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Predictive relevance of programmed cell death protein 1 and tumor-infiltrating lymphocyte expression in papillary thyroid cancer

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Background. Co-signaling molecule programmed cell death 1 ligand 1 has been shown to induce potent inhibition of T cell-mediated antitumoral immunity. Our study aimed to investigate the prognostic value of programmed cell death 1 ligand 1 expression and tumor-infiltrating lymphocyte density as biomarkers in specimens from patients with papillary thyroid cancer.

Methods. We retrospectively analyzed the data and tissue samples of 75 patients with papillary thyroid cancer. Stained cells were counted manually and analyzed for clinical and histopathologic correlations and disease-free survival.

Results. Programmed cell death 1 ligand 1 expression was significantly correlated with increased incidence of lymphovascular invasion (P = .038), extrathyroidal extension (P = .026), and concurrent lymphocytic thyroiditis (P = .003). Patients with low CD8+ and CD3+ expression presented with a significantly higher incidence of lymph node metastasis (P = .042) and extrathyroidal extension (P = .015). The subgroup of cases with positive programmed cell death 1 ligand 1 expression and low CD8+ T cell infiltration demonstrated a significantly increased incidence of lymph node metastasis (P = .031). Univariate and multivariate analysis confirmed that a high CD8+ T cell density was significantly associated with favorable disease-free survival (P = .017). Subanalysis revealed that the shortest disease-free survival was evident in the programmed cell death 1 ligand 1^{+}/$CD8^{low}$ group (P = .004).

Conclusion. Our findings indicate that CD8+ tumor-infiltrating lymphocyte density and programmed cell death 1 ligand 1 expression may serve as valuable predictive biomarkers in patients with papillary thyroid cancer.

Thyroid gland carcinoma is the most common malignant endocrine tumor, irrespective of ethnicity or geographic location, comprising ≈1% of all human malignancies.[1] For the past 4 decades, the reported incidence of thyroid cancer has consistently increased in most areas throughout the world.[2] While the majority of patients with differentiated thyroid cancer (DTC) respond well to existing therapies, 20% to 30% of papillary thyroid cancer (PTC) patients develop recurrence and/or metastases after primary thyroidectomy, most frequently in the locoregional lymph nodes.[3] Limited treatment options are currently available for inoperable or radioactive iodine-refractory metastatic DTC, which is associated with poor survival.[4] Additionally, 2% of these patients advance to develop anaplastic thyroid cancer, contributing to 50% of all thyroid cancer-related deaths annually.[5] Therapeutic routes are presently limited for these patients, who demonstrate a median survival of 3 to 5 months.[6]

Programmed cell death protein 1 (PD-1) is a member of the B7-CD28 family of coregulatory molecules.[7] PD-1 is selectively expressed on a number of hematopoietic cells following T cell receptor signaling and cytokine stimulation, and interacts with 2 binding ligands: PD-L1 (B7H1; CD274) and PD-L2 (B7DC; CD273).[8] PD-L1 is expressed constitutively on hematopoietic cells, including B cells, DCs, macrophages, bone marrow-derived mast cells, and T cells, and is further upregulated after cell-specific stimulation by cytokines including interferon γ and tumor necrosis factor α.[8] Additionally, PD-L1 is the primary ligand expressed on the surface of tumor cells in various solid malignancies.[9]

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The engagement of PD-1 on T cell surfaces by co-signaling molecule PD-L1 has been demonstrated to convey inhibitory signals that decrease T cell proliferation, cytokine production, and cytolytic function.[10] PD-1/PD-L1 interactions also increase T cell susceptibility to apoptosis, thereby preventing immune-mediated lysis of tumor cells.[10]

Antibodies inhibiting the interaction between PD-1 and PD-L1 have been shown to conserve T cell activation and restore their antitumor activity.[11] It has been proposed that the most accurate predictive models for the effectiveness of PD-1/PD-L1 antibody blockade will involve integrating PD-L1 expression with supplementary factors, including the type, location, and degree of tumor-infiltrating immune cells present.[12] Therefore, we examined PD-L1 expression and tumor-infiltrating lymphocyte (TIL) density in biopsy samples from PTC patients and investigated their association with clinicopathologic characteristics and clinical outcomes.

Methods

Patients

This study was approved by the South West Sydney Local Health District Human Research Ethics Committee. In the study, 75 surgically resected PTCs of various stages were obtained from patients who underwent hemithyroidectomy ($n = 4$), total thyroidectomy ($n = 42$), and total thyroidectomy with central neck dissection ($n = 29$) in the South West Sydney Local Health District from 2006–2015. Patient demographics and clinicopathologic parameters were acquired through retrospective medical record review (Table I). Standard American Joint Committee on Cancer, 7th edition, tumor-node-metastases scoring was used for thyroid cancer staging.

