High Expression of Lactate Dehydrogenase A is a Potential Promoter of Malignant Behaviour in Extramammary Paget's Disease

Kohei OGAWA[1], Keiji SHIMADA[2], Toshihiro TAKAI[3], Yasuhiro MITSUI[1], Masamitsu KUWAHARA[4] and Hideo ASADA[1]

[1]Department of Dermatology and [4]Division of Plastic Surgery, Nara Medical University School of Medicine, 840 Shijo-cho, Kashihara, Nara 634-8522, [2]Department of Diagnostic Pathology, Nara City Hospital, Nara, and [3]Department of Dermatology, Hyogo Cancer Centre, Hyogo, Japan. E-mail: khogawa@naramed-u.ac.jp; hamsan2387@yahoo.co.jp
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Extramammary Paget's disease (EMPD) is a rare cutaneous adenocarcinoma that most commonly affects the anogenital area in elderly patients. EMPD usually progresses slowly and is often diagnosed as carcinoma *in situ*. However, in the case of invasive carcinoma, it frequently metastasizes and exhibits a poor prognosis.

Lactate dehydrogenase (LDH) is a major enzyme involved in glycolysis when oxygen resources are limited. As the tumour environment is highly hypoxic, cancer cells have a heightened anaerobic metabolism (1), known as the Warburg effect (2). Increased lactate concentrations have been shown to predict tumour malignancy, recurrence, survival and metastasis in patients with cancer (3, 4). Lactate dehydrogenase A (LDHA) is a subunit of LDH that is predominantly involved in anaerobic or aerobic glycolysis, which may be associated with the Warburg effect in neoplastic cells; however, its roles are unclear in EMPD. This study examined the expression pattern of LDHA in EMPD by immunohistochemical analysis.

MATERIALS AND METHODS

A total of 37 patients with primary EMPD, diagnosed between September 2009 and August 2019 at Nara Medical University and Hyogo Cancer Centre, Japan, were included in this study. Clinical data for all patients were extracted from the medical records and the data were anonymized. The clinical data is summarized in **Table I**. The 37 patients comprised 20 cases of invasive extramammary Paget's carcinoma (EMPC), 9 cases of EMPD with microinvasion (mi-EMPD) and 8 cases of non-invasive EMPD (ni-EMPD). In the 20 patients with EMPC, regional lymph node metastases were detected in 12 patients and distant metastases were observed in 11 patients during the course of the disease. Seven of the 20 patients died due to EMPC despite multidisciplinary treatment. In contrast, the disease courses of patients with mi-EMPD and ni-EMPD were uneventful after total resection. In the process of diagnosis, immunohistochemical evaluation was performed. CK7 was found to be diffusely positive in 32 patients. CK20 was focally positive in 12 of 27 patients, and negative in 15. GCDFP was diffusely positive in 2 of 19 patients, focally positive in 9, and negative in 8.

Formalin-fixed paraffin-embedded (FFPE) tissue sections of primary tumour lesions and metastatic regional lymph nodes were used for histopathological and immunohistochemical analysis. Normal visceral tissues were stained as controls. Immunohistochemical staining was performed using Bond-Max (Leica Microsystems Co. Ltd, Wetzlar, Hesse, Germany). Paraffin-embedded tissue sections were deparaffinized, and hydrated in xylene and a graded alcohol series. The antigen was then activated with a heat-induced epitope retrieval (HIER) method (citrate base, pH 6.0) at 100°C for 20 min. Rabbit-polyclonal anti-human LDHA (anti LDHA-specific antibody; Cosmo Bio, Tokyo, Japan, 1:200) was used as the primary antibody. High LDHA expression was detected in skeletal muscle, liver (5), and spleen. Semi-quantitative analysis of LDHA was scored using the "Allred Score" (6). The Allred score is a useful evaluation tool to confirm the levels of immunohistochemical expression for oestrogen and progesterone receptors to assess the indication for endocrine therapy for breast cancer (7, 8). Briefly, a proportion score was assigned representing the estimated proportion of positively stained tumour cells (0=none; 1<1/100; 2=1/100 to <1/10; 3=1/10 to <1/3; 4=1/3 to 2/3; 5>2/3). The mean estimated intensity of staining in positive cells was assigned an intensity score (0=none; 1=weak; 2=intermediate; 3=strong). The proportion score and intensity score were added to obtain a total score that ranged from 0 to 8. Slides were scored by a pathologist (KS) who did not have knowledge of the clinical information.

Serum LDH levels were measured in 37 patients using blood samples obtained before total resection (Japan Society of Clinical Chemistry (JSCC) transferable method).

RESULTS

Immunohistochemical staining for LDHA was performed for all 37 cases of primary lesions and 3 cases of regional lymph node metastasis. mi-EMPD and ni-EMPD were grouped together because they had an almost equally good prognosis. LDHA expression was detected in 19 of 20 EMPC cases and in 7 of 17 cases of EMPD (**Fig. 1**A, B). LDHA was also expressed in the metastatic tumour cells in the regional lymph nodes in all 3 cases (Fig. 1C). Allred scores of LDHA were compared between 20 cases of invasive EMPC and 17 cases of EMPD. The mean Allred

Table I. Clinical characteristics and course of 37 cases of extramammary Paget's disease (EMPD) and extramammary Paget's carcinoma

