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PD-1/PD-L1 expressions in medullary thyroid carcinoma: Clinicopathologic and prognostic analysis of Chinese population

Yalan Bi [1], Xinyu Ren [1], Xiaohui Bai, Yunxiao Meng, Yufeng Luo, Jinling Cao, Yuhan Zhang, Zhiyong Liang*

Department of Pathology, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100730, China

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ABSTRACT

Introduction: Few studies have focused on PD-L1 expression in medullary thyroid carcinoma (MTC). Expressions of PD-1 and PD-L1 and their clinicopathologic and prognostic relevance were therefore further investigated on a relatively large population of MTC patients.

Materials and methods: Surgical specimens were obtained from 87 MTC patients during a median follow-up of 37.7 months. PD-1 and PD-L1 expressions on tumor and associated immune cells were studied immunohistochemically using >1% positive cells as a threshold for positivity. Their correlations with clinicopathologic and prognostic feature were analyzed.

Results: PD-1 and PD-L1 were positively stained in 22 and 19 MTC patients. Most PD-L1-positive cases (18/19) showed weak to moderate staining intensity. PD-1 and PD-L1 were co-expressed in 11 patients. PD-L1 positivity was significantly correlated with distant metastases at surgery (21.1% vs 1.5%, $P = 0.007$). Coexpression of PD-1 and PD-L1 in MTC was correlated with advanced pathologic TNM stage III/IV ($P = 0.040$) and distant metastases at surgery ($P = 0.013$). However, there was no other clinicopathologic and prognostic relevance regarding to PD-1, PD-L1 or their coexpression in our MTC patients.

Conclusion: PD-1/PD-L1 pathway was expressed in MTC patients and was significantly correlated with the distant metastases at surgery, which may shed light on PD-1/PD-L1 as a promising therapeutic target in MTC. Future better understanding of PD-1/PD-L1 expression and their relationship with immunotherapy response may provide direct evidence for management of refractory MTC.

Introduction

Medullary thyroid carcinoma (MTC) is a rare malignancy of parafollicular cell origin that distinct it from other types of thyroid carcinoma. It accounts for less than 10% of all thyroid tumors [1], but represents up to 13% of all thyroid cancer-related death [2,3]. Surgical resection remains the only curative treatment for most MTC patients [4]. However, almost 50% of patients will still have residual or recurrent disease even after aggressive treatment [5]. Palliative chemotherapy and/or radiotherapy are only recommended for unresectable patients [6], which necessitated alternative treatment options.

Immunotherapy targeting immune checkpoint molecules such as programmed cell death protein 1 (PD-1) has been rapidly explored as a promising alternative for cancer management, including thyroid carcinomas [7,8]. Expressions of PD-1 and its ligand, programmed death-ligand 1 (PD-L1), have been demonstrated in various human cancers and were associated with the prognosis of the patients [9–15]. PD-L1 was suggested to act as a predictive marker for clinical response to PD-1/PD-L1 blockage [16,17]. Identification of predictive biomarkers may be helpful in patient selection for better patient management and utilization of healthcare resources. In MTC, blocking the PD-1/PD-L1interaction using an anti-PD-1 agent, Nivolumab, has been reported to achieve a partial response in one patient [18]. However, to our best knowledge, only one study has reported PD-L1 expression in MTC patients, with very low or almost no expression of PD-L1 in tumor

* Corresponding author.
E-mail address: liangzhiyong1220@yahoo.com (Z. Liang).
[1] Yalan Bi and Xinyu Ren contributed equally to this work.

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cells and tumor-infltrating immune cells [19]. These results prompted us to further investigate the prevalence and prognostic significance of PD-1/PD-L1 expressions in a comparatively large population of MTC patients.

