



Research Paper

Programmed Death - Ligand 1 Expression Distinguishes Invasive Encapsulated Follicular Variant of Papillary Thyroid Carcinoma from Noninvasive Follicular Thyroid Neoplasm with Papillary-like Nuclear Features



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ABSTRACT

Background: The noninvasive Encapsulated follicular variant of papillary thyroid cancer (EFVPTC) has been reclassified as Noninvasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP) without a significant risk for malignant behavior. However the evaluation remains a challenge for clinicians. We sought to determine whether programmed death-ligand 1 (PD-L1) expression may serve as a biomarker to predict invasiveness of EFVPTC and assist to distinguish these neoplasms from NIFTP.

Methods: Immunohistochemical staining of PD-L1 expression was performed in sections of 174 Formalin-fixed paraffin-embedded (FFPE) tissue blocks from surgery removed thyroid nodules.

Results: Cytoplasmic PD-L1 expression was significantly increased in invasive EFVPTC (4.76 ± 1.49) as compared to NIFTP (3.06 ± 2.16 , $p < 0.001$). Increased cytoplasmic PD-L1 expression was associated with invasiveness in EFVPTC ($p < 0.001$); PD-L1 positive EFVPTC cases were at 3.16 folds higher risk in developing invasion than the PD-L1 negative cases. No significant difference in cytoplasmic PD-L1 expression was observed between NIFTP and benign nodules.

Conclusion: PD-L1 expression may serve as a useful biomarker in predicting invasiveness of EFVPTC and distinguishing NIFTP from invasive EFVPTC. To our knowledge this is the first report suggesting the application of a protein biomarker to confirm NIFTP as benign indolent neoplasms.

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1. Introduction

The global increasing incidence of thyroid cancer in part attributed to improved diagnostic tools and surveillance has culminated in over-diagnosis and over-treatment (Vaccarella et al., 2016). The follicular variant of papillary thyroid carcinoma (FVPTC) is a heterogeneous group of tumors, including the infiltrating and encapsulated variants (Shi et al., 2016). The diagnosis of encapsulated FVPTC (EFVPTC) without invasion as a malignant lesion remains controversial (Hodak et al.,

2016; Nikiforov et al., 2016; Thompson, 2016). The microscopic diagnostic criteria for EFVPTC are follicular architecture, widespread nuclear features of papillary carcinoma, some additional features (papillae, fibrous bands, dense eosinophilic colloid), and the presence of capsule (Shi et al., 2016).

The noninvasive EFVPTC lesions having follicular growth pattern, particular microscopic architecture and nuclear features of PTC with low risk of malignancy have been reclassified as noninvasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP) (Nikiforov et al., 2016; Strickland et al., 2016). The assessment of such borderline lesions is challenging for clinical practice as in this “grey zone” the intra-observer agreement may vary from 17% to 100% (Elsheikh et al., 2008). Recently Nikiforov et al. proposed diagnostic criteria for NIFTP which improved the overall diagnostic accuracy of NIFTP to 85.0–94.3% (Nikiforov et al., 2016). In EFVPTC, invasion rather than nuclear features influence tumor progression, patient management and prognosis (Ganly et al., 2015).

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Molecular testing may yield markers helpful in distinguishing invasive EFVPTC versus NIFTP where histological assessment of invasion is difficult or equivocal and ultimately may aid in differentiating these tumors by the fine needle aspirate (FNA) biopsy. Thus, ancillary methods to assess the invasive behavior of thyroid neoplasms are necessary to arrive at appropriate clinical decision to reduce the necessity of surgery and potential radioactive iodine (RAI) treatment. Biomarker-based ancillary screening may be a great asset in improving the diagnosis of NIFTP in pathology practice.

Recently, we reported programmed cell death ligand 1 (PD-L1) expression on tumor cells correlated with poor prognosis in thyroid cancer (Chowdhury et al., 2016). PD-L1 is a ligand for Programmed cell death 1 (PD-1/B7-H1) receptor to function as negative immune regulator. PD-1 is expressed on the surface of lymphocytes, such as T cells, B cells and natural killer T (NKT) cells. PD-L1 binding to PD-1 suppresses T-cell proliferation and cytokine release, such as IL-2 (Dong et al., 2002; Boussiotis, 2016). Its overexpression in a malignant neoplasm prevents malignant cells from being attacked by immune system due to a suppression of cytotoxic T cells. Thus, higher expression of PD-L1 in tumors will interfere with anti-tumor immunological attack and facilitate tumor growth and metastases. However, PD-L1 overexpression has not been examined to date to determine whether it can distinguish NIFTP from EFVPTC.

