

*Short Communication***LAT1 Is a Central Transporter of Essential Amino Acids in Human Umbilical Vein Endothelial Cells**Keitaro Hayashi¹, Promsuk Jutabha¹, Takao Kamai², Hitoshi Endou³, and Naohiko Anzai^{1,*}¹Department of Pharmacology and Toxicology, ²Department of Urology, Dokkyo Medical University School of Medicine, Mibu, Tochigi 321-0293, Japan³J-Pharma Co., Ltd., Yokohama, Kanagawa 230-0046, Japan

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Abstract. Endothelial cell proliferation supporting angiogenesis requires sufficient nutrient supply because of facilitated intracellular metabolism. However, little is known about the mechanism for the promotion of nutrient incorporation in proliferating endothelial cells. Here we show that L-type amino acid transporter 1 (LAT1) is a major transporter of essential amino acids in human umbilical vein endothelial cells (HUVECs). Growing HUVECs express a certain level of LAT1. A LAT1-specific inhibitor suppressed leucine uptake, cell proliferation, and tube formation of HUVECs. Therefore, LAT1 acts to support effective uptake of amino acids, which is critical for the optimal function of HUVECs for angiogenesis.

Keywords: LAT1, amino acid transporter, HUVEC

Angiogenesis is active in tumor tissue, inflamed tissue or fetus, and requires proliferation of endothelial cells (1). Since cell proliferation accompanies facilitated energy consumption and intracellular biosynthesis, maximum incorporation of nutrients is demanded in proliferating cells. Organic acids such as amino acids and glucose are regularly incorporated into cells via their specific transporters. Therefore, proliferating cells must express special transporters that uptake nutrients effectively. However, molecular mechanisms by which endothelial cells proceeding blood vessel expansion promote the incorporation of amino acids have not been determined.

LAT1 (L-type amino acid transporter 1) is a transporter for essential amino acids and expressed in a wide range of cancer cells (2, 3). However, only a few reports have indicated the role of LAT1 in normal cells (4) because of its extremely low expression in normal tissues (5, 6). In this study, we show that LAT1 is a major transporter of essential amino acids in human umbilical vein endothelial cells (HUVECs). HUVECs express a certain level of LAT1 protein. JPH203, a specific inhibitor of LAT1,

suppressed leucine uptake and proliferation of HUVECs. Furthermore, the tube formation of HUVECs on matrigel was prevented by JPH203. Therefore, our results suggest that LAT1 is necessary for human endothelial cells to ensure smooth angiogenesis.

Human umbilical vein endothelial cells were purchased from Lonza (Basel, Switzerland). The cells were cultured in EGM-2 medium (Lonza) including provided growth factors and 2% FCS.

Western blot for LAT1 detection was described previously (7). S2 cells stably transfected with empty vector, human LAT1, or LAT2 were described previously (8).

JPH203 was described previously (8).

[¹⁴C]-L-Leucine uptake was initiated by incubating the cells in HBSS (Invitrogen, Carlsbad, CA, USA) containing 1.0 μM [¹⁴C]-L-leucine (Moravek, Brea, CA, USA) and JPH203 at 37°C for 1 min. Uptake was terminated by washing the cells 3 times with ice-cold HBSS. Cells were lysed with 0.1 N NaOH and radioactivity was measured using an LSC-5100 β-scintillation counter (Aloka, Tokyo).

The tube formation assay was performed by culturing HUVEC at a concentration of 2×10^4 cells per well in a matrigel-coated 96-well plate for 16 h (Becton Dickinson, Franklin Lakes, NJ, USA).

