

Unique and selective expression of L-amino acid transporter 1 in human tissue as well as being an aspect of oncofetal protein

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Summary. Dysregulated expression of L-type amino acid transporter 1 (LAT1), which transports large neutral amino acids, is a characteristic of various human cancers and possibly offers a molecular target for chemotherapy. LAT2, in contrast, shows lower expression in neoplasms. LAT1 is presumed to be a biomarker of many cancers, suggesting a kind of oncoprotein. However, no precise analysis of LAT1 and LAT2 expression has been performed in systemic normal tissues. To see characteristics of LAT1 and LAT2, immunohistochemical expression of LAT1 and LAT2 was assessed and compared in normal human systemic organs and tissues from 3 adults, 3 children and 3 fetuses in the present study. Cardiac muscles, hepatocytes, thymic epithelial cells and primitive neuroectodermal cells in fetus were positive with LAT1, whereas no expression was found in the respective adult tissues, indicating an aspect of oncofetal protein. In adult tissues, LAT1 was found to be expressed proximal to proliferative zones in gastrointestinal mucosa by double immunostaining of LAT1 and Ki-67. Testicular Sertoli cells, ovarian follicular cells, and pancreatic islet cells showed strong expression. Although the systemic capillary endothelium did not express LAT1, but did express LAT2, capillaries corresponding to the blood-brain, blood-follicle, and blood-retinal barriers demonstrated strong LAT1 immunoreactions. In conclusion, LAT1 was expressed in gonad tissues and several kinds of cells having special functions, as well as being discovered to be an aspect of

oncofetal protein. In addition, ubiquitous LAT2 expression was confirmed immunohistochemically in systemic tissues, indicating constitutional function.

Key words: Human tissue, L-amino acid transporter, Oncofetal protein

Introduction

System L is a major nutrient transport system, with transporters expressed on cell membranes transferring large neutral amino acids into cells. L-type amino acid transporter 1 (LAT1) is the isoform that transports larger amino acids. On the other hand, it has been reported to be expressed on cancer cells (Kanai et al., 1998; Nakamura et al., 1999; Prasad et al., 1999; Yanagida et al., 2001; Chrostowski et al., 2009; Ohno et al., 2009). Recently, we demonstrated that LAT1 expression of cancer cells could be an independent prognostic marker in prostate (Sakata et al., 2009), stomach (Ichinoe et al., 2011), lung and pancreas cancers (Nakanishi et al., 2006; Kaira et al., 2009a; Imai et al., 2009, 2010; Yanagisawa et al., 2012). Particularly, in prostate and pancreas cancers, LAT1 expression was correlated to the malignancy grade (Sakata et al., 2009; Yanagisawa et al., 2012). It has recently been shown that increased amino acid uptake in cells via LAT1 expression promotes

Abbreviations. BBB, Blood-brain barrier; BFB, Blood-follicle barrier; BRB, Blood-retinal barrier; BTB, Blood-testis barrier; DAB, Diaminobenzidine; FITC, Fluorescein-isothiocyanate; LAT1, L-amino acid transporter 1; LAT2, L-amino acid transporter 2; PBS, Phosphate buffered saline

mammalian target of rapamycin complex 1 (mTORC1) signaling and cell growth in human prostate cancer cell lines (Wang et al., 2011). The PI3K/PTEN/Akt pathway is activated in prostate cancer. PTEN is frequently mutated in prostate cancer and activates Akt, leading to activation of mTORC1 (Stambolic et al., 1998; Maehama and Dixon, 1998; Guertin and Sabatini, 2007; Blando et al., 2009). Further, we showed that a proto-oncogene, c-Myc, is a critical positive regulator of LAT1 expression in MIA Paca-2 human pancreatic cancer cell lines (Hayashi et al., 2012). LAT2 is a second isoform which transports smaller amino acids and is considered to exhibit broad-spectrum expression in the human body (Segawa et al., 1999; Pineda et al., 1999; Rossier et al., 1999; Babu et al., 2003; Fernandez et al., 2003; Fraga et al., 2005). LAT1 has prospects for application as a diagnostic biomarker of cancer, a molecular target of chemotherapy with LAT1 inhibitors, and a target of tumor-selective diagnosis and radiation therapy using selective drug accumulation, such as positron emission tomography and boron neutron capture therapy (Nawashiro et al., 2006; Kim et al., 2008; Yamauchi et al., 2009; Kaira et al., 2009b; Oda et al., 2010; Wang et al., 2011). In fact, we recently developed a specific inhibitor of synthetic small molecule compound targeting LAT1. It inhibited 16C-Leucin uptake and proliferation of HT-29 cells in vitro culture system, and growth of transplanted HT-29 cells in nude mice (Oda et al., 2010). Although partial descriptions of LAT1 and LAT2 expression have been reported, to our knowledge, a systemic examination of these two proteins has not yet been documented. To identify LAT1 as one aspect of oncofetal protein, we evaluated its location in normal human tissues (adult, child, and fetus) using immunohistochemistry with our recently developed novel monoclonal antibody against a synthetic N-terminal polypeptide of LAT1 (Sakata et al., 2009). The specificity of our monoclonal antibody for LAT1 was confirmed with absorption test using synthetic peptides and Western blotting (Sakata et al., 2009). Furthermore, in contrast, we also examined LAT2 expression to confirm its broad-spectrum expression in the human body.