We hypothesized that PD-L1 expression could be a useful biomarker to distinguish NIFTP from invasive EFVPTC and thereby serve as to improve the accuracy of histopathological diagnosis of “grey zone” lesions which are in fact benign.

2. Materials and methods

2.1. Patients

This retrospective study was approved by Research Ethics Board (REB #07-0212-E) of Sinai Health System, Mount Sinai Hospital (MSH), a University of Toronto-affiliated hospital and a prime referral centre for thyroid diseases in Toronto, Canada. A total of 1859 cases of surgically removed thyroid nodules were carried out at Mount Sinai Hospital from Jan 2010 to Jun 2015. After analysis of clinical charts, surgical pathology reports and histological slides, 174 cases of surgically resected thyroid nodules were selected for this retrospective study using following inclusion criteria: the presence of a recorded chart with the gender and age of the patient at the time of diagnosis, recurrence episodes, size of the nodule, focality, area of fibrosis, capsular/vascular invasion, lymph node metastasis, as well as the existence of enough biological material in the paraffin blocks. Formalin-fixed paraffin-embedded (FFPE) tissue blocks from the selected 174 thyroid nodules were retrieved from MSH tumor bank. H&E stained sections were reviewed by two pathologists (CM, OP) and classified into four specific diagnostic subgroups- 52 NIFTPs and 45 cases of EFVPTC with invasion for comparison with 40 benign nodules and 37 cases of FVPTC with coexisting advanced lymphocytic or Hashimoto's thyroiditis (LT) (van Heerden et al., 1992; Nikiforov et al., 2012; Seethala et al., 2014; Nikiforov et al., 2016).

2.2. Inclusion/exclusion criteria

To identify NIFTP subgroup the inclusion criteria included major features (encapsulation/clear demarcation, follicular growth pattern, nuclear features of PTC: enlargement, crowding/overlapping, elongation, irregular contours, grooves, pseudoinclusions, chromatin clearing) and minor features (dark colloid, irregularly shaped follicles, intratumoral fibrosis, “sprinkling” sign, follicles cleft from stroma, multinucleated giant cells within follicles). The exclusion criteria for NIFTP were observed presence of “true” papillae > 1%, psammoma bodies, infiltrative border, tumor necrosis, high mitotic activity, cell/morphologic characteristics of other variants of PTC (Nikiforov et al., 2016).

To identify EFVPTC with invasion we used the features as encapsulated or well circumscribed nodule, follicular architecture, full thickness capsular invasion/vascular invasion or extrathyroidal extension, and 2–3 nuclear features of PTC using a 3-point nuclear scoring scheme (Nikiforov et al., 2016) (enlargement, crowding/overlapping, elongation, irregular contours, grooves, pseudoinclusions, chromatin clearing) (Seethala et al., 2014). Lymphocytic thyroiditis (LT) was separated from the FVPTC cases which did not satisfy the inclusion criteria for NIFTP and had no definite capsular or vascular invasion and had histological features of coexisting advanced lymphocytic or Hashimoto's thyroiditis. (van Heerden et al., 1992; Nikiforov et al., 2012; Seethala et al., 2014). All microcarcinomas (≤ 1 cm) were excluded from the study. Two pathologists independently reviewed all cases and separated them into 4 groups based on these inclusion/exclusion criteria.

2.3. Specimens and immunohistochemistry

FFPE tissue sections (5 μ m thickness) were deparaffinized and rehydrated with series of graded alcohol. Antigen retrieval for PD-L1 was performed in Tris-EDTA buffer (pH = 9.0). After blocking the non-specific binding with background punisher (Biocare Medical, LLC, Concord, CA), the sections were incubated overnight (4 °C) with the monoclonal rabbit anti-PD-L1 antibody at 1:100 dilution (E1L3N, Cell Signalling Technology, Inc., Danvers, MA). The VECTASTAIN rapid protocol (Vector Labs, Burlington, ON, Canada) for immunostaining was performed. Diaminobenzidine (DAB) was used as the chromogen and counterstained with hematoxylin as described by us (Chowdhury et al., 2016). FFPE sections of classical variant of PTC were used as a positive control and histiocytes as internal positive controls. The thyroid cancer tissue incubated with an isotype specific IgG in place of the primary antibody was used as a negative control for PD-L1 staining in each batch of immunostaining.

