



Expression of PD-L1/PD-1 in lymphoepithelioma-like carcinoma of the thymus

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Abstract

Poorly differentiated non-keratinizing squamous cell carcinoma of the thymus, also known as lymphoepithelioma-like carcinoma, is a rare primary malignant neoplasm of thymic origin. The mainstay of treatment for these tumors is surgical and they tend to respond poorly to chemotherapy. The checkpoint programmed cell death ligand-1 protein (PD-L1) bound to its receptor (PD-1) has been demonstrated to be an important therapeutic target for many different tumors. Expression of PD-L1/PD-1 in lymphoepithelioma-like carcinoma of the thymus may indicate that these tumors are potential targets for inhibitor therapy. Twenty-one cases of lymphoepithelioma-like carcinoma of the thymus were collected and reviewed. Tissue microarrays were created using triplicate 2 mm cores for each case. PD-L1/PD-1 staining pattern (neoplastic cells versus tumor infiltrating lymphocytes) was documented for each case. Out of 21 cases, 15 (71.4%) showed various degrees of membranous PD-L1 staining. Of the positive cases, 48% showed high expression of PD-L1 (>50% of tumor cells) and 24% showed low expression (<50%). PD-1 staining showed focal positivity in 12/20 (60%) cases among tumor infiltrating lymphocytes. PD-L1/PD-1 inhibitor therapy has been applied successfully in other solid malignant tumors with high expression of PD-L1/PD-1. The high level of PD-L1 expression in our cases indicates that PD-L1 may play a role in the pathogenesis of these tumors and that PD-L1/PD-1 blockade may be a viable therapeutic option for patients with lymphoepithelioma-like carcinoma of the thymus who have failed other first-line therapies.

Introduction

Poorly differentiated non-keratinizing squamous cell carcinoma, also known as ‘lymphoepithelioma-like carcinoma’ is a rare subtype of thymic carcinoma comprising approximately 5–10% of all thymic carcinomas [1–3]. These tumors represent a distinct histologic subtype of thymic carcinoma which is characterized by highly atypical tumor cells that grow in cords, nests, and trabeculae and often contain a dense lymphoplasmacytic infiltrate in the stroma [4]. Recently, several studies have identified increased expression of PD-L1 and PD-1 in thymic epithelial neoplasms including thymomas and thymic carcinomas suggesting that these lesions may be responsive to

PD-L1/PD-1 inhibitor therapy [5–14]. However, the expression of PDL-1 in lymphoepithelioma-like carcinoma of the thymus has not yet been specifically addressed.

Programmed cell death-1 (PD-1) and its receptor, programmed cell death-1 ligand-1 (PD-L1), constitute a regulatory immune checkpoint which plays an important role in self-tolerance and local inflammatory responses. PD-1 was shown to be expressed on T and B lymphocytes, while PD-L1 has been shown to be variably expressed in many stromal tissue types as well as upregulated in many types of neoplastic cells [14, 15]. The discovery of increased PD-L1 expression in many tumor types has led to the understanding that tumor cells may increase PD-L1 as a method of escaping immune surveillance and evading localized inflammatory responses which may help prevent tumorigenesis [16]. More recently, PD-L1/PD-1 immune complex inhibitor therapy has been shown to be efficacious in many different tumor types including lung, skin, bladder, renal, and some hematologic malignancies [15–19].

To this date, no study has specifically examined lymphoepithelioma-like carcinoma of the thymus with regard to expression of PD-L1/PD-1. These lesions are

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Table 1 Antibody information

Antibody	Clone	Dilution	Vendor	City, State, Country
PD-L1	SP142	1:30	Spring Biosciences	Pleasanton, CA, USA
PD-1	NAT105	1:50	Biocare Medical	Boston, MA, USA
CD20	L26	Ready to use	Dako-Agilent	Santa Clara, CA, USA
CD3	Polyclonal	Ready to use	Dako-Agilent	Santa Clara, CA, USA
Tdt	Polyclonal	Ready to use	Cell Marque	Darmstadt, Germany

characterized by having large numbers of mature stromal B and T lymphocytes, as well as plasma cells that are intimately associated with the tumor cells. The close association of the tumor cells with dense lymphocytic infiltrates in these tumors, unlike what is seen in other histologic subtypes of thymic carcinoma, suggests that immune checkpoint regulation may play a role in the pathogenesis of these lesions.

