Expression of Programmed Death Ligand 2 in Patients with Thymoma and Thymomatous Myasthenia Gravis

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Research Article

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Abstract

Purpose: The purpose of this study was to examine the expression of PD-L2 in thymoma and thymomatous myasthenia gravis (MG).

Methods: The records of 70 patients with thymoma who underwent surgical resection between January 2017 and December 2018 were retrospectively reviewed. Thymoma PD-L2 expression was evaluated by immunohistochemistry (IHC) staining. Associations between PD-L2 expression and clinicopathological features were examined.

Results: PD-L2 expression was positive in 41 patients (58.6%) and negative in 29 patients (41.4%). Of the patients, 33 had thymomatous MG. Patients with MG were more likely to be ≤ 50 years of age (69.70% vs. 35.14%), have higher WHO type (84.85% vs. 64.86%), smaller tumor size (4.09 ± 2.33 vs. 6.47 ± 2.42 cm), have positive PD-L2 expression (78.79% vs. 40.54%), and have higher PD-L2 expression intensity, ratio, and score (all, $P < 0.05$). Positive PD-L2 expression was associated with higher WHO type, higher Masaoka-Koga stage, smaller tumor size, ectopic thymus, and MG (all, $P < 0.05$). Factors significantly associated with MG were age > 50 years (OR = 0.09, 95% CI: 0.02 to 0.39), tumor size > 5 cm (OR = 0.22, 95% CI: 0.06 to 0.82), and positive PD-L2 expression (OR = 9.25, 95% CI: 1.93 to 44.30) (all, $P < 0.05$; Table 3).

Conclusion: Thymoma PD-L2 expression is significantly associated with thymomatous MG and WHO histological type B2 and B3.

Introduction

Thymoma is the most common anterior mediastinal mass in adults [1]. About 30% of patients with thymoma have myasthenia gravis (MG: thymomatous MG)[2]. Almost all patients with thymomatous MG have anti-acetylcholine receptor antibodies [3]. However, the pathogenesis of thymomatous MG, and the signaling pathway that results in auto-antibodies are not clear. Studies have revealed that T follicular helper (TFH) cells, a specialized subset of CD4 + T cells, play a fundamental role in humoral immunity due to their ability to promote germinal center formation, B cell differentiation into plasma cells and memory cells, and antibody production in secondary lymphoid tissues [4]. Chen et al. [5] reported that programmed cell death protein 1 (PD-1) expression on T cells and programmed death-ligand 2 (PD-L2) expression on B cells controls the number of TFH cells and PC numbers. PD-1 also regulates germinal center B cell survival and the formation and affinity of long-lived plasma cell [5]. Thymic TFH cells may be involved in the pathogenesis of MG with thymoma [6, 7].

PD-1 is highly expressed in TFH cells; however, the roles of PD-1/PD-L1 and PD-1/PD-L2 signaling in the pathogenesis of thymomatous MG has been virtually unstudied. On the other hand, studies have shown that PD-L1 protein expression is not associated with MG status [8, 9]. As such, PD-L2 may be a valuable prognostic marker. PD-L2 is highly expressed in some organs such as the lung, liver, and heart, and exhibits low expression in other organs such as the spleen, lymph nodes, and thymus [10]. In addition,

studies have shown that PD-L2 is expressed on solid tumors, anaphase-promoting complex (APC), which regulates PD-1, PD-1, and PD-L1 expression during normal and autoimmune responses, and medullary thymic epithelial cells [10]. There is also growing evidence of a relation PD-1 and various PD-Ls and autoimmune diseases [11]. Based on the aforementioned data, we postulated that thymoma PD-L2 may play a role in the development and course of MG.

Thus, the purpose of this study was to examine the expression of PD-L2 in thymoma and thymomatous MG, and characterize associations of PD-L2 expression and the clinicopathological features of patients with MG.