Disease-free survival (DFS) was defined as the time period between completion of primary treatment and detection of residual disease, recurrent disease, or death. After initial therapy, criteria for excellent response (absence of persistent tumor) was defined as (1) no clinically detectable thyroid cancer, (2) no imaging evidence of tumor by radioactive iodine imaging and/or neck ultrasound (US), or (3) low serum thyroglobulin (Tg) during thyroid stimulating hormone suppression (Tg <0.2 ng/mL) or following stimulation (Tg <1 ng/mL) with negative thyroglobulin antibodies.[13] The patients who did not experience tumor persistence were censored at the last follow-up contact.

Table I
Clinical summary of patients

Characteristics	IHC *n* (%)
Age, y	
Median age at diagnosis (range), y	51 (24–83)
<45	22 (29.3)
≥45	53 (70.7)
Sex	
Female, *n* (%)	66 (88.0)
Male, *n* (%)	9 (12.0)
Tumor types	
Papillary thyroid cancer	
Classic form PTC	48 (64.0)
Follicular variant PTC	27 (36.0)
Stage	
I	62 (82.7)
II	3 (4.0)
III	9 (12.0)
IV	1 (1.3%)
Tumor size (cm)	
≤1	39 (52.0)
1–2	20 (26.7)
2–4	11 (14.7)
≥4	5 (6.7)
Lymph node metastasis	15 (20.0)
Extrathyroidal extension	15 (20.0)
Multifocality	14 (18.7)
Lymphovascular invasion	6 (8.0)
Lymphocytic thyroiditis	33 (44.0)

IHC, immunohistochemistry.

Immunohistochemical analysis

Tissue blocks were cut into 4-μm sections for analysis. Preheated tissue sections together with positive controls were deparaffinized. Antigen retrieval was performed using Envision FLEX Target Retrieval Solution High pH (DAKO, DM828) for 30 minutes at 98°C. The sections were sequentially incubated with mouse anti-human PD-L1, clone 22C3, 1:100 (DAKO) for 60 minutes, then FLEX+ mouse link (DAKO, K8002) for 15 minutes, FLEX/HRP (DAKO, K8002) for 20 minutes, and finally FLEX DAB+ (DAKO, K8002) for 5 minutes.

The tissue sections together with positive controls were loaded on Leica Bond III Immuno-autostainer for CD3, CD4, CD8, and CD20 immunohistochemical staining. The slides were incubated for 15 minutes for the following primary antibodies separately: rabbit anti-human CD3 1:200 (DAKO, A0452, Denmark), mouse anti-human CD4 clone 4B12, 1:100 (Leica Microsystems, Newcastle, United Kingdom), mouse anti-human CD8 clone C8/144B, 1:100 (DAKO, Denmark), and mouse anti-human CD20 clone L26, 1:400 (DAKO, Denmark), then bond polymer refine detection system (Leica, DS9800) for 16 minutes.

Immunohistochemistry scoring

Scoring of CD3+, CD8+, CD4+, and CD20+ TILs and PD-L1 expression was performed based on the percentage of immunopositive-stained cells and staining intensity as described previously.[6,14] First, a quantitative score based on the estimated percentage of immunopositive-stained cells among total cells was specified according to the following scale: 1 (<1% cells); 2 (1%–10% cells); 3 (11%–33% cells); 4 (34%–66% cells); and 5 (67%–100% cells). Immunopositive cells were defined as those showing partial or complete staining within the cytoplasm and/or plasma membrane. Second, staining intensity was scored as follows: 0 (none), 1^+ (mild), 2^+ (moderate), and 3^+ (intense). Finally, Allred scores (ranging from 1–8) were calculated by adding the percentage positivity scores and the intensity scores for each of the sections. The median value was used to separate the patient cohort into 2 groups with either low or high CD3+, CD8+, CD4+, or CD20+ expression. Patients were divided into 3 groups based PD-L1 expression: negative, moderate, and diffuse. Sample PD-L1 expression and TIL subset presence was evaluated in the following 3 regions; the center of the tumor, the tumor periphery, and the normal tissue adjacent to the tumor. Two pathologists (T.Y. and S.A.) blinded to tumor clinicopathologic characteristics and patient outcomes independently scored all slides, with discrepancies resolved by consensus.

Statistical analysis

Statistical evaluation was conducted with GraphPad Prism v.7 (La Jolla, CA) and SPSS software (version 22; SPSS, Inc., Chicago, IL). Clinical characteristics and associations with biomarkers were analyzed using Fisher exact test and Cochran-Armitage test for trend. DFS was calculated using the Kaplan-Meier method, and the significance of these results was assessed using the log-rank test. Multivariate regression was performed using the Cox proportional hazards model. All reported *P* values are 2-sided.

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