Statistical significance was tested with Student's *t*-test

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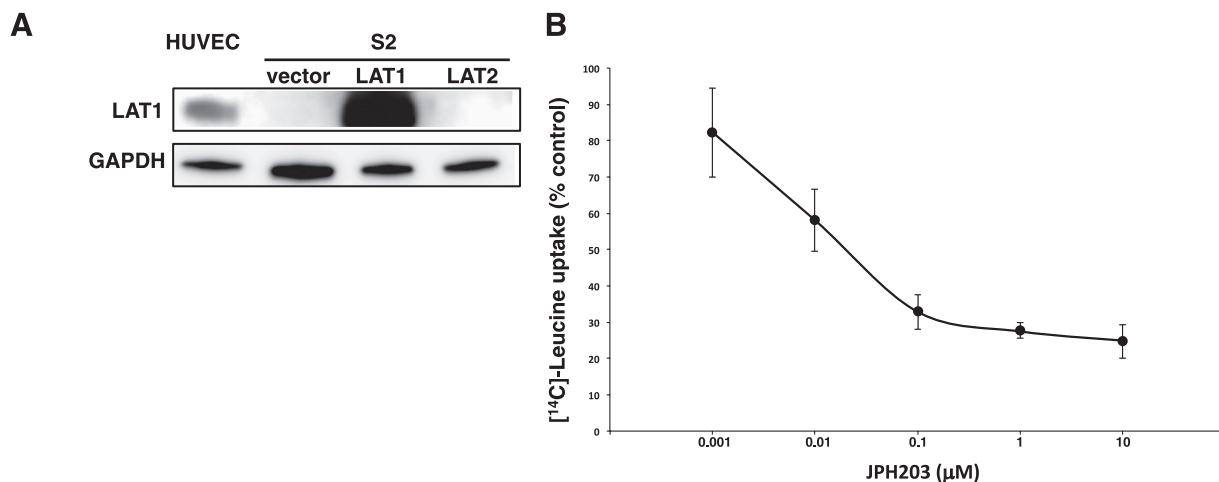


Fig. 1. LAT1 is central transporter of essential neutral amino acids in HUVEC. A) HUVEC (1×10^3 cells) were lysed and LAT1 protein was determined by western blot analysis. For controls, the lysate from S2 cells (1×10^3 cells) introduced with empty vector (negative control), LAT1 (positive control), and LAT2 (negative control) were also used. B) Impaired incorporation of essential neutral amino acid by JPH203. [^{14}C]-L-leucine and JPH203 were added into HUVEC and leucine uptake was determined by measuring radioactivity. Data expressed as the mean \pm S.D.

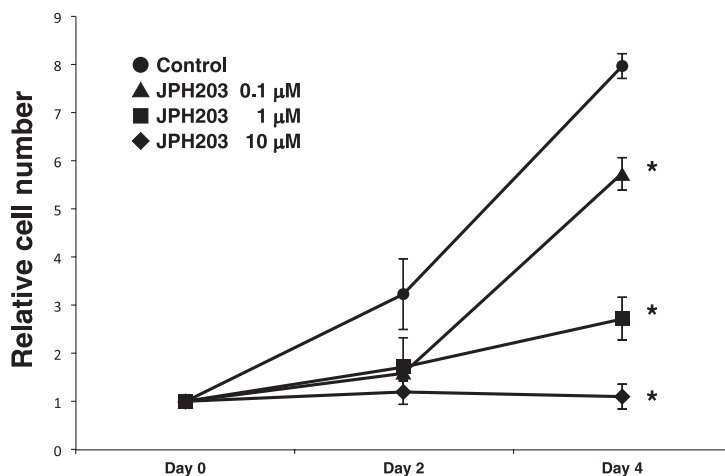


Fig. 2. Effect of JPH203 on the growth of HUVEC. HUVECs were cultured with JPH203 for the indicated days. The number of the cells was counted, and relative cell number to day 1 is shown in the graph. Data expressed as the mean \pm S.D. * $P < 0.01$. The P -value between groups with one-way ANOVA is 0.00002.

by comparing JPH203-treated samples with a control sample. In Figs. 2 and 3, the P -value between groups with one-way ANOVA was also tested.

We analyzed the expression of LAT1 in HUVECs by western blotting. Figure 1A shows that HUVECs express LAT1 protein. This result prompted us to examine the functional importance of LAT1 as a transporter of essential amino acids in these cells. To verify this, we examined the effect of a LAT1-specific inhibitor on the uptake of amino acid in HUVECs. JPH203 is a LAT1-specific inhibitor and we initially tested the influence of JPH203 on the uptake of leucine. HUVEC are incubated with or without JPH203 for 1 min and uptake of [^{14}C]-labeled L-leucine was determined. The uptake of leucine was impaired by JPH203 in a dose-dependent manner (Fig. 1B). This result suggests that LAT1 functions as a

central transporter of essential amino acids in HUVECs.

HUVECs proliferate best when cultured with some growth factors such as VEGF. Since cell growth accompanies the increase of intracellular metabolism which requires high amount of amino acids, we examined the effect of LAT1 inhibition on HUVEC proliferation. The cells were cultured with or without JPH203 and cell number was counted. HUVEC growth was dramatically reduced by JPH203 (Fig. 2). These results indicate that incorporation of a maximum amount of amino acids mediated by LAT1 is necessary for growth of HUVECs.

