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The Expression of Insulin-Like Growth Factor 2 Messenger RNA-Binding Protein 3 in Langerhans Cell Histiocytosis and Langerhans Cell Sarcoma

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Langerhans cell neoplasms, which include Langerhans cell histiocytosis and Langerhans cell sarcoma, are tumors that originate from dendritic cells. Langerhans cell sarcoma is defined as a high-grade neoplasm with overtly malignant cytological features and the Langerhans cell-like phenotype, and generally has a poorer prognosis and more aggressive phenotype than Langerhans cell histiocytosis. Insulin-like growth factor 2 messenger RNA-binding protein 3 (IGF2BP3 or IMP3) is an oncofetal protein that is expressed in various cancer types; its expression is often associated with a poor prognosis and aggressive phenotype. Here, we used immunohistochemistry to evaluate IGF2BP3 expression in Langerhans cell neoplasms. IGF2BP3 expression was scored as negative (< 1%) or positive (≥ 1%) by immunohistochemistry. All 4 patients with Langerhans cell sarcoma (100%) and 6 of 22 pediatric (age < 18 years) patients with Langerhans cell histiocytosis (27.3%) had positive results for IGF2BP3; however, 16 of 22 pediatric patients with Langerhans cell histiocytosis (72.7%) and all 15 adult (age ≥ 18 years) patients with Langerhans cell histiocytosis (100%) had a negative result. Among patients with Langerhans cell histiocytosis, IGF2BP3 expression was independent of sex, location, prognosis, and BRAF V600E staining results. Taken together, these results indicate that IGF2BP3 expression may be a helpful marker for distinguishing Langerhans cell sarcoma from Langerhans cell histiocytosis in adult patients.

Keywords: IGF2BP3; immunohistochemistry; Langerhans cell histiocytosis; Langerhans cell neoplasm; Langerhans cell sarcoma
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Introduction

Langerhans cell neoplasms (LCNs), including Langerhans cell histiocytosis (LCH) and Langerhans cell sarcoma (LCS), are tumors that originate from dendritic cells, expressing the Langerhans cell markers, such as

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CD1a, CD207 (Langerin), and S-100 protein (Weiss et al. 2017). LCS is defined as a high-grade Langerhans cell neoplasm with overtly malignant cytological features, including hyperchromatic nuclei, prominent nucleoli, and frequent mitotic figures (Harmon and Brown 2015; Nakamine et al. 2016; Weiss et al. 2017; Yi et al, 2019). Despite their immunohistochemical similarities, the prognoses of LCS are completely different from those of LCH. LCS is an aggressive tumor with a mortality rate greater than 50%, whereas the mortality rate of unifocal LCH is less than 1%. Nevertheless, cases that are difficult to distinguish according to these features do exist (Weiss et al. 2017).

Insulin-like growth factor 2 mRNA-binding protein 3 (IGF2BP3 or IMP3) is a known oncofetal protein. It is expressed in embryos but is absent in most adult cells, except gonads and germinal centers. IGF2BP3 expression is associated with tumorigenesis, and it has been identified in various carcinomas, sarcomas, (Do et al. 2008; Cornejo et al. 2012; Yamamoto et al. 2014; Bellezza et al. 2016; Shooshtarizadeh et al. 2016; Takizawa et al. 2016; Burdelski et al. 2018; Okabayashi et al. 2020) and benign tumors, such as pyogenic granuloma and follicular adenoma (Kulacoglu and Erkilinc 2015; Okabayashi et al. 2020). Among lymphoid neoplasms, IGF2BP3 is expressed in Hodgkin lymphoma and some types of non-Hodgkin lymphoma (King et al. 2009; Hartmann et al. 2012; Zhang and Aguilera 2014; Masoud et al. 2019). However, IGF2BP3 expression has not been described in LCNs.

The aim of this study was to determine whether IGF2BP3 is an immunohistochemical marker useful for distinguishing between LCH and LCS.