Materials and methods

Materials

Three autopsy cases each of adults and of children without multiple organ failure, severe infectious disease or chromosomal abnormality were chosen from case files in Kitasato University Hospital (Table 1). In all cases, the postmortem autopsy was performed within 12 h of death and formalin-fixation time was less than 72 h. Formalin (10%)-fixed and paraffin-embedded tissue blocks of each available organ were selected in these six autopsy cases. For both adults and children, organs which were not available from the autopsy cases were collected from surgically operated cases which matched

the ages of the autopsy cases. Three fetal samples without degeneration or maceration, which were incidentally found in specimens of missed abortion, were also collected.

Immunohistochemistry

After paraffin-embedded tissue sections (4 μ m) were deparaffinized and dehydrated, endogenous peroxidase activity was blocked by incubation with 0.3% H_2O_2 for 15 minutes. For LAT1 immunostaining, the slides were then placed in citrate buffer (pH6.0) and heated for antigen retrieval using a microwave oven for 15 minutes. A mouse monoclonal antibody against LAT1 (2 μ g/ml; J-Pharma, Tokyo, Japan) and a rabbit polyclonal antibody against LAT2 (2 μ g/ml; Trans Genic, Kumamoto, Japan) were applied to the slides at 4°C for overnight in a humid chamber. After washing with Tris or phosphate buffered saline (PBS) buffer, the slides were incubated with peroxidase-labeled polymer (Envision, DakoCytomation, Kyoto, Japan) for 30 min. Finally, reactions were visualized with 3-3'-diaminobenzidine (DAB; Dojindo, Masushiro, Japan) as the chromogen. All slides were counterstained with hematoxylin or methyl-green to facilitate histological assessment. Normal kidney and normal pancreas tissues were used as positive controls for LAT1 and LAT2, respectively. For an internal control of LAT1, infiltrating lymphoid cells were used, of which activated T-cells are positive for LAT1 (Nii et al., 2001; Hayashi et al., 2012). Stromal fibroblasts were used as negative controls for both LAT1 and LAT2. To evaluate the immunoreactivity, the highest LAT1 intensity of more than 10% cells was adopted in each tissue. Four categories were defined: intensity score 0, no staining; 1, weak membrane staining; 2, moderate complete membrane staining; 3, intense complete membrane staining (Sakata et al., 2009; Ichinoe et al., 2011; Yanagisawa et al., 2012). Two pathologists (N. N. and T. M.) determined the intensity of LAT1 expression 0-3 of each organ and tissue by observing them together under the discussion

Table 1. Clinicopathological information for six autopsy cases and three fetuses.

Patient	Age	Gender	Autopsy diagnosis
Adult	18 years	M	Acute myeloid leukemia
	69 years	F	Idiopathic interstitial pneumonia
	71 years	M	Mediastinal carcinoid and squamous cell carcinoma
Child	2 days	F	Intracranial hemorrhage
	1 month	M	Disseminated intravascular coagulation
	1 month	M	Respiratory distress syndrome
Fetus	11 weeks	M	Missed abortion
	12 weeks	U	Missed abortion
	20 weeks	U	Missed abortion

M, male; F, female; U, unknown. In two fetal cases, sex organs were undefinable.

microscope and determining the score (Fig. 1).

Double immunohistochemistry for LAT1 and Ki-67 was carried out to identify LAT1 positive cells and proliferative cells on histological sections for gastrointestinal mucosa as follows. After immunohistochemical staining with a combination of anti-LAT1 antibody and DAB reaction, histologic sections were treated for antigen retrieval and blocking crossreactivity using a microwave oven for 5 min according to the methods of Lan et al. (1995). Then, mouse monoclonal anti-Ki-67 antibody (MIB-1, x100 diluted, Dako-Cytomation, Japan) was applied to the slides overnight at 4°C. After washing the slides were incubated with peroxidase-labeled polymer (Envision, Dako-Cytomation) for 30 min. Finally, reactions were visualized with a mixture of 0.07% NiCl_2 (Junseikagaku, Tokyo, Japan) and DAB as the chromogen. Counterstain was performed with methyl green.

Double immunofluorescence staining

Double immunofluorescence staining was performed to identify LAT1 and LAT2 positive cells on histological sections for selected organs. After deparaffinized histological sections were incubated with the primary anti-LAT1 antibody overnight at 4°C, they were exposed to Alexa Fluor 488 F(ab')₂ fragment of goat anti-mouse IgG (H+L) (x1,000 diluted; Molecular Probes, Invitrogen, Carlsbad, CA, USA), at room temperature for 30 min. After washing with PBS, they were again incubated with the anti-LAT2 antibody overnight at 4°C and subsequently exposed to rhodamine-conjugated goat anti-rabbit IgG (x200 diluted; Molecular Probes, Invitrogen) at room temperature for 30 min. For staining of LAT1, microwave treatment in 0.01M citrate buffer (5 min, pH 6.0) was performed before incubation with the primary antibody. After washing with PBS, the slides were observed under a fluorescence microscope (Olympus BX61 + UCB + DP71, Tokyo, Japan).

Results

Both LAT1 and LAT2 showed expression on cell membranes. The findings in each tested organ are summarized in Table 2. We focused particular attention on the following tissues.

Gastrointestinal mucosa

In the adult, child, and fetus, both LAT1 and LAT2 showed expression in gastrointestinal mucosa. LAT1 was generally located in the lower zones or crypt base of the gastric, small intestine, and colonic mucosa, while LAT2 was found at the upper epithelial surfaces. In gastric mucosa, fundic gland also expressed both LAT1 and LAT2 (Fig. 1A-a,b). In the esophageal mucosa, the lower zone was immunopositive for both LAT1 and LAT2 (Fig. 1B-a,b).

