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Original Article

Expression of L-type amino acid transporter 1 in various skin lesions

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ABSTRACT

L-type amino acid transporter 1 (LAT1) is a Na^+-independent neutral amino acid transporter that has an essential role in cell proliferation. Although the involvement of LAT1 in human carcinogenesis has been investigated by immunohistochemistry in various organs, LAT1 expression in skin has not been reported yet. Therefore, in the present study, immunohistochemistry for LAT1 was performed in 15 keratoacanthoma (KA), 10 seborrheic keratosis, 16 Bowen's disease, 11 basal cell carcinoma (BCC), and 9 squamous cell carcinoma (SCC) cases as well as 61 normal epidermis as control. It was demonstrated that LAT1 expression limited to the basal layer was occasionally observed in normal epidermis while its expression was significantly decreased in the epithelium of seborrheic keratosis and Bowen's disease ($P < 0.05$). By contrast, a significantly higher rate of LAT1 expression was observed in the epithelium of KA, BCC, and SCC than in normal epidermis ($P < 0.05$). Although LAT1 expression was limited to the basal layer or rim of the nests in KA, LAT1 expression was also observed in the center of the nests in BCC and SCC ($P < 0.001$). Thus, LAT1 is differentially expressed in various skin lesions and may be an especially useful marker to distinguish KA from SCC.

Introduction

L-type amino acid transporter 1 (LAT1) is a Na^+-independent neutral amino acid transporter first cloned from rat glioma cells [11], which mediates amino acid uptake across the plasma membrane [3,11,29]. LAT1 plays a critical role in proliferation, since it preferentially transports large neutral amino acids including the essential ones [11,27]. LAT1 mRNA expression is normally restricted to organs such as the brain, thymus, placenta, and testis in humans, but LAT1 has been found to be highly expressed in various tumor cell lines [27].

Immunohistochemical analysis of LAT1 expression in normal squamous epithelium as well as squamous cell carcinoma (SCC) of the esophagus and oral mucosa has previously been reported [13,14]. Furthermore, we have recently reported differential LAT1 expression in uterine cervical carcinogenesis [25]. However, to our knowledge, LAT1 expression in normal and diseased skin tissues has not been reported yet. Therefore, the present study was designed to assess LAT1 expression in normal epidermis as well as epithelium of various skin diseases.

In this manuscript, we focused on 4 major topics: (1) the influence of aging on LAT1 expression in normal epidermis, which has been reported in normal muscle [5]; (2) whether LAT1 expression gradually increases from normal epidermis through dysplastic epithelium to invasive SCC as reported in oral tissue [13], or decreases in dysplastic epithelium and carcinoma in situ compared with normal epithelium and invasive SCC as shown in the uterine cervix [25]; (3) the usefulness of LAT1 immunostaining in differentiating between keratoacanthoma (KA) and SCC, which is often difficult [12]; and (4) the comparison of LAT1 expression with cell proliferation by Ki-67 immunostaining. Here, we show that immunostaining for LAT1 in various skin lesions provides solutions to these biological questions.

Material and methods

Patients and tissues

In the present study, resected specimens were taken by standard procedures from all analyzed patients. After obtaining consent

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from patients, the tissues were used for diagnostic work-up and transferred to archival files at Kyorin University Hospital, Tokyo, according to the data and ethical protection rules of the Medical Faculty. Included in the present study were all the excised specimens diagnosed with seborrheic keratosis ($n = 10$), Bowen's disease ($n = 16$), KA ($n = 15$), SCC ($n = 9$), and basal cell carcinoma (BCC, $n = 11$) by two experienced pathologists between 2007 and 2010. These patients ($n = 61$) included 32 males and 29 females (33–97 years old [y.o.], mean 71.2 ± 13.6 y.o.). All the specimens contained normal epidermis. All the tissues were fixed by immersion in 10% formalin for 24 h and embedded in paraffin using standard techniques. One representative diseased tissue sample as well as normal epidermis from each case was selected for the immunohistochemical study.

Immunohistochemistry

LAT1 expression was determined by immunohistochemistry with an affinity-purified polyclonal rabbit anti-human LAT1 antibody [27]. Briefly, an oligopeptide corresponding to amino acid residues 497–507 of human LAT1-C (CQKLMQVVPQET) was synthesized, a C-terminal cysteine residue was introduced for conjugation with keyhole limpet hemocyanin, and anti-peptide antibody was produced as described elsewhere [3]. Specificity of the immunoreaction including immunoabsorption testing was established in a previous study [20], and the same antibody was used in the present study. This antibody is now commercially available (J-Pharma, Tokyo, Japan). However, to verify the specificity of immunoreactions in normal skin by immunoabsorption experiments, tissue sections were treated with primary antibody in the presence of antigen peptide (200 μg/ml).