Patients and methods

Patients

MTC patients that underwent surgical resection in the Peking Union Medical College Hospital during February 2001 to January 2016 were retrospectively reviewed. Pathological diagnoses were established by experienced pathologists according to the World Health Organization [20]. Paraffin embedded tumor samples were obtained from all eligible patients for histopathological analysis. The corresponding peritumoral normal tissues were also analyzed. Clinicopathological and follow-up information were retrieved from the medical records of the patients. This study was approved by the Ethical Committee of Peking Union Medical College Hospital. Informed consent was waived for this retrospective study.

PD-1/PD-L1 immunohistochemistry

A rabbit anti-human PD-L1 monoclonal antibody (clone SP263, Ventana Medical Systems, Tucson, USA) was used to assess PD-L1 expression on tumor cells and tumor-infiltrating immune cells. Expression of PD-1 in the tumor-infiltrating immune cells was detected by a mouse monoclonal antibody (MRQ-22, OriGene Technologies, Rockville, MD). Paraffin embedded tumor samples were deparaffinized and rehydrated with descending ethanol. Heat-induced antigen retrieval was achieved by boiling at 100 °C for 30 min. Endogenous peroxidase activity was quenched by incubation with 3% H_2O_2 for 5 min. The sections were then incubated with anti-bodies against PD-1 and PD-L1 at 37 °C for 16 min and followed by incubation with the herseradish peroxidase-conjugated second antibodies. Immunoreactivity was visualized with diaminobenzidine. The sections were counterstained with haematoxylin and mounted.

Immunohistochemistry results were interpreted by two experienced pathologists. Only the membranous staining was considered as positive for tumoral PD-L1 expression, while either membranous or cytoplasmic staining was considered for tumor infiltrating immune cells [21]. The percentages of PD-1 or PD-L1 positive cells within the tumor area were scored. A threshold of >1% positive cells was considered as PD-1 or PD-L1 positivity [19].

Statistical analysis

Differences in clinicopathological data between PD-1 or PD-L1 positive and negative groups were analyzed by *t*-test, Mann–Whitney *U* test, and Chi-square or Fisher Exact test as appropriate. Progression-free survival (PFS) was defined as the interval from the date of surgical resection to the date of progression (recurrence/distant metastases), death, or the date of last follow up. Overall survival (OS) was defined as the interval from the date of surgical resection to the date of death or last follow-up. Univariate COX analysis was performed to evaluate the progression free and overall survival of the patients. A multivariate Cox proportional hazards model was established to evaluate the independent predictors of progression or overall mortality using a forward stepwise method, with all variables in the univariate analysis included (age, sex, MTC type, pathologic TNM stage, unilateral distribution, multifocality, adjuvant therapy, PD-1 and PD-L1 expressions). All tests were two sided. A P value of <0.05 was considered as statistically significant (SPSS 22.0, SPSS, Chicago, IL).

Results

Clinical characteristics of MTC patients

A total of 87 patients with MTC were identified in this study. The median age of the patients was 47 years (range: 21–73 years). Forty-four patients were male, with an equal male-to female ratio. Most patients were diagnosed with sporadic MTC (68/87, 78.2%). Most tumors were unifocal (58/87, 66.7%), unilateral (70.1%), and without capsular invasion (74.7%). Forty-three patients (49.4%) had lymph node metastasis (LNM). Enlarged thyroid gland was the major complaints of the patients (29.9%), followed by compression (10.3%) and high blood pressure (6.9%). By contrast, 37 patients (42.5%) were asymptomatic. Thyroid surgery was carried out in all 87 patients. Five patients had distant metastases at presentation (4 pulmonary and 1 liver). Fourteen patients were further treated by adjuvant therapy (10 with radiotherapy, 3 with chemotherapy, and 1 with chemoradiotherapy). Eight patients died during a median follow-up of 37.7 months.

