Original papers

PD-L1, PDK-1 and p-Akt are correlated in patients with papillary thyroid carcinoma

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation;
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Abstract

Background. Papillary thyroid carcinoma (PTC) is the most common type of thyroid carcinoma.

Objectives. To investigate the clinical significance of programmed death ligand 1 (PD-L1) and phosphoinositide-dependent protein kinase 1 (PDK1) in PTC.

Material and methods. A total of 194 PTC patients were recruited. Contralateral normal thyroid tissues were obtained and used as controls (n = 80). The expression levels of PD-L1, PDK1 and p-Akt were determined using immunohistochemistry.

Results. The PD-L1, PDK1 and p-Akt were upregulated in cancer tissues compared to the normal tissues. The mean optical density (MOD) values of PD-L1, PDK1 and p-Akt were significantly higher in the PTC tissues. The expression of PD-L1 positively correlated with the levels of PDK1 and p-Akt. In addition, the expression of PD-L1, PDK1 and p-Akt in PTC patients without chronic lymphocytic thyroiditis (CLT) was significantly higher than the expression of those proteins in the CLT patients. The patients with higher expression levels of PD-L1, PDK1 or p-Akt had remarkably larger tumors and higher rates of TNM III—IV, capsular infiltration, lymph node metastasis, and of recurrence. The Kaplan—Meier curve showed that patients with lower expression of PD-L1, PDK1 or p-Akt had significantly longer recurrence-free time. The logistic regression analysis revealed that only CLT, PD-L and capsular infiltration were risk factors for patients' five-year recurrence.

Conclusions. The PD-L1, PDK1 and p-Akt were found to be positively correlated with a poor prognosis in PTC.

Key words: prognosis, papillary thyroid carcinoma, p-Akt, sPD-L1, PDK-1

Introduction

Papillary thyroid carcinoma (PTC) is the most common type of thyroid carcinoma.[1,2] It accounts for about 70–85% of all thyroid carcinoma cases.[3,4] Compared to other types of thyroid carcinomas, such as follicular thyroid carcinoma (FTC) and anaplastic thyroid carcinoma (ATC), PTC has a better prognosis with a five-year survival rate of over 90%.[5,6] However, lymph node metastasis and recurrence are very common in PTC patients.[7] Recurrence has been reported in 15–30% of cases and lymph node metastasis occurs in 5–10% of PTC patients.[8,9] For these patients, the five-year survival rate is only about 50%.[10]

Chronic lymphocytic thyroiditis (CLT), also known as Hashimoto's thyroiditis, is an autoimmune disease characterized by a painless and diffuse goiter, occasionally accompanied by atypical symptoms, such as hyperthyroidism, exophthalmos, hypothyroidism, etc.[11] The relationship between CLT and PTC has been reported in recent years. Previous studies have shown that PTC patients with concurrent CLT had better prognoses than those without CLT.[12,13] However, a deeper understanding of the relationship between PTC and CLT is still needed.

Programmed death ligand 1 (PD-L1), also known as CD274, is a cell surface glycoprotein that belongs to the B7 family.[14] It plays important roles in many cancers, such as non-small-cell lung cancer and pancreatic cancer.[15,16] Recent evidence has revealed that PD-L1 was upregulated in PTC emerging from CLT and was correlated with metastasis.[17] However, the clinical significance of PD-L1 in PTC remains unclear. In addition, another study found that PD-L1 was correlated with the phosphoinositide/dependent protein kinase 1 (PDK1), the phosphorylation of Akt in ovarian cancer.[18] However, to the best of our knowledge, no study has focused on the relationship between PD-L1, PDK1 and p-Akt in PTC patients with or without CLT.

