学　位　論　文

Immunoreactivity of receptor and transporters for lactate located in astrocytes and epithelial cells of choroid plexus of human brain

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Immunoreactivity of receptor and transporters for lactate located in astrocytes and epithelial cells of choroid plexus of human brain

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Abstract

Glucose metabolism produces lactate and hydrogen ions in an anaerobic environment. Cerebral ischemia or hypoxia is believed to become progressively lactacidemic. Monocarboxylate transporters (MCTs) in endothelial cells are essential for the transport of lactate from the blood into the brain. In addition, it is considered that MCTs located in astrocytic and neuronal cells play a key role in the shuttling of energy metabolites between neurons and astrocytes. However, roles of lactate in the brain remain to be clarified. In this study, the localization of lactate transporters and a receptor for cellular uptake of lactate was immunohistochemically examined in autopsied human brains. Immunoreactivity for MCT1 was observed in the apical cytoplasmic membrane of some epithelial cells in the choroid plexus as well as astrocytes and the capillary wall, whereas that for MCT4 was found in the basolateral cytoplasmic membrane of small number of epithelial cells as well as astrocytes and the capillary wall. In addition, immunoreactivity for the hydroxy-carboxylic acid 1 receptor (HCA1 receptor), a receptor for cellular uptake of lactate, was also found on the basolateral cytoplasmic membrane of epithelial cells as well as astrocytic and neuronal cells. Immunoreactivity for lactate dehydrogenase (LDH)-B was observed in the cytoplasm of epithelial cells in

the choroid plexus as well as astrocytes and the capillary wall.

These immunohistochemical findings indicate the localization of MCT1, MCT4, the HCA1 receptor, and LDH-B in epithelial cells of the choroid plexus as well as astrocytes, and suggest the transport of intravascular lactate into the brain through epithelial cells of the choroid plexus as well as cerebral vessels and the possibility of lactate being utilized in epithelial cells.

INTRODUCTION

Monocarboxylate transporter 1 (MCT1), MCT2, MCT3, and MCT4 are known to catalyze the proton-coupled transport of lactate, pyruvate, and ketone bodies across the plasma membrane of several organs [1,2]. MCT1, MCT2, and MCT4 are widely expressed in the brain [3,4]. In the brain, MCT1 is mainly detected on endothelial cells of the blood-brain barrier in rodents and humans [5,6]. Both MCT1 and MCT4 are known to be involved in lactate release by astrocytes, in which energy supply depends on glycolysis [7-9]. The low-affinity and high-capacity transporter MCT4 conveys lactate from astrocytes to other brain cells. In contrast, MCT2, which is known to be mainly located in neurons, transports lactate as an efficient oxidative energy substrate, and contributes to the establishment of the astrocyte-neuron lactate shuttle concept [10-12]. In this way, the cellular distribution of MCTs in the brain suggests a key role of these transporters in the shuttling of energy metabolites between neurons and astrocytes [10, 12-14]. On the other hand, the expression of MCT3 is restricted to cells in the retinal pigment and choroid plexus epithelium [15,16]. Regarding the expression of MCTs other than MCT3 in the choroid plexus, weak and diffuse staining for MCT1 was reported to be observed in epithelial cells of the rat choroid plexus, although the

immunohistochemical images were not shown [17]. A quantitative targeted absolute proteomics study using the plasma membrane in the isolated choroid plexus of rats and humans showed low-level expression of MCT1 in rats and humans, low-level expression of MCT3 in rats, and very low-level expression of MCT4 in humans [18]. These findings suggest low expression of MCT1 and MCT4 as well as MCT3 in the choroid plexus. In addition, a recent quantitative study involving analysis by quantitative targeted absolute proteomics-based liquid chromatography-tandem mass spectrometry using the porcine choroid plexus showed that MCT1 was expressed in plasma membranes of the porcine choroid plexus [19]. However, it remains unclear, especially in human brains, whether MCT1 and MCT4 are significantly expressed in epithelial cells of the choroid plexus; moreover, it remains to be clarified where they are expressed in cytoplasmic membranes of epithelial cells.

A hydroxy-carboxylic acid 1 receptor (HCA1 receptor), a receptor for cellular lactate, is primarily expressed in white and brown adipocytes [20,21]. Immunohistochemical studies indicated that the HCA1 receptor is highly concentrated in principal neurons, such as hippocampal pyramidal and Purkinje cells, and in interneurons in multiple brain regions, whereas the receptor is also expressed to a lesser extent in astrocytes and the vascular endothelium [16,22]. However, at present, it

remains unclear whether the HCA1 receptor is expressed in epithelial cells of the choroid plexus. Accordingly, in this study, we immunohistochemically examined whether receptors for the cellular uptake of lactate as well as MCTs are localized in epithelial cells of the choroid plexus as well as astrocytes of autopsied human brains.