Immunohistochemistry has been reliably used to identify tumors in which PD-L1/PD-1 status has prognostic and therapeutic significance, such as in melanoma and small cell lung carcinoma [16–19]. Herein we examine PD-L1 and PD-1 status in 21 cases of lymphoepithelioma-like carcinoma of the thymus by immunohistochemistry using a clinically validated PD-L1 antibody (Clone SP142) [16–19]. The presence of increased PD-L1 expression may indicate that these lesions are amenable to PD-L1/PD-1 inhibitor therapy, especially in patients who are refractory to chemotherapy.

Materials and methods

Twenty-one cases of lymphoepithelioma-like carcinoma were obtained from the surgical pathology files of the Department of Pathology at the Beth Israel Deaconess Medical Center, Boston, MA, and the Medical College of Wisconsin, Milwaukee, WI, as well as from the personal consultation files of one of the authors (SS). Clinical information was obtained from the patient's records or by contacting the treating physicians. On each case, 2 to 13 histologic glass slides stained with hematoxylin and eosin were available for review. Representative paraffin blocks were chosen from each case for the creation of tissue microarrays. Detailed clinicopathological findings of these cases have been recently described separately [20].

Tissue microarrays were created using the 'TMA Grandmaster' platform (3DHISTECH LTD, Budapest, Hungary) using tumor tissue previously fixed in 10% buffered formalin and embedded in paraffin. Cases were represented in triplicate with 2 mm cores to ensure adequate tumor tissue was available for immunohistochemical studies. Then, 4 µm thick sections were cut from the tissue microarrays or individual blocks, deparaffinized in xylene, hydrated in descending dilutions of ethanol, and exposed to heat-induced epitope retrieval. Following pretreatment with

DAKO Target Retrieval Solution, tissue was blocked with peroxidase-blocking reagent for 5 min and incubated with the primary antibody at room temperature using antibodies listed in Table 1 on the DAKO AutostainerPlus instrument. Signals were detected using the Dako FLEX detection kit. Counterstaining was performed with Envision FLEX hematoxylin for 7 min at room temperature. Appropriate positive and negative controls were run concurrently for all antibodies tested.

A cutoff for the staining of PD-L1 of 50% was chosen to divide cases into either low expression (<50% staining in tumor cells) or high expression (>50% staining in tumor cells). Previous studies examining PD-L1 expression in non-small cell lung cancer have used similar cut-offs [19]. Only cases with appropriate membranous staining patterns were scored. In addition, staining for CD3, CD20, and TdT was performed to characterize the lymphocyte population.

Results

The patients were 17 men and 4 women, ranging in age from 20 to 85 years (mean = 60 years). All the tumors were located within the anterior mediastinum and measured from 2.0 cm to 13.5 cm in greatest dimension. By modified Masaoka [21] staging, 6 of the tumors presented as stage I, 8 of the tumors presented as stage II, 3 of the tumors presented at stage III, and 2 of the tumors presented at stage IV.

Histologically, the tumors were characterized by nests, islands, and cords of cohesive tumor cells separated by abundant lymphoplasmacytic stroma (Fig. 1a). On higher magnification, the tumor cells showed large, vesicular nuclei with single prominent eosinophilic nucleoli and a rim of amphophilic cytoplasm (Fig. 1b). Mitotic activity was high, and varied from 3 to 28 mitoses per 10 high-powered fields (mean = 8). Focal central areas of comedo-like necrosis were identified in all cases (Fig. 1c). Prominent lymphoid follicles with reactive germinal centers could be observed in 3 cases. The tumors were divided into lymphoid stroma predominant (lymphoepithelioma-like), lymphoid stroma-poor predominant (desmoplastic), and mixed.