We aimed to perform an observational study to investigate the clinical significance of PD-L1, PDK1 and p-Akt in PTC patients. Our data showed that PD-L1, PDK1 and p-Akt were upregulated in PTC patients, especially those without CLT, and that the high expression of PD-L1, PDK1 and p-Akt was correlated with a poor prognosis of PTC. These findings might provide clinical evidence for the potential application of PD-L1, PDK1 and p-Akt as biomarkers in PTC diagnosis.

Material and methods

Patients

The present study included 194 patients with PTC who went to our hospital between April 2011 and October 2014, and met the inclusion criteria. All patients were first diagnosed with PTC using histological analysis. The exclusion criteria were as follows: receiving chemotherapy or radiotherapy before the study; other thyroid diseases apart from CLT, such as nodular goiter; other immune diseases; and other cancers or papillary thyroid microcarcinoma. In addition, 80 contralateral normal thyroid tissue samples were obtained from the same patients and all were confirmed to be healthy tissues through histological analysis. The study was approved by the Ethic Committee of Shanghai Xuhui Center Hospital, China.

Immunohistochemistry

The expression levels of PD-L1, PDK1 and p-Akt were determined using immunohistochemistry (IHC). Briefly, tissues were collected immediately after resection and stored at –20°C before being used. After the samples were fixed with 10% formalin, embedded in paraffin and sectioned, hematoxylin and eosin (H&E) staining was performed. The samples were then immersed in 3% H_2O_2 followed by incubation with primary antibodies – anti-PD-L1 (ab205921; Abcam, Cambridge, UK), anti-PDK1 (ab90444; Abcam), or anti-p-Akt (ab38449; Abcam) – at 4°C overnight. After being incubated with a corresponding second antibody at 37°C for 30 min, the samples were stained with diaminobenizidine (DAB). Some sections were treated with phosphate-buffered saline (PBS) instead of primary antibody and were used as negative controls. Sections with a high expression of PD-L1, PDK1 or p-Akt served as positive controls. The Allred scoring system was used for pathological scoring. The degrees of staining intensity were the following: 0 (no staining); 1 (weak staining); 2 (moderate staining); and 3 (strong staining). Scores for the percentage of stained area were as follows: 0 (none); 1 (<1%); 2 (1–10%); 3 (10–33%); 4 (33–66%); and 5 (>66%). Both were used as the final IHC score: 0–2 (negative); 3–4 (weak); 5–6 (moderate); and 7–8 (strong). To further determine the expression levels of PD-L1, PDK1 and p-Akt, semi-quantitative analysis was conducted by calculating the mean optical density (MOD) using Image Pro Plus v. 6.0 software (Media Cybernetics, Rockville, USA). Briefly, photographs were collected at a magnification of ×400 under the same exposure conditions. Five random photographs were captured for each slice. The integrated optical density (IOD) was calculated. For pictures with no blank area, MOD = IOD/picture area; for pictures with a blank area, MOD = IOD/(picture area – blank area). The MOD value of 1 slice was calculated as the mean value of the 5 randomly selected fields in the slice.

Data collection

The basic characteristics of all patients were collected, including age, sex and body mass index (BMI). The clinical variables – including TNM (tumor–nodule–metastasis) stage, tumor diameter, capsular infiltration, tumor number, and lymph node metastasis – were also recorded.

All patients were followed up for 5 years from admission. To analyze the recurrence rate, the recurrence-free time was calculated during the study period from admission to death or to the end of follow-up.

Statistical analysis

The measurement data are expressed as means ± standard deviation (SD). Comparisons between 2 groups of continuous data were performed using Student's t-test. The χ^2 test was used to compare the categorical variables. Pearson's correlation analysis was performed to analyze the correlation of PD-L1, PDK1 and p-Akt. For recurrence analysis, Kaplan–Meier curve was used with the log-rank test. For logistic analysis, logistic regression was conducted using the stepwise method. A p-value <0.05 was considered to be statistically significant. All data analysis was performed using SPSS v. 22.0 software (IBM Corp., Armonk, USA).

