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The characteristic expression of B7-associated proteins in Langerhans cell sarcoma

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ABSTRACT

Langerhans cell sarcoma (LCS) is a rare malignancy derived from dendritic cells of the epidermis that is characterized by cytological atypia, frequent mitoses, and aggressive clinical behavior. Cancer-associated B7 molecules including B7-H1, B7-DC, B7-H3 and B7-H4 are thought to be involved in the immunoescape of cancer cells and to function as prognostic markers. However, the expression and distribution of these molecules in LCS have not been described. Here we report that all of these molecules were observed in LCS sample sections by immunohistochemistry analysis. At the cellular level, they were found on the cell membrane and in the cytoplasm. Fluorescence dual staining indicated that B7-H1, B7-H3 and B7-H4 were principally associated with Langerin$^+$ tumor cells. More interestingly, B7-H1, B7-H3 and B7-H4 were co-expressed on the same tumor cells. Z39Ig, the novel B7-related protein, was also found in the LCS sample sections. Fluorescence dual staining showed that Z39Ig was restricted on CD68$^+$ macrophages. Our results suggest that B7-H1, B7-H3 and B7-H4 may be potential biomarkers to identify LCS, and a clear understanding of their functional roles may further elucidate the pathogenesis of this carcinoma and potentially contribute to the development of novel immunotherapeutic strategies.

Langerhans cell sarcoma (LCS) is a rare malignancy of dendritic cells of the epidermis that is characterized by cytological atypia, frequent mitoses, and aggressive clinical behavior. The most effective treatment protocol for LCS is a combination of radiotherapy with chemotherapy. Some patients are treated with surgery and local radiotherapy as well, but the results are less positive (Uchida et al., 2008). The diagnostic specificity of LCS has not been well defined, although Langerin has been suggested to be a potential biomarker (Nakayama et al., 2010). It is essential to identify more specific markers for the diagnosis of LCS.

Co-signaling by B7/CD28 family members can regulate the initiation, maintenance, and termination of immune responses (Greenwald et al., 2005). In addition to their constitutive expression on macrophages and dendritic cells (DCs), co-inhibitory ligands such as B7-H1 (PD-L1 or CD274), B7-DC (PD-L2 or CD273), B7-H3 (CD276), and B7-H4 (B7x or B7S1) are ectopically expressed in non-lymphoid tissues, including various human cancers (Driessens et al., 2009). These cancer-associated B7 molecules are thought to be involved in the immunoescape of cancer cells and to function as prognostic markers. Many types of carcinomas, including breast cancer, ovarian carcinoma, oral squamous cell carcinoma, nonsmall cell lung carcinoma (NSCLC), clear cell renal cell carcinoma (ccRCC), gastric carcinoma, and esophageal cancer, have been reported to express B7-H1, and the expression is strongly associated with cancer progression and poor patient survival rate (Flies and Chen, 2007).

B7-H3, a type I transmembrane protein with ~20% sequence homology to B7.1, has been reported to be expressed on activated macrophages, DCs, monocytes and a variety of human cancers (Chapoval et al., 2001; Loos et al., 2010). The precise role of B7-H3 in tumor immunity is unclear, whereas the expression of B7-H3 in some tumor types has been linked to a poor prognosis, in other cancers, the opposite effect has been observed. For example, the 5-year survival rate of gastric cancer patients was significantly lower in patients with high B7-H3 expression than in patients with low expression, demonstrating that B7-H3 appears to be a useful biomarker for predicting gastric tumor progression (Arigami et al., 2011). However, in pancreatic cancer, B7-H3 may potentially stimulate an antitumor immune response, as its expression significantly correlates with prolonged postoperative survival (Loos et al., 2009; Yamato et al., 2009).

B7-H4 is a GPI-linked protein that has been demonstrated to be a coinhibitor of T cell responses *in vitro* by inhibiting T cell proliferation, cell cycle progression and cytokine production (Sica

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Table 1
Primary antibodies and their source.

Ab	Dilution	Clone	Source
B7-H1	1:100	Monoclonal mouse IgG (5H1)	Kindly provided by Dr. Lieping Chen (Department of Immunobiology, Yale University School of Medicine, USA)
	1:100	Monoclonal mouse IgG (29E.2A3)	Kindly provided by Dr. Gordon J Freeman (Department of Medical Oncology, Dana-Farber Cancer Institute, Harvard Medical School, USA)
	1:50	Polyclonal Rabbit IgG (H-130)	Santa cruz
B7-DC	1:200	Monoclonal mouse IgG (24F.7G12)	Kindly provided by Dr. Gordon J Freeman
		Polyclonal Goat IgG	R&D system
Z39Ig	1:50	Polyclonal Goat IgG (Y-18)	Santa cruz
	1:100	Monoclonal mouse IgG (6H8)	Kindly provided by Dr. Won-Ha Lee (Department of Genetic Engineering, Kyungpook National University, Korea)
B7-H3	1:100	Polyclonal Goat IgG	R&D system
B7-H4	1:100	Polyclonal Rabbit IgG (H-108)	Santa cruz
CD3	1:50	Monoclonal mouse IgG (F7.2.38)	DAKO
CK-18	1:400	Monoclonal mouse IgG (C-04)	Santa cruz
CD68	1:50	Monoclonal mouse IgG (3F103)	Santa cruz
CD31	1:50	Monoclonal mouse IgG (10G9)	Santa cruz
Langerin	1:200	Monoclonal mouse IgG (12D6)	Abcam
CD1a	1:200	Monoclonal mouse IgG (7A7)	Abcam
CD44	1:50	Monoclonal mouse IgG (3G5)	R&D system