Immunohistochemical staining for PD-L1 revealed that 15 out of 21 cases (71.4%) showed some degree of

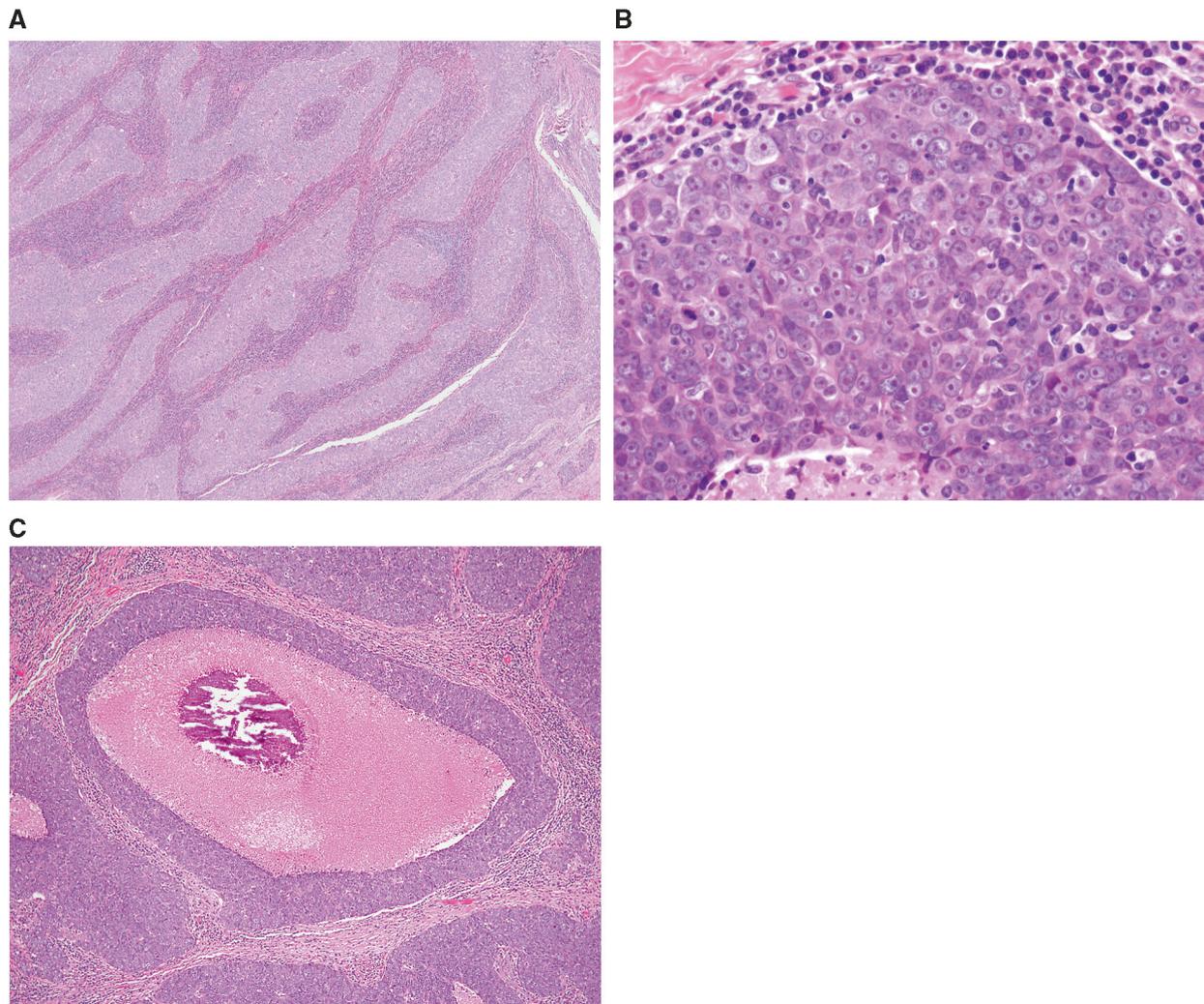


Fig. 1 **a** Low-power view of lymphoepithelioma-like carcinoma of the thymus showing nests, cords, and islands of poorly differentiated carcinoma cells embedded in a dense lymphoplasmacellular stroma. **b** High-power view showing large atypical cells with prominent