Results

Basic characteristics of all patients

The basic characteristics of the patients are listed in Table 1. Among the 194 PTC patients, 68 had CLT and 126 were without concurrent CLT. The ratio of female patients in the PTC+CLT group was significantly higher and the mean age of the patients was significantly lower than in the patients without CLT (p < 0.05; Table 1). The PCT

Table 1. Basic characteristics of all patients

Variables	PTC without CLT, n = 126	PTC+CLT, n = 68	p-value
Age [years]	48.76 ±9.48	42.30 ±10.27	<0.001
Sex, female (%)	86 (68.25)	57 (83.82)	0.008
BMI [kg/m²]	22.92 ±3.00	23.39 ±3.09	0.307
TNM stage, n (%)			
I–II	67 (53.17)	50 (73.53)	0.002
III–IV	59 (46.83)	18 (26.47)	
Mean diameter [cm]	2.21 ±0.69	1.92 ±0.61	<0.001
Capsular infiltration, n (%)			
yes	79 (62.70)	25 (36.76)	<0.001
no	47 (37.30)	43 (63.24)	
Number of tumors, n (%)			
≥6	17 (13.49)	10 (14.71)	0.684
<6	109 (86.51)	58 (85.29)	
Lymph node metastasis, n (%)			
yes	69 (54.76)	27 (39.71)	0.034
no	57 (45.24)	41 (60.29)	

BMI – body mass index; PTC – papillary thyroid carcinoma; CLT – chronic lymphocytic thyroiditis.

patients with CLT showed significantly lower rates of TNM III–IV, capsular infiltration and lymph node metastasis, as well as markedly smaller tumor diameter than those without CLT (p < 0.05). However, there was no significant difference in the number of tumors between the 2 groups.

Expression of PD-L1, PDK1 and p-Akt was upregulated and positively correlated in PTC patients

The expression levels of PD-L1, PDK1 and p-Akt were determined with IHC and the MOD values were assessed. As shown in Fig. 1, the expression levels of PD-L1, PDK1 and p-Akt were upregulated in the cancer tissues and remained low in the normal tissues. Similarly, the MOD values of PD-L1, PDK1 and p-Akt were significantly higher in PTC tissues compared with the controls (p < 0.05). Moreover, the expression of PD-L1, PDK1 and p-Akt in PTC patients without CLT was markedly higher than that in patients with CLT (p < 0.05). The Pearson's analysis revealed that the levels of PD-L1 and PDK1, PD-L1 and p-Akt, and PDK1 and p-Akt were positively correlated in all tissues (p < 0.05; Table 2).

Table 2. Expression of PD-L1 and PDK1, PD-L1 and p-Akt, and PDK1 and p-Akt were positively correlated

Proteins	Statistical variables	PD-L1	PDK1	p-Akt
PD-L1	Pearson's correlation	1	0.542	0.585
	p-value	–	0.000	0.000
PDK1	Pearson's correlation	0.542	1	0.556
	p-value	0.000	–	0.000
p-Akt	Pearson's correlation	0.585	0.556	1
	p-value	0.000	0.000	–

PD-L1 – programmed death ligand 1; PDK1 – phosphoinositide-dependent protein kinase 1.