Methods

Patients

In this study, we retrospectively reviewed the records of patients with thymoma who received surgical resection at The First Affiliated Hospital of Sun-yat-Sen University between January 2017 and December 2018. This study was approved by Institutional Review Board of the First Affiliated Hospital of Sun Yat-sen University, and because of the retrospective nature of the study the requirement of informed patient consent was waived.

Data extracted from the medical records included patient age and sex, tumor size, World Health Organization (WHO) histological type, the presence of MG, Masaoka-Koga disease stage, and ectopic thymus. For the patients with MG, the Myasthenia Gravis Foundation of America (MGFA) Clinical Classification at diagnosis was also recorded. Paraffin-embedded tumor specimens were used to assess the expression of PD-L2 by immunohistochemistry (IHC) analysis.

IHC

IHC studies were performed on formalin-fixed, paraffin-embedded tissue sections of each tumor. Each section was cut to a thickness of 4-μm. Sections were stained with anti-PD-L2 mouse immunoglobulin G monoclonal antibody (clone UMAB223; ZSGB Bioscience) diluted to 1:100 as the primary antibody. IHC staining was performed automatically with a Ventana BenchMark XT Stainer (Roche Diagnostics, Basel, Switzerland).

Scoring of PD-L2 positivity

Two pathologists independently evaluated the expression of PD-L2, and then discussed their findings and the final proportion of PD-L2 positive tumor cells was determined by consensus. A tumor cell was defined as “PD-L2 positive” when the cell membranes were partially or completely stained [12] (Fig. 1). In contrast, PD-L2 staining in the cytoplasm of a tumor cell was defined as “negative.” PD-L2 positive immune cells, such as lymphocytes and macrophages, were excluded from the cell counts.

Tumor cells were quantified by evaluating the ratio of stained to unstained tumor cells. PD-L2 positivity was evaluated based on the proportion of PD-L2 positive tumor cells. A PD-L2 expression rate of 1% or greater was defined as PD-L2 positive; any value < 1% was defined as PD-L2 negative. The intensity of PD-L2 staining of tumor cells was also evaluated based on the following scale: 0, negative; 1, very weak; 2, moderate; and 3, strong. Mean PD-L2 expression scores were calculated by multiplying the percentage of tumor area stained by the staining intensity.

Statistical analysis

Continuous data were presented as mean $\pm$ standard deviation, and categorical data as number and percentage (%). Comparisons of means between groups was performed with Student's independent t-test or Mann-Whitney U test, depending on the normality assumption. Categorical data compared with the chi-square test or Fisher's exact test (if an expected value ≤ 5 was found). Univariate and multivariate logistic regression models were used to investigate factors associated with MG. Independent variables which were significant in the univariate analysis were entered into a multivariate model, and variables significant in the multivariate model were considered factors associated with MG. Data were reported as estimated odds ratio (OR) and 95% confidence interval (CI). All significant associated factors were used to build a multivariate logistic model to predict the estimated probability of MG. Receiver operating characteristic (ROC) curve analysis was performed using the probabilities of MG as independent continuous variables, and the area under the ROC curve (AUC) was calculated. All statistical analyses were 2-tailed, and values of $P < 0.05$ were considered to indicate statistical significance. Statistical analyses were performed with SPSS version 25 software (SPSS Statistics, IBM Corporation, Somers, New York). Statistical software R (version 3.5.2) and the 'rms' package was used to create a nomogram.

Results

Patient characteristics

A total of 70 patients who received surgery for thymoma were included in this study. The mean patient age was 49.03 ± 12.99 years, and there were 42 males and 28 females (ratio, 1:0.67). Of the patients, 33 had thymomatous MG. There were no significant differences between patients with MG and those without MG with respect to sex, age, and length of disease (Table 1). However, patients with MG were more likely to be ≤ 50 years of age (69.70% vs. 35.14%), have higher WHO type (84.85% vs. 64.86%), smaller tumor size (4.09 ± 2.33 vs. 6.47 ± 2.42 cm), have positive PD-L2 expression (78.79% vs. 40.54%), and have higher PD-L2 expression intensity, ratio, and score (all, $P < 0.05$).