Correlation of PD-1/PD-L1 expression with the clinical characteristics

We did not detect PD-1 or PD-L1 expression in any of peritumoral normal thyroid tissues. By contrast, immunostainings of PD-L1 and PD-1 were identified in tumor and associated immune cells. Representative patterns of PD-1 and PD-L1 expressions are shown in Fig. 1. PD-1 was positively stained in 22 cases of immune cells infiltrated MTC (25.3%). Nineteen (21.8%) patients had positive PD-L1 staining on tumor and associated immune cell, respectively. Most PD-L1-positive MTC showed weak to moderate staining intensity (18 in tumor cells and 17 in immune cells), which resulted in the strong positive staining of PD-L1 in 1 and 2 cases of tumor cells and associated immune cells. Concomitant expressions of immune PD-1 and tumor PD-L1, as well as immune and tumor PD-L1 were observed in 11 patients. There was a significant correlation between immune and tumor PD-L1 positivity ($r = 0.461$, $P < 0.001$), and the percentage of PD-L1 positive cells in tumor and tumor-infiltrating immune cells (Supplemental Fig. 1). No microsatellite instability was observed in our study based on the immunohistochemical analysis of MSH-2, MSH-6, PMS-2, MLH-1, and SDHB expressions.

Correlation of PD-1, PD-L1 or their coexpression with clinicopathological characteristics were then explored as shown in Table 1. Both PD-L1 expressions in tumor cells and associated immune cells (Supplemental Table 1) were significantly associated with distant metastases at surgery, with a higher rate of metastasis observed in PD-L1 positive ones (21.1% vs 1.5%, $P = 0.007$). No significant correlation was evident between PD-1/PD-L1 expression and other clinicopathological data (Table 1). Coexpression of PD-1 and PD-L1 was presented in patients with more advanced disease, including pathologic TNM stage III/IV ($P = 0.040$) and distant metastasis at surgery ($P = 0.013$). However, there was no significant association with respect to other clinicopathological data, as shown in Table 1.

Survival analysis of patients with MTC

The median follow-up time was 37.7 months (IQR: 15.7–72.4 months). During the follow-up, 19 patients had disease progression (recurrence and/or metastasis) and 8 patients died. The estimated 5-year progression free and overall survival rates were $72.4 \pm 6.2\%$ and $86.0 \pm 4.8\%$, respectively. No significant effect of PD-1 and PD-

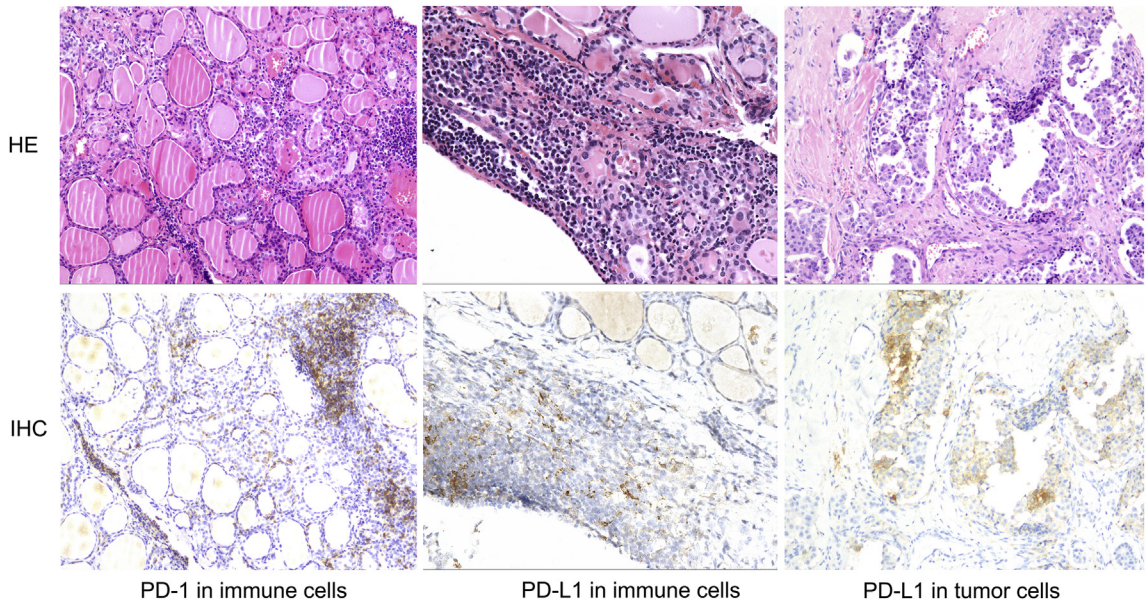

Fig. 1. Histopathological analysis of PD-1 and PD-L1 expressions in medullary thyroid carcinoma patients.