Double immunohistochemistry demonstrated that LAT1-positive cells in the mucosa of digestive organs, including esophagus, stomach, and intestines were located proximal to Ki-67-positive epithelium but clearly different from Ki-67 positive cells (Fig. 1B-c, 1C-c).

Fetal tissues

In several fetal tissues, characteristic expression of LAT was observed. Hepatocytes expressed LAT1 in fetal tissue (Fig. 2a) but not at other ages. Extramedullary hematopoietic cells showed strong LAT1 expression. Low LAT2 expression was found in hepatocytes (Fig. 2b). Although neurons and glial cells in the adult and child were negative for LAT1, primitive neuroectodermal cells of the fetal brain showed weak expression. Although cardiac muscle cells of the fetus showed LAT1 expression (Fig. 2c), those of the adult and child did not. In thymic epithelial cells, the LAT1 intensity score for the fetus was 2 (Fig. 2d), while that for the child was 0-1 and that for the adult was 0. The LAT1 intensity score for lymphocytes in the thymus varied from 1 to 3. In the placenta, syncytiotrophoblasts showed high immunoreactivity for LAT1 (Fig. 2f), while cytotrophoblasts showed a somewhat lower intensity.

Blood vessels

LAT1 expression was not found in the capillary endothelium, whether of arteriole, venule, muscular artery and vein, of any of the organs, but was clearly positive in that of the ovary (Fig. 3a), brain (Fig. 3b), and retina (Fig. 3c). LAT2 was generally expressed in the endothelium of vessels. Double immunofluorescence staining also showed that LAT1 and LAT2 were partially co-expressed in the endothelium of vessels in the brain (Fig. 4a-c).

Testis and ovary

LAT1 expression could be demonstrated in spermatogonia, spermatids, and Sertoli cells of the testis but not in sperm (Fig. 3d). In the ovary, LAT1 was expressed in the follicular epithelium, including primary follicles. Seminiferous tubules of fetal testis also showed strong expression of LAT1 (Fig. 2e).

Kidney

Strong LAT1 expression was observed on basal plasma membranes of distal tubules while LAT2 was strongly found in the same sites in proximal tubules. LAT1 did not show immunostaining in glomeruli, while LAT2 showed weak immunostaining in glomeruli and arteriole. Distal tubules also showed immunoreactivity for LAT2. Double immunofluorescence staining clearly showed their complementary expression (Fig. 4d-f).