nucleoli, vesicular chromatin pattern, high mitotic activity, and indistinct cytoplasmic borders. **c** Lymphoepithelioma-like carcinoma displaying areas of comedo-like necrosis within nests of tumor cells

membranous staining of the tumor cells. Of these cases, 10 (48%) displayed strong staining (>50% staining in tumor cells) and 5 (24%) displayed weak staining (<50% staining in tumor cells) (Fig. 2a, b). Six (28%) cases showed no staining with PD-L1 in the tumor cells. PD-L1 showed no expression in the tumor-associated lymphocytes. The cases showing positive staining for PD-L1 all contained abundant stromal lymphoplasmacytic infiltrates in the cores examined except for two cases (cases 5 and 11, see Table 2).

PD-1 staining in 12 out of 20 cases (60%) showed focal staining within scattered small lymphocytes in the stroma (Fig. 3a, b). Of these cases, 8 (40%) showed staining in 0–33% of tumor cells, 3 (15%) cases showed staining in 34–66% of tumor cells, and only 1 (5%) case showed staining in >67% of lymphocytes. In addition, 8 cases (40%) showed no staining for PD-1 in the lymphoid cell component. PD-1 staining did not appear to parallel PD-L1

expression but was also seen mostly in the cores that contained abundant lymphoplasmacytic stroma. The lymphoid infiltrate showed positivity for CD20 and CD3 and was negative for TdT consistent with a mature mixed B- and T-lymphocyte population. Most the lymphoid cells in the lymphoid stroma-rich cases were composed of mature B cells and plasma cells, with only a few scattered mature CD3+ T lymphocytes. The pattern of staining for PD-1 overlapped with the pattern of staining for the CD3+ small lymphocytes.

Discussion

Poorly differentiated non-keratinizing squamous cell carcinoma of the thymus, also known as lymphoepithelioma-like carcinoma of the thymus, is a rare tumor with limited

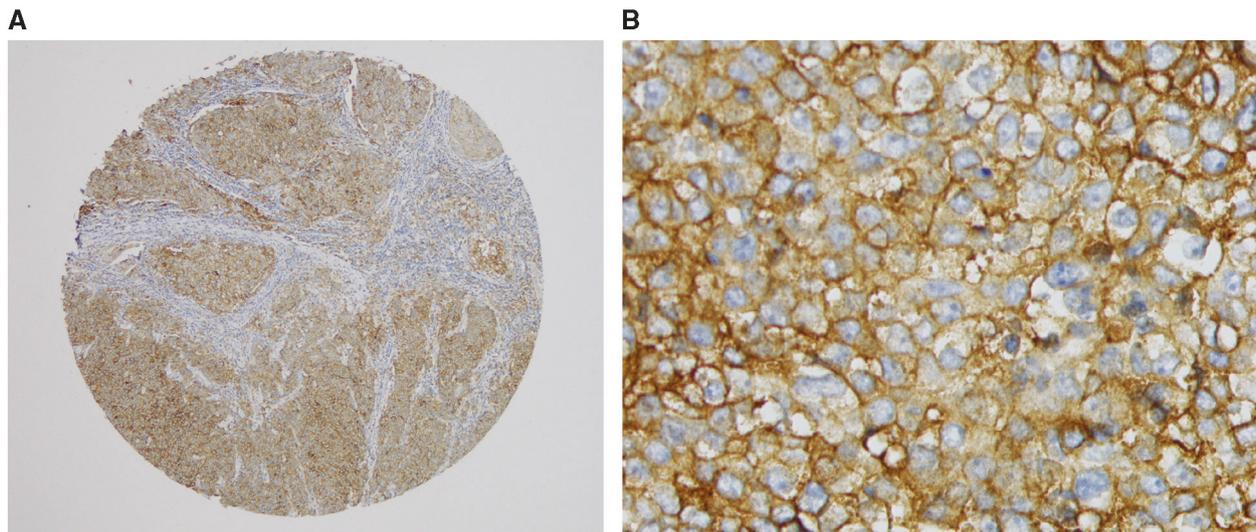


Fig. 2 **a** Tissue microarray showing strong membranous staining of PD-L1 in a high percentage of tumor cells. **b** High-power view of membranous staining pattern for PD-L1