Materials and Methods

Human brain samples were obtained at autopsy from 6 patients with or without neurological abnormalities in Kagawa University Hospital, as previously reported [23-25]. The main diagnosis of each case was established according to the clinical and autopsy findings shown in Table 1. This study using human brains was approved by the institutional ethics committee of the Faculty of Medicine, Kagawa University. The brains were fixed in 10% formalin and processed for immunohistochemical examination. The brain samples were embedded in paraffin and sectioned at a 4-μm thickness. The antibodies used in this study were mouse antibodies for MCT1 (1:100, Abcam, ab90582) [26], GFAP (1:200, ab10062) [24], and early endosome antigen 1 (EEA1) (1:100, Novus, NBP2-36568) [24] and rabbit antibodies for MCT1 (1:100, Merck, AB3538P) [27], MCT4 (SLC16A3) (1:200, NBP1-81251, Novus Biologicals,

Littleton, CO, USA) [28], MCT4 (1:50, Abcam, ab244385) [29], HCA1 receptor (1:200, NLS-2095, Novus Biologicals) [30], lactate dehydrogenase A (LDH-A) (1:200, Abcam, ab125683) [31], LDH-B (1:200, Abcam, ab75167) [32], GFAP (1:1000, DAKO, Z0334) [24], and EEA1 (1:100, Novus, NBP1-30914) [24]. Before incubation with these antibodies, antigen retrieval was performed by heating sections in 10 mM sodium citrate buffer (pH 6) at 95°C for 20 min. After treatment with hydrogen peroxide and blocking with 2% bovine serum albumin in PBS for 30 min, the sections were incubated with the primary antibodies at 4°C overnight. Staining was achieved with Simple Stain kit (Nichirei, Tokyo, Japan) and developed with 3,3'-diaminobenzidine tetrahydrochloride (DAB) and hydrogen peroxide (Nichirei) at room temperature for 5-7 min. The sections were counterstained with hematoxylin.

For morphometrical analyses, the average numbers of epithelial cells positive for immunostaining of MCT1 (ab90582), MCT4 (NBP1-81251), HCA1-R (NLS-2095), and LDH-B (ab75167) as percentages of the cells examined in ten randomly selected areas were calculated in six human brains.

For fluorescent immunostaining, the sections were incubated overnight with the mouse antibody for MCT1 (ab90582), GFAP (ab10062), or EEA1 (NBP2-36568) and the rabbit antibody for GFAP (Z0334), MCT4 (NBP1-81251 or ab244385), HCA1

receptor (NLS-2095), LDH-B (ab75167), or EEA1 (NBP1-30914), followed by incubation at RT for 60 min in Alexa Fluor 488-anti-mouse IgG and Alexa Fluor 594-conjugated anti-rabbit IgG antibodies (1:200, Molecular Probes, Eugene, OR, USA), respectively. Then, the sections were incubated for 60 min at RT in Monomeric Cyanine Nucleic Acid Stain (TO-PRO-3, Molecular Probes, Eugene, OR, USA), which was diluted to 2.5 μM in PBS. The fluorescent signals were viewed under a confocal microscope (Carl Zeiss LSM700, Oberkochen, Germany).

For confirmation of the specificity of the antibodies used in this study, the antibodies for MCT1 (ab90582), MCT1 (AB3538P), MCT4 (NBP1-81251), MCT4 (ab244385), HCA1-R (NLS-2095), and LDH-B (ab75167) were preincubated with an excess of recombinant peptides or proteins, which were antigens for MCT1 (ab152689), MCT1 (H00006566-P01, Abnova), MCT4 (NBP-1-81251PEP), MCT4 (NBP-1-81251PEP), HCA1-R (amino acid sequence of synthetic peptide; SPSFPKFYNKLKICSLKPK), and LDH-B (ab96765), respectively, at 4°C overnight, according to the procedure reported previously [25]. Then, immunohistochemical examination using the antibodies preincubated with the antigens were performed. In addition, Western blot analysis was also performed using human carcinoma cell lines to confirm the specificity of the antibodies for MCT1 and MCT4. The human cervical

carcinoma cell line (HeLa) and colorectal adenocarcinoma cell line (HT-29) were obtained from the American Type Culture Collection (Manassas, VA, USA). HT-29 cells were maintained in Dulbecco's modified Eagle's medium supplemented with 10% FBS, 100 mU/mL penicillin, and 100 μg/mL streptomycin at 37°C under a 5% CO_2 - 95% atmosphere. Eagle's Minimum Essential Medium supplemented with 10% FBS, 100 mU/mL penicillin, and 100 μg/mL streptomycin was used for HeLa cells. Preparation of whole-cell lysates subjected to Western blot and subsequent Western blot analysis using the antibodies for MCT1 (ab90582) and MCT4 (NBP1-81251) were performed according to the procedure reported previously [33].

RESULTS

Preabsorption of the antibodies for MCT1, MCT4, HCA1 receptor, and LDH-B with their antigens abolished the immunoreactivity on the cytoplasmic membrane of epithelial cells of the choroid plexus (Supplemental Fig. 1A-F). In addition, Western blot analysis with the antibody for MCT1 (ab90582) using human carcinoma cell lines revealed immunoreactive signal bands with molecular masses around 53 and 47 kDa, whereas that for MCT4 (NBP1-81251) revealed an immunoreactive signal band with a