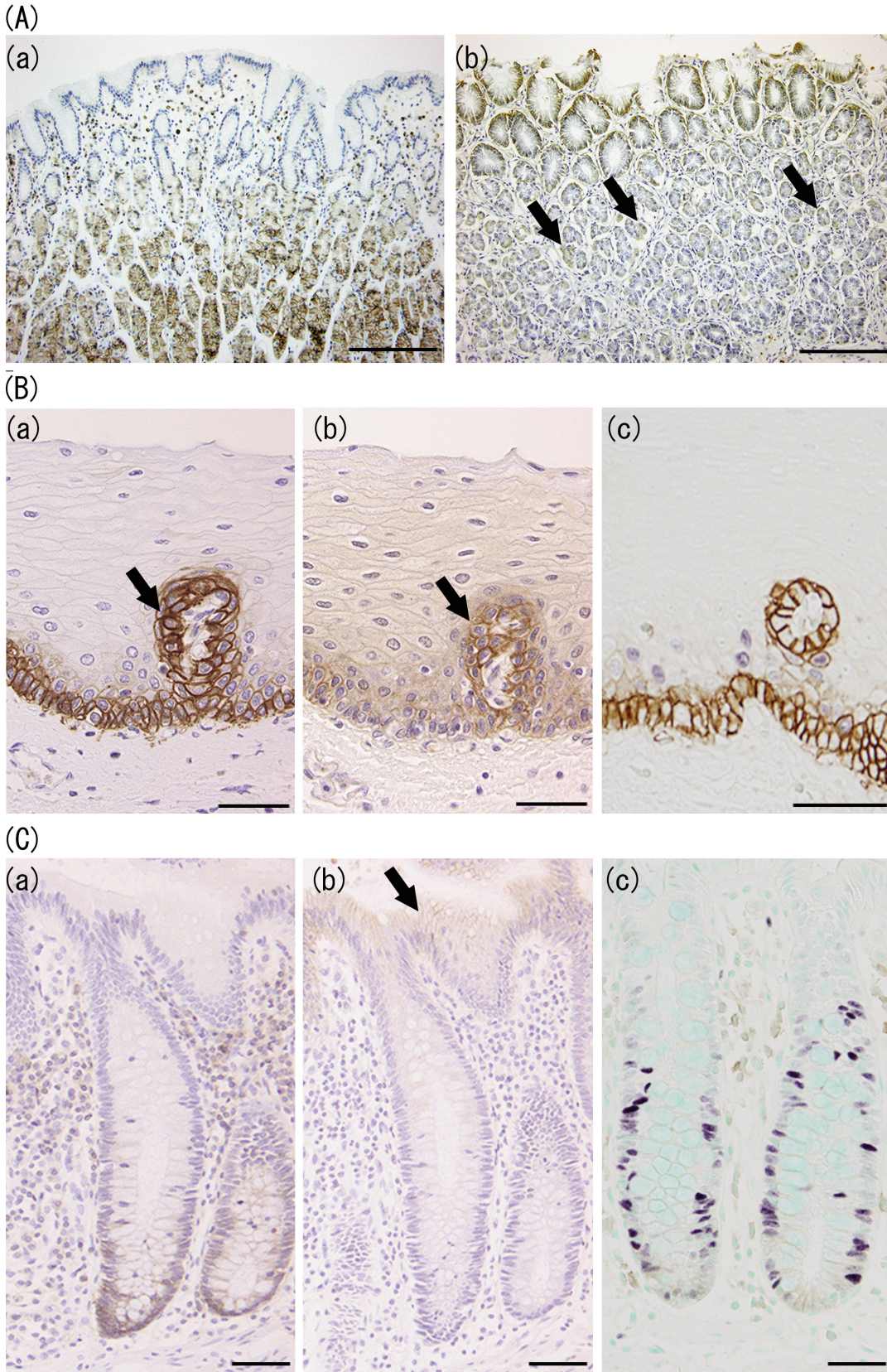


Fig. 1. A. LAT1 staining (a) of adult gastric mucosa, with LAT2 staining (b). Note that parietal cells are positive for LAT2 (arrows). **B.** Serial sections of an adult esophageal mucosa, stained for LAT1 (a; arrow, intensity score 3) and LAT2 (b; arrow, intensity score 2). Both LAT1 and LAT2 are clearly positive in the basal layer. Double immunohistochemistry stained for LAT1 and Ki-67 (c). Although LAT1 positive cells (brown color-cell membrane) are located at the basal layer, Ki-67 positive cells (blue nickel color-nucleus) are distributed at the parabasal layer, different from LAT1-positive cells. **(C)** Serial sections of an adult colonic mucosa, stained for LAT1 (a) and LAT2 (b; arrow, intensity score 1). The cell membrane at the crypt base is positive with brown color. Double immunohistochemistry for LAT1 and Ki-67 (c) shows that LAT1 positive cells (brown color-cell membrane) and Ki-67 positive cells (blue nickel color-nucleus) are clearly separated at the crypt base. Bars: A, 200 μm ; B and C, 50 μm .

Table 2. LAT1 and LAT2 immunoreactive intensity scores for fetuses, children, and adults.

Organ		LAT1 score			LAT2 score		
		Adult	Child	Fetus	Adult	Child	Fetus
Heart	Cardiac muscle	0	0	1-2	1	1	0-1
Lung	Alveolar epithelium	0	0	NA	2	1	0-1
	Bronchiolar epithelium	0	1	1-2	1-2	1	1
Esophagus	Epithelium	3*	3*	2-(n=1)*	2-3*	1-2*	0 (n=1)*
Stomach	Epithelium	2-3†	3†	1-2†	2-3†	2†	0-1†
	Fundic gland	1-2†	2†	NA	1†	1-2†	NA
Small intestine	Epithelium	2†	2-3†	2-3†	1-3†	2-3†	1-3†
Colon	Epithelium	2-3†	2†	1-2†	1-3†	2-3†	1-3†
Liver	Hepatocyte	0	0	1-2	1	1-2	0-1
	Bile duct epithelium	0	0	0	1	1	0
	Extramedullary hematopoiesis	NA	NA	2-3	NA	NA	0-1