Table 2 Percentage of PD-L1 staining in 21 cases of poorly differentiated non-keratinizing squamous cell carcinoma of the thymus

Case no.	PD-L1 staining percent staining	Stromal pattern
1	>50%	Lymphoepithelioma-like
2	0	Desmoplastic
3	>50%	Lymphoepithelioma-like
4	>50%	Lymphoepithelioma-like
5	<50%	Desmoplastic
6	0	Desmoplastic
7	>50%	Lymphoepithelioma-like
8	<50%	Lymphoepithelioma-like
9	>50%	Lymphoepithelioma-like
10	>50%	Lymphoepithelioma-like
11	<50%	Desmoplastic
12	0	Desmoplastic
13	0	Desmoplastic
14	<50%	Lymphoepithelioma-like
15	>50%	Lymphoepithelioma-like
16	>50%	Lymphoepithelioma-like
17	0	Desmoplastic
18	>50%	Lymphoepithelioma-like
19	<50%	Lymphoepithelioma-like
20	>50%	Lymphoepithelioma-like
21	0	Desmoplastic

Lymphoepithelioma-like: cores were characterized by dense lymphoplasmacytic stroma; Desmoplastic: cores showed a lymphoid cell-poor stroma

treatment options outside of surgery [4, 20]. In the largest study to date of lymphoepithelioma-like carcinoma of the thymus, it was found that these tumors tend to respond poorly to chemotherapy with an overall survival of

18 months [4]. First-line chemotherapeutic agents most often consist of carboplatin and paclitaxel followed by other platinum-based regimens as second-line therapy, with limited data available on the efficacy of immune checkpoint inhibitors [22]. PD-L1/PD-1 inhibitor therapy has been used in other solid malignant tumors with high expression of PD-L1/PD-1 to great success. In many of these tumor types, PD-L1 and PD-1 expression have been demonstrated to predict responsiveness to treatment with immune checkpoint inhibitor drugs such as pembrolizumab, atezolizumab, and nivolumab [15–19]

Herein, we have used immunohistochemistry to assess the expression of PD-L1 and PD-1 in lymphoepithelioma-like carcinoma of the thymus. To our knowledge, this is the first study to specifically evaluate PD-L1 and PD-1 expression in these tumors as most of the previous studies have primarily evaluated well-differentiated squamous cell carcinomas. Well-differentiated forms of squamous cell carcinoma of the thymus have been reported to be associated with tumor infiltrating lymphocytes; however, the lymphoid infiltrate is rarely as prominent as compared to lymphoepithelioma-like thymic carcinoma [4]. Previous studies have also reported a variable relationship of PD-L1 and PD-1 expression to overall survival; and the relationship to treatment response of immune checkpoint inhibitors has yet to be properly studied in these tumors [12–14].

In our study we found that approximately 71.4% of tumors expressed some degree of positivity for PD-L1 staining and 60% of tumors showed some degree of PD-1 positivity. Of our cases, 48% displayed PD-L1 staining in greater than 50% of malignant tumor cells, placing them into the clinically actionable category identified in similar studies of solid tumors in other organ systems [17–19].