2.4. Evaluation of immunostaining

Immunostaining scores were based on the percentage positivity and staining intensity as described previously (Chowdhury et al., 2016). Slides were scored as positive if epithelial cells showed immunoreactivity in the cytoplasm. Percentage positive scores were assigned according to the following scale: 0 \leq 10%; 1 \geq 11–30%; 2 \geq 31–50%; 3 \geq 51–70%; and 4 \geq 71%. Staining intensity was scored semi-quantitatively as follows: 0 (none); 1 (mild); 2 (moderate) and 3 (intense) (Ralhan et al., 2015; Chowdhury et al., 2016). A total score was then obtained (ranging from 0 to 7) by adding the percentage positivity scores and intensity scores for each section. The IHC scoring was blinded from the histopathology report and was performed by two evaluators independently and used for subsequent analyses. The final score was given using following formula: [(percentage score 1 + intensity score 1) + (percentage score 2 + intensity score 2)]/2.

2.5. Statistical analysis

The data collected in a database designed for this study were analyzed using The SPSS 24.0 (SPSS, Chicago, IL, <http://www.ibm.com/analytics/us/en/technology/spss/>) program. A descriptive analysis of the population was performed. A multivariate analysis performed and the quantitative variables were described with central tendency measures: means, standard error and dispersion standard deviation, range to 95%. Means of PD-L1 scores were compared among the different diagnostic subgroups using one-way ANOVA test. The significance of the association between cytoplasmic PD-L1 positivity and invasiveness was examined by Fisher's exact test. The results were considered significant when the obtained 2-tailed values were $p < 0.05$.

3. Results

3.1. Patient characteristics and clinical-pathological parameters

The clinical and pathologic characteristics of the study cohort are summarized in Table 1. Among the 174 cases meeting the inclusion criteria, 40 cases were classified as benign nodules, 52 as NIFTP, 45 as EFVPTC with capsule or vessel invasion and 37 cases with lymphocytic thyroiditis (LT). Based on the staging classification system according to the 7th edition of American Joint Committee on Cancer (AJCC) (Edge and Compton, 2010), AJCC stages of NIFTPs and EFVPTCs were classified according to the pathology findings at resection as shown in Table 1.

3.2. Cytoplasmic expression of PD-L1 is decreased in NIFTP

Immunohistochemical evaluation of cytoplasmic PD-L1 staining revealed significant differences in its expression between NIFTP and EFVPTC with invasion subgroups (Table 2). Increasing cytoplasmic PD-L1 immunostaining was observed from NIFTP (Fig. 1a & b) and benign nodules (Fig. 2a & b) to EFVPTC with invasion (Fig. 1c & d) and LT (Fig. 2c & d, Table 3). The negative and positive controls are shown in Fig. 3a & b, respectively. Multivariate analyses showed cytoplasmic PD-L1 expression was significantly increased in EFVPTC with invasion (mean \pm standard deviation, 4.76 ± 1.49 ; 95% CI, 4.32–5.21) as compared to NIFTP (3.06 ± 2.16 ; 95% CI, 2.46–3.67, $p < 0.001$) as well as benign nodules (2.53 ± 1.34 ; 95% CI, 2.10–2.96, $p < 0.001$). There was no significant difference shown between benign nodules and NIFTP with $p = 0.554$. A significant increase in PD-L1 cytoplasmic expression was observed in EFVPTC with lymphocytic thyroiditis (4.51 ± 1.39 ; 95% CI: 0.79–3.31) comparing to benign ($p < 0.001$) as well as NIFTP subgroups ($p < 0.001$) (Tables 2 & 3). Based on cutoff point ≥ 4.5 for PD-L1 cytoplasmic score (sensitivity 91%, specificity 85%) (Chowdhury et al., 2016), 31 of 45 cases (69%) EFVPTC with invasion and 16 of 52 (31%) NIFTP cases were positive (Table 4). Fisher's exact test revealed that increased

Table 1
Demographics and clinicopathologic features of patient cohort.