Gallbladder	Epithelium	0	0	NA	1-2	1	NA
Pancreas	Exocrine acini	0	0	0	1-2	0-1	1
	Islet cell	2-3	2-3	1 (n=2)	1-2	1	1-2 (n=2)
Pituitary	Anterior lobe	1	NA	1	1	NA	1-2
	Posterior lobe	1	NA	1 (n=1)	1	NA	1 (n=1)
Thyroid	Follicular epithelium	0	0	NA	1-2	0-1	NA
Adrenal gland	Cortex	1-2	1-2	0-1 (n=2)	1-2	1-2	1 (n=2)
	Medulla	0	1-2	2 (n=1)	1	1	1-2 (n=1)
Bone marrow	Hematopoietic cell	1	2-3**	2-3**	1-2	1**	1**
Spleen	Lymphocyte	2	2	2-3	0	0	0
Thymus	Thymic epithelial cell	0	0-1	2	1	1-2	0-1
	Lymphocyte	2	1-2	1-2	1	0	0-1
Kidney	Proximal tubule	0	0	0 (n=2)	3	2-3	1-2 (n=2)
	Distal tubule	3	2	2 (n=2)	0-1	1	1 (n=2)
	Glomerulus	0	0	0 (n=2)	0-1	0	0 (n=2)
Urinary bladder	Urothelial epithelium	0-1	0-1	NA	1-2	1	NA
Testis	Spermatogonia	2	2-3	NA	1-2	1	NA
	Spermatid	2	2	NA	1-2	1	NA
	Sperm	0	0	NA	0	0	NA
	Sertoli cell	2-3	3	3 (n=1)	1	1	1 (n=1)
	Leydig cell	0	0	0 (n=1)	0	0	0 (n=1)
	Epigenital tubule (primitive)	NA	NA	3	NA	NA	1
Prostate	Prostatic gland	0-1	NA	NA	1-2	NA	NA
Breast	Ductal epithelium	0-1	NA	NA	1-2	NA	NA
Ovary	Ovum	0	NA	NA	0	NA	NA
	Theca cell	0	NA	NA	1	NA	NA
	Follicular epithelium	2	NA	NA	0-2	NA	NA
Endometrium	Epithelium	2-3	NA	NA	1-2	NA	NA
proliferative phase	Stroma	0	NA	NA	1	NA	NA
Endometrium	Epithelium	0-1	NA	NA	2	NA	NA
secretory phase	Stroma	0	NA	NA	1	NA	NA
Placenta	Syncytiotrophoblast	3	NA	NA	1	NA	NA
	Cytotrophoblast	2	NA	NA	1-2	NA	NA
Brain	Glial cell	0	0	NA	0	0	NA
	Neuron	0	0	NA	0	0	NA
	Ependymal cell	2-3	0 (n=1)	0-1	1-2	1 (n=1)	0-1
	Primitive neuroectodermal cells	NA	NA	1	NA	NA	0
Capillary	Brain	3	3	3	1	1-2	0-1
	Ovary	2	1 (n=1)	NA	1	1 (n=1)	NA
	Choroid plexus	0	0	NA	1	1	NA
	Retina	2	2	NA	1-2	1	NA
	Others	0	0	0	1	1	1
Skin	Epidermis	2-3	NA	NA	1	NA	NA
	Sebaceous gland	2-3	NA	NA	1	NA	NA
	Sweat gland	0	NA	NA	1	NA	NA

