Upregulated expression of glucose transporter isoform 1 in invasive and metastatic extramammary Paget's disease

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Abstract. Glucose transporter isoform 1 (GLUT1), which is upregulated in a variety of malignant tumors, facilitates cellular glucose uptake to boost rapid tumor growth and progression. In several types of cancer, inhibition of GLUT1 suppresses tumor proliferation and metastasis, indicating that GLUT1 is a potential target of anticancer therapy. The present study performed immunohistochemistry to analyze GLUT1 expression levels in 51 patients with extramammary Paget's disease (EMPD), including 23 with only intraepidermal lesions and 28 with dermal-invasive lesions. Of the 28 patients with dermal invasion, nine had available samples of lymph node metastasis. GLUT1 staining scores were significantly higher in dermal-invasive (P<0.0001) and metastatic lesions (P=0.0008) compared with in intraepidermal lesions. GLUT1 is upregulated during the transition from preinvasive to invasive or metastatic tumor in EMPD. Moreover, GLUT1 staining scores were statistically higher in intraepidermal tumor cells of dermal-invasive EMPD compared with tumor cells of only *in situ* EMPD (P=0.0338). GLUT1 is upregulated even during the preinvasive phase in patients with invasive EMPD, suggesting that GLUT1 immunostaining can predict the risk of dermal invasion. The present study provides novel evidence to pursue *in vitro* and *in vivo* studies to confirm that upregulated expression of GLUT1 enhances tumor aggressiveness in EMPD.

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Abbreviations: EMPD, extramammary Paget's disease; GLUT1, glucose transporter isoform 1

Key words: extramammary Paget's disease, glucose transporter, GLUT1, invasion, metastasis, skin cancer

Introduction

Glucose transporter isoform 1 (GLUT1) is expressed in a variety of human and animal tissues such as blood vessels, muscles, liver, and skin (1). It plays an important role in transporting glucose into the cytoplasm (1). Various malignant tumors upregulate the expression of GLUT1 to facilitate cellular glucose uptake to boost their rapid growth and progression (2-5). The expression of GLUT1 is associated with [^{18}F]-2-fluro-2-deoxy-D-glucose uptake in positron emission tomography in various types of cancers such as cholangiocellular carcinoma (6) and ovarian cancer (7). Furthermore, GLUT1 expression is associated with tumor proliferation, angiogenesis and survival; GLUT1 is regarded as a prognostic marker in several types of cancer (2,5,8). Baer *et al* (9) investigated GLUT1 expression in several types of skin tumors. This study showed positive staining in cutaneous squamous cell carcinoma. By contrast, GLUT1 staining was negative in benign nevi and seborrheic keratosis (9). Other studies have reported that metastatic melanoma lesions have stronger GLUT1 immunostaining compared with primary lesions (10,11). In addition, GLUT1 suppression significantly inhibits melanoma cell growth and hepatic metastases in mouse models (10).

Although the association between GLUT1 expression levels and tumor aggressiveness has been investigated in a variety of cancers, to the best of our knowledge, it has not been studied in extramammary Paget's disease (EMPD). Once EMPD tumor cells invade the dermis, they frequently metastasize to lymph nodes and other organs, resulting in a worse prognosis (12). Thus, the present study investigated the relationship between GLUT1 expression levels and the degree of tumor progression in EMPD.

Materials and methods

Patient samples. All samples used in this study were obtained from patients who underwent surgery or biopsy in the Department of Dermatology, Hirosaki University Hospital between 2005 and 2018. All patients with EMPD whose tumor samples were available were included in this study. The relationship between GLUT1 expression levels and the degree of tumor progression was investigated in 51 patients with EMPD,

including 23 with only intraepidermal lesions and 28 with dermal-invasive lesions. Among 51 patients, 27 (52.9%) were females and 24 (47.1%) were males. Age ranged from 51 to 93 years (median, 73 years) (Table SI). Of 28 patients with dermal invasion, 13 had lymph node metastasis. Overall, nine of the patients had available samples of lymph node metastasis (Table I). The present study was approved by the institutional review board of Hirosaki University Graduate School of Medicine (Hirosaki, Japan; approval no. 2021-116). This study was conducted in accordance with the ethical principles of the Declaration of Helsinki.