Table 1
Clinical characteristic of the patients with MTC and their associations with PD-1 and PD-L1 expressions.

	PD-1 in immune cells			PD-L1 in tumor cells			PD-1/PD-L1		
	PD-1- (n = 65)	PD-1+ (n = 22)	*P*	PD-L1- (n = 68)	PD-L1+ (n = 19)	*P*	Coexp (n = 11)	Non-coexp (n = 76)	*P*
Age (years)	47.79 ± 11.7	47.68 ± 13.27	0.973	47.09 ± 12.59	50.16 ± 9.77	0.329	49.82 ± 10.48	47.46 ± 12.29	0.547
Gender n (%)			0.950			0.470			0.354
Male	33 (50.8)	11 (50.0)		33 (48.5)	11 (57.9)		7 (63.6)	37 (48.7)	
Female	32 (49.2)	11 (50.0)	0.950	35 (51.5)	8 (42.1)	0.470	4 (36.4)	39 (51.3)	0.354
Sporadic MTC n (%)	51 (79.7)	17 (81.0)	1.000	52 (78.8)	16 (84.2)	0.752	8 (72.7)	60 (81.1)	0.686
Pathologic TNM stage n (%)			0.242			0.310			**0.040**
I/II	33 (50.8)	8 (36.4)		34 (50.0)	7 (36.8)		2 (18.2)	39 (51.3)	
III/IV	32 (49.2)	14 (63.6)		34 (50.0)	12 (63.2)		9 (81.8)	37 (48.7)	
Tumor size (cm)	2.36 ± 1.52	2.41 ± 1.71	0.902	2.35 ± 1.61	2.43 ± 1.44	0.845	2.42 ± 1.95	2.36 ± 1.53	0.912
Multifocality n (%)	5 (7.7)	1 (4.5)	1.000	6 (8.8)	0 (0.0)	0.332	5 (7.4)	1 (5.3)	1.000
Bilateral distribution n (%)	19 (29.2)	7 (31.8)	0.819	22 (32.4)	4 (21.1)	0.408	4 (36.4)	22 (29.0)	0.727
Capsular invasion n (%)	16 (24.6)	6 (27.3)	0.804	15 (22.1)	7 (36.8)	0.19	4 (36.4)	18 (23.7)	0.459
LNM n (%)	31 (59.6)	12 (60.0)	0.976	32 (57.1)	11 (68.8)	0.565	7 (63.6)	36 (47.5)	0.297
Distant metastases at surgery n (%)	2 (3.1)	3 (13.6)	0.100	1 (1.5)	4 (21.1)	**0.007**	3 (27.3)	2 (2.6)	**0.013**
Adjuvant therapy n (%)	9 (14.1)	5 (22.7)	0.336	10 (14.9)	4 (21.1)	0.500	3 (27.3)	11 (14.5)	0.377

Note: MTC: Medullary thyroid carcinoma; LNM: Lymph node metastasis; Coexp: Coexpression.

L1 expression on the overall and progression free survival was observed in our patients. Coexpression of PD-1 and PD-L1 was significantly associated with the worse overall survival (HR = 5.67, 95%CI: 1.10–29.31, $P = 0.038$), but not with the worse progression free survival of patients ($P = 0.915$).