PD-L1, PDK1 and p-Akt levels correlated with patients' clinical outcomes

To further study the roles of PD-L1, PDK1 and p-Akt in PTC, the clinical characteristics of all patients were analyzed. The PTC patients were divided into high/low PD-L1 groups according to the MOD value of PD-L1 (0.023), as well as high/low PDK1 groups and high/low p-Akt groups according to the MOD values of PDK1 (0.021) and p-Akt (0.022). The results showed that patients with high expression of PD-L1, PDK1 or p-Akt had significantly more cases of TNM III–IV (p < 0.05; Tables 3–5). Furthermore, the percentage of patients with capsular infiltration, lymph node metastasis and five-year recurrence
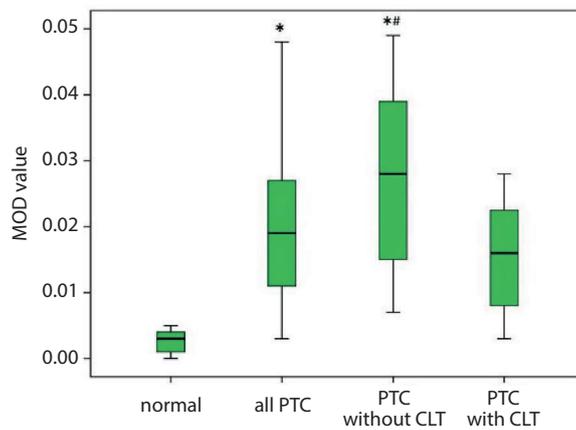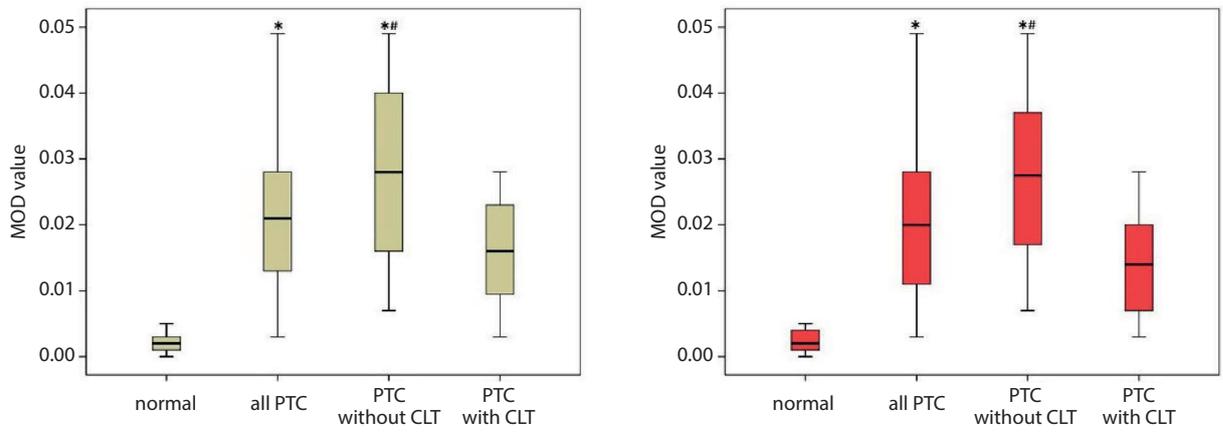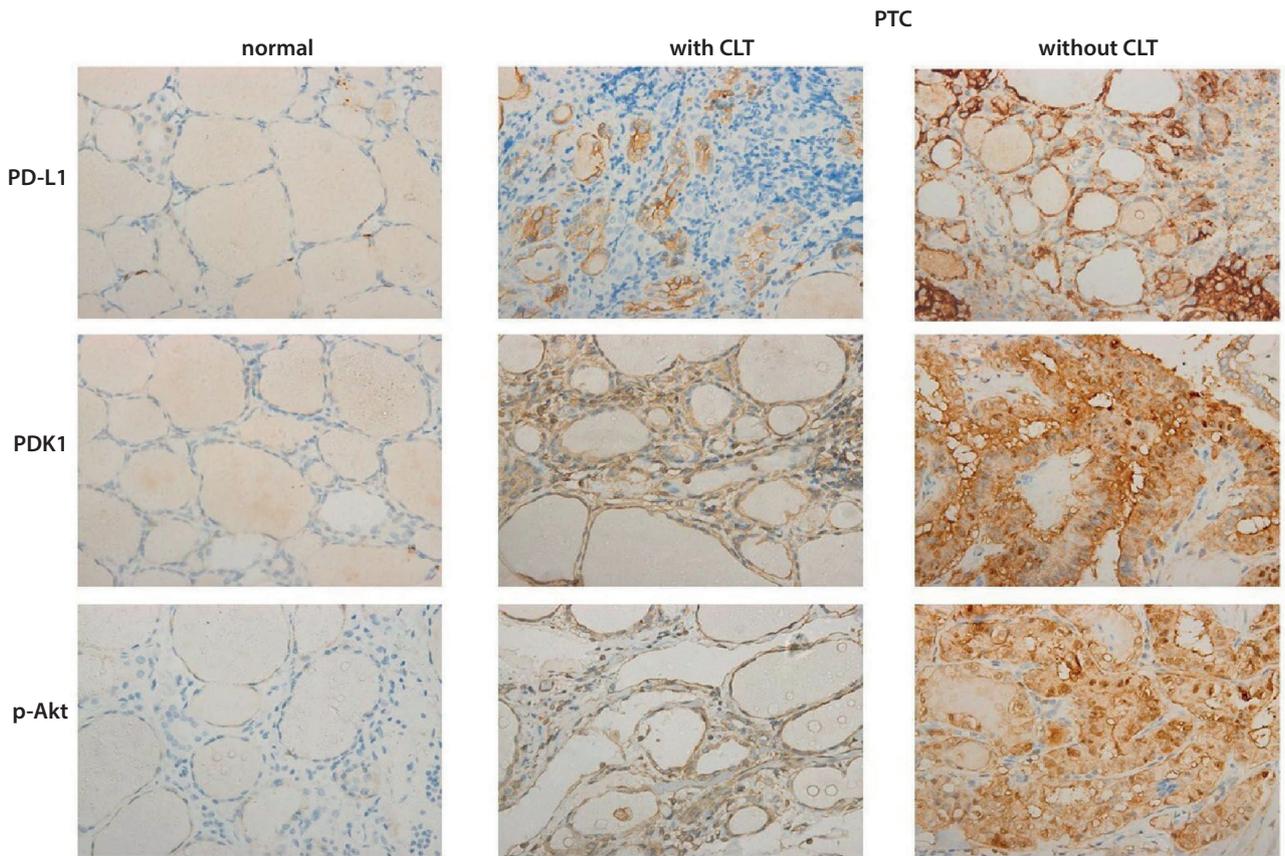
Fig. 1. Expressions of PD-L1, PDK1 and p-Akt were upregulated and positively correlated in PTC patients

