leucine to cancer cells and suppresses the growth of cancer cells [26]. As LAT1 inhibitors have a different mechanism of action from conventional anti-tumor drugs, they can be co-administered with currently used treatment paradigms. Moreover, LAT1 inhibitors alone may be effective for cancer patients who do not respond to current treatments. In addition, combining LAT1 inhibitors with conventional anti-tumor drugs may allow for reduced doses of conventional anti-tumor drugs to be used, consequently reducing the side effects of such drugs. A study on canine malignant melanoma reported that selective LAT1 inhibitors such as 2-amino-2-nor bornane-carboxylic acid (BCH) or melphalan (LPM) inhibited cell growth and amino acid uptake, and that the tumor growth inhibitory effects of BCH and LPM were enhanced when combined with conventional anti-cancer agents such as carboplatin, cyclophosphamide, and dacarbazine [4]. In human medicine, synergistic effects between LAT1 inhibitors and anti-tumor agents such as cisplatin, gemcitabine, 5-FU, gefitinib, and bicalutamide have been reported [8, 12, 29, 30].

In this study, LAT1 was upregulated in both primary and secondary intracranial tumors in dogs and cats, suggesting that it is related to activity of metabolism. Further study is needed on this point. This suggests that LAT1 may be a molecular target for the treatment of primary and secondary intracranial tumors in dogs and cats, and that LAT1 inhibitors are expected to be effective against intracranial tumors in dogs and cats.

POTENTIAL CONFLICTS OF INTEREST. The authors have no conflict of interests to declare.

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