Univariate survival analysis showed that multifocality (HR = 5.11, 95%CI: 1.03–25.36, $P = 0.046$), distant metastases at surgery (HR = 10.86, 95%CI: 2.57–45.93, $P = 0.001$) and adjuvant therapy (HR = 4.69, 95%CI: 1.17–18.80, $P = 0.029$) were also significantly associated with overall survival of the patients (Table 2). However, no significant factor that related the overall survival was identified when pathologic TNM stage was entered (without lymph node metastasis and distant metastasis at surgery) into a multivariate model (Table 2). One thing worth noting is that variables including pathologic TNM stage and lymph node metastasis were not analyzed in the univariate COX model due to the small sample size (Supplemental Fig. 2).

Meanwhile, progression free survival of the patients were significantly associated with the pathologic TNM stage III/IV (HR = 6.93, 95%CI: 1.98–24.28, $P = 0.002$), multifocality (HR = 4.86, 95%CI: 1.35–17.52, $P = 0.016$), lymph node metastasis (HR = 13.06, 95%CI: 1.69–101.31, $P = 0.014$), distant metastases at surgery (HR = 6.70, 95%CI: 1.86–24.10, $P = 0.004$), and adjuvant therapy (HR = 8.88, 95%CI: 3.25–24.27, $P < 0.001$). In multivariate Cox analysis, however, only pathologic TNM stage III/IV (HR = 8.19, 95% CI = 1.80–37.17, $P = 0.0006$) and adjuvant therapy (HR = 7.67, 95% CI = 2.52–23.29, $P < 0.001$) were independent predictors of worse progression free survival (Table 3 and Fig. 2).

Discussion

PD-1 and PD-L1 expressions in MTC patients were evaluated here in a comparatively large sample of the population. Our study revealed for the first time a high rate of PD-1 expression (25.3%, 22/87) on the tumor-infiltrating immune cells in MTC patients. Meanwhile, PD-L1 was positively stained by tumor and associated

Table 2
Overall survival of the patients with medullary thyroid carcinoma.

	Univariate analysis	
	HR (95% CI)	*P*
Age ≥45 years	5.00 (0.61–40.88)	0.133
Male	2.83 (0.57–14.05)	0.203
Familial MTC	0.03 (0.00–50.11)	0.364
Pathologic TNM stage III/IV	-	-
Unilateral distribution	0.45 (0.11–1.81)	0.262
Multifocality	**5.11(1.03–25.36)**	**0.046**
LNM	-	-
Distant metastases at surgery	**10.86 (2.57–45.93)**	**0.001**
Adjuvant therapy	**4.69 (1.17–18.80)**	**0.029**
Immune PD-1+	1.59 (0.32–7.92)	0.574
Tumor PD-L1+	3.58 (0.85–15.14)	0.083
Immune PD-L1+	2.65 (0.63–11.14)	0.183
PD-1/PD-L1 coexpression	**5.67 (1.10–29.31)**	**0.038**

Note: MTC: Medullary thyroid carcinoma; LNM: Lymph node metastasis. Univariate analysis of TNM stage and LNM is not performed since all patients died were at TNM stage III/IV or with LNM.

immune cells in 19 patients (21.8%), respectively. We showed lack of PD-1 and PD-L1 expression in the corresponding peritumoral non-tumor tissues. These results were much higher than those reported by M Bongiovanni et al., which indicated PD-L1 expressions in 6.25% (1/16) and 6.25% (1/16) of MTC cases on tumor and accompanying inflammatory cells, respectively [19]. Besides, our study showed a higher expression of PD-L1 in patients with distant metastasis at surgery (21.1% vs 1.5%, $P = 0.007$), and co-expression of PD-1 and PD-L1 was significantly associated with the advanced TNM stage (stage III/IV, $P = 0.040$) and distant metastasis at surgery ($P = 0.013$). However, no significant association was observed with respect to other clinicopathological characteristics. Moreover, we failed to demonstrate any prognostic relevance of PD-1, PD-L1 or their coexpression in our multivariate analysis. These results were partially consistent with no prognostic significance of PD-L1 reported by M Bongiovanni et al. [19]. Despite of that, our study showed that pathologic TNM stage III/IV and adjuvant therapy were independent predictors of worse progression free survival in our MTC patients. Our results highlighted the MTC as a distinctive type that is always refractory to adjuvant therapy. Therefore, significant associations between advanced TNM stage and PD-L1 or PD-1/PD-L1 coexpression, as well as refractory to adjuvant therapy of MTC observed in our study may shed light on immunotherapy against PD-1/PD-L1 checkpoint as an alternative treatment.