Next we tested the significance of LAT1 on the angiogenesis in vitro proceeded by HUVECs. We examined three-dimensional structure tube formation assay of endothelial cells using a culture plate coated with matrigel, a commonly used model of angiogenesis in

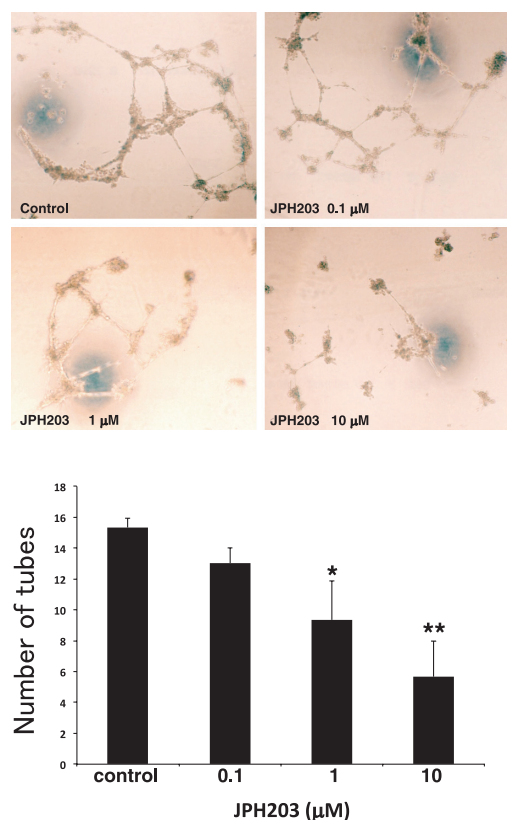


Fig. 3. LAT1 is critical for tube formation of HUVEC in vitro. HUVEC was cultured on a matrigel-coated plate in the presence or absence of JPH203 for 16 h and the number of complete tubes was counted. Data expressed as the mean \pm S.D. * $P = 0.059$, ** $P = 0.015$. The P -value between groups with one-way ANOVA is 0.00085.

vitro. HUVECs were cultured on a matrigel-coated plate in the presence or absence of JPH203 for 16 h and the number of formed tubes within one visual field was assessed by microscopy. The formation of tubes from HUVECs treated with high concentration JPH203 was markedly decreased compared to control cells (Fig. 3). These results indicate that LAT1 is crucial for angiogenesis composed by HUVECs, at least in vitro.

Here we show that LAT1 is a crucial transporter of amino acids for proliferation and tube formation of HUVECs. Our results raise the possibility that the LAT1 inhibitor may act to prohibit angiogenesis in vivo. Of note, the concentration of JPH203 for inhibition of tube formation in vitro is higher than that for growth inhibition of HUVECs. This suggests that the mechanism of functional inhibition of HUVECs by JPH203 is different between physiological phenomena. Nevertheless, our results suggest that a LAT1 inhibitor would be beneficial for the treatment of cancer because active angiogenesis is essential for growing cancer cells to receive adequate nutrients. In addition, LAT1 inhibitor could be a potential drug for treating aberrant angio-

genesis such as diabetic retinopathy. However, we have to consider quite carefully about the utility of LAT1 inhibitor in clinical practice, since we cannot exclude the possibility that LAT1 functions in normal blood vessels. Nonetheless, we presume that the blood vessels in cancer tissue express higher level of LAT1 compared to normal blood vessels, since LAT1 is preferentially expressed in proliferating cells such as cancer and activated T cells (4). If such is the case, JPH203 could be an attractive drug for clinical therapy by selectively preventing angiogenesis in a lesion area but not in normal tissues. In addition, the JPH203 concentration for suppression of HUVEC proliferation is lower than that of cancer cells proliferation (K. Hayashi, unpublished data). This suggests that even though a low concentration of JPH203 cannot kill the cancer cells directly, a tumor could be treatable with a low concentration of JPH203 as it inhibits the growth of blood vessels that feed cancers. Our ongoing experiment of LAT1 expression in blood vessels of normal and cancer tissue would reveal further availability of JPH203 as a clinical drug for treatment of undesirable angiogenesis.

Conflicts of Interest

The authors indicated no potential conflicts of interest.

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