Immunohistochemistry. Tumor tissue samples were fixed in 10% buffered formalin for 24-48 h at room temperature. GLUT1 immunohistochemistry was performed on formalin-fixed, paraffin-embedded samples using anti-GLUT1 primary antibody (ready-to-use solution; cat. no. #760-4526; Roche Diagnostics). Briefly, tissue samples were cut into 5-μm thick sections and deparaffinized using xylene and a graded alcohol series. For antigen retrieval, sections were autoclaved in 10 mM sodium citrate buffer (pH 6.0) for 10 min at 125˚C. These sections were washed with distilled water three times and incubated in 0.3% H_2O_2 at room temperature for 10 min to block endogenous peroxidase activity. Sections were incubated with primary antibody at 4˚C overnight. Next, the sections were incubated with secondary antibody (anti-rabbit Poly-HRP-IgG, ready-to-use solution, BOND Polymer Refine Detection Kit, cat. no. DS9800; Leica Biosystems) at room temperature for 30 min. The sites of GLUT1 localization were visualized with diaminobenzidine. Sections were counterstained with hematoxylin for 10-20 sec at room temperature for microscopic examination. A total of five random fields of view on each slice were evaluated under high magnification (magnification, x400) using a light microscope (BX43; Olympus Corporation). Staining intensity was categorized as negative, weak or strong with reference to the immunostaining of erythrocytes, which were scored as strong, as described previously (Fig. 1) (10). The percentage of positively stained cells was rated using a semiquantitative scale as 0-10%, 11-50% or 51-100%. Staining results were scored from 0 to 4 according to a previously reported scoring system based on the intensity and percentage of positively stained cells (Table II) (6). Quantification was performed by two independent investigators in a blinded manner. Staining results were independently scored for intraepidermal, dermal-invasive and metastatic lesions in each sample.

Statistical analysis. The Wilcoxon matched-pair signed-rank test was used for comparison between intraepidermal and dermal-invasive lesions. One-way analysis of variance with Friedman's test and Dunn's post-hoc multiple comparisons test were used for comparisons of intraepidermal lesions vs. dermal-invasive lesions vs. metastatic lesions. The Mann-Whitney U test was used to analyze the significance of the differences between patients with only intraepidermal lesions vs. those with both intraepidermal and dermal-invasive lesions. Multiple logistic regression was performed to assess the relationship between GLUT1 scores and dermal invasion. There was adjustment for age and sex as confounding factors. Statistical analyses were performed with GraphPad Prism software, version 8.4.3 (Dotmatics). $P<0.05$ was considered to indicate a statistically significant difference.

Results

GLUT1 staining scores are higher in dermal-invasive lesions compared with intraepidermal lesions. GLUT1 staining in intraepidermal tumor nests was faint compared with staining in surrounding normal keratinocytes (Fig. 2, upper panels). GLUT1 staining was stronger in dermal-invasive cells compared with in intraepidermal tumor cells. GLUT1 staining was positive in both the plasma membrane and cytoplasm (Fig. 2, middle panels, black arrow). Among patients who had dermal-invasive EMPD, GLUT1 staining scores were statistically higher in invasive lesions compared with those in intraepidermal lesions (n=28; $P<0.0001$; Fig. 3A). Multiple logistic regression analysis revealed that GLUT1 staining scores were associated with dermal invasion after adjusting for age and sex (odds ratio, 2.399; 95% confidence interval, 1.149-5.612; P=0.0282).

GLUT1 staining scores are higher in metastatic lesions compared with intraepidermal lesions. In most of the current metastatic samples, GLUT1 staining was strong, particularly in the plasma membrane and the cytoplasm (Fig. 2, lower panels). Among patients from whom intraepidermal, dermal-invasive and metastatic samples were all available, GLUT1 staining scores were significantly higher in the metastatic lesions compared with the intraepidermal lesions (n=9; P=0.0008; Fig. 3B). Because of the small sample size, survival analysis was not performed. Among nine patients with lymph node metastases available for analysis, at least five patients died of EMPD progression; four of these five patients had metastatic lesions with GLUT1 staining scores of 4 (Table I). On the other hand, a patient who achieved 5-year recurrence-free survival after lymph node dissection had a GLUT1 score of 2, even in metastatic lesions (patient 9).