et al., 2003). B7-H4 mRNA was found in both lymphoid and non-lymphoid organs; however, its protein expression is restricted to activated T cells, B cells, monocytes, DCs and numerous types of carcinomas, including prostate cancer, ccRCC, NSCLC and gastric carcinoma. In addition, the presence of B7-H4 is useful for predicting the progression of cancer (Yi and Chen, 2009; Quandt et al., 2011; Simon et al., 2006). For example, gastric cancer-associated B7-H4 expression was significantly correlated with depth of tumor invasion and lymph node metastasis. Furthermore, the 5-year survival rate was significantly lower in patients with B7-H4 expression than in those without B7-H4 expression (Arigami et al., 2010). In breast cancer, B7-H4 expression in invasive ductal carcinomas may decrease tumor infiltrating lymphocyte (TIL) infiltration, suggesting that B7-H4 overexpression may help cancers avoid immune detection (Mugler et al., 2007).

Z39Ig (also called complement receptor-IgG (CRIg) or V-set and Ig domain-containing 4 (VSIG4)), is a recently identified B7-related protein. Z39Ig was first reported to be a receptor for complement C3, which mediates the clearance of pathogens (Helmy et al., 2006). Recent research indicated that Z39Ig has the capacity to inhibit $CD4^+$ and $CD8^+$ T cell proliferation and IL-2 production through the activation of an unidentified receptor on T cells (Vogt et al., 2006). Z39Ig mRNA is highly expressed in various tissues, whereas its protein expression is restricted to the surface of macrophages (Helmy et al., 2006). Recent studies showed that $Z39Ig^+$ cells were found in tissues from rheumatoid arthritis (RA), atherosclerosis (AS) and chronic hepatitis B virus (HBV), indicating that Z39Ig might be involved in the pathogenesis of such inflammatory diseases (Lee et al., 2006; Guo et al., 2010). However, the expression and function of Z39Ig in cancer is as of yet unknown.

It appears that cancer-associated B7 molecules (B7s) may serve as potent prognostic biomarkers. We performed immunohistochemical analyses to characterize the expression of B7s in LCS, to measure the expression of these molecules on neoplastic cells and to determine whether they might serve as useful biomarkers for LCS.

Materials and methods

Samples collection

Two LCS samples and one Langerhans cell histiocytosis (LCH) sample were collected at the Department of Pathology, 150th Hospital (Luoyang, Henan Province, China); the other two LCS samples were collected at the Department of Pathology, Xinqiao Hospital, Third Military Medical University (Chongqing, China). The origin of 3 cases was from skin and one was from lymph nodes. The tissues were fixed in 10% neutral buffered formalin and paraffin embedded. This study protocol was approved by the Ethics Committee of the Third Military Medical University review board.

Immunohistochemistry

The protocol used for immunohistochemistry has been published previously, with slight modifications (Alonso-Magdalena et al., 2009). Briefly, paraffin-embedded tissue blocks were cut into 2–5 μm sections and mounted on polylysine-charged glass slides. After the sections were dewaxed and rehydrated, antigen retrieval was performed by microwaving sections in 10 mM citrate buffer (pH 6.0). After cooling the sections to room temperature, endogenous peroxidase was blocked by incubation with a solution of 0.5% hydrogen peroxidase in 50% methanol for 1 h. Sections were incubated in 3% BSA plus 0.1% Nonidet P-40 in PBS for 1 h at room temperature to block nonspecific binding. Sections were incubated overnight at 4 °C with primary antibodies (Table 1) that had been diluted in 1% BSA plus 0.1% Nonidet P-40 in PBS. After washing, sections were incubated with the corresponding secondary antibodies for 1 h at RT. The Vectastain ABC kit (Vector Laboratories) was used for the avidin–biotin complex (ABC) method according the manufacturer's instructions. Peroxidase activity was visualized with DAB (DAKO). The sections were lightly counterstained with hematoxylin, dehydrated through an ethanol series to xylene, and mounted. Sections incubated with isotype-matched, concentration-matched immunoglobulin without primary antibodies were used as isotype controls. Reactivity was detected with a DAB Elite kit (K3465, Dako); brown coloration of tissues represented positive staining. Sample sections were viewed using a light microscope.

Immunofluorescence staining

For immunofluorescence staining, sections were incubated with mouse monoclonal anti-Langerin, anti-CD3, anti-CD31, anti-CK-18, anti-B7-H1, anti-B7-H4, anti-B7-H3, anti-B7-DC and anti-Z39Ig Abs at 4 °C overnight. Sections were then incubated with Alexa 568-conjugated goat anti-mouse/rat/rabbit IgG antibodies or fluorescein isothiocyanate (FITC)-conjugated mouse anti-goat IgG antibodies (Jackson ImmunoResearch, West Grove, PA, USA) for 1 h. Sections incubated with the appropriate isotype control primary antibodies and fluorescently labeled secondary antibodies