Table 3. PD-L1 correlated with patients' clinical outcomes

Variables	High PD-L1 (n = 92)	Low PD-L1 (n = 102)	p-value
Age [years]	47.35 ±10.46	45.64 ±9.94	0.247
Sex, female (%)	69 (75.00)	74 (72.55)	0.694
BMI [kg/m²]	23.19 ±2.86	22.98 ±3.22	0.638
TNM stage, n (%)			
I–II	47 (51.09)	70 (68.63)	0.011
III–IV	45 (48.91)	32 (31.37)	
Mean diameter [cm]	2.13 ±0.67	2.09 ±0.69	0.672
Capsular infiltration, n (%)			
yes	59 (64.13)	45 (44.12)	0.005
no	33 (35.87)	57 (55.88)	
Number of tumors, n (%)			
≥6	12 (13.04)	15 (14.71)	0.733
<6	80 (86.95)	87 (85.29)	
Lymph node metastasis, n (%)			
yes	61 (66.30)	35 (34.31)	0.000
no	32 (34.70)	67 (65.69)	
MOD of PDK1	0.023 ±0.011	0.021 ±0.013	0.155
MOD of p-Akt	0.025 ±0.012	0.020 ±0.011	0.005
Five-year recurrence, n (%)	17 (17.53)	7 (6.86)	0.021

BMI – body mass index; MOD – mean optical density;
PD-L1 – programmed death ligand 1.