Patients with invasive EMPD had higher intraepidermal tumor cell GLUT1 staining scores compared with patients with only in situ EMPD. The present study compared GLUT1 staining scores of intraepidermal lesions between EMPD lesions with vs. without dermal invasion (i.e., intraepidermal lesion only) to evaluate whether GLUT1 expression was upregulated in preinvasive lesions. GLUT1 scores were significantly higher in intraepidermal tumor cells of dermal-invasive EMPD (n=28) compared with tumor cells of only *in situ* EMPD (n=23) (P=0.0338; Fig. 3C).

GLUT1 staining scores in EMPD are independent of glycometabolism. The present study evaluated the association between diabetes mellitus and GLUT1 staining scores because GLUT1 was reported to be upregulated by blood glucose and insulin (13). The GLUT1 scores of intraepidermal tumors were compared between patients with diabetes (n=10) and without diabetes (n=39) (Table SI). Two patients were excluded because of missing data. There were

Table I. Outcomes and GLUT1 staining scores in patients who have EMPD with lymph node metastasis.

				GLUT1 staining score		
Patient no.	Age (years)	Sex	Outcome	Intraepidermal lesion	Invasive lesion	Metastatic lesion
3	70	M	Died of EMPD	1	4	4
6	66	F	Died of EMPD	2	3	4
7	79	F	Died of EMPD	0	1	NA
8	70	F	Survived for 3 years after surgery without recurrence	1	4	NA
9	78	M	Completed 5-year follow-up without recurrence	1	2	2
10	51	F	NA	0	0	4
11	76	M	NA	1	2	2
12	63	F	Died of EMPD	0	0	4
13	82	F	NA	0	1	3
14	67	M	Died of EMPD	1	3	4
20	74	M	Died of EMPD	0	1	4
26	66	M	NA	1	4	NA
27	77	F	NA	2	2	NA

GLUT1, glucose transporter isoform 1; EMPD, extramammary Paget's disease; F, female; M, male; NA, data not available.

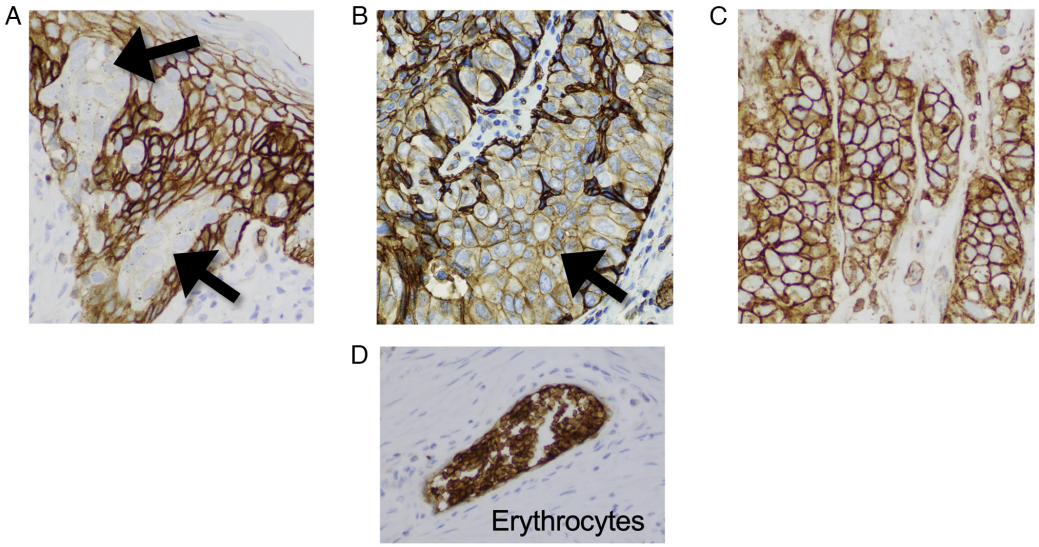

Figure 1. Representative images of three different GLUT1 staining intensities in EMPD. (A) Negative staining in intraepidermal tumor nests (arrows); (B) weak staining in intraepidermal tumor nests (arrow); and (C) strong staining in dermal invasive tumor cells. Strongly stained cells in (A) and (B) are epidermal keratinocytes. (D) Strong staining of GLUT1 in erythrocytes was used as an internal control. Magnification, x200 for (A)-(C) and x400 for (D). EMPD, extramammary Paget's disease; GLUT1, glucose transporter isoform 1.