molecular mass around 42 kDa (Supplemental Fig. 1G). Immunohistochemical examination using the antibody for MCT1 (ab90582) showed immunoreactivity not only in granular structures in the cytoplasm of astrocytic cells and cerebral vessel wall, but also in the apical cytoplasmic membrane of some epithelial cells in the choroid plexus of human brains (Fig. 1A, B). Immunoreactivity for MCT1 (AB3538P) was also observed in the apical cytoplasmic membrane of some epithelial cells in the choroid plexus (left inset in Fig. 1B). Immunohistochemical examination using the antibody for MCT4 (NBP1-81251) showed immunoreactivity not only in the cytoplasm of astrocytic cells and cerebral vessel wall, but also in the basolateral cytoplasmic membrane of small number of epithelial cells in the choroid plexus (Fig. 1C, D). Immunoreactivity for MCT4 (ab244385) was also observed in the basolateral cytoplasmic membrane of small number of epithelial cells in the choroid plexus (left inset in Fig. 1D). Immunohistochemical examination using an antibody for the HCA1 receptor showed strong immunoreactivity in neuronal cells located in the hippocampus and subiculum and weak immunoreactivity in the cytoplasm of some astrocytic cells (Fig. 1E). In addition, immunoreactivity for the HCA1 receptor was noted in the basolateral cytoplasmic membrane of epithelial cells in the choroid plexus (Fig. 1F). Immunoreactivity for LDH-B was clearly observed in the cytoplasm of astrocytic cells

and faintly in the vessel wall (Fig. 1G). In addition, immunoreactivity for LDH-B was found in the cytoplasmic membrane and cytoplasm of epithelial cells in the choroid plexus (Fig. 1H). Most epithelial cells in the choroid plexus were not stained with the antibody for LDH-A (data not shown). The average numbers of epithelial cells positive for immunostaining of MCT1, MCT4, HCA1-R, and LDH-B as percentages of the total cells examined are shown in Table 1.

Double immunofluorescence examination using antibodies for GFAP, a marker of astrocytes, and MCT1, MCT4, the HCA1 receptor, or LDH-B was performed. Granular immunoreactivity for MCT1, MCT4, and the HCA1 receptor was partially colocalized with that of GFAP (Fig. 2A-C), whereas immunoreactivity for LDH-B was observed in the cytoplasm of astrocytes showing immunoreactivity for GFAP (Fig. 2D). In addition, granular immunoreactivity for MCT1 and MCT4 was partially colocalized with that for EEA1 (Fig. 2E, F). Granular immunoreactivity for MCT1 was colocalized with that for MCT4 (Fig. 2G). In the choroid plexus, immunoreactivity for MCT1 and MCT4 was observed in the apical and basolateral cytoplasmic membrane of small number of epithelial cells, respectively (Fig. 2H). From the findings in this study, the hypothesized localization of MCT1, MCT4, HCA1-receptor, and LDH-B and movement of lactate in epithelial cells of the choroid plexus are shown in Fig. 2I.

DISCUSSION

In this study, immunohistochemistry was performed using antibodies that had been used for immunohistochemical examination [24,26-30,32]. The immunostaining absorption tests confirmed the specificity of the antibodies used in this study. In addition, Western blot analysis with antibodies for MCT1 (ab90582) and MCT4 (NBP1-81251) using human carcinoma cell lines revealed immunoreactive signal bands with molecular masses compatible with findings reported previously [34,35]. Immunohistochemical findings of MCT1, MCT4, and the HCA1 receptor on astrocytes and endothelial or neuronal cells were consistent with previous results [2-4]. In addition, new findings in the choroid plexus of human brains were found. Immunoreactivity for MCT1, MCT4, and the HCA1 receptor was observed on the apical or basolateral cytoplasmic membrane of epithelial cells of the choroid plexus. However, it is unclear why these transporters and receptors are located in the apical or basolateral cytoplasmic membrane of small number, some, or almost all epithelial cells. Diversity in the ultrastructure and molecular expression among epithelial cells of the choroid plexus has been reported [36,37]. Each epithelial cell of the choroid plexus

might have an individual molecular expression profile to fulfill its function.

It is known that LDH-A is involved in lactate production from pyruvic acid, whereas LDH-B catalyzes the conversion of lactate into pyruvic acid in a metabolic pathway [38]. Expression of LDH-B in cells is convenient to supply energy by metabolizing lactate. Although it had already been reported that the intensity of enzymatic staining showing LDH activity was located in choroid plexus epithelial cells [39], it was unclear whether LDH-B existed in epithelial cells of the choroid plexus. In this study, expression of LDH-B was immunohistochemically confirmed to be localized in the epithelial cells of the choroid plexus as well as in astrocytes. Granular immunoreactivity for MCT1 or MCT4 was partially colocalized with that for EEA1 (Fig. 2E, F). The findings suggest the colocalization of MCT1 and MCT4 in the endosomal membrane of glial cells, as reported in an efflux transporter for iron [24]. Smith et al. [40] reported the internalization of MCT1 from the plasma membrane into early endosomes in rat cerebrovascular endothelial cells. Lauritzen et al. [22] reported the endocytotic internalization of MCTs by lactate through the HCA1 receptor into cytosolic membranes of organelles. Accordingly, analogous internalization of MCTs into endosomal structures depending on the energy supply status may also occur in astrocytes.

The proton and lactate are translocated through MCT1 across the membrane and released on the other side [4]. This may represent either the influx or efflux of lactate depending of the intra- and extracellular lactate concentrations and existing pH gradient across the plasma membrane. MCT4 is considered to be involved in the efflux of lactate from tissues to prevent its intracellular accumulation [41]. On the other hand, it is well-known that GLUT1, a representative transporter of glucose, is located in the basolateral cytoplasmic membrane of epithelial cells in the choroid plexus. In addition, sodium/glucose cotransporter 2 (SGLT2) was recently confirmed to be expressed in epithelial cells of the choroid plexus [25]. Based on these findings, MCTs, GLUT1, and SGLT2 are considered to work cooperatively to transport glucose and lactate in choroid plexus epithelial cells to supply energy. Further studies using brains with an increased lactate utilization state are needed to clarify the significance of MCTs in the choroid plexus. As several kinds of transporters are known to be present in the choroid plexus, the disturbance of the choroid plexus may affect the brain parenchyma in periventricular regions, including the hippocampus, near the choroid plexus and lead to the exacerbation of cognitive dysfunction.