Table 1
Patient characteristics

Parameters	No-MG (n = 37)	MG (n = 33)	All (N = 70)	P
Sex				0.171
Male	25 (67.57)	17 (51.52)	42 (60.00)	
Female	12 (32.43)	16 (48.48)	28 (40.00)	
Age, year,	51.86 ± 12.66	45.85 ± 12.80	49.03 ± 12.99	0.052
Age group				0.004
≤ 50 years	13 (35.14)	23 (69.70)	36 (51.43)	
> 50 years	24 (64.86)	10 (30.30)	34 (48.57)	
Disease length	4.70 ± 7.19	6.09 ± 11.00	5.36 ± 9.14	0.530
MGFA classification				-
I	-	10 (30.30)	-	
IIA	-	4 (12.12)	-	
IIB	-	17 (51.52)	-	
IIIA	-	1 (3.03)	-	
IIIB	-	1 (3.03)	-	
MGFA classification (grouped)				-
I + IIA	-	14 (42.42)	-	
IIB + IIIA + IIIB	-	19 (57.58)	-	
WHO type				0.025
A	7 (18.92)	1 (3.03)	8 (11.43)	
AB	6 (16.22)	4 (12.12)	10 (14.29)	
B1	9 (24.32)	3 (9.09)	12 (17.14)	
B2	11 (29.73)	20 (60.61)	31 (44.29)	
B3	4 (10.81)	5 (15.15)	9 (12.86)	
WHO type (grouped 1)				0.056

MG, myasthenia gravis; MGFA, Myasthenia Gravis Foundation of America; PD-L2, programmed death-ligand 2; STAT6, signal transducer and activator of transcription 6; WHO, World Health Organization.

Data are presented as mean ± standard deviation, or count (percentage).

Parameters	No-MG (n = 37)	MG (n = 33)	All (N = 70)	P
A + AB	13 (35.14)	5 (15.15)	18 (25.71)	
B1 + B2 + B3	24 (64.86)	28 (84.85)	52 (74.29)	
WHO type (grouped 2)				0.003
A + AB + B1	22 (59.46)	8 (24.24)	30 (42.86)	
B2 + B3	15 (40.54)	25 (75.76)	40 (57.14)	
Masaoka-Koga stage				0.126
I	26 (70.27)	24 (72.73)	50 (71.43)	
IIA	5 (13.51)	6 (18.18)	11 (15.71)	
IIB	0 (0.00)	1 (3.03)	1 (1.43)	
IIIA	1 (2.70)	2 (6.06)	3 (4.29)	
IIIB	3 (8.11)	0 (0.00)	3 (4.29)	
IV	2 (5.41)	0 (0.00)	2 (2.86)	
Masaoka-Koga stage (grouped)				0.820
I	26 (70.27)	24 (72.73)	50 (71.43)	
IIA to IV	11 (29.73)	9 (27.27)	20 (28.57)	
Tumor size, cm	6.47 ± 2.42	4.09 ± 2.33	5.35 ± 2.65	< 0.001
Tumor size group				0.004
≤ 5 cm	13 (35.14)	23 (69.70)	36 (51.43)	
> 5 cm	24 (64.86)	10 (30.30)	34 (48.57)	
Ectopic thymus				0.249
No	32 (86.49)	25 (75.76)	57 (81.43)	
Yes	5 (13.51)	8 (24.24)	13 (18.57)	
PD-L2 expression				0.001
Negative	22 (59.46)	7 (21.21)	29 (41.43)	
Positive	15 (40.54)	26 (78.79)	41 (58.57)	

MG, myasthenia gravis; MGFA, Myasthenia Gravis Foundation of America; PD-L2, programmed death-ligand 2; STAT6, signal transducer and activator of transcription 6; WHO, World Health Organization.

Data are presented as mean ± standard deviation, or count (percentage).