	Sex	Average age, years (range, median)	Site	Regional lymph node metastasis in the period of follow-up	Distant metastasis in the period of follow-up	Death related to EMPD	Average observation period, months (range, median)
i-EMPC (*n*=20)	15 M, 5 F	70.9 (57–89, 69)	19 in the genital area, 1 in the axilla	12	11	7	28.8 (3–66, 20.5)
mi-EMPD (*n*=9)	7 M, 2 F	75.0 (62–88, 72.5)	9 in the genital area	0	0	0	49.2 (19–78, 55)
ni-EMPD (*n*=8)	5 M, 3 F	75.4 (69–86, 75)	6 in the genital area, 2 in the axilla	0	0	0	27.25 (8–57, 21)

i-EMPC: invasive extramammary Paget's carcinoma; mi-EMPD: microinvasive EMPD; ni-EMPD: non-invasive EMPD; EMPC: extramammary Paget's carcinaoma.
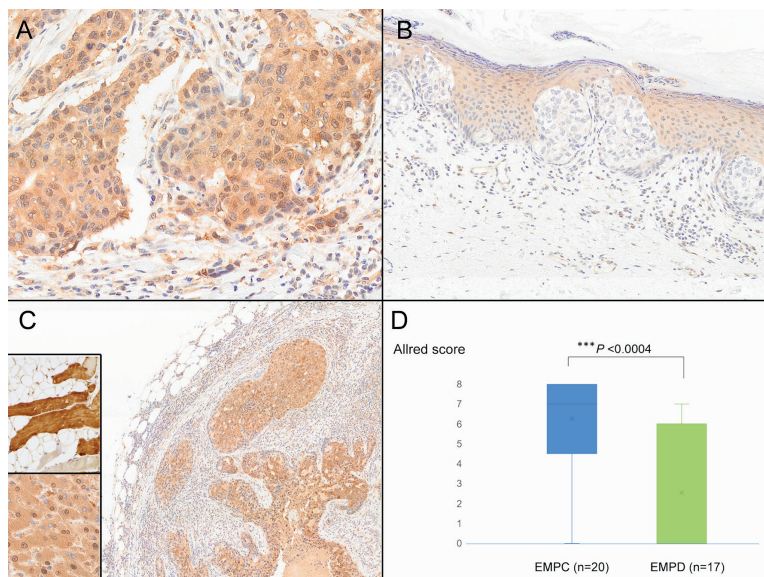
Fig. 1. Lactate dehydrogenase A (LDHA) protein expression in tumour cells of extramammary Paget's carcinoma (EMPC) and extramammary Paget's disease (EMPD). (A) A case of EMPC. Invasive tumour cells in the deep dermis overexpressed LDHA. The total Allred score was 8 (proportion score 5, intensity score 3) (LDHA, ×200). (B) A case of non-invasive EMPD (ni-EMPD). Paget's cells forming nests in the epidermis did not exhibit LDHA expression. The total Allred score was 0 (LDHA, ×100). (C) Sentinel lymph node of EMPC. Metastatic tumour cells were strongly positive for LDHA (LDHA, ×40). LDHA staining for normal skeletal muscle and liver are shown as positive controls (*inset*). (D) Statistical comparison of the Allred score between EMPC and EMPD groups. The mean Allred score in 20 cases of EMPC was 6.25 (median 7; range 0–8), and 2.53 (median 0; range 0–7) in 17 cases of EMPD. LDHA levels in tumour cells were significantly higher in invasive EMPC than in EMPD ($p < 0.0004$).

score in cases of EMPC was 6.25 (median 7; range 0–8), and in cases of EMPD it was 2.53 (median 0; range 0–7). The 2 groups were compared using the Welch test, and the LDHA levels in tumour cells were significantly higher in invasive EMPC than in EMPD ($p < 0.0004$) (Fig. 1D). On the other hand, the mean serum LDH level in patients with EMPC was 187 U/l (median 178; range 130–272), and 208 U/l (median 184; range 138–542) in patients with EMPD. There was no significant difference between these groups.

DISCUSSION

Abnormal LDHA upregulation is a common characteristic of tumours. LDHA levels are directly related to lactic acid levels (3), and an acidic extracellular pH promotes cancer development, maintenance and metastasis (1). Therefore, LDHA is an attractive novel target for cancer therapies (9, 10).

This study demonstrated that LDHA protein expression is significantly higher in tumour cells of invasive EMPC than in those of *in situ* lesions. Furthermore, LDHA was highly expressed in tumour cells that metastasized to regional lymph nodes. This suggested that LDHA is involved in the process of invasion and metastasis of EMPD. Thus, it may promote the malignant transformation of EMPD. The results of this study are consistent with a recent finding by Urata K et al. (11), demonstrating high levels of LDHA

mRNA in EMPD. However, in their study, LDHA mRNA levels were mainly compared between EMPD and non-lesional tissue, and only 4 cases of invasive EMPD were investigated. In addition, all 4 cases of invasive carcinoma were included in the high LDHA group. The current study is a strong complement to the previous report, and revealed that the high expression of LDHA protein is due to proliferating and invasive tumour cells. In addition, overexpression of LDHA in tumour cells may influence the local environment, but it was not considered sufficient to increase levels of serum LDH.

In conclusion, LDHA protein is markedly increased upon transition from *in situ* to invasive lesions in EMPD. This suggests that LDHA plays an important role in the initial stage of transformation to the invasive phenotype and may be essential for the development of EMPD. LDHA therefore has the potential as a new therapeutic target in EMPC.

The authors have no conflicts of interest to declare.

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