Characteristics	Total cases (n = 174)
Age, mean (range)	50.2 (16–80)
Gender	n (%)
Male	
<45 years	10 (6)
≥ 45 years	26 (15)
Female	
<45 years	50 (29)
≥ 45 years	88 (50)
Pathological diagnosis	n (%)
Benign	
Multiple hyperplastic nodules	34 (20)
Cyst	4 (2)
Follicular adenoma	2 (1)
Neoplasm	
NIFTP	52 (30)
EFVPTC	45 (26)
Lymphocytic thyroiditis (LT) ^a	37 (21)
AJCC stages of neoplasms^b	n (%)
NIFTP	
Stage 0	2 (1)
Stage I	31 (60)
Stage II	12 (23)
Stage III	5 (10)
EFVPTC	
Stage I	27 (60)
Stage II	13 (29)
Stage III	5 (11)

^a Lymphocytic thyroiditis (LT) was separated from other categories because of coexisting advanced Lymphocytic or Hashimoto's thyroiditis.

^b AJCC staging for neoplasms of NIFTP and EFVPTC was based upon pathology findings at resection.

Table 2

Comparison of cytoplasmic PD-L1 expression among subgroups of Benign, NIFTP, EFVPTC and Lymphocytic thyroiditis.

(I) Groups	(J) Groups	Mean difference (I–J)	Std. error	P value	95% Confidence interval	
					Lower bound	Upper bound
Benign	NIFTP	–0.61	0.041	0.554	–1.79	0.58
	EFVPTC	–2.33 ^a	0.43	0.000	–3.54	–1.12
	Lymphocytic thyroiditis	–2.05 ^a	0.45	0.000	–3.31	–0.79
NIFTP	Benign	0.61	0.42	0.554	–0.58	1.79
	EFVPTC	–1.72 ^a	0.36	0.000	–2.73	–0.71
	Lymphocytic thyroiditis	–1.44 ^a	0.38	0.003	–2.51	–0.37
EFVPTC	Benign	2.33 ^a	0.43	0.000	1.12	3.54
	NIFTP	1.72 ^a	0.36	0.000	0.71	2.73
	Lymphocytic thyroiditis	0.28	0.39	0.915	–0.82	1.38
Lymphocytic thyroiditis	Benign	2.01 ^a	0.45	0.000	0.79	3.31
	NIFTP	1.43 ^a	0.38	0.003	0.37	2.51
	EFVPTC	–0.28	0.39	0.915	–1.38	0.82

^a The mean difference is significant at $p < 0.05$ level (ANOVA).

cytoplasmic PD-L1 expression statistically correlated with capsular invasion ($p < 0.001$). There was no strong association between cytoplasmic PD-L1 expression and age of patient, with a cut off 45 years old ($p = 0.196$, Fisher's exact test).

The comparison of PD-L1 expression between benign subgroup and EFVPTC with invasion significantly correlated with invasion risk [Odds ratio (OR) 19.93, 95% CI: 1.59–3.07; $p < 0.001$]. The relative risk for cases with positive PD-L1 cytoplasmic expression was 3.16 folds higher than the PD-L1 negative cases.

4. Discussion

This study revealed cytoplasmic PD-L1 immunohistochemical analysis may serve as a potential ancillary method for distinguishing invasive EFVPTC from NIFTP and as an invasion predictor in EFVPTC to improve the accuracy of histopathological diagnosis of “grey zone” thyroid nodules thereby preventing patients from overtreatment and overdiagnosis. The four key novel findings are: (i) PD-L1 levels were significantly lower in NIFTP as compared to invasive EFVPTC; (ii) PD-L1 expression in benign and NIFTP subgroups was similar; (iii) EFVPTC cases with positive cytoplasmic PD-L1 expression were at higher risk of invasion; (iv) Lymphocytic infiltration enhanced PD-L1 expression.

To our knowledge, this is the first report to demonstrate the use of a protein biomarker to objectively distinguish NIFTP from EFVPTC with invasion. The pathological criteria recommended by Nikiforov et al. (2016) for identifying NIFTP are based on morphological features and adequate sampling of the tumor capsule interface to exclude invasion. The assessment of the whole capsule and evaluation of invasiveness could be challenging in some cases (e.g. sharp tumor bud invades into but not through the capsule, follicles aligned perpendicular, mushroom-shaped tumor within but not through the capsule, partially preserved capsule, FNA biopsy cytology features (Maletta et al., 2016)). The use of a protein biomarker PD-L1 expression, combining with histopathology, will facilitate an accurate distinction between NIFTP and EFVPTC with invasion and thereby aid in selection of NIFTP patients for lobectomy without RAI or observation only, and invasive EFVPTC management by completion thyroidectomy \pm RAI (Haugen et al., 2016). Recently, we reported increasing PD-L1 expression in thyroid cancer cells correlated with poor prognosis (Chowdhury et al., 2016). Upon binding to its specific receptor PD-1, it acts as an immune checkpoint regulator. This PD-L1/PD-1 pathway played a role in inhibiting T-cell receptor (TCR) mediated cell growth and cytokine production, resulting in peripheral tolerance of cancer cells and a failure to recognize