PD-1/PD-L1 axis is an immunoregulator that has been widely investigated as a potential target for tumor immunotherapy [22]. Expressions of PD-1 and/or PD-L1 have been studied in various cancers to predict immunotherapy response. In thyroid tumors, however, only few studies have been carried out, but mainly focused on follicular cell-derived tumors, such as papillary thyroid carcinoma (PTC), follicular thyroid carcinoma (FTC) and anaplastic thyroid carcinoma (ATC) [12,23–29]. Tumor PD-L1 expression has been reported to range from 6.1% to 82.5% in PTC, 7.6%–87.5% in FTC, and 22.2%–75.0% in ATC (Table 4). With respect to PD-L1 expression in the tumor-infiltrating immune cells, few studies are available. The frequencies of PD-L1 positivity on tumor-infiltrating immune cells were 28.5% (93/326) for PTC, 9.1% (6/66) for FTC, 11.1% (1/9) for ATC according to the study by Ahn et al. [25]. However, a higher immune cell PD-L1 positivity of 43.75% (7/16) was also noted in ATC patients [27]. Potential explanations for these discrepancies include different population selected, antibodies utilized, and discordant scoring and interpretation of immunohistochemical results [30]. PD-L1 was suggested to be highly expressed in advanced thyroid cancer, such as FTC and ATC [25]. As for MTC, only one study has reported on PD-L1 expression. Very low expression of PD-L1 was observed in MTC patients. PD-L1 was found to be expressed on tumor and associated immune cells in each one (6.25%) of their MTC patients [19]. A slightly higher expression of PD-L1 was observed in our study, even though the same antibody (clone SP263) and same methods for scoring and data interpretation were applied. Only the membranous staining of PD-L1 was counted for tumor cells and the percentage of positive cells >1% was applied as a threshold for determining PD-1/PD-L positivity in our study [19,25].

MTC is known to have a tendency of early metastasis [31], which may consequently contribute to the comparatively poor prognosis of the patients [32]. In our study, positivity of PD-L1, but not PD-1, was more frequent in MTC patients with distant metastasis at surgery. Coexpression of PD-1 and PD-L1 was significantly associated with advanced tumor stage (TNM III/IV) and distant metastasis at surgery. However, there was no significant association between prognosis and PD-1, PD-L1 or their expression in a multivariate Cox analysis. These results were partially consistent with the previous report by Bongiovanni et al., their study showed no correlation between PD-L1 expression and clinicopathological stage or survival of the MTC patients [19]. Association of PD-L1 status with clinicopathologic features and clinical outcome has been studied in other types of thyroid cancer. The results were conflicting with the study population and tumor types. Some studies showed a strong correlation between PD-L1 expression and clinicopathologic variables

Table 3
Progression free survival of the patients with medullary thyroid carcinoma.

	Progression free survival			
	Univariate analysis		Multivariate analysis	
	HR (95% CI)	*P*	HR (95% CI)	*P*
Age ≥45 years	0.72 (0.27–1.87)	0.494		
Male	0.67 (0.25–1.79)	0.428		
Familial MTC	0.41 (0.09–1.79)	0.234		
Pathologic TNM stage III/IV	**6.93 (1.98–24.28)**	**0.002**	**8.19 (1.80–37.17)**	**0.006**
Unilateral distribution	0.80 (0.30–2.18)	0.668		
Multifocality	**4.86 (1.35–17.52)**	**0.016**		
LNM	**13.06 (1.69–101.31)**	**0.014**		
Distant metastases at surgery	**6.70 (1.86–24.10)**	**0.004**		
Adjuvant therapy	**8.88 (3.25–24.27)**	**<0.001**	**7.67 (2.52–23.29)**	**<0.001**
Immune PD-1+	1.79 (0.58–5.58)	0.314		
Tumor PD-L1+	1.41 (0.40–4.98)	0.594		
Immune PD-L1+	2.11 (0.74–6.03)	0.162		
PD-1/PD-L1 coexpression	1.12 (0.15–8.60)	0.915		

Note: MTC: Medullary thyroid carcinoma; LNM: Lymph node metastasis.

