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

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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 EMPD, 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 EMPD 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 focally 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 LDH 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. 1A, 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 scores of invasive extramammary Paget’s carcinoma; mi-EMPD: microinvasive EMPD; ni-EMPD: non-invasive EMPD; EMPC: extramammary Paget’s carcinoma.

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-EMPC (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 carcinoma.

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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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