CONFLICT OF INTEREST

There is no conflict of interest.

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References

[1] A.P. Halestrap, The SLC16 gene family – structure, role and regulation in health and disease, Mol. Aspects Med. 34 (2013) 337-349.

[2] T. Iwanaga, A. Kishimoto, Cellular distribution of monocarboxylate transporters: a review, Biomed. Res. 36 (2015) 279-301.

[3] K. Pierre, L. Pellerin, Monocarboxylate transporters in the central nervous system: distribution, regulation and function, J. Neurochem. 94 (2005) 1-14.

[4] N. Vijay, M.E. Morris, Role of monocarboxylate transporters in drug delivery to the brain, Curr. Pharm. Des. 20 (2014) 1487-1498.

[5] J.P. Smith, A.L. Uhernik, L. Li, Z. Liu, L.R. Drewes, Regulation of Mct1 by cAMP-dependent internalization in rat brain endothelial cells, Brain Res. 1480 (2012) 1-11.

[6] A.L. Uhernik, L. Li, N. LaVoy, M.J. Velasquez, J.P. Smith, Regulation of monocarboxylic acid transporter-1 by cAMP dependent vesicular trafficking in brain microvascular endothelial cells, PLoS One 9 (2014) e85957, doi: 10.1371/journal.pone.0085957.

[7] S. Bröer, B. Rahman, G. Pellegri, L. Pellerin, J.L. Martin, S. Verleysdonk, B.

Hamprecht, P.J. Magistretti, Comparison of lactate transport in astroglial cells and monocarboxylate transporter 1 (MCT1) expressing Xenopus laevis oocytes. Expressin of two different monocarboxylate transporters in astroglial cells and neurons, J. Biol. Chem. 272 (1997) 30096-30102.

[8] E. Maekawa, K. Minehira, K. Kadomatsu, L. Pellerin, Basal and stimulated lactate fluxes in primary cultures of astrocytes are differentially controlled by distinct proteins, J. Neurochem. 107 (2008) 789-798.

[9] K. Rostafio, L. Pellerin, Oxygen tension controls the expression of the monocarboxylate transporter MCT4 in cultures mouse cortical astrocytes via a hypoxia-inducible factor-1alpha-mediated transcriptional regulation, Glia 62 (2014) 477-490.

[10] L. Pellerin, G. Pellegri, P.G. Bittar, Y. Charnay, C. Bouras, J.L. Martin, N. Stella, P.J. Magistretti, Evidence supporting the existence of an activity-dependent astrocyte-neuron lactate shuttle, Dev. Neurosci. 20 (1998) 291-299.

[11] L. Pellerin, A.K. Bouzier-Sore, A. Aubert, N. Stella, M. Merle, R. Costalat, P.J. Magistretti, Activity-dependent regulation of energy metabolism by astrocytes: an update, Glia 55 (2007) 1251-1262.

[12] J. Pérez-Escuredo, V.F. van Hée, M. Sboarina, J. Falces, V.L. Payen, L. Pellerin, P.

Sonveaux, Monocarboxylate transporters in the brain and in cancer. Biochim. Biophys. Acta 1863 (2016) 2481-2497.

[13] P.G. Bitter, Y. Chanay, L. Pellerin, C. Bouras, P.J. Magistretti, Selective distribution of lactate dehydrogenase isoenzymes in neurons and astrocytes of human brain, J. Cereb. Blood Flow Metab. 16 (1996) 1079-1089.

[14] L. Pellerin, Brain energetics (thought needs fond). Curr. Opin. Clin. Nutr. Metab. Care 11 (2008) 701-705.

[15] N.J. Philp, H. Yoon, L. Lombardi, Mouse MCT3 gene is expressed preferentially in retinal pigment and choroid plexus epithelia, Am. J. Physiol. Cell. Physiol. 280 (2001) C1319-C1326.

[16] L.H. Bergersen, Lactate transport and signaling in the brain: potential therapeutic targets and roles in body-brain interaction. J. Cereb. Blood Flow. Metab. 35 (2015) 176-185.

[17] L.M. Roberts, D.S. Black, C. Raman, K. Woodford, M. Zhou, L.E. Haggerty, A.T. Yan, S.E. Cwirla, K.K. Grindstaff, Subcellular localization of transporters along the rat blood-brain barrier and blood-cerebral-spinal fluid barrier by *in vivo* biotinylation, Neuroscience 155 (2008) 423-438.

[18] Y. Uchida, Z. Zhang, M. Tachikawa, T. Terasaki, Quantitative targeted absolute

proteomics of rat blood-cerebrospinal fluid barrier transporters: comparison with a human specimen, J. Neurochem. 134 (2015) 1104-1115.