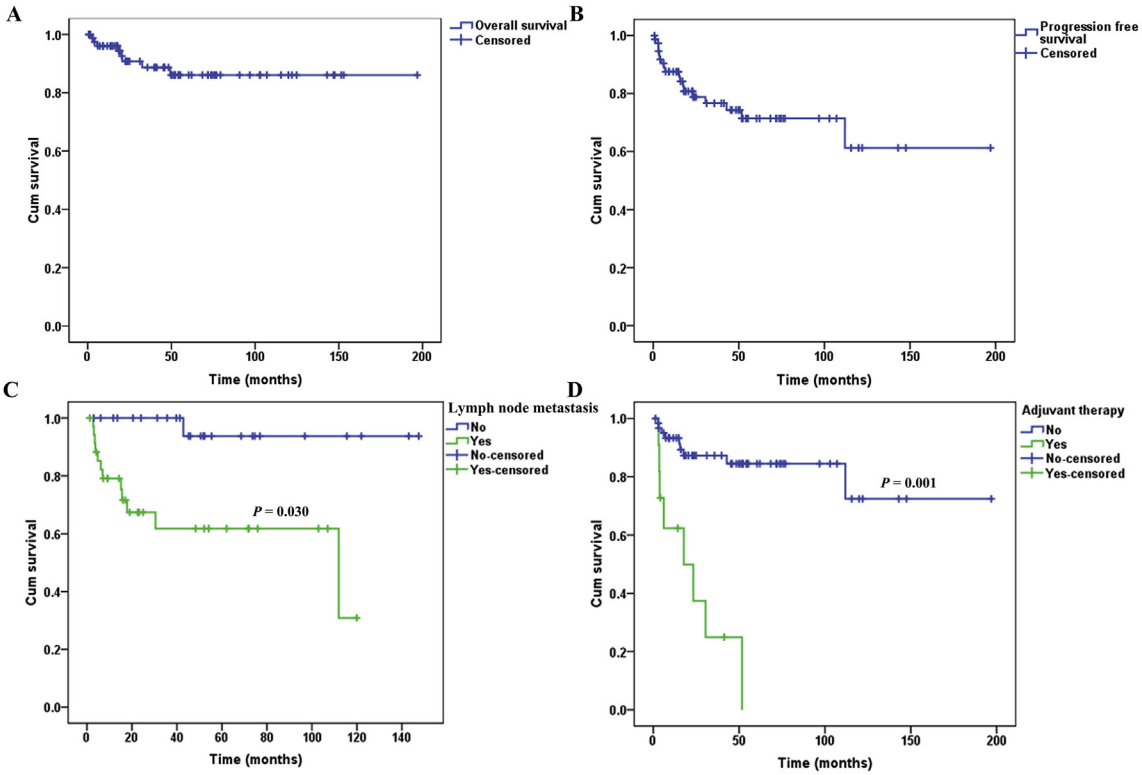

Fig. 2. Survival analysis of the patients with medullary thyroid carcinoma. Overall survival (A); Progression free survival (B) and that stratified by pathologic TNM stage (C) and adjuvant therapy (D).

Table 4
PD-L1 quantification by routine immunohistochemistry in thyroid carcinomas.