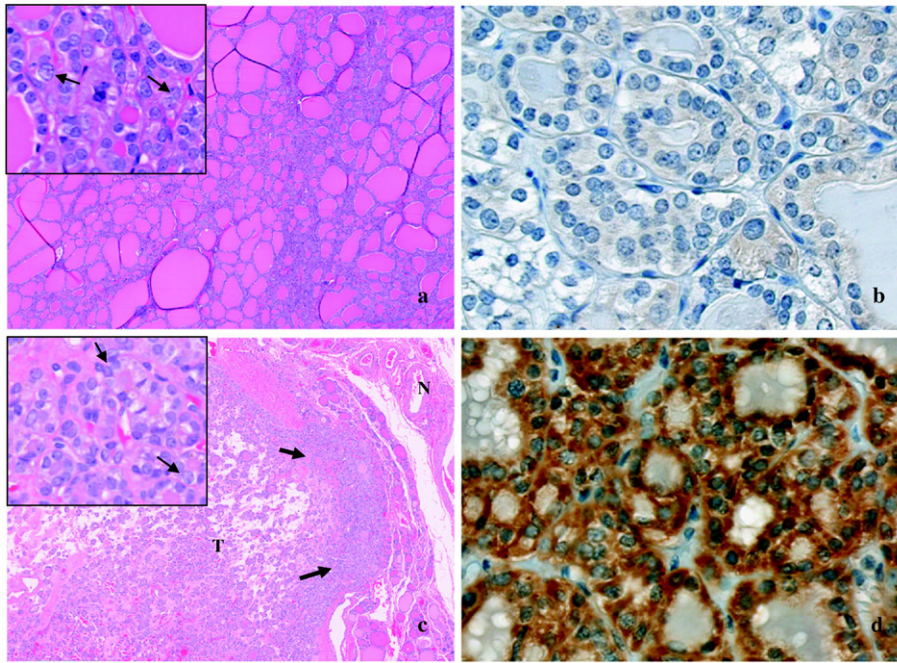


Fig. 1. Histology and immunohistochemical staining of PD-L1 expression in NIFTP and EFVPTC tissues. (a), H&E stained tissue section of NIFTP showed follicular architecture. Insert: PTC cytological features: nuclear enlargement, grooves, micronuclei, pale to clear chromatin and crowding (thin black arrows). (b), Immunostaining of tissue section of NIFTP. Faint cytoplasmic PD-L1 immunostaining was detected in NIFTP. (c), H&E stained tissue section of EFVPTC showed the focal capsular invasion (thick black arrow), the follicular growth pattern of the tumor (T) as compared to normal thyroid (N). Insert: typical PTC cytological features: enlarged nuclei with central clearing, grooves and crowding (thin black arrows). (d), Immunostaining of tissue section of EFVPTC with capsular invasion. Moderate to strong cytoplasmic PD-L1 expression was observed in EFVPTC with invasion. Image magnifications are $\times 20$ for (a) and (c), $\times 200$ for (a) and (c) inserts, and $\times 400$ for (b) and (d), respectively.

and destroy the foreign cancer cells (Freeman et al., 2000; Blank et al., 2005; Okazaki and Honjo, 2007). Accordingly, the extent of PD-L1 expression on tumor cells largely emerges as a predictive biomarker in anti-PD-L1 directed cancer immunotherapy (Topalian et al., 2015). In

the present study, we demonstrated that the expression of PD-L1 can be a predictor for distinguishing NIFTP from invasive EFVPTC.