[19] Y. Uchida, R. Goto, H. Takeuchi, M. Łuczak, T. Usui, M. Tachikawa, T. Terasaki, Abundant expression of OCT2, MATE1, OAT1, OAT3, PEPT2, BCRP, MDR1, and xCT transporters in blood-arachnoid barrier of pig and polarized localizations at CSF- and blood-facing plasma membranes, Drug Metab. Dispos. 48 (2020) 135-145.

[20] D.K. Lee, T. Nguyen, K.R. Lynch, R. Cheng, W.B. Vanti, O. Arkhitko, T. Lewis, J.F. Evans, S.R. George, B.F. O'Dowd, Discovery and mapping of ten novel G protein-coupled receptor genes, Gene 275 (2001) 83-91.

[21] S. Offermanns, Hydroxy-carboxylic acid receptor actions in metabolism, Trends Endocrinol. Metab. 28 (2017) 227-236.

[22] K.H. Lauritzen, C. Morland, M. Puchades, S. Holm-Hansen, K. Andersson, E.M. Hagelin, F. Lauritzen, H. Attramadal, J. Storm-Mathisen, A. Gjedde, L.H. Bergersen, Lactate receptor sites link neurotransmission, neurovascular coupling, and brain energy metabolism, Cereb. Cortex 24 (2014) 2784-2795.

[23] R. Murakami, Y. Chiba, K. Tsuboi, K. Matsumoto, M. Kawauchi, R. Fujihara, M. Mashima, K. Kanenishi, T. Yamamoto, M. Ueno, Immunoreactivity of glucose

transporter 8 is localized in the epithelial cells of the choroid plexus and in ependymal cells, Histochem. Cell Biol. 146 (2016) 231-236.

[24] K. Yanase, N. Uemura, Y. Chiba, R. Murakami, R. Fujihara, K. Matsumoto, G. Shirakami, N. Araki, M. Ueno, Immunoreactivities for hepcidin, ferroportin, and hephaestin in astrocytes and choroid plexus epithelium of human brains, Neuropathology 40 (2020) 75-83.

[25] Y. Chiba, Y. Sugiyama, N. Nishi, W. Nonaka, R. Murakami, M. Ueno, Sodium/glucose cotransporter 2 is expressed in choroid plexus epithelial cells and ependymal cells in human and mouse brains, Neuropathology 40 (2020) 482-491.

[26] A. Schutkowski, N. Wege, G.I. Stangl, B. König, Tissue-specific expression of monocarboxylate transporters during fasting in mice, PLoS One 9 (2014) e112118, 2014.

[27] F. Lopes-Coelho, C. Nunes, S. Gouveia-Fernandes, R. Rosas, F. Silva, P. Gameiro, T. Carvalho, M. Gomes da Silva, J. Cabeçadas, S. Dias, L.G. Gonçalves, J. Serpa, Monocarboxylate transporter 1 (MCT1), a tool to stratify acute myeloid leukemia (AML) patients and a vehicle to kill cancer cells, Oncotarget 8 (2017) 82803-82823.

[28] E.S. Silagi, Z.R. Schoepflin, E.L. Seifert, C. Merceron, E. Schipani, I.M. Shapiro,

M.V. Risbud, Bicarbonate recycling by HIF-1-dependent carbonic anhydrase isoforms 9 and 12 is critical in maintaining intracellular pH and viability of nucleus pulposus cells, J. Bone Mineral Res. 33 (2018) 338-355.

[29] K. Duan, Z.J. Liu, S.Q. Hu, H.Y. Huo, Z.R. Xu, J.F. Ruan, Y. Sun, L.P. Dai, C.B. Yan, W. Xjong, Q.H. Cui, H.J. Yu, M. Yu, Y. Qin, Lactic acid induces transport and glycolysis/OXPHOS interconversion in glioblastoma, Biochem. Biophys. Res. Commun. 503 (2018) 888-894.

[30] E.H. Jeninga, A. Bugge, R. Nielsen, S. Kersten, N. Hamers, C. Dani, M. Wabitsch, R. Berger, H.G. Stunnenberg, S. Mandrup, E. Kalkhoven, Peroxisome proliferator-activated receptor δ regulates expression of the anti-lipolytic G-protein-couples receptor 81 (*GPR81/Gpr81*), J. Biol. Chem. 284 (2009) 26385-26393.

[31] L. Yu, Y. Sun, J. Li, Y. Zhu, Y. Shi, X. Fan, J. Zhou, Y. Bao, J. Xiao, K. Cao, P. Cao, Silencing the girdin gene enhances radiosensitivity of hepatocellular carcinoma via suppression of glycolytic metabolism, J. Exp. Clin. Cancer Res. 36 (2017) 110. doi: 10.1186/s13046-017-0580-7.

[32] D. Usoskin, A. Furlan, S. Islam, H. Abdo, P. Lönnerberg, D. Lou, J. Hjerling-Leffler, J. Haeggström, O. Kharchenko, P.V. Kharchenko, S. Linnarsson, P. Ernfors, Unbiased classification of sensory neuron types by large-scale single-cell

RNA sequencing, Nat. Neurosci. 18 (2015) 145-153.

[33] A. Itoh, Y. Nonaka, T. Ogawa, T. Nakamura, N. Nishi, Galectin-9 induces atypical ubiquitination leading to cell death in PC-3 prostate cancer cell, Glycobiology 29 (2019) 22-35.