Table 4. PDK1 correlated with patients' clinical outcomes

Variables	High PDK1 (n = 97)	Low PDK1 (n = 97)	p-value
Age [years]	47.42 ±10.36	45.57 ±10.04	0.209
Sex, female (%)	69 (71.13)	74 (76.29)	0.407
BMI [kg/m²]	22.93 ±3.14	23.24 ±2.94	0.480
TNM stage, n (%)			
I–II	51 (52.58)	66 (68.04)	0.025
III–IV	46 (47.42)	31 (31.96)	
Mean diameter [cm]	2.24 ±0.72	1.98 ±0.61	0.008
Capsular infiltration, n (%)			
yes	59 (60.82)	45 (46.39)	0.041
no	38 (39.18)	52 (53.61)	
Number of tumors, n (%)			
single	82 (84.54)	85 (87.63)	0.528
multiple	15 (15.46)	12 (12.37)	
Lymph node metastasis, n (%)			
yes	56 (57.73)	39 (40.21)	0.013
no	41 (42.27)	58 (59.79)	
MOD of PD-L1	0.027 ±0.012	0.020 ±0.012	0.001
MOD of p-Akt	0.025 ±0.013	0.021 ±0.011	0.063
Five-year recurrence, n (%)	18 (18.56)	6 (6.19)	0.008

BMI – body mass index; MOD – mean optical density;
PDK1 – phosphoinositide-dependent protein kinase 1.

Table 5. p-Akt correlated with patients' clinical outcomes

Variables	High p-Akt (n = 96)	Low p-Akt (n = 98)	p-value
Age [years]	48.12 ±10.34	44.90 ±9.89	0.028
Sex, female (%)	68 (70.83)	75 (76.53)	0.360
BMI [kg/m²]	22.75 ±2.94	23.42 ±3.10	0.120
TNM stage, n (%)			
I–II	49 (51.04)	68 (69.39)	0.008
III–IV	47 (48.96)	30 (30.61)	
Mean diameter [cm]	2.20 ±0.70	2.01 ±0.64	0.054
Capsular infiltration, n (%)			
yes	59 (61.46)	45 (45.92)	0.028
no	37 (38.54)	53 (54.08)	
Number of tumors, n (%)			
single	81 (84.38)	86 (88.76)	0.472
multiple	15 (15.63)	12 (12.24)	
Lymph node metastasis, n (%)			
yes	54 (56.25)	41 (41.84)	0.042
no	42 (43.75)	57 (58.16)	
MOD of PD-L1	0.026 ±0.013	0.021 ±0.012	0.003
MOD of PDK1	0.025 ±.0012	0.020 ±0.011	0.008
Five-year recurrence, n (%)	17 (17.71)	7 (7.14)	0.023

BMI – body mass index; MOD – mean optical density.

were significantly higher in patients with high expression levels of PD-L1, PDK1 or p-Akt than the ones with low expression (p < 0.05). The mean diameter of PTC tumors in patients with high expression of PDK1 was significantly higher than that in the group with low PDK1 expression, while no significant difference in tumor diameter was observed in the patients with high/low PD-L1 or high/low p-Akt (p < 0.05). Additionally, the age of the patients with lower expression of p-Akt was markedly lower than the age of the patients with high p-Akt expression (p < 0.05). All of these results indicate that PD-L1, PDK1 and p-Akt expression levels correlate with the clinical outcomes of PTC patients.

PD-L1, PDK1 and p-Akt expression correlated with patients' recurrence

Finally, we analyzed the correlation between PD-L1, PDK1 and p-Akt expression, and the recurrence of PTC patients. The recurrence-free time was analyzed using the Kaplan–Meier curve (Fig. 2). Patients with lower expression levels of PD-L1, PDK1 or p-Akt had significantly longer recurrence-free time compared to the ones with high expression levels (p < 0.05). Then logistic regression showed that only CLT, PD-L1 and capsular infiltration were risk factors for patients' five-year recurrence (Table 6).