no significant differences in GLUT1 staining scores between the two groups (P=0.858; Fig. 3D). This finding suggests that GLUT1 staining scores in EMPD were independent of glycometabolism.

Discussion

In the present immunohistochemical analyses, GLUT1 was upregulated during the transition from preinvasive tumor to invasive or metastatic tumor in EMPD. GLUT1 was already upregulated even during the preinvasive phase in patients with invasive EMPD, suggesting that GLUT1 immunostaining can predict the risk of dermal invasion. Collectively, the expression of GLUT1 is upregulated in EMPD tumor cells that have more aggressive and malignant features. However, the number of patients was not large enough to perform a statistical analysis of prognosis. Thus, further studies with larger cohorts and longer follow-up periods are needed to confirm that GLUT1 expression levels are associated with prognosis in patients with EMPD.

Table II. Scoring system for GLUT1 immunohistochemistry.

	Staining intensity	
Stained cells (%)	Weak	Strong
0-10	0	2
11-50	1	3
51-100	2	4

GLUT1, glucose transporter isoform 1.

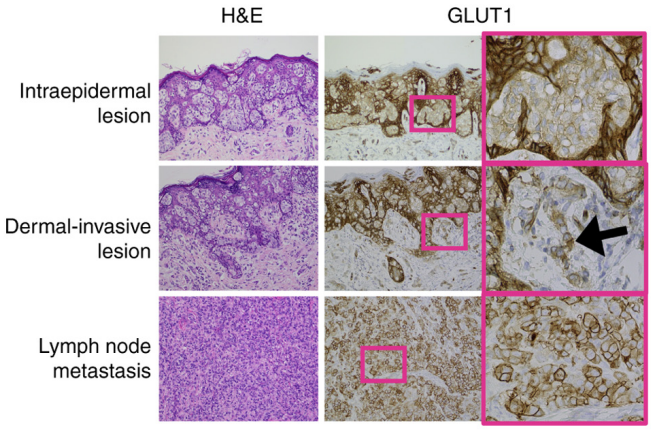

Figure 2. Immunohistochemical staining for GLUT1 in EMPD. Representative images of intraepidermal lesions (upper panels), dermal-invasive lesions (middle panels) and lymph node metastasis (lower panels). The right column demonstrates enlarged images of the indicated area in the images of the middle column. The black arrow indicates tumor cells that have invaded the dermis. Magnification, x400 for the right panels and x100 for the middle and left panels. EMPD, extramammary Paget's disease; GLUT1, glucose transporter isoform 1; H&E, hematoxylin and eosin.