[34] S. Kershaw, J. Cummings, K. Morris, J. Tugwood, C. Dive, Optimisation of immunofluorescence methods to determine MCT1 and MCT4 expression in circulating tumour cells, BMC Cancer 15 (2015) 387. doi: 10.1186/s12885-015-1382-y.

[35] S. Xu, X. Jiang, Q. Liu, J. Xu, J. Huang, S. Gan, W. Lu, F. Zhuo, M. Yang, S. Sun, Role of rno-miR-124-3p in regulating MCT1 expression in rat brain after permanent focal cerebral ischemia, Gene & Diseases 6 (2019) 398-406.

[36] M.A. Lobas, L. Helper, C.G. Vernon, D. Schreiner, Y. Zhang, M.J. Holtzman, D.R. Thedens, J.A. Weiner, Molecular heterogenity in the choroid plexus epithelium: the 22-member γ-protocadherin family is differentially expressed, apically localized, and implicated in CSF regulation, J. Neurochem. 120 (2012) 913-927.

[37] T.C. Mathew, Diversity in the surface morphology of adjacent epithelial cells of the choroid plexus: an ultrastructural analysis, Mol. Cell Biochem. 301 (2007) 235-239.

[38] T. Duka, Z. Collins, S.M. Anderson, M.A. Raghanti, J.J. Ely, P.R. Hof, D.E. Wildman, M. Goodman, L.I. Grossman, C.C. Sherwood, Divergent lactate dehydrogenase isoenzyme profile in cellular compartments of primate forebrain structures, Mol. Cell Neurosci. 82 (2017) 137-142.

[39] F. Ferrante, F. Amenta, Enzyme histochemistry of the choroid plexus in old rats, Mech. Ageing Dev. 41 (1987) 65-72.

[40] J.P. Smith, A.L. Uhernik, L. Li, Z. Liu, L.R. Drewes, Regulation of Mct1 by cAMP-dependent internalization in rat brain endothelial cells, Brain Res. 1480 (2012) 1-11.

[41] K.S. Dimmer, B. Friedrich, F. Lang, J.W. Deitmer, S. Broer, The low-affinity monocarboxylate transporter MCT4 is adapted to the export of lactate in highly glycolytic cells, Biochem. J. 350 (2000) 210-227.

Figure legends

Fig. 1

Representative microphotographic images of immunoreactivity for MCT1 (A, B, and right inset in B: ab90582, left inset in B: AB3538P), MCT4 (C, D, and right inset of D: NBP1-81251, left inset of D: ab244385), HCA1 receptor (E,F: NLS-2095), and LDH-B (G,H: ab75167) in sections of human brains are shown. The inset of each image shows an enlarged image (A-H). Scale bars indicate 100 μm.

Fig. 2

Representative confocal microscopic merged images (A-D) of glial cells immunostained with anti-MCT1 (A), MCT4 (B), HCA1-R (C), and LDH-B (D) antibodies (visualized as green) and the anti-GFAP antibody (visualized as red) and with nuclear staining by TO-PRO-3 (visualized as blue) are shown. Representative confocal microscopic merged images (E and F) of glial cells immunostained with anti-MCT1 (E) or MCT4 (F) antibodies (visualized as green) and the anti-EEA1 antibody (visualized as red) and with nuclear staining by TO-PRO-3 (visualized as blue) are shown. Representative confocal microscopic merged images (G and H) of glial cells immunostained with the anti-MCT1 antibody (visualized as green) and the anti-MCT4 antibody (visualized as red) and with nuclear staining with TO-PRO-3 (visualized as blue) are shown. The insets of each

image (A-H) show enlarged green, red, and merged images of cells indicated by arrows. Scale bars indicate 50 μm. Hypothesized localization of MCT1, MCT4, HCA1-R, and LDH-B and movement of lactate in the cytoplasm of the choroid plexus epithelium is shown (I).

Supplemental Fig. 1

Validation of the specificity of the antibodies used in the present study. Immunoreactivities of the antibodies for MCT1 (ab90582), MCT1 (AB3538P), MCT4 (NBP1-81251), MCT4 (ab244385), HCA1-receptor, and LDH-B without preabsorption with their antigens are shown in (A-1), (B-1), (C-1), (D-1), (E-1), and (F-1), respectively, whereas immunoreactivities of these antibodies with preabsorption with their antigens are shown in (A-2), (B-2), (C-2), (D-2), (E-2), and (F-2), respectively. Preabsorption of the antibodies with their antigens abolishes the immunoreactivity on the cytoplasmic membrane of epithelial cells in the choroid plexus. The inset of each image shows an enlarged image (A-F). Western blot analyses of MCT1 (ab90582) and MCT4 (NBP1-81251) using human cell lines are shown in (G) and (H), respectively. Whole-cell lysates of HeLa and HT-29 cells were subjected to Western blot analysis. M: molecular weight marker proteins.