Table 6. PD-L1 and PDK1 correlated with patients' recurrence

| Variable	Wald	OR	95% CI	p-value
Age	2.261	0.039	1.040 (0.988–1.094)	0.133
BMI	1.190	−0.105	0.900 (0.746–1.087)	0.275
Diameter	0.119	0.128	1.137 (0.549–2.355)	0.730
Sex	1.294	0.835	2.306 (0.547–9.727)	0.255
CLT	6.622	1.943	0.143 (0.033–0.629)	0.010
TNM stage	1.066	−0.549	0.578 (0.204–1.637)	0.302
Capsular infiltration	3.926	1.232	3.428 (1.013–11.596)	0.048
Number of tumors	0.476	0.487	1.627 (0.408–6.484)	0.490
Lymph node metastasis	0.327	−0.318	0.728 (0.245–2.162)	0.567
PD-L1	7.808	60.801	12.545 (7.66–8.453)	0.005
PDK1	3.660	43.209	5.829 (0.347–9.794)	0.056
p-Akt	0.658	15.821	1.902 (0.000–2.975)	0.417

BMI – body mass index; CLT – chronic lymphocytic thyroiditis; PD-L1 – programmed death ligand 1; PDK1 – phosphoinositide-dependent protein kinase 1; OR – odds ratio; 95% CI – 95% confidence interval.

Discussion

With the development of surgical and chemotherapy methods, the five-year survival condition for PTC patients has been greatly improved.[19] However, lymph node metastasis commonly occurs in PTC patients, who often have poor prognosis. Thus, the early diagnosis of PTC is of great significance. In this study, we found that PD-L1, PDK1 and p-Akt were upregulated in PTC tissues, especially in patients without CLT, and that this upregulation correlated with a poor prognosis of the PTC patients.

The involvement of PD-L1 has been reported in many cancers. Tanaka et al. demonstrated that ERO1-α promoted immune escape by elevating PD-L1 in breast cancer.[20] It was also reported that poly(ADP-ribose)polymerase (PARP) inhibitor enhanced cancer-associated immunosuppression by upregulating PD-L1.[21] Chintakuntlawar et al. demonstrated that high PD-L1 expression in anaplastic thyroid cancer trended toward worse progression-free survival and overall survival.[22] In a more recent study, upregulation of PD-L1 was found in PTC patients and the high expression of PD-L1 predicted shorter disease-free time in these patients.[23] In medullary thyroid carcinoma, however, the expression of PD-L1 was low, indicating the controversial role of PD-L1 in human cancers.[24] In this study, we found that PD-L1 was upregulated in PTC patients. Moreover, the expression of PD-L1 was higher in PTC patients without CLT. PD-L1 was also found to be positively correlated with PDK1 expression, and high expression of PD-L1 was related to poor prognosis in PTC patients.