Materials and Methods

Pathologic specimens

For this study, we extracted data on 37 patients with LCH and 7 patients with LCS from the surgical pathology files of several institutions. All LCH samples had been fixed in 10% neutral buffered formalin for 12-72 h at room temperature and embedded in paraffin. All patients signed the Kyoto University Hospital Informed Consent Form for the Non-therapeutic Use of Histopathological Materials, and the signed forms were uploaded into each patient's electronic health record. Pathologic materials were reviewed on the basis of the methods described in Weiss et al. (2017), and the diagnosis of LCH was confirmed. The LCH cases were previously reported in Takei et al. (2017) and Matsuoka et al. (2020), and the LCS cases were previously reported in Murakami et al. (2014), Nakamine et al. (2016), and Katsuragawa et al. (2020). Clinical information is summarized in Table 1A, B. Clinical data and samples were used with the approval of the Institutional Review Board of Kyoto University Hospital.

Immunohistochemical analysis

Tissue sections (3 μm thick) were deparaffinized with xylene, rehydrated, and pretreated with 0.3% hydrogen peroxide for 5 min. For IGF2BP3 staining, the sections were steam heated for 20 min in EDTA buffer (pH 9.0), anti-IMP3 antibodies (Abs; 1:75, mouse monoclonal clone 69.1; DAKO Cytomation, Glostrup, Denmark) were added, and the sections were incubated for 15 min at room temperature after blocking the background stains with Protein Block (X0909; DAKO Cytomation). For CD1a (a diagnostic marker for LCN) and Cyclin D1 staining, the sections were steam heated for 60 min in EDTA buffer (pH 8.5), anti-CD1a antibodies (Abs; 1:50, mouse monoclonal clone O10; Abcam, Cambridge, MA, USA) or anti-Cyclin D1 antibodies (Prediluted, rabbit monoclonal clone SP4-R; Roche, Basel, Switzerland) were added, and the sections were incubated for 32 min at room temperature. For BRAF V600E (a poor prognostic marker for LCN) staining, the sections were steam heated for 20 min in EDTA buffer (pH 9.0), anti-BRAF V600E antibodies (Abs; 1:100, mouse monoclonal clone VE1; Abcam) were added, and the sections were incubated for 15 min at room temperature. For Ki-67 staining, the sections were steam heated for 60 min in EDTA buffer (pH 8.5), anti-Ki-67 antibodies (Abs; 1:100, mouse monoclonal clone MIB-1; DAKO Cytomation) were added, and the sections were incubated for 32 min at room temperature. Staining for IGF2BP3 and BRAF V600E was performed with a BOND III automated stainer (Leica Biosystems, Richmond, IL, USA), and that for CD1a, Cyclin D1, and Ki-67 was performed with a BenchMark ULTRA (Roche) according to the manufacturer's instructions. The stained sections were imaged with a BX45 microscope (Olympus, Tokyo, Japan) equipped with a DP26 digital camera (Olympus).

To confirm the specificity of the antibody against IGF2BP3, we utilized lymph node samples, including lymph follicles (as positive controls) and interfollicular areas (as negative controls). The degree of IGF2BP3 staining was scored as follows: negative (< 1%) or positive (≥ 1%), according to the previous reports (Hartmann et al. 2012; Yamamoto et al. 2014; Kulacoglu and Erkilinc 2015; Takizawa et al. 2016; Burdelski et al. 2018; Okabayashi et al. 2020). This index is calculated by number of IGF2BP3-positive tumor cells/total number of tumor cells × 100.

Statistical analysis

Between-group differences were examined for statistical significance with the chi-squared test. Microsoft Excel (Redmond, WA, USA) was used to perform all statistical analyses.

Results

IGF2BP3 expression in patients with LCH

IGF2BP3 staining was positive in 6 of 37 patients with LCH (16.2%) (Table 1A). IGF2BP3-positive and -negative patients showed a statistically significant difference in age (2.50 ± 2.59 years vs. 21.2 ± 16.3 years, mean ± standard deviation, $p = 0.0087$). Representative cases are shown in Fig. 1 (positive case, upper panels; negative case, middle

Table 1. Details of patients with Langerhans cell neoplasms (LCNs).

(A) Langerhans cell histiocytosis (LCHs).