Parameters	No-MG (n = 37)	MG (n = 33)	All (N = 70)	P
PD-L2 expression intensity				0.002
0	22 (59.46)	7 (21.21)	29 (41.43)	
1	8 (21.62)	8 (24.24)	16 (22.86)	
2	5 (13.51)	6 (18.18)	11 (15.71)	
3	2 (5.41)	12 (36.36)	14 (20.00)	
PD-L2 expression ratio	14.16 ± 25.07	51.82 ± 38.54	31.91 ± 37.08	< 0.001
PD-L2 score	23.27 ± 55.48	124.09 ± 117.30	70.80 ± 102.74	< 0.001
STAT6 expression				0.157
Negative	30 (81.08)	31 (93.94)	61 (87.14)	
Positive	7 (18.92)	2 (6.06)	9 (12.86)	
MG, myasthenia gravis; MGFA, Myasthenia Gravis Foundation of America; PD-L2, programmed death-ligand 2; STAT6, signal transducer and activator of transcription 6; WHO, World Health Organization.				
Data are presented as mean ± standard deviation, or count (percentage).				

Pd-L2 Expression And Characteristics

The characteristics of patients with positive and negative PD-L2 expression were compared (Table 2). Positive PD-L2 expression was associated with higher WHO type, higher Masaoka-Koga stage, smaller tumor size, ectopic thymus, and MG (all, $P < 0.05$). Positive PD-L2 expression was also associated with a greater PD-L2 expression intensity, ratio, and score (all, $P < 0.05$).

Table 2
Characteristics of patients with negative vs. positive PD-L2 expression

Parameters	PD-L2 negative (n = 29)	PD-L2 positive (n = 41)	All (n = 70)	P
Sex				0.488
Male	16 (55.17)	26 (63.41)	42 (60.00)	
Female	13 (44.83)	15 (36.59)	28 (40.00)	
Age, years	47.00 ± 13.49	50.46 ± 12.59	49.03 ± 12.99	0.275
Age group				0.311
≤ 50 years	17 (58.62)	19 (46.34)	36 (51.43)	
> 50 years	12 (41.38)	22 (53.66)	34 (48.57)	
Course	3.76 ± 6.67	6.49 ± 10.48	5.36 ± 9.14	0.221
MGFA class				0.608
I	2 (28.57)	8 (30.77)	10 (30.30)	
IIA	2 (28.57)	2 (7.69)	4 (12.12)	
IIB	3 (42.86)	14 (53.85)	17 (51.52)	
IIIA	0 (0.00)	1 (3.85)	1 (3.03)	
IIIB	0 (0.00)	1 (3.85)	1 (3.03)	
MGFA class (grouped)				0.422
I + IIA	4 (57.14)	10 (38.46)	14 (42.42)	
IIB + IIIA + IIIB	3 (42.86)	16 (61.54)	19 (57.58)	
WHO type				< 0.001
A	1 (3.45)	7 (17.07)	8 (11.43)	
AB	6 (20.69)	4 (9.76)	10 (14.29)	
B1	11 (37.93)	1 (2.44)	12 (17.14)	
B2	9 (31.03)	22 (53.66)	31 (44.29)	
B3	2 (6.90)	7 (17.07)	9 (12.86)	
PD-L2, programmed death-ligand 2; STAT6, signal transducer and activator of transcription 6; WHO, World Health Organization.				
Data are presented as mean ± standard deviation, or count (percentage).				