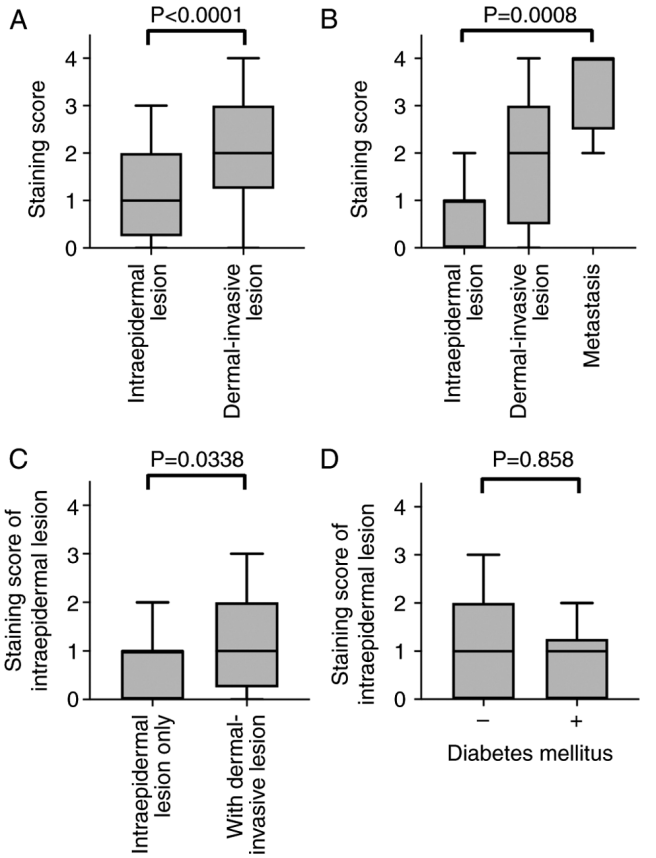

Figure 3. GLUT1 staining scores in dermal-invasive or metastatic EMPD. (A) GLUT1 staining scores were significantly higher in dermal-invasive lesions compared with the intraepidermal lesions (n=28). (B) GLUT1 staining scores were significantly higher in metastatic lesions compared with that in the intraepidermal lesions of the same patient (n=9). (C) GLUT1 staining scores were significantly higher in intraepidermal lesions in patients with invasive tumors (n=28) compared with in patients with only intraepidermal tumors (n=23). (D) GLUT1 staining scores were similar in patients with (n=10) and without (n=39) diabetes mellitus. GLUT1, glucose transporter isoform 1.

Previous studies have reported that metastatic melanoma lesions have stronger GLUT1 immunostaining than primary lesions (10,11). In addition, shRNA-suppression of GLUT1 inhibits melanoma cell growth and hepatic metastases in mouse models (10). Treatment with a specific small molecule inhibitor of GLUT1, WZB117, caused a dose-dependent reduction in glucose consumption, proliferation and apoptosis resistance in melanoma and lung cancer cell lines (10,14). Furthermore, GLUT1 plays a role in the development of resistance to anticancer agents in some types of cancer such as laryngeal cancer and breast cancer (15,16). Inhibition of GLUT1 using the natural flavonoid apigenin results in overcoming chemoresistance to cisplatin (15,17). Based on these data, GLUT1 is a promising target molecule for personalized therapeutic approaches to treating patients with invasive or metastatic EMPD.

To the best of our knowledge, this is the first report on GLUT1 upregulation in invasive and metastatic EMPD. The current study has several limitations. This is a retrospective study. In addition, information on follow-up and comorbidities was not available for several patients. Thus, the present study was not able to perform survival analyses. The data provides new evidence to pursue future *in vitro* and *in vivo* studies to confirm that GLUT1 expression enhances tumor aggressiveness in EMPD.

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Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

MM, HN, EA and DR contributed to the conception and design of the study. MM, TS and HM acquired the data. MM, YM, DS and DR interpreted the data and conducted statistical