The PDK1 is an important protein in the development of multiple cancers. Zhang et al. reported that dicumarol
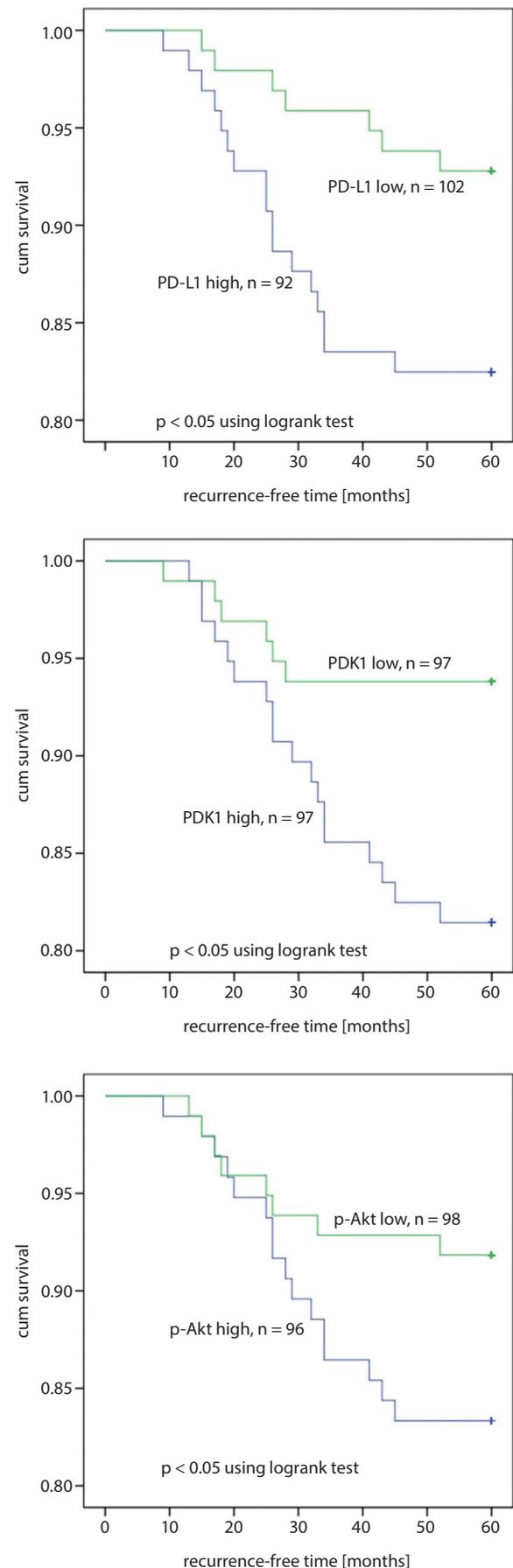
Fig. 2. The recurrence-free time in PTC patients was analyzed using Kaplan–Meier curve

facilitated the progression of ovarian cancer by suppressing PDK1.[25] It was found that PDK1 induced the invasion and migration of gallbladder cancer.[26] Fujiwara et al. reported that the inhibition of PDK1 could also be considered as a novel therapeutic target in multiple myeloma.[27] In this study, we demonstrated for the first time that a high expression level of PDK1 correlated with poor prognosis and positively correlated with the expression of PD-L1 in PTC patients.

Additionally, the relationship between PD-L1 and PDK1 was also observed in several previous studies. Recent data showed that aberrant upregulation of PDK1 was correlated with the overexpression of PD-L1,[18] which was consistent with our data. The p-Akt is the downstream protein of PDK1, which is also reported to play important roles in PTC. A recent study found that Akt inhibitor suppressed the proliferation of PTC cells.[28] Also, the activation of the Akt signaling pathway promoted the development of PTC.[29] Consistent with the above in vitro studies, we showed that the expression of p-Akt was upregulated in PTC tissues and that it predicted worse prognosis of PTC. We also found that the expression of p-Akt was higher in patients without CLT. Further investigations are needed to elucidate the molecular mechanism underlying the regulation of PD-L1, PDK1 and p-Akt in PTC progression.

The present study has some limitations. Firstly, the sample size was small. Secondly, only PTC patients were included. Other histological types of thyroid carcinoma were not investigated. Thirdly, the molecular mechanisms of PD-L1, PDK1 and p-Akt in PTC development are not clear yet and their relationship with CLT needs to be clarified.

Conclusions

This observational study evaluated the clinical significance of PD-L1, PDK1 and p-Akt in PTC. The results show that PD-L1, PDK1 and p-Akt were upregulated in PTC patients, and their expression levels were higher in patients without CLT. The high expression of PD-L1, PDK1 and p-Akt correlated with poor prognosis of PTC. This study provided clinical evidence for the potential use of PD-L1, PDK1 and p-Akt as novel potential biomarkers in PTC diagnosis.

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