Parameters	PD-L2 negative (n = 29)	PD-L2 positive (n = 41)	All (n = 70)	P
WHO type (grouped 1)				0.800
A + AB	7 (24.14)	11 (26.83)	18 (25.71)	
B1 + B2 + B3	22 (75.86)	30 (73.17)	52 (74.29)	
WHO type (grouped 2)				0.006
A + AB + B1	18 (62.07)	12 (29.27)	30 (42.86)	
B2 + B3	11 (37.93)	29 (70.73)	40 (57.14)	
Masaoka-Koga stage				0.028
I	20 (68.97)	30 (73.17)	50 (71.43)	
IIA	6 (20.69)	5 (12.20)	11 (15.71)	
IIB	0 (0.00)	1 (2.44)	1 (1.43)	
IIIA	0 (0.00)	3 (7.32)	3 (4.29)	
IIIB	3 (10.34)	0 (0.00)	3 (4.29)	
IV	0 (0.00)	2 (4.88)	2 (2.86)	
Masaoka-Koga stage (grouped)				0.701
I	20 (68.97)	30 (73.17)	50 (71.43)	
IIA to IV	9 (31.03)	11 (26.83)	20 (28.57)	
Tumor size, cm	6.24 ± 2.78	4.72 ± 2.39	5.35 ± 2.65	0.017
Tumor size group				0.353
≤ 5 cm	13 (44.83)	23 (56.10)	36 (51.43)	
> 5 cm	16 (55.17)	18 (43.90)	34 (48.57)	
Ectopic thymus				0.035
No	27 (93.10)	30 (73.17)	57 (81.43)	
Yes	2 (6.90)	11 (26.83)	13 (18.57)	
Myasthenia gravis				0.001

PD-L2, programmed death-ligand 2; STAT6, signal transducer and activator of transcription 6; WHO, World Health Organization.

Data are presented as mean ± standard deviation, or count (percentage).

Parameters	PD-L2 negative (n = 29)	PD-L2 positive (n = 41)	All (n = 70)	P
Negative	22 (75.86)	15 (36.59)	37 (52.86)	
Positive	7 (24.14)	26 (63.41)	33 (47.14)	
PD-L2 expression intensity				< 0.001
0	29 (100.00)	0 (0.00)	29 (41.43)	
1	0 (0.00)	16 (39.02)	16 (22.86)	
2	0 (0.00)	11 (26.83)	11 (15.71)	
3	0 (0.00)	14 (34.15)	14 (20.00)	
PD-L2 expression ratio	1.72 ± 9.28	53.27 ± 34.40	31.91 ± 37.08	< 0.001
PD-L2 score	0.00 ± 0.00	120.88 ± 109.57	70.80 ± 102.74	< 0.001
STAT6 expression				0.289
Negative	27 (93.10)	34 (82.93)	61 (87.14)	
Positive	2 (6.90)	7 (17.07)	9 (12.86)	
PD-L2, programmed death-ligand 2; STAT6, signal transducer and activator of transcription 6; WHO, World Health Organization.				
Data are presented as mean ± standard deviation, or count (percentage).				

Factors Associated With Mg

Univariate and multivariate logistic regression models were used to investigate the associations of independent variables to MG. Factors significantly associated with MG were age > 50 years (OR = 0.09, 95% CI: 0.02 to 0.39), tumor size > 5 cm (OR = 0.22, 95% CI: 0.06 to 0.82), and positive PD-L2 expression (OR = 9.25, 95% CI: 1.93 to 44.30) (all, P < 0.05; Table 3).

Table 3

Logistic regression analysis of independent variables associated with myasthenia gravis

Univariate			Multivariate	
Parameters	OR (95% CI)	P	OR (95% CI)	P
Sex				
Male	ref.	-		
Female	1.96 (0.74 to 5.17)	0.173		
Age group				
≤ 50 years	ref.	-	ref.	-
> 50 years	0.24 (0.09 to 0.64)	0.005	0.09 (0.02 to 0.39)	0.001
Disease course				
	1.02 (0.96 to 1.07)	0.532		
WHO type				
A + AB + B1	ref.	-	ref.	-
B2 + B3	4.58 (1.63 to 12.86)	0.004	3.66 (0.93 to 14.45)	0.064
Masaoka-Koga stage				
I	ref.	-		
IIA to IV	0.89 (0.31 to 2.51)	0.820		
Tumor size group				
≤ 5 cm	ref.	-	ref.	-
> 5 cm	0.24 (0.09 to 0.64)	0.005	0.22 (0.06 to 0.82)	0.023
Ectopic thymus				
No	ref.	-		
Yes	2.05 (0.60 to 7.03)	0.255		
PD-L2 expression				
Negative	ref.	-	ref.	-
Positive	5.45 (1.88 to 15.75)	0.002	9.25 (1.93 to 44.30)	0.005
STAT6 expression				
Negative	ref.	-		

PD-L2, programmed death-ligand 2; WHO, World Health Organization.