	PD-L1 IHC		PD-L1 positivity				
	Ab	Scoring system	Tumor cells	Immune cells	Control	Clinical association	Prognosis
Cunha et al. [26]	pAb (ab82059)	>0% in cytoplasm of tumor cells	209/253 (82.5%) PTC, 35/40 (87.5%) FTC		2/5 (33.3%) normal and 92/114 (80.7%) benign	Negatively with LNM	None
Angell et al. [24]	pAb (4059)		18/33 (54.5%) PTC				
Wu et al. [23]	mAb (5H1)		3/13 (23.1%) ATC				
Chowdhury et al. [29]	mAb (E1L3N)	>0% in cytoplasm or membrane	74/187 (40.0%) and 123/187 (66.5%) PTC*		6/66 (9.1%) benign	None	Recurrence and worse DFS
Bastman et al. [34]	mAb (SP142)	Allred scores ≥3	7/14 (50.0%) DTC, 6/8 (75.0%) ATC	10/13 (76.9%) PD-L1+ Tumor		Positively with LNM	
Shi et al. [28]	mAb (ab174838/ MABC290)	Immunoreactivity score ≥4	136/260 (52.3%) PTC		96/260 (36.9%) peritumoral	Positively with multifocality and ETE	Worse RFS
Chintakuntlawar et al. [27]	mAb (E1L3N)	Allred scores ≥3	13/16 (81.25%) ATC	7/16 (43.75%)		None	Worse PFS and OS
Ahn et al. [25]	mAb (SP142)	>1% or 5% in membrane of tumor cells	20/326 (6.1%) PTC, 5/66 (7.6%) FTC, 2/9 (22.2%) ATC	93/326 (28.5%) PTC, 6/66 (9.1%) FTC, 1/9 (11.1%) ATC		None	None
Bongiovanni et al. [19]	mAb (SP263)	>1% in membrane of tumor cells	1 (6.25%) MTC	1/16 (6.25%) MTC		None	None

Note: *Membranous and cytoplasmic expressions, respectively; mAb: Monoclonal antibody; pAb: Polyclonal antibody; LNM: Lymph node metastasis; PTC: Papillary thyroid cancer; FTC: Follicular thyroid cancer; DTC: Differentiated thyroid cancer; ATC: Anaplastic Thyroid Cancer; MTC: Medullary thyroid carcinoma; ETE: Extrathyroidal extension; DFS: Disease free survival; RFS: Recurrence free survival; PFS: Progression free survival; OS: Overall survival.

and/or disease progression. However, the opposite results of no significant association were also reported (Table 4). The difference in tumor types and sample sizes may partially contribute to the heterogeneous results observed in these studies. Added to this is the problem of possible variation in antibodies utilized. All of these differences, combined with the lack of standardized criteria for PD-L1 quantification, mean that prognostic value of PD-L1 in thyroid cancers will still be controversial. A harmonized procedure is urgently needed for PD-L1 evaluation in thyroid cancers. One thing worth noting in our study is that PD-L1 and its coexpression with PD-1 were significantly associated with the distant metastasis at surgery and/or advanced pathologic TNM stage (III/IV). The latters were significantly associated with overall survival or progression free survival of the patients. MTC is always known to be refractory to conventional chemotherapy and radiotherapy, as demonstrated by our data and previous study of 1252 MTC patients by Roman and

colleagues, which indicated that adjuvant radiation therapy was an independent factor that is associated with poor prognosis of the MTC patients [33]. However, these results must be interpreted cautiously in view of the small patient number. Nevertheless, based on these results, we suggested that PD-1/PD-L1 may be a promising therapeutic target for MTC management. Future studies are therefore warranted to understand better the relationship between PD-L1 expression and immune response.

In conclusion, present study extended previous observations by demonstrating high PD-1 and PD-L1 expressions in MTC patients. Although no prognostic significance of PD-1/PD-L1 was observed in our MTC patients, we showed for the first time a significant correlation between PD-L1 positivity and distant metastasis at surgery, which may shed light on PD-1/PD-L1 as a promising therapeutic target for MTC management. Future studies are needed to understand better the relationship between PD-L1 expression and immune response.

Declarations of interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejso.2018.10.060.

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