NA, not available. *: In esophageal mucosa, both LAT1 and LAT2 were located in the lower zone. †: In gastric mucosa, small intestine, and colonic mucosa, LAT1 was located in lower zones, while LAT2 was found on mucosal surfaces. **: In fetus and child cases, the intensity score for hematopoietic cells was evaluated in liver as extramedullary hematopoiesis.

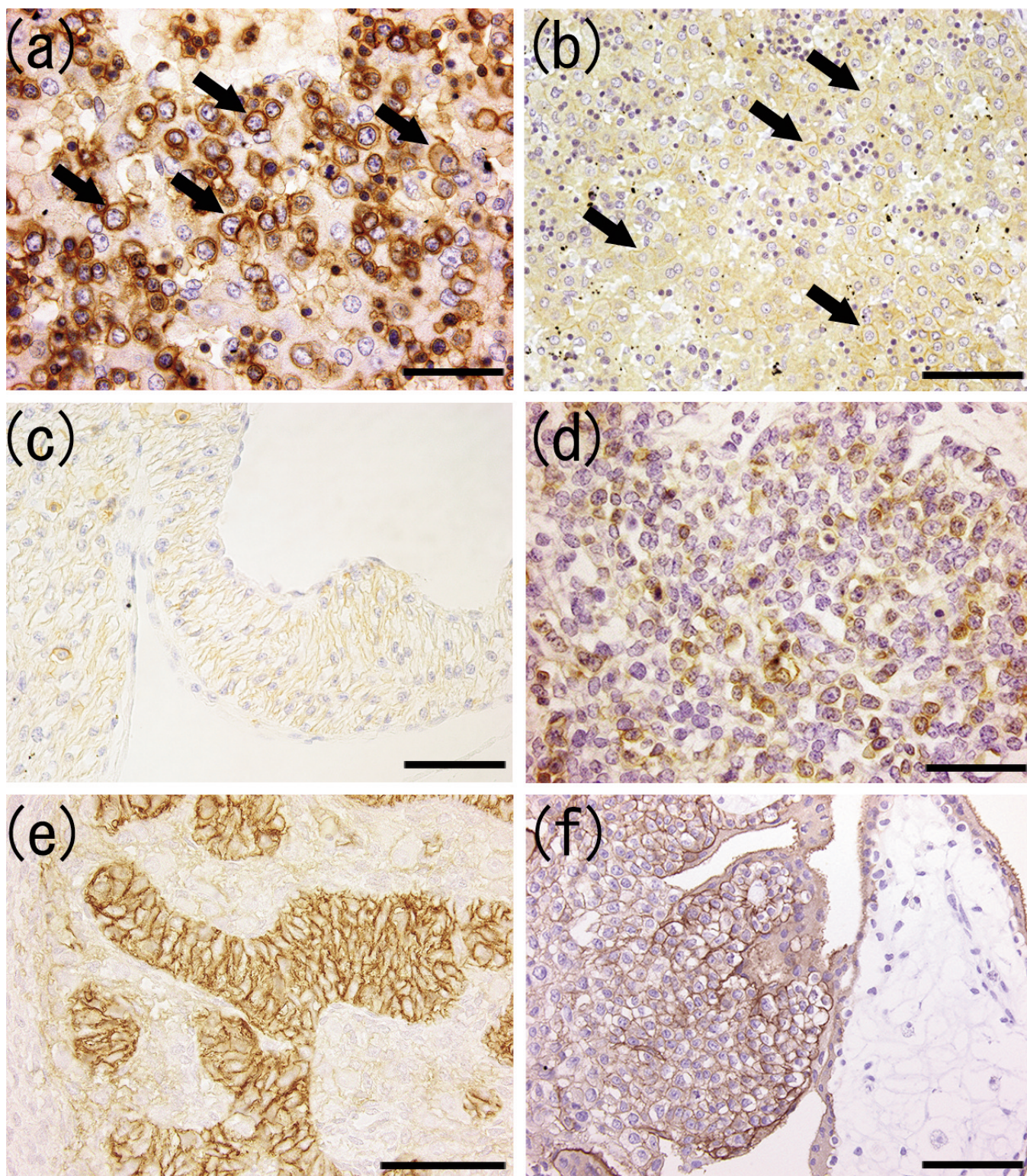


Fig. 2. Fetal hepatocytes (arrows) are strongly immunoreactive for LAT1 (a) but weakly for LAT2 (b). Cardiac muscle cells of a fetus show weak expression of LAT1 (c). Thymic epithelial cell of a fetus showed moderate expression of LAT1 (d). Seminiferous tubules of a fetus showed strong expression of LAT1 (e). Trophoblasts showing strong LAT1 expression (f). Bars: a, d, 25 μ m; b, c, e and f, 50 μ m.