Case	Age (years)	Sex	BRAF V600E	Site	IGF2BP3
1	0	m	-	Skin (trunk)	+ (100%)
2	0	m	-	Skin (shoulder)	+ (90%)
3	1	f	-	Skull	+ (90%)
4	3	f	-	Femur	
5	3	f	+	Femur	+ (100%)
6	4	m	-	Tibia	-
7	4	f	-	Clavicle	-
8	4	f	-	Humerus	-
9	4	f	-	Femur	-
10	5	f	-	Femur	+ (50%)
11	6	m	-	Skin (back)	-
12	6	f	-	uncertain	-
13	6	f	-	Skull	+ (100%)
14	9	m	-	Humerus	-
15	10	f	-	Femur	-
16	10	m	-	Femur	-
17	10	m	+	Fibia	-
18	10	m	-	Scapula	-
19	11	f	+	Skull	-
20	11	f	+	Skull	-
21	15	m	-	Ilium	-
22	16	m	-	Rib	-
23	21	f	-	Lung	-
24	22	m	+	Femur	-
25	23	m	-	Vertebra	-
26	25	m	-	Vertebra	-
27	25	f	+	Ischium	-
28	26	m	+	Vertebra	-
29	27	m	-	Skull	-
30	35	m	-	Lung	-
31	38	f	-	Dura	-
32	38	f	+	Lung	-
33	42	m	-	Mandible	-
34	42	m	+	Mandible	-
35	43	m	-	Lung	-
36	56	f	+	Stomach	-
37	62	m	-	Maxilla	-

(B) Langerhans cell sarcomas (LCSs).

Case	Age (years)	Sex	BRAF V600E	Site	IGF2BP3
1	70	m	-	Scalp	+ (100%)
2	72	f	+	Salivary gland	+ (100%)
3	73	m	N.D.	Lymph node	+ (30%)
4	76	m	-	Scalp	+ (10%)

f, female; m, male; N.D., not determined.

+ (%) for IGF2BP3 means the percentage of IGF2BP3-positive tumor cells/all tumor cells.

Seven cases of LCSs were prepared, though three cases which were completely negative for Ki67 in any cells were excluded in the current study.

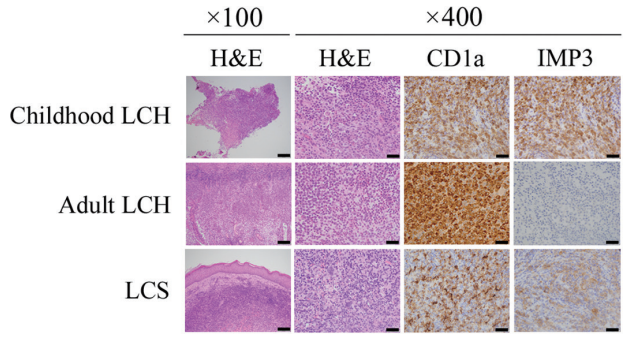

Fig. 1. IGF2BP3 (IMP3) expression in Langerhans cell neoplasms. Immunohistochemical images are shown (magnifications ×100 [Scale bars: 200 μm] and ×400 [Scale bars: 50 μm]). Hematoxylin-eosin staining (H&E) and immunohistochemistry of CD1a and IGF2BP3 in representative three cases are shown: childhood Langerhans cell histiocytosis (LCH) (upper panels), adult LCH (middle panels), and Langerhans cell sarcoma (LCS) (lower panels). The CD1a staining is shown as a positive marker for tumor cells of LCH and LCS.

Table 2. Summary of patients with Langerhans cell neoplasms (LCNs).

	Langerhans cell histiocytosis (LCH)		Langerhans cell sarcomas (LCSs)
IGF2BP3	Positive	Negative	Positive
Cases	6	31	4
Age (years)*	2.50 ± 2.59	21.2 ± 16.3	72.8 ± 2.50
m : f**	2 : 4	18 : 13	2 : 2
BRAF V600E#	1 : 5	9 : 22	1 : 2##

*Data are shown as mean ± standard deviation.
**male : female.
#positive cases : negative cases of BRAF V600E.
##Case 3 of LCSs was not determined in its BRAF V600E status.

panels). All IGF2BP3-positive patients were children with childhood LCH. All other clinical parameters (sex, site, and BRAF V600E status) were statistically similar between IGF2BP3-positive and -negative patients (Table 2).