	Univariate	Multivariate
Positive	0.28 (0.05 to 1.44)	0.127
PD-L2, programmed death-ligand 2; WHO, World Health Organization.		

Roc Analysis And Nomogram

The probabilities that thymoma patients would have MG were estimated by a multivariate logistic regression model that included age group, tumor size group, and PD-L2 expression. The adjusted R-square (Nagelkerke R-Square) reached 49.76%. The estimated probabilities were used in the ROC analysis predict the likelihood of MG. As shown in Fig. 2, the AUC = 0.859 (95% CI: 0.772 to 0.946; $P < 0.001$), indicating excellent predictability. A nomogram using the 3-factor model was established for clinical use (Fig. 3).

Discussion

Few studies have examined PD-L2 expression in thymoma. In humans, PD-L2 is expressed on dendritic cells and medullary thymic epithelial cells [10]. High PD-L2 expression may promote tumor metastasis, and predict an unfavorable prognosis of solid tumor cancer patients after surgery [13]. Previous studies have indicated that PD-L2 plays an important role in immune tolerance via dampening and modulating the T helper type 2 (Th2) cell response [14]. MG is an autoimmune disease associated with pathologies of the thymus, and in this study we investigated the association between PD-L2 expression in thymoma and MG. The main result of this study was that thymoma PD-L2 expression was significantly associated with MG and WHO histological types B2 and B3.

The relation between thymoma and MG is complex. Study has shown that MG is uncommon in patients with type A and type C thymoma [15]. A propensity score matching trial that included 470 patients reported that MG was not an independent or direct prognostic factor of thymoma [16]. On the other hand, age, Masaoka stage, and recurrence after surgery were found to be prognostic factors of overall survival in patients with MG and thymoma [17]. Recent study has also indicated genetic differences in thymoma between patients with MG and those without [18].

We found that approximately 58.6% of all patients with thymoma had positive PD-L2 expression. However, Rouquette et al. reported that thymic epithelial tumors did not stain positive for PD-L2, but positive expression of PD-L2 was associated with thymoma B2/B3 histological type [19]. It is important to note that that antibodies used, and cutoff values for positive staining vary among studies, and may lead to varying results. Few studies have examined PD-L2 expression in thymoma, but many studies have shown that high expression of PD-L1 in thymoma is associated with the B2/B3 histological type [20, 21]. A meta-analysis, however, indicated that in solid tumor cancer patients, high PD-L2 expression was

associated with an unfavorable prognosis [13]. We did not explore prognosis, but rather investigated the association of PD-L2 expression and MG.

Patients with a B2 histological type thymoma accounted for the greatest percentage of patients in this study (44.3%), and MG was associated with the B2/B3 histological type. Maggi et al. [22] reported that AB and B2 thymomas were the most frequently observed histological types in patients with MG. Ströbel et al. [23] also observed that the B2 histological type was the most frequently in patients with MG. Other studies have reported that the B1 and B2 histological types are the most common [24, 25].

Our results showed that positive PD-L2 expression was associated with a smaller tumor size, and the tumor size was smaller in patients with MG than in those without MG. This may be due to an earlier diagnosis because of the presence of MG [26]. In addition, we found that PD-L2 expression was associated with ectopic thymus. An ectopic thymus may play an important role in the pathogenesis of MG in patients with thymoma because of the TFH cells [6]. Furthermore, in a prior study we found that ectopic thymus was a poor prognosis factor in patients with MG [27]. In the current study, multivariable analysis showed that positive PD-L2 expression was significantly associated with MG in patients with thymoma.