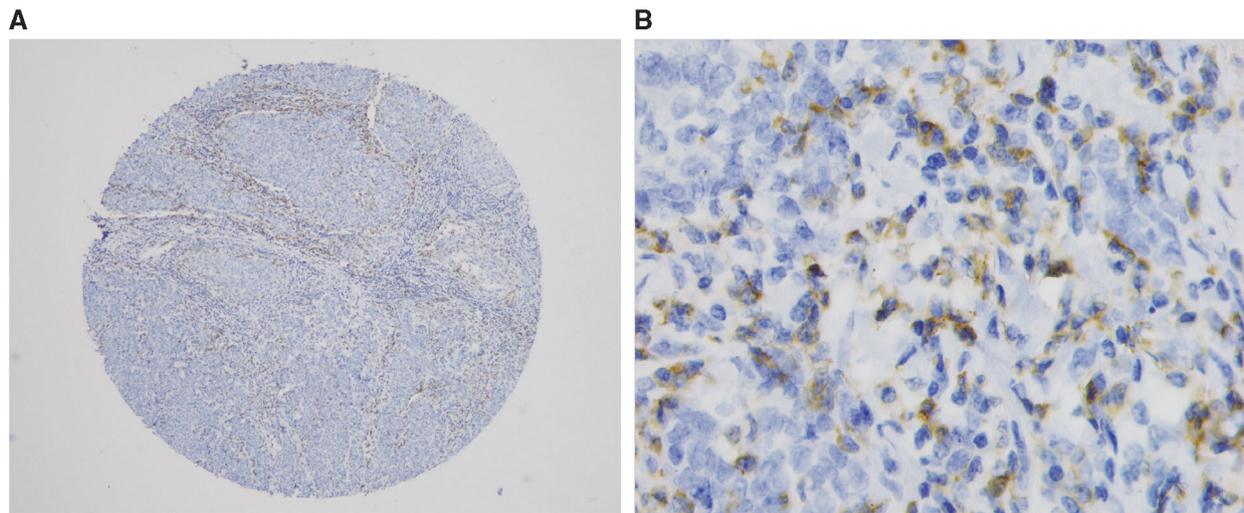


Fig. 3 **a** Tissue microarray showing the predominant pattern of PD-1 expression with weak intensity of staining in a low-to-moderate percentage of tumor-associated lymphocytes in the stroma. **b** High-power magnification of PD-1 staining in lymphocytes adjacent to tumor cells

These findings are also in keeping with previous studies of PD-L1 and PD-1 in thymic carcinoma and thymoma that have found increased PD-L1/PD-1 expression in thymic epithelial neoplasms [12–14]. Lymphoepithelioma-like carcinoma of the thymus has recently been shown to present with two distinct histologic variants: lymphoepithelioma-like (with dense lymphoplasmacytic stroma) and desmoplastic (devoid of lymphocytes in the stroma) [20]. It is of interest that all the cases in our study showing expression of PD-L1 contained abundant lymphocytes in the stroma. This is in keeping with the observation that tumors associated with a prominent lymphoid host response are the ones most likely to express PD-L1 in the tumor cells. Testing for PD-L1 is therefore recommended in cases of poorly differentiated non-keratinizing squamous cell carcinoma of the thymus of the lymphoepithelioma-like variant. Our findings support the notion that some of these lesions may be amenable to immune checkpoint therapy.

Most cases in this study demonstrated weak staining with PD-1 in a low percentage of tumor-associated lymphocytes. Only a single case with high expression of PD-L1 showed strong staining for PD-1 in the associated tumor infiltrating lymphocytes. This is not unexpected as PD-1 is mostly expressed in cytotoxic T cells rather than in mature B lymphocytes and plasma cells, in keeping with the predominance of mature B cells and plasma cells seen in the stroma of lymphoepithelioma-like carcinoma.

We have demonstrated that lymphoepithelioma-like carcinoma of the thymus, a lesion often associated with dense lymphoplasmacytic infiltrates in the stroma, displays high expression of PD-L1 on tumor cells in nearly half of the cases evaluated. Previous studies on solid and

hematologic tumors at other sites have shown that PD-L1 expression can predict clinical response to immune checkpoint inhibitor therapies in a range of advanced or refractory tumors, including small cell carcinoma of the lung, renal cell carcinoma, urothelial carcinoma, and melanoma [15–19]. Our finding of increased PD-L1 expression in LELCA of the thymus suggests that these poorly differentiated tumors may be amenable to immune checkpoint therapy, particularly in patients who have failed first-line therapy with platinum-based chemotherapy agents.

Compliance with ethical standards

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

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