Other organs

Both LAT1 and LAT2 proved positive in islet cells of the pancreas of the adult, child, and fetus. Low LAT1 expression was found in pituitary and adrenal glands, but not in thyroid tissue. Low LAT2 expression was found in epithelium of pancreatic duct, pancreatic acini, pituitary, and adrenal glands. Double immunofluorescence staining revealed that pancreatic islet cells partially co-expressed LAT1 and LAT2 (Fig. 4g-i).

Endometrial epithelium in the proliferative phase expressed LAT1 strongly. Weak expression was observed in the early secretory phase. In stromal cells, LAT1 was not expressed in any phase.

Ductal epithelium of the breast showed no or low

expression (score 0-1) of LAT1 but low or moderate expression (score 1-2) of LAT2. Moderate expression was found in myoepithelium of the breast.

Discussion

In the present study, all tissues which expressed LAT1 or LAT2 demonstrated their location on the cell membranes, in line with their function as amino acid transporters into cells. In some tissues, such as small intestine, colon, and tubules of kidney, LAT1 and LAT2 showed inverse expression patterns, the former being generally found in lower zones close to proliferative zones, while LAT2 expression was located in mature cells at mucosal surfaces. The exception was the lower

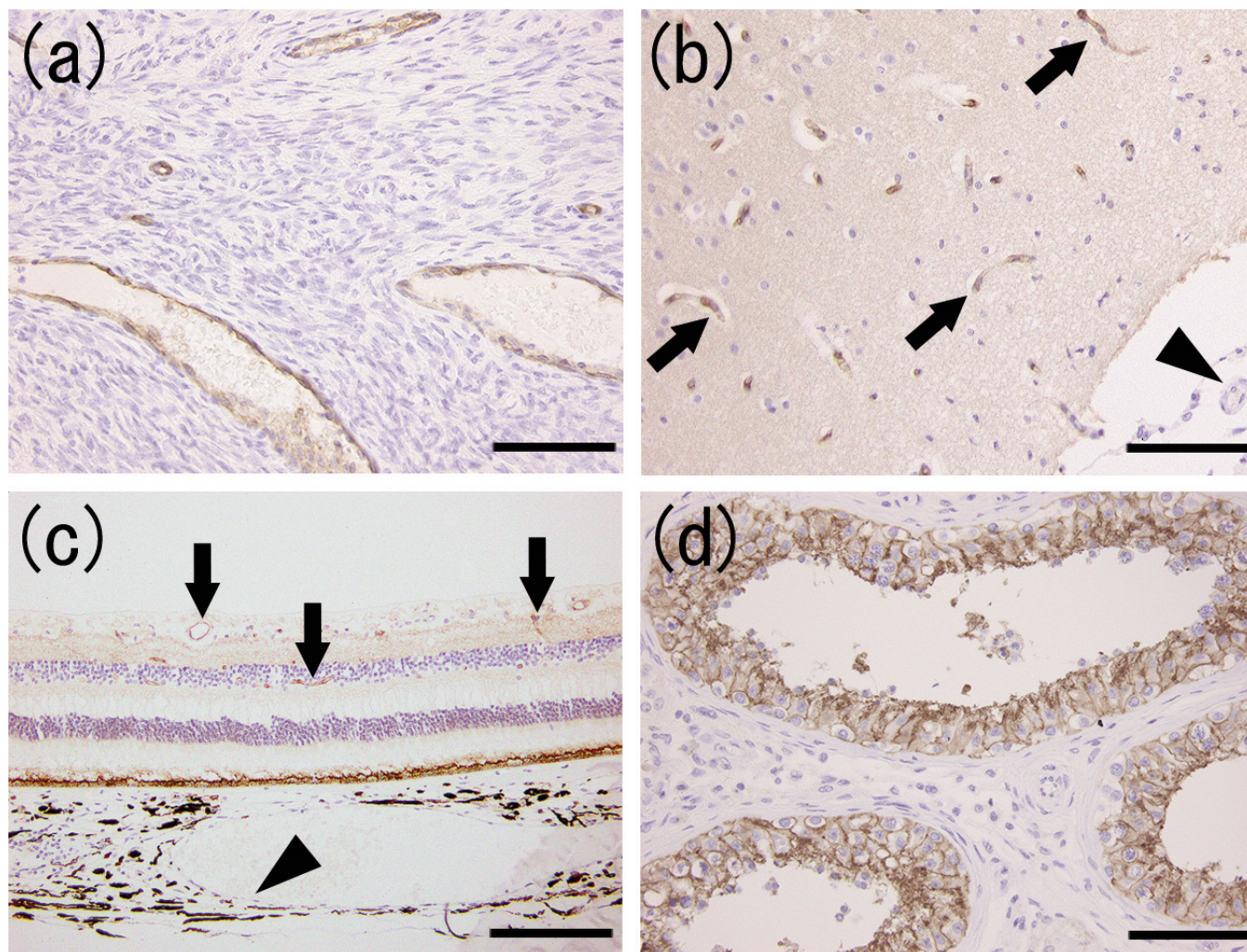


Fig. 3. LAT1 staining of an adult ovary (a). Note the immunoreactions in the endothelium. Ovarian stromal cells are negative for LAT1. An LAT1 stained adult brain is shown (b). While the endothelium of small vessels in the subarachnoid space is negative (arrowhead), the endothelium of capillaries in the brain parenchyma is positive (arrows). LAT1 staining of an adult retina (c). The endothelium of capillaries shows moderate LAT1 expression (arrows), while the endothelium of capillaries in choroid does not show LAT1 expression (arrowhead). Spermatogonia, spermatids, and Sertoli cells of an adult showing strong LAT1 expression, although Leydig cells are negative (d, intensity score 0). Bars: a, b and d, 100 μ m; c, 200 μ m.

zone of esophageal mucosa, positive for both. In the stomach, parietal cells were additionally positive for LAT2. Since LAT2 was reported to be involved in gastric acid secretion (Kirchhoff et al., 2006), these findings were compatible with the function. It is possible that immature cells in the crypt base and basal cells need larger amino acids for proliferation, while cells in surface areas need other amino acids for secretion. In this context, it should be mentioned that surface cells in squamous epithelium would not be expected to need amino acids after differentiation. On the other hand, LAT1-positive cells in the crypt base of intestinal mucosa and the basal layer of esophageal mucosa were negative for Ki-67 labeling, which indicates cell

proliferative activity. Those results suggest that LAT1 is necessary for not only cell proliferation but also another special function in immature cells. In the kidney, LAT2 is reported to be responsible for amino acid reabsorption in tubules (Rossier et al., 1999; Fernandez et al., 2003).

Although LAT1 and LAT2 expression in adult and child tissues was similar, their expressions in fetal tissues varied in several organs. LAT1 was positive in several kinds of fetal cells, such as cardiac muscle, hepatocytes, primitive neuroectodermal cells, and thymic epithelial cells but negative in those kinds of adult cells. On the other hand, it is reported that strong LAT1 expression was observed in cancer cells of various organs and showed prognostic significance, including

