



Contents lists available at ScienceDirect

Journal of Pharmacological Sciences

journal homepage: www.elsevier.com/locate/jphs

Short communication

LAT1 acts as a crucial transporter of amino acids in human thymic carcinoma cells

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ARTICLE INFO

Article history:

Received 28 March 2016

Received in revised form

27 June 2016

Accepted 11 July 2016

Available online xxx

Keywords:

Thymic carcinoma

Anticancer drug

Transporter

ABSTRACT

L-type amino acid transporter 1 (LAT1, SLC7A5) incorporates essential amino acids into cells. Recent studies have shown that LAT1 is a predominant transporter in various human cancers. However, the function of LAT1 in thymic carcinoma remains unknown. Here we demonstrate that LAT1 is a critical transporter for human thymic carcinoma cells. LAT1 was strongly expressed in human thymic carcinoma tissues. LAT1-specific inhibitor significantly suppressed leucine uptake and growth of Ty82 human thymic carcinoma cell lines, suggesting that thymic carcinoma takes advantage of LAT1 as a quality transporter and that LAT1-specific inhibitor might be clinically beneficial in therapy for thymic carcinoma.

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Thymic carcinomas are rare malignant tumors and are often in an advanced stage when detected (1). Although chemotherapy is the mainstay of treatment for thymic carcinomas, patients frequently show resistance to drugs and more efficacious treatment should therefore be established.

LAT1 is a transporter that incorporates essential amino acids into cells. A unique feature of LAT1 is its extremely high expression in many human cancers (2,3), whereas only a small amount of LAT1 is detected in a healthy body (4,5), though LAT1 still has a clear role in normal tissues (6–8).

A LAT1-specific inhibitor has shown powerful suppressive effects on many cancer cell lines *in vitro* and *in vivo* (9) and is currently under evaluation in a clinical trial of cancer patients. Although a wide range of human cancers express LAT1, little is known about LAT1 in thymic carcinomas. In this study, we investigated the role of LAT1 in human thymic carcinoma.

Ty82 human thymic carcinoma cells were purchased from Japanese Collection of Research Bioresources Cell Bank (Ibaraki). The cells were cultured in RPMI1640 containing 10% FCS. S2 cells stably transfected with empty vector, human LAT1 and LAT2 were described previously (9).

Anti-LAT1 antibody (mouse monoclonal) was kindly provided by J-Pharma (Tokyo).

Anti-β-actin antibody (clone C4) was purchased from Merck Millipore (Darmstadt, Germany).

For human tissue staining, surgically excised thymic carcinoma or thymoma was fixed with formalin, embedded with paraffin, and sliced. The samples were stained with anti-LAT1 antibody and detected by DAB. The study using human tissue was approved by Dokkyo Medical University Bioethics Committee.

For western blot analysis, cells were lysed with lysis buffer (50 mM Tris–HCl pH 7.8, 150 mM NaCl, 5 mM EDTA, 1% Tween-20, 10 mM NaF, 1 mM Na₃VO₄, 3 mM β-glycerophosphate, 5 mM pyrophosphate, protease inhibitor cocktail (Roche Diagnostics, Basel, Switzerland)). Protein amount was determined using BCA Protein Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA). Western blot was performed with 3.5 μg of total protein as described previously (10).

JPH203 was described previously (9).

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Peer review under responsibility of Japanese Pharmacological Society.

<http://dx.doi.org/10.1016/j.jphs.2016.07.006>

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[^{14}C]-L-leucine uptake was initiated by incubating the cells in HBSS containing 1.0 μM [^{14}C]-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 0.1N NaOH and radioactivity was measured using an LSC-5100 β -scintillation counter (Aloka, Tokyo).

For apoptosis analysis, the cells were stained with annexin V-FITC and propidium iodide (Medical & Biological Laboratories, Nagoya) and analyzed by FACS (Becton Dickinson, Franklin Lakes, New Zealand).

For cell cycle analysis, the cells were initially fixed with 70% ethanol. After washing the cells with PBS, cells were treated with RNase A (100 $\mu\text{g}/\text{ml}$) for 30 min at 37 °C and further incubated with 2 $\mu\text{g}/\text{ml}$ of propidium iodide for 10 min at room temperature. Cell cycle was analyzed with FACS.

All statistical significance was tested with Student's t-test by comparing JPH203-treated samples with control sample.

To understand the role of LAT1 in thymic carcinoma, we initially analyzed the expression of LAT1 in human thymic carcinoma tissues. Strong expression of LAT1 was detected in thymic carcinoma

(Fig. 1A). On the other hand, LAT1 was hardly found in thymoma (non-cancer) tissue (Fig. 1A). We next examined LAT1 expression in Ty82 human thymic carcinoma cell line. Ty82 cells expressed LAT1 at a high level (Fig. 1B). These results indicate that LAT1 expression is facilitated in human thymic carcinoma cells.