Importantly, no significant difference in cytoplasmic PD-L1 expression between benign and NIFTP subgroups suggests that NIFTPs are

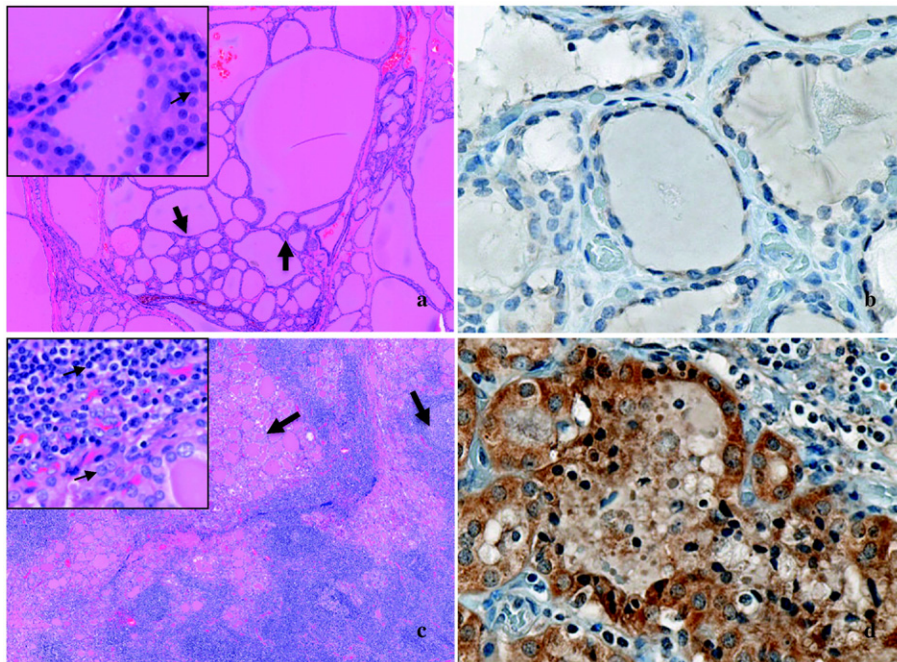


Fig. 2. Histology and immunohistochemical staining of PD-L1 expression in benign nodules and EFVPTC with lymphocytic thyroiditis tissues. (a), H&E stained tissue section of benign hyperplastic nodule showed follicles of various sizes (thick black arrows). Insert: uniform round nuclei (thin black arrows). (b), Immunostaining of tissue section of benign neoplasm. No detectable cytoplasmic PD-L1 expression was observed in benign nodules. (c), H&E stained tissue section of Lymphocytic thyroiditis showed severe lymphoplasmacytic infiltration with germinal center formation and follicles (thick black arrows). Insert: extensive lymphocytic and plasmacytic infiltrate with oxyphilic metaplasia of follicular cells (thin black arrows). (d), Immunostaining of tissue section of EFVPTC with lymphocytic thyroiditis. Mild to moderate cytoplasmic PD-L1 expression was observed. Image magnifications are $\times 40$ for (a) and (c), $\times 200$ for (a) and (c) inserts, and $\times 400$ for (b) and (d), respectively.

Table 3
Cytoplasmic PD-L1 expression in subgroups of Benign, NIFTP, EFVPTC and Lymphocytic thyroiditis.

Subgroups	N	Mean \pm SD	95% Confidence interval	
			Lower bound	Upper bound
Benign	40	2.53 \pm 1.34	2.10	2.96
NIFTP	52	3.06 \pm 2.16	2.46	3.67
EFVPTC	45	4.76 \pm 1.49	4.32	5.21
Lymphocytic thyroiditis	37	4.51 \pm 1.39	4.04	4.97

more close to benign nodules, supporting the new paradigm for classification of NIFTP as non-malignant neoplasms (Maletta et al., 2016; Nikiforov et al., 2016; Patel, 2016; Thompson, 2016). The EFVPTC with invasion subgroup in which the cases were mainly classified as stage I and II at resection showed increased PD-L1 cytoplasmic staining. Using a completely different patient cohort in our previous study, a significant increase in both membrane and cytoplasmic PD-L1 expression was shown in patients with stage IV disease and correlated with high risk of aggressive disease, distant metastasis or death (Chowdhury et al., 2016). The correlation of cytoplasmic PD-L1 expression with risk of invasion in EFVPTC is in accord with our recent report of PD-L1 as a marker of thyroid cancer prognosis and aggressiveness (Chowdhury et al., 2016) and other cancers (Hamanishi et al., 2007; Leite et al., 2015). The cytoplasmic PD-L1 expression was significantly increased in EFVPTC with invasion comparing to NIFTP subgroup. These findings support the concept that NIFTP behaves primarily as a benign neoplasm. Based on several studies, including our previous report (Chowdhury et al., 2016), we classified cases of FVPTC without detectable capsular/vascular invasion and having coexisting diffuse lymphocytic infiltration into a separate LT subgroup since the mononuclear infiltration can influence the expression of PD-L1 and lead to a false positive result. The level of cytoplasmic PD-L1 expression was significantly higher in cases with lymphocytic infiltration than benign thyroid nodules, but similar to EFVPTC with invasion. This emphasizes that overexpression of PD-L1 in benign thyroid nodules should be interpreted with caution and that coexistence of chronic lymphocytic thyroiditis or Hashimoto's thyroiditis can cause a misleading false positive result (Taube et al., 2012; Cunha et al., 2013).