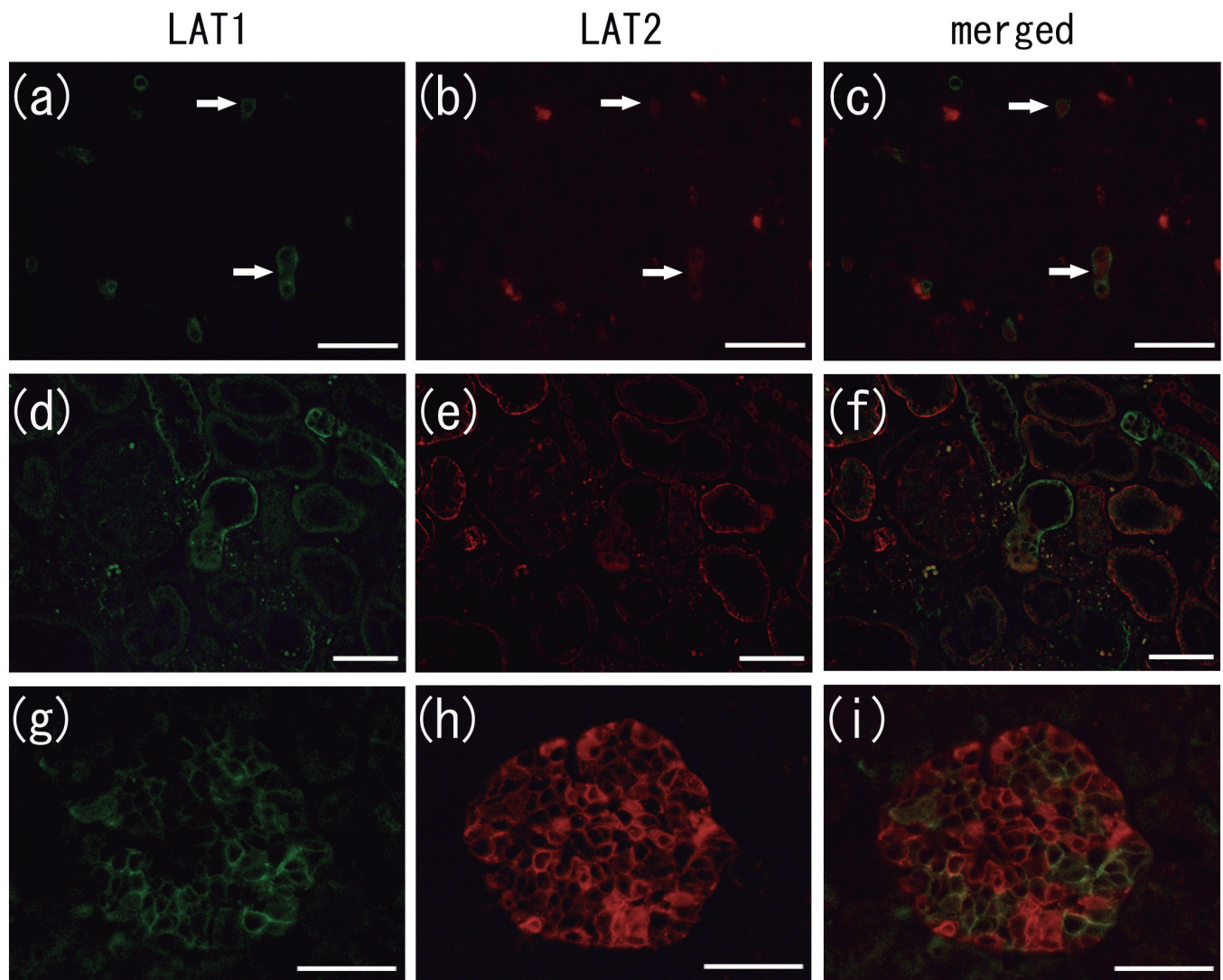


Fig. 4. Double immunofluorescence-stained images showing LAT1 (a, d, g; FITC, green) and LAT2 (b, e, h; rhodamine, red) (c, f, i; merged). Brain vessels (arrows) strongly express LAT1 and partially co-express both LAT1 and LAT2 (a, b, c). Note that LAT1 and LAT2 expression is complementary between the distal and proximal tubules (d, e, f). Pancreatic islet cells partially co-express both LAT1 and LAT2 (g, h, i). Bars: a, b and c, 50 μ m; d, e, f, g, h and i, 100 μ m.

prostate (Sakata et al., 2009), stomach (Ichinoe et al., 2011), and lung (Nakanishi et al., 2006; Kaira et al., 2009a; Imai et al., 2009, 2010). Further, recently we discovered that activated human T cells strongly express LAT1, which is partly regulated with oncogene, c-Myc (Hayashi et al., 2012). These facts indicate that LAT1 has an aspect of oncofetal protein.

Importantly, LAT1 expression was limited to the capillaries of the brain, retina, and ovary, which are components of the blood-brain barrier (BBB), blood-retinal barrier (BRB), and blood-follicle barrier (BFB), respectively, suggesting some essential role of LAT1 for maintenance of endothelial cells as a barrier (Boado et al., 1999; Kageyama et al., 2000; Matsuo et al., 2000; Mann et al., 2003; Tomi et al., 2005; Makrides et al., 2007; Zhou et al., 2007). In addition, Sertoli cells which showed strong LAT1 expressions are considered to be a component of the blood-testis barrier (BTB) (Pelletier and Byers, 1992; Yazama, 2008). Although LAT1 expression may be related to cell proliferation, the capillaries of these organs do not show proliferative activity under normal conditions. Therefore, LAT1 might have alternative functional roles. For the BRB and BBB, it has been reported that LAT1 transports neutral amino acids for maintaining neurotransmitters (Boado et al., 1999; Kageyama et al., 2000; Matsuo et al., 2000; Tomi et al., 2005). BTB and BFB formation may be related with spermatogenesis and folliculogenesis (Lui et al., 2003; Zhou et al., 2007). In this context, LAT1 expression may also be correlated with sperm formation and ovarian follicular growth in gonad organs. Our results, including moderate to strong immunoreactive LAT1 expression of placenta, testis, bone marrow, and fetal liver and brain are in line with our previous study using Northern blot analysis (Yanagida et al., 2001).

Islet cells and syncytiotrophoblasts of placenta showed strong expression of LAT1. This suggests that LAT1 could be important to maintain special functions.

LAT1 requires 4F2 heavy chain (4F2hc, CD98) for its functional expression. Since expression of 4F2hc is basically ubiquitous in various tissues by Northern blot analysis (Yanagida et al., 2001) and LAT1 expression in cancer cells is correlated closely with 4F2hc expression (Kaira et al., 2008), we did not evaluate 4F2hc expression in the present study. High expression of LAT1 in the placenta, kidney and testis is in line with high level 4F2hc expression of these tissues by Northern blot analysis in our previous report (Yanagida et al., 2001).

Considering possible side effects of LAT1 inhibitors, injury to the gastrointestinal tract, renal dysfunction, suppression of bone marrow activity, immune suppression, hypogonadism, sterility, visual disorders, and neural problems all may appear conceivable. In addition, LAT1 transports some drugs which have similar structures to amino acids, such as L-DOPA, gabapentin, α -methyl-DOPA, and thyroid hormone (Kageyama et al., 2000; Gomes and Soares-da-Silva, 2002; Uchino et al., 2002; Kuhne et al., 2007). In this

study, ubiquitous LAT2 expression was confirmed immunohistochemically in the systemic tissues. Additionally, our Northern blot analysis demonstrated significant hybridization signals for LAT2 in many tissues (unpublished data). Repeated injections of our LAT1 inhibitors inhibited tumor cell growth without any side effects in vivo mouse experiment (Oda et al., 2010). Therefore, it may be possible that when LAT1 was blocked, cell nutrition would be preserved by LAT2. Although several factors need to be taken into account with clinical applications of LAT1-related drugs, our present results do provide pointers to important anatomical sites.

In conclusion, this is the first report evaluating LAT1 and LAT2 expression in the systemic human organ, and comparing among adult, child, and fetus. LAT1 has a unique and selective expression in human adult tissues. Its expression is not identical to merely proliferative cells, implying specific roles in adult organs such as endothelial barrier and endocrine character. Stronger LAT1 expression in the fetus indicates an aspect of an oncofetal protein of LAT1.

Acknowledgements. We thank Robert E. Brandt, Founder, CEO, and CME of MedEd Japan, for editing the manuscript.

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Accepted June 27, 2013