In this study of patients with thymoma, MG was associated with the B2/B3 histological types, but there was no association with sex or Masaoka-Koga stage. There was, however, a tendency of MG in younger patients, but the result did not reach significance ($P = 0.052$). Kondo et al. [26] reported a higher percentage of patients with thymoma were females, and that patients with MG were significantly younger than those without MG. Shen et al. [28] reported that in patients with thymoma MG was associated with early clinical stage and AB, B1, and B2 type histological types, and suggested that there is an interrelation between WHO histological type, Masaoka-Koga stage, and MG. In our patients, PD-L2 scores were much higher in patients with MG than without MG (124.1 vs 23.3, $P < 0.001$). We did an analysis of PD-L2 scores in patients with MG only. We found that there were no significantly difference in PD-L2 scores regarding MGFA stage, Masaoka-Koga stage, tumor size or ectopic thymus, but a tendency toward high PD-L2 scores in patients with B2/B3 types.

It is speculated that T-lymphocyte development is involved in the pathogenesis of MG in patients with thymoma, and the different proportions of T-lymphocytes in different WHO histological types may contribute different frequencies of MG [29]. Thymomas in patients with MG are enriched in autoreactive T cells with specificity for acetylcholine receptors, and the export of autoreactive CD4 + T cells is of pathogenic relevance for MG because they may be involved in a signaling pathway for the pathogenesis of MG [30]. Study has shown a positive association between the percentage of CD4 + T cells and MG severity [4]. Other study has shown a marked decrease in anti-acetylcholine receptor antibodies after thymectomy for thymoma [31]. Furthermore, PD-1 is highly expressed in TFH cells and the PD-1 signaling pathway is involved decreased GC death and increase TFH cell cytokine production, i.e., PD-1 regulates GC and B cell survival and the formation and affinity of long-lived plasma cells [5]. We hypothesize that the relation of thymoma to the pathogenesis of MG is that PD-L2 co-stimulates PD-1 signaling through

exporting autoreactive T cells that assist in GC formation, B cell differentiation into plasma cells and memory cells, and antibody production in secondary lymphoid tissues. In future study we plan to explore the potential mechanism of the PD-L2 signaling pathway in thymoma-related MG pathogenesis using immunofluorescence and flow cytometry.

There are limitations to this study that should be considered. This was a retrospective study performed at a single institution, and the number of patients was relatively small. In addition, we did not investigate the association of PD-L2 expression with survival or other potential complications.

Conclusions

In conclusion, high thymoma PD-L2 expression in thymoma was significantly associated with MG and WHO histological types B2 and B3. These results suggest that PD-L2 may play a role in the pathogenesis of thymomatous MG.

Declarations

Funding: None declared.

Author Contributions: Chunhua Su conceived and designed the study. Haoshuai Zhu, Jianyong Zou, Bo Zeng, and Wei Yang acquired and analyzed the clinical data. Jiefei Xiao, and Xin Zhang conducted the statistical analysis. All authors wrote and reviewed the manuscript, and all authors approved the final version of the manuscript.

Availability of data and material: All data generated or analyzed during this study are included in this published article.

Code availability: Not applicable.

Compliance with Ethical Standards

Conflict of interest: The authors declare that they have no conflict of interest.

Ethical approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent: because of the retrospective nature of the study the requirement of informed patient consent was waived.

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Figures

Figure 1

Programmed death ligand 2 (PD-L2) immunohistochemical staining of thymoma. In this case, the tumor cells had stained cell membranes, and this tumor was defined as PD-L2 positive. The proportion of PD-L2 positive tumor cells was 95%, and staining intensity score = 3. (magnification, ×200).

Figure 2

Receiver operating characteristic (ROC) curve analysis of the probabilities of factors associated with MG. The area under the ROC curve (AC) = 0.859 (95% confidence interval [CI]: 0.772 to 0.946; $P < 0.001$).

Figure 3

Nomogram for prediction of myasthenia gravis established using the associated factors of age group, tumor size group, and PD-L2 expression.