Table 1. Summary of clinical profiles and percentage of immuno-positive epithelial cells in the subjects

No.	Age/sex	Main diagnosis	MCT1	MCT4	HCA1R	LDH-B
1	30-39/F	Brainstem hemorrhage	71%	13%	97%	98%
2	70-79/M	Cerebellar tuberculosis, LI, DM	73%	16%	96%	99%
3	60-69/M	Dissecting aneurysm, DM	79%	13%	96%	99%
4	70-79/F	Pneumonia	66%	12%	95%	98%
5	70-79/M	Unstable angina, DM	65%	14%	96%	98%
6	70-79/M	Lung cancer, DM	68%	16%	95%	99%

DM, diabetes mellitus; F, female; LI, lacunar infarction; M, male

Fig. 1

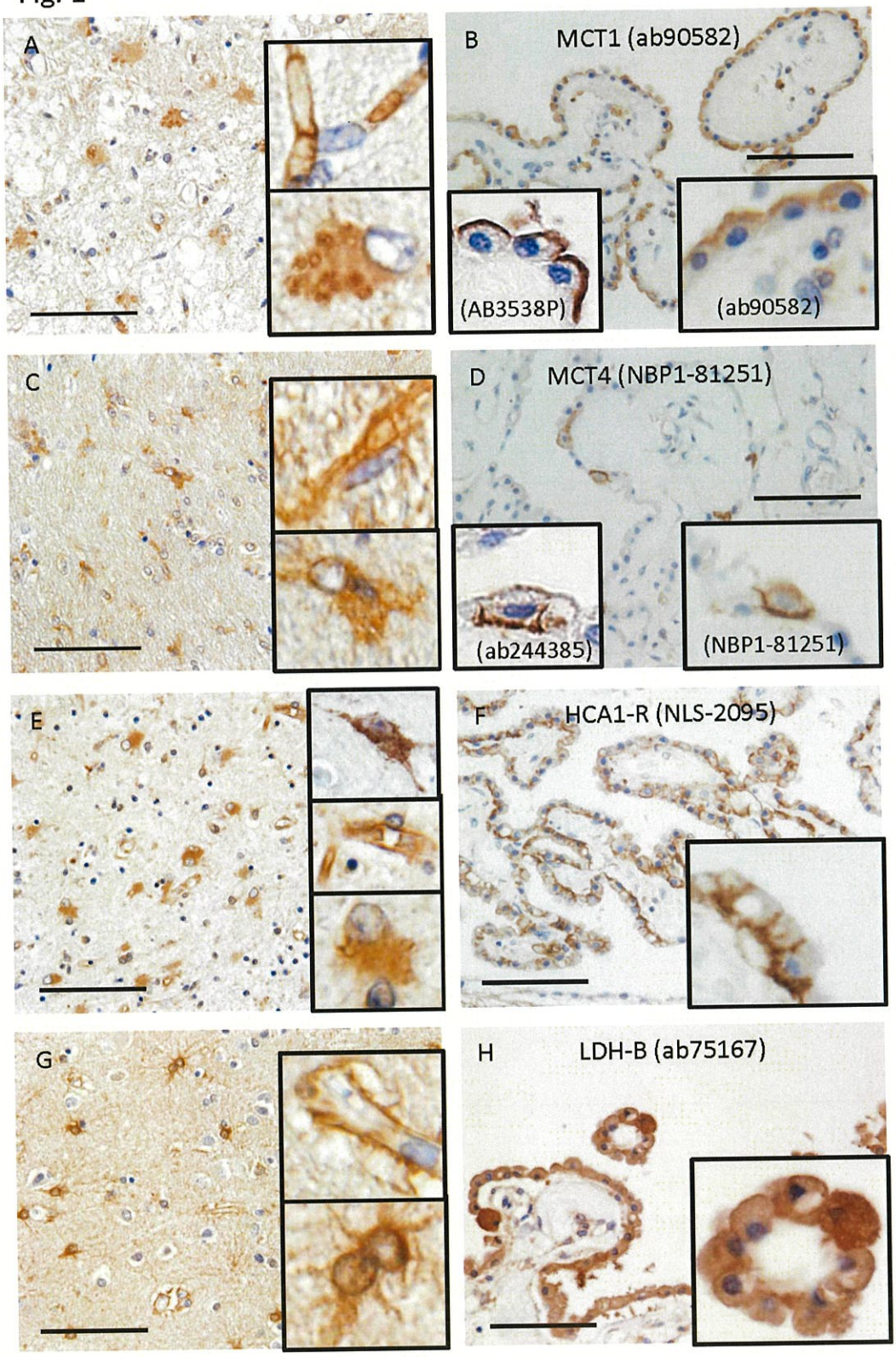
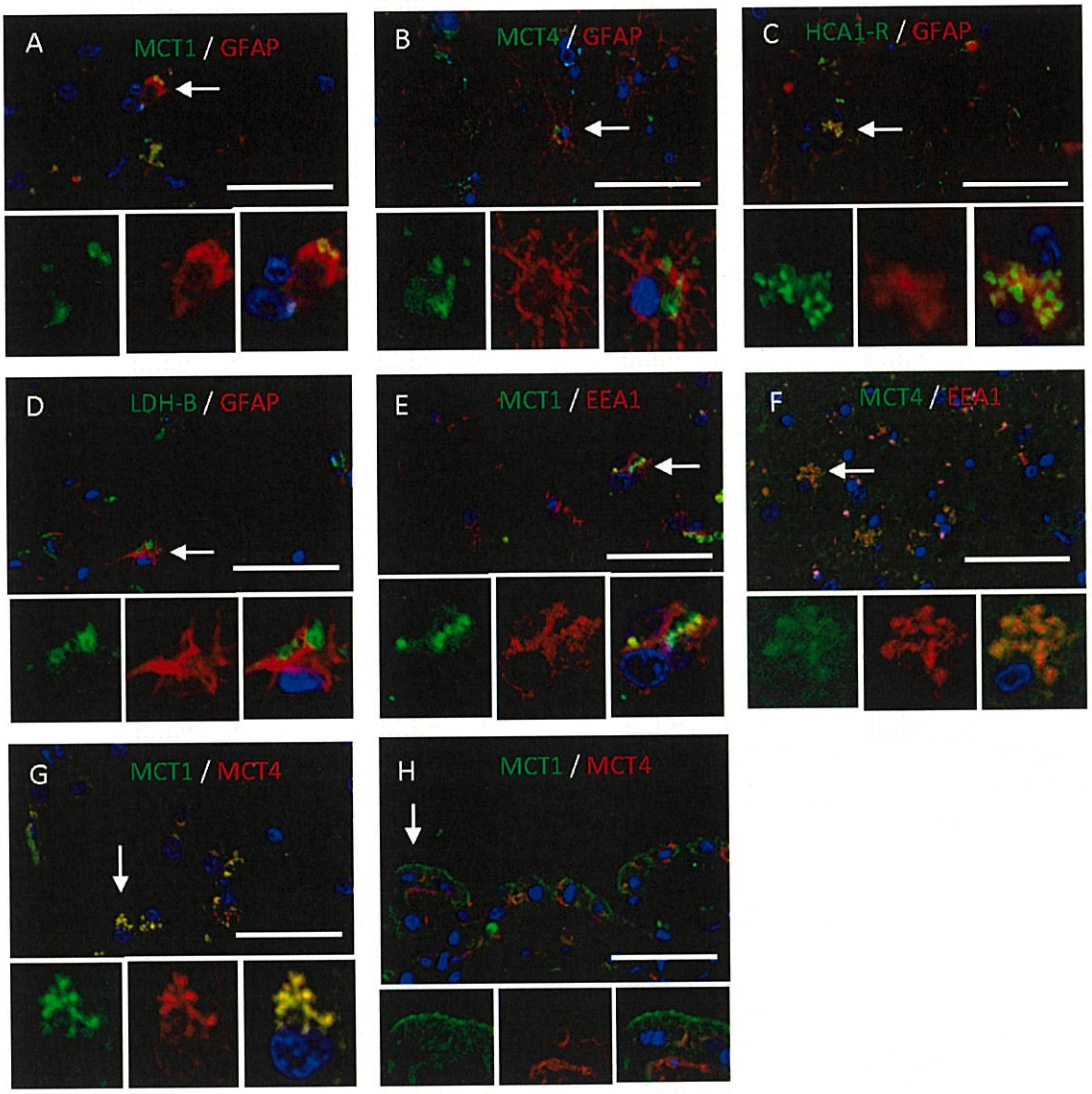
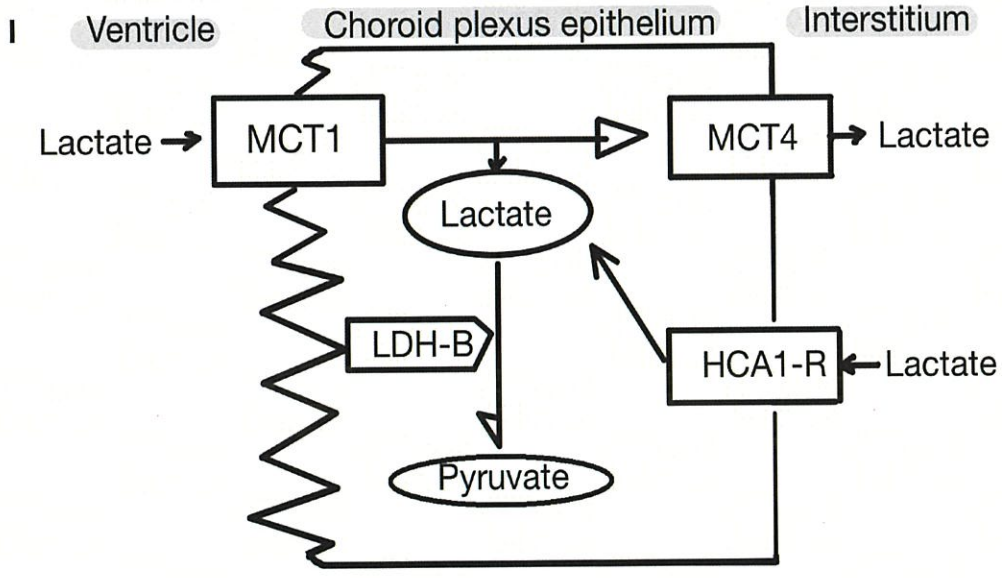

A
B
MCT1 (ab90582)
(AB3538P)
(ab90582)
C
D
MCT4 (NBP1-81251)
(ab244385)
(NBP1-81251)
E
F
HCA1-R (NLS-2095)
G
H
LDH-B (ab75167)

Fig. 2


A
MCT1 / GFAP
B
MCT4 / GFAP
C
HCA1-R / GFAP
D
LDH-B / GFAP
E
MCT1 / EEA1
F
MCT4 / EEA1
G
MCT1 / MCT4
H
MCT1 / MCT4


I
Ventricle
Choroid plexus epithelium
Interstitium
Lactate
MCT1
MCT4
Lactate
Lactate
LDH-B
HCA1-R
Lactate
Pyruvate

Supplemental Fig. 1