Immunohistochemical staining was performed on paraffin sections using a polymer peroxidase method (Signet's ACUITY detection system, Signet Laboratories/Covance, Dedham, MA, USA). Briefly, deparaffinized and rehydrated 3 μm sections were treated with 3% hydrogen peroxide in methanol for 15 min to block endogenous peroxidase activity. To expose antigens, sections were autoclaved in EDTA buffer (pH 9.0) for 5 min and cooled for 30 min. After rinsing in 0.05 M Tris-buffered saline containing 0.1% Tween-20 (pH 7.6), the sections were incubated with affinity purified anti-LAT1 antibody (1:600) overnight at 4 °C. Horseradish peroxidase labeled polymer conjugated to mixture of goat anti-mouse and anti-rabbit immunoglobulin antibody was used as secondary antibody. The peroxidase reaction was performed using 0.02% 3,3′-diaminobenzidine tetrahydrochloride and 0.01% hydrogen peroxide in 0.05 M Tris–HCl buffer (pH 7.4). Finally, nuclear counterstaining was performed with Mayer's hematoxylin. As negative control, the incubation step with the primary antibody was omitted. Immunostaining for anti-Ki-67 (MIB-1, 1:200, Dako, Glostrup, Denmark) was also performed in the same manner except antigen retrieval with citrate buffer solution (pH 6.0) was used.

Interpretation of immunohistochemical results

Light microscopy performed with a Carl Zeiss HAL 100 instrument and W-PI 10×/23 ocular lens was used to analyze and quantitate the immunohistochemical data. In assessing LAT1-positive epithelial cells, positivity was given only to those cells showing linear cytomembranous staining, since it has been established that LAT1 functions only when it covalently binds to the membrane-associated heavy chain of the 4F2 antigen (CD98) [9,15,20]. Therefore, granular cytoplasmic staining was regarded as non-specific staining. Indeed, in the immunoabsorption experiments, granular cytoplasmic staining remained, although cytomembranous staining was diminished (Fig. 1). To assess LAT1 expression in normal epidermis, the average counts of LAT1-positive basal cells per total analyzed basal cells (LAT1 expression ratio) were calculated in at least 3 separate regions of 1 mm longitudinal length in each specimen. In diseased epithelium, at least 5 sites of 1/16 mm^2 squares were chosen, and LAT1-positive cell counts per total analyzed cell counts (LAT1 expression ratio) were calculated at the basal layer or rim of the invasive islands (nests) in each specimen, although the latter was performed to check whether or not LAT1 expression was preserved in the center of the invasive islands. For Ki-67 in epithelial cells, patterns of positive nuclear staining were assessed as either peripheral (positive nuclear staining limited to the basal and parabasal layers) or diffuse as well as scattered or confluent. Furthermore, immunopositive cell area was compared between LAT1 and Ki-67 immunostainings.

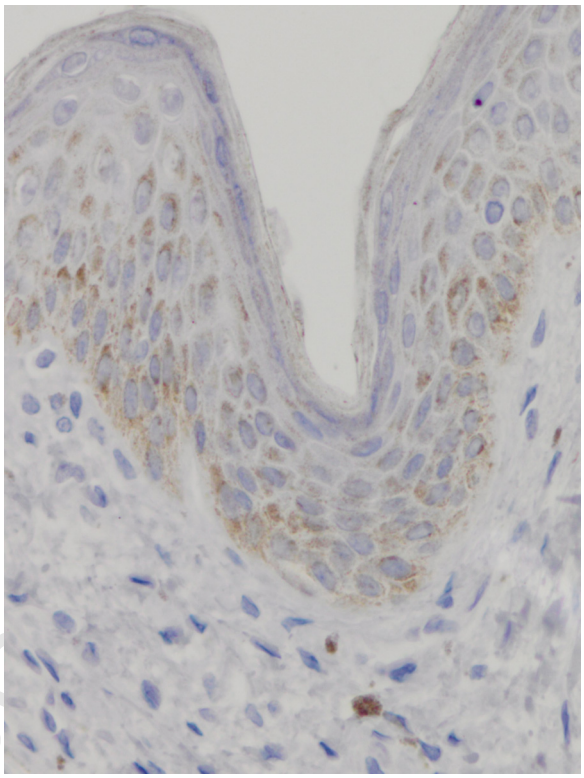

Fig. 1. In the immunoabsorption experiments, the cytomembranous LAT1 immunostaining in the epidermis was diminished, while granular cytoplasmic staining was retained.

Statistical analysis

Based on the LAT1-positive cell ratios described above, statistical analyses using Pearson product–moment correlation was performed with the SPSS software package (standard version, release 10.0.7J). LAT1-positive cell ratio in normal epidermis was compared with the patients' age and LAT1-positive cell ratio in each disease group. Average LAT1-positive cell ratio between males and females, or between resection from face and extrafacial sites was examined by Student's *t*-test. Furthermore, Fisher's exact probability test was used, when necessary, to compare presence or absence of LAT1 expression in the center of the nests in different disease groups. The level of significance was set at $P < 0.05$.

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