We are cognizant of the limitations that our findings are based on patients from a single clinical center; validation in independent patient cohorts is needed. Further, limited follow up period (1.5–5 years) did not permit a clear statement regarding the relationship of cytoplasmic PD-L1 overexpression with progression of invasive EFVPTC, their aggressiveness and/or recurrence.

In conclusion, examination of PD-L1 expression provided an add-on approach in distinguishing invasive EFVPTC from NIFTP and supported the concept that NIFTP is a non-malignant neoplasm. NIFTP patients showing high PD-L1 expression require follow up monitoring to exclude

Table 4
Comparison of cytoplasmic PD-L1 expression in NIFTP and EFVPTC.

		Groups		
		NIFTP	EFVPTC	Total
PD-L1 cytoplasmic expression	Negative cases	36 (69%)	14 (31%)	50
	Positive cases	16 (31%)	31 (69%)	47
No. of total cases (%)		52 (100%)	45 (100%)	97

Notes: (1) Negative cases were defined by score of PD-L1 cytoplasmic expression <4.5 ; Positive cases were defined by score of PD-L1 cytoplasmic expression ≥ 4.5 .

(2) Fisher's exact test showed the positivity of cytoplasmic PD-L1 expression statistically associated with capsular invasiveness with $p < 0.001$.

a risk of invasiveness. The level of PD-L1 expression may be useful in guiding their future management by improving the accuracy of histopathological diagnosis of "grey zone" thyroid nodules and pre-surgical cytological diagnosis thereby allowing a better selection of patients requiring surgery to prevent overtreatment and distress for patients.

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Disclosure statement

PGW and RR are shareholders in Proteocyte Diagnostics Inc. All the other authors (GF, OP and CM) have nothing to disclose.

Authors' contributions

RR and PGW conceptualized the study, contributed to the study design and to the manuscript. GF stained the slides, GF and OP conducted the experimental work. OP provided the clinical parameters and the follow-up data. GF and OP are equal contributors. CM performed the histopathology reporting of all the patients' tissues analyzed. OP and GF did the statistical analysis. RR and PGW provided the infrastructural support for this study. The manuscript was drafted by GF, OP and RR, and submitted for comments to all the authors. All authors approved the final version of the manuscript. RR and PGW are both senior and corresponding authors of this manuscript.

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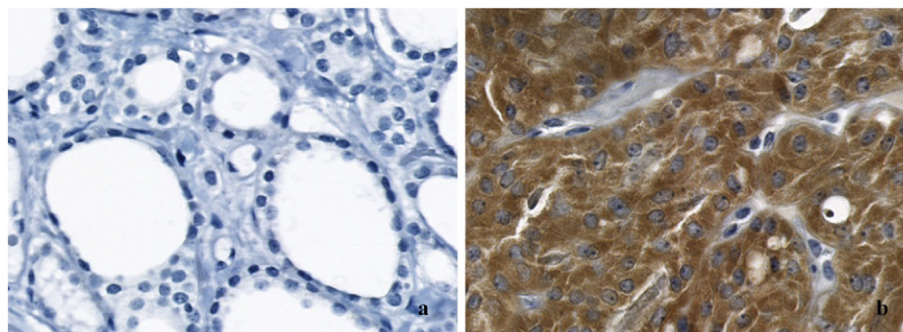


Fig. 3. Negative and positive controls for immunohistochemical staining of PD-L1 expression. (a), Negative control, a benign thyroid nodule incubated with isotype specific IgG in place of the anti-PD-L1 antibody did not show detectable immunostaining. (b), Positive control, an example of classic aggressive PTC section randomly selected for anti-PD-L1 antibody staining showed moderate to strong immunostaining. Image magnifications are $\times 400$ for (a) and (b).

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Presentations

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