IGF2BP3 expression in patients with LCS

We examined IGF2BP3 expression in 7 patients with LCS. Three of these patients had completely negative results for Ki67 in all cells (including inflammatory cells); these patients also had negative results for IGF2BP3. The remaining 4 patients had positive results for IGF2BP3 (Tables 1B and 2). Representative case is shown in Fig. 1 (lower panels).

Discussion

In this study, we evaluated IGF2BP3 expression in 7 patients with LCS. Among these, 3 had completely negative results for Ki67 in all cells (including inflammatory cells). These patients also had negative results for IGF2BP3. Ki67 immunoreactivity is significantly reduced by inadequate fixation or long-term storage (Hendricks and Wilkinson 1994; Wester et al. 2000). Therefore, we believe that the specimens from these patients would have been inadequately processed and should be excluded from this study. Similarly, IGF2BP3 immunoreactivity may be also significantly reduced by inadequate fixation or long-term storage.

According to the current WHO classification, differential diagnosis between benign and malignant Langerhans cell neoplasms depends on morphologic phenotypes, such as nuclear atypia and frequent mitotic figures (Weiss et al. 2017). Our current study indicates that IGF2BP3 expression can be an additional helpful marker for distinguishing between LCH and LCS in adult patients but not in pediatric patients. Markers for distinguishing between LCH and LCS have been proposed, such as aberrant CD56 expression and p16 deletion (Kawase et al. 2005; Xerri et al. 2018). Among the patients with LCS described here, 3 of 4 showed p16 deletion, and none showed CD56 expression (data not shown). The combination of p16 deletion, aberrant CD56 expression, and IGF2BP3 expression may help distinguish between LCH and LCS. This study seems to have a limitation by the rarity of LCS cases. Considering the previous reports on other malignant tumors, however, our results would warrant further examination. Accumulation of more LCS cases evaluated should be necessary to conclude the utility of IGF2BP3 staining to distinguish between LCH and LCS.

IGF2BP3 is expressed in malignant cells with aggressive or proliferative phenotypes (Do et al. 2008; King et al. 2009; Cornejo et al. 2012; Yamamoto et al. 2014; Zhang and Aguilera 2014; Kulacoglu and Erkilinc 2015; Bellezza et al. 2016; Shooshtarizadeh et al. 2016; Takizawa et al. 2016; Burdelski et al. 2018; Masoud et al. 2019; Okabayashi et al. 2020). LCS shows aggressive or proliferative phenotypes when compared with those of LCH, and the current results seem to be acceptable. IGF2BPs are associated with the expression levels of cyclin D1, cyclin D3, and cyclin G1 (Rivera et al. 2014). However, we observed 2 of 4 LCS cases were cyclin D1-negative by immunohistochemistry (data not shown). IGF2BP3 might promote LCS proliferation via other cyclin(s) or other molecule(s).

We found that some patients with childhood LCH expressed IGF2BP3, in contrast to IGF2BP3 negativity in adult patients with LCH. This is understandable because some benign lesions have shown IGF2BP3 staining (Kulacoglu and Erkilinc 2015; Okabayashi et al. 2020). We also observed that non-neoplastic Langerhans cells in the skin samples from pediatric patients had mostly positive results for IGF2BP3, and those obtained from adult skin samples had negative results (data not shown). This might be explained by the origin of Langerhans cells. Langerhans cells in the skin of children originate from the yolk sac, whereas those in adult skin originate from aorta-gonad-

mesonephros and/or bone marrow (Collin and Milne 2016). Neoplastic and non-neoplastic Langerhans cells that originate from the yolk sac may be IGF2BP3-positive.

Taken together, IGF2BP3 expression would be a useful marker for distinguishing LCS from LCH in adult patients, though accumulation of more LCS cases evaluated should be necessary to conclude this utility.

Conflict of Interest

The authors declare no conflict of interest.

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