To investigate the functional significance of LAT1 in thymic carcinoma, we assessed the effects of JPH203, a LAT1-specific inhibitor, on the incorporation of amino acids in Ty82 cells. The cells were incubated with or without JPH203 for 1 min and uptake of [^{14}C]-labeled L-leucine was analyzed. JPH203 impaired the uptake of leucine in a dose-dependent manner (Fig. 2). These results indicate that LAT1 is crucial transporter of amino acids in Ty82 cells.

We next examined the effect of a LAT1-specific inhibitor on growth of Ty82 cells. The cells were cultured in the presence or absence of JPH203 and the number of cells was counted. The number of Ty82 cells was clearly reduced by JPH203 (Fig. 3A). To determine whether the reduction of Ty82 cells by JPH203 arises from aberrance of the cell cycle or cell death, we first analyzed the survival rate of Ty82 cells treated with JPH203 by annexin V and

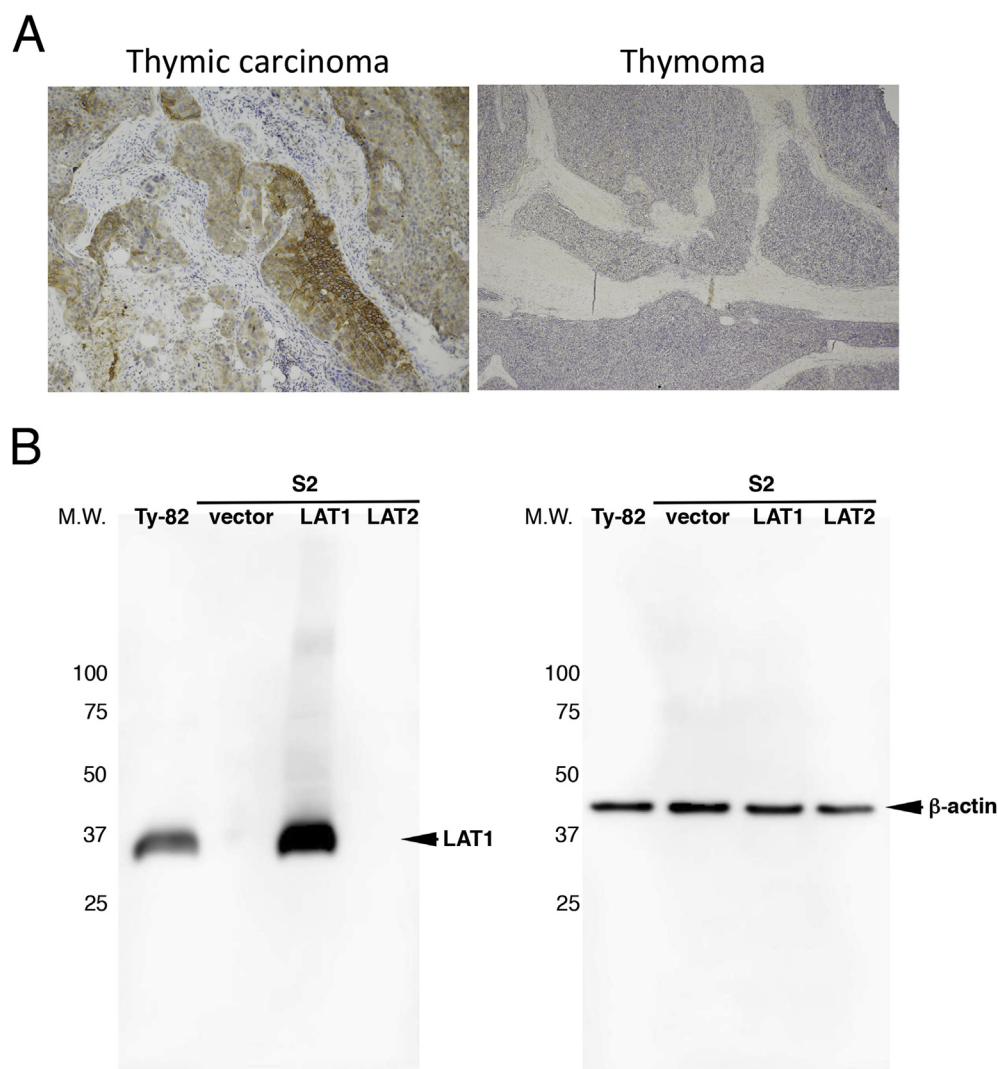


Fig. 1. LAT1 is a critical transporter of amino acids in thymic carcinoma cells. (A) Tissue staining of human thymic carcinoma (left) and thymoma (right) with anti-LAT1 antibody. (B) Ty82 cells were lysed and LAT1 protein was detected by western blot. As controls, lysates from S2 cells stably transfected with an empty vector (negative control), LAT1 (positive control), and LAT2 (negative control) were also used.

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