Clinicopathologic and Prognostic Implications of Programmed Death Ligand 1 Expression in Thymoma

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Background. **Programmed death ligand 1 (PD-L1) has been reported to be expressed in various malignancies and is considered to be a prognostic factor and an immunotherapeutic target. The aim of this study was to characterize PD-L1 expression in thymoma and determine statistical associations between this expression and clinical features.**

Methods. **We reviewed formalin-fixed, paraffin-embedded tissue specimens from 82 thymoma cases accumulated at Kurume University, the majority of which achieved surgical complete resection. Expression of PD-L1 was evaluated by immunohistochemistry. Statistical associations between PD-L1 expression and clinicopathologic features were evaluated by using χ^2 test and Fisher's exact test. Disease-free survival and overall survival curves were established by the Kaplan-Meier method and compared using a log-rank test. Predictive factors for disease-free survival after complete resection were analyzed by using a Cox proportional hazards model in univariate and multivariate analysis.**

Results. **Overall, 44 thymoma cases (54%) revealed high PD-L1 expression. High PD-L1 expression was statistically associated with Masaoka stage III/IV disease ($p = 0.043$) and World Health Organization type B2 or B3 thymoma ($p = 0.044$). Disease-free survival after complete resection in high PD-L1 expression was significantly worse than that in low PD-L1 expression ($p = 0.021$), although there was no significant difference in overall survival ($p = 0.957$). Multivariate analysis also revealed high PD-L1 expression as an independent risk factor for recurrence ($p = 0.008$).**

Conclusions. **Characterization of PD-L1 expression in thymoma should enable more effective clinical approaches, including prognostic stratification of patients and potential use of anti-PD-L1 antibody immunotherapy.**

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Thymoma is a relatively rare malignant mediastinal tumor. Complete resection is recommended for resectable thymoma and usually results in a good prognosis, although thymoma sometimes displays local invasion and distant metastases [1, 2]. In contrast, in unresectable thymoma cases, the average survival duration is reported to be short (16 to 60 months) even when chemotherapy is administered [3, 4]. Standard therapy for unresectable cases remains controversial because of the rarity of the disease, although cisplatin/doxorubicin-based regimens yield favorable response rates of 62% to 100% as initial treatments [3]. Therefore, improved therapeutic strategies are absolutely required.

Programmed death ligand 1 (PD-L1) is an immunomodulatory glycoprotein expressed on antigen-presenting cells [5]. Binding of PD-L1 to the programmed death 1 (PD-1) receptor, which is present on the surface of T cells, plays a significant role in the regulation of immune responses through the suppression of cytokine production, T-cell proliferation, and T-cell adhesion [6]. The PD-1/PD-L1 pathway has been described to function as a negative regulator not only in normal immune responses but also in antitumor immunity mediated by T cells [7]. Expression of PD-L1 by tumor cells has also been suggested to allow those cells to escape from antitumor immunity associated with the PD-1/PD-L1 pathway [8].

Anti-PD-L1 antibody (anti-PD-L1 Ab), a recently developed therapy, inhibits the interaction between PD-L1 and PD-1, leading to blockade of the PD-1/PD-L1 pathway responsible for the escape of PD-L1–positive tumor cells from antitumor immune responses [8]. Apparent antitumor efficacy of anti-PD-L1 Abs has been clinically observed in various PD-L1–positive malignant tumors [9].

In this study, we retrospectively investigated PD-L1 expression in thymoma, as well as statistical associations

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Abbreviations and Acronyms

anti-PD-L1 Ab = anti-PD-L1 antibody
DFS = disease-free survival
IHC = immunohistochemistry
OS = overall survival
PD-1 = programmed death 1
PD-L1 = programmed death ligand 1
WHO = World Health Organization

between PD-L1 expression and clinicopathologic features including Masaoka stage and World Health Organization (WHO) classification.

Material and Methods

Patients

We reviewed formalin-fixed, paraffin-embedded tissue specimens from 82 thymoma cases resected at Kurume University between 2000 and 2013. All cases were initially diagnosed, and pathologic diagnosis was performed by two pathologists (O.K. and M.H.) based on the 2004 WHO classification [10]. Clinical data were obtained by reviewing patients' medical charts. All of the patients had undergone clinical follow-up including computed tomography assessment at least every other year after surgery. This study was approved by the Research Ethics Committee of Kurume University, and conforms to the ethical guidelines of the Declaration of Helsinki. All of the participants provided written informed consent for use of their tissue samples.