analyses. MM drafted the original manuscript. DR, HN, EA and DS provided supervision. DR critically revised the manuscript. MM and DR confirmed the authenticity of the raw data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was conducted in accordance with the Declaration of Helsinki and was approved by the institutional review board of Hirosaki University Graduate School of Medicine (approval no. 2021-116). An opt-out approach was used to obtain informed consent from study participants.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Olson AL and Pessin JE: Structure, function, and regulation of the mammalian facilitative glucose transporter gene family. Annu Rev Nutr 16: 235-256, 1996.
2. Wang J, Ye C, Chen C, Xiong H, Xie B, Zhou J, Chen Y, Zheng S and Wang L: Glucose transporter GLUT1 expression and clinical outcome in solid tumors: A systematic review and meta-analysis. Oncotarget 8: 16875-16886, 2017.
3. Kitamura K, Hatano E, Higashi T, Narita M, Seo S, Nakamoto Y, Yamanaka K, Nagata H, Taura K, Yasuchika K, *et al*: Proliferative activity in hepatocellular carcinoma is closely correlated with glucose metabolism but not angiogenesis. J Hepatol 55: 846-857, 2011.
4. Macheda ML, Rogers S and Best JD: Molecular and cellular regulation of glucose transporter (GLUT) proteins in cancer. J Cell Physiol 202: 654-662, 2005.
5. Semaan A, Munkarah AR, Arabi H, Bandyopadhyay S, Seward S, Kumar S, Qazi A, Hussein Y, Morris RT and Ali-Fehmi R: Expression of GLUT-1 in epithelial ovarian carcinoma: Correlation with tumor cell proliferation, angiogenesis, survival and ability to predict optimal cytoreduction. Gynecol Oncol 121: 181-186, 2011.
6. Paudyal B, Oriuchi N, Paudyal P, Higuchi T, Nakajima T and Endo K: Expression of glucose transporters and hexokinase II in cholangiocellular carcinoma compared using [18F]-2-fluro-2-deoxy-D-glucose positron emission tomography. Cancer Sci 99: 260-266, 2008.
7. Kurokawa T, Yoshida Y, Kawahara K, Tsuchida T, Okazawa H, Fujibayashi Y, Yonekura Y and Kotsuji F: Expression of GLUT-1 glucose transfer, cellular proliferation activity and grade of tumor correlate with [F-18]-fluorodeoxyglucose uptake by positron emission tomography in epithelial tumors of the ovary. Int J Cancer 109: 926-932, 2004.
8. Kim TH, Kwak Y, Song C, Lee HS, Kim DW, Oh HK, Kim JW, Lee KW, Kang SB and Kim JS: GLUT-1 may predict metastases and death in patients with locally advanced rectal cancer. Front Oncol 13: 1094480, 2023.
9. Baer SC, Casaubon L and Younes M: Expression of the human erythrocyte glucose transporter Glut1 in cutaneous neoplasia. J Am Acad Dermatol 37: 575-577, 1997.
10. Koch A, Lang SA, Wild PJ, Gantner S, Mahli A, Spanier G, Berneburg M, Müller M, Bosserhoff AK and Hellerbrand C: Glucose transporter isoform 1 expression enhances metastasis of malignant melanoma cells. Oncotarget 6: 32748-32760, 2015.
11. Wachsberger PR, Gressen EL, Bhala A, Bobyock SB, Storck C, Coss RA, Berd D and Leeper DB: Variability in glucose transporter-1 levels and hexokinase activity in human melanoma. Melanoma Res 12: 35-43, 2002.
12. Fujisawa Y, Yoshino K, Kiyohara Y, Kadono T, Murata Y, Uhara H, Hatta N, Uchi H, Matsushita S, Takenouchi T, *et al*: The role of sentinel lymph node biopsy in the management of invasive extramammary Paget's disease: Multi-center, retrospective study of 151 patients. J Dermatol Sci 79: 38-42, 2015.
13. Laybutt DR, Thompson AL, Cooney GJ and Kraegen EW: Selective chronic regulation of GLUT1 and GLUT4 content by insulin, glucose, and lipid in rat cardiac muscle in vivo. Am J Physiol 273: 1309-1316, 1997.
14. Liu Y, Cao Y, Zhang W, Bergmeier S, Qian Y, Akbar H, Colvin R, Ding J, Tong L, Wu S, *et al*: A small-molecule inhibitor of glucose transporter 1 downregulates glycolysis, induces cell-cycle arrest, and inhibits cancer cell growth in vitro and in vivo. Mol Cancer Ther 11: 1672-1682, 2012.
15. Xu YY, Wu TT, Zhou SH, Bao YY, Wang QY, Fan J and Huang YP: Apigenin suppresses GLUT-1 and p-AKT expression to enhance the chemosensitivity to cisplatin of laryngeal carcinoma Hep-2 cells: An in vitro study. Int J Clin Exp Pathol 7: 3938-3947, 2014.
16. Chen Q, Meng YQ, Xu XF and Gu J: Blockade of GLUT1 by WZB117 resensitizes breast cancer cells to adriamycin. Anticancer Drugs 28: 880-887, 2017.
17. Kowalczyk A, Bodalska A, Miranowicz M and Karłowicz-Bodalska K: Insights into novel anticancer applications for apigenin. Adv Clin Exp Med 26: 1143-1146, 2017.