Immunohistochemistry

Primary antibodies used for immunohistochemistry (IHC) included anti-PD-L1 Ab (rabbit monoclonal, clone: EPR1161[2], Abcam, Cambridge, UK), anti-cytokeratin (mouse monoclonal, clones: AE1/AE3, Dako, Glostrup, Denmark), and anti-CD3 (mouse monoclonal, clone: F7.2.38, Dako). The IHC protocol for PD-L1 is detailed here. Paraffin-embedded specimens sectioned at a thickness of 2.5 μm were deparaffinized in xylene followed by 95% alcohol. After rehydration with water, sections were pretreated with the aim of antigen retrieval in a microwave oven at 95°C for 20 minutes in EDTA buffer, pH 8.0. Then, 3% H_2O_2 solution was used to block endogenous peroxidase activity. The primary antibody was diluted 1:200 for an overnight incubation in a humidified chamber at 4°C. The secondary antibody of the Dako REAL EnVision Detection System (Dako) was incubated for 30 minutes at room temperature. The immunoreaction was visualized using 3,3′-diaminobenzidine chromogen (Dako) for 3 minutes.

Assessment of Programmed Death Ligand 1 Expression

DEFINITION OF PROGRAMMED DEATH LIGAND 1–POSITIVE RATE. Calculation of the PD-L1–positive rate included the following steps (Fig 1): (1) The value of the PD-L1–positive area was measured in each case by counting the number of pixels in the PD-L1–positive region of IHC samples by using ImageJ (National Institutes of Health, Bethesda, MD) [11]. (2) In the same procedure, the value of the cytokeratin-positive area in the next serial section after PD-L1 was measured. (3) We divided the value of the PD-L1–positive area by that of the cytokeratin-positive area, and declared the result of the calculation the PD-L1–positive rate. Two pathologists (O.K. and M.H.) evaluated the PD-L1– and cytokeratin-positive areas in IHC samples without knowledge of any clinical characteristics. For type AB thymoma, the PD-L1–positive rate was evaluated in the field containing both components almost equally.

DETECTION OF THE PROGRAMMED DEATH LIGAND 1–POSITIVE RATE CUTOFF VALUE. A receiver operating characteristic curve and Youden's index were used to determine the statistically optimal cutoff value of the PD-L1–positive rate (Fig 2) [12, 13]. In preparing this curve, we used Masaoka stage to classify each case as either stage I and II (I/II) or stage III and IV (III/IV) as a dichotomous variable, with the PD-L1–positive rate as a continuous variable. The optimal cutoff point of the PD-L1–positive rate was calculated to be 38% by Youden's index. Cases with a PD-L1–positive rate of at least 38% were defined as high PD-L1 expression cases, whereas cases with a rate of less than 38% were defined as low PD-L1 expression cases.

Statistical Analysis

Statistical comparisons between clinicopathologic features and PD-L1 expression were evaluated using a χ^2 test, whereas Fisher's exact test was added as necessary during the statistical analysis. In the analysis of recurrence and survival, the start point was defined as the day resection was performed. The end of the disease-free survival (DFS) period was defined as the day of recurrence, and that of overall survival (OS) period was defined as the day confirmed alive or dead, respectively. Estimation of DFS and OS was calculated by the Kaplan-Meier method, and the curves were compared using a log-rank test. In univariate and multivariate analysis, the influence of anti-acetylcholine receptor (anti-AchR) antibody titer and the PD-L1–positive rate were evaluated as continuous variables, and possible risk factors for recurrence were analyzed with a Cox proportional hazards model, whereas that for OS could not be analyzed owing to few fatalities. To assess whether PD-L1 expression independently predicts recurrence, multivariate analysis was performed adjusting for selected confounding factors including Masaoka stage and WHO classification. A probability value less than 0.05 was recognized as representing statistical significance. All statistical analyses in this study were performed using JMP, version 11 software (SAS Institute, Tokyo, Japan).

Results

Clinicopathologic Characteristics

All patient characteristics are presented in Table 1. The median postoperative follow-up term was 37 months (range, 1 to 144 months). A total of 32 men (39%) and