Papillary thyroid carcinoma (PTC) is the most common thyroid cancer and represents the first most prevalent malignancy in Korea, especially in Korean women. PTC often occurs in conjunction with Hashimoto’s thyroiditis (HT), which is a common form of chronic autoimmune thyroid disease and clinically characterized by circulating autoantibodies to primary thyroid antigens including thyroglobulin and thyroid peroxidase. HT is pathologically characterized by the presence of diffuse plasma and lymphocytic cell infiltration, oxyphilic cells, the formation of lymphoid follicles and reactive germinal centers, and parenchymal atrophy. The pathogenesis of PTC coexisting with HT is uncertain. The prognostic impact of the coexistence of HT with PTC has also been controversial. Some investigators have reported that PTC coexisting with HT is associated with good prognosis, lower recurrence rate, and a less aggressive disease at presentation. However, others have reported that the coexistence of HT has no such protective effect on PTC patient outcome. Moreover, the significance of this coexistence with respect to lymph node metastasis continues to be debated.

P2X<sub>7</sub> receptor (P2X<sub>7</sub>-R) is plasma membrane receptor, functionally expressed in human T cells, and plays a critical role in...
cell survival and growth. Because the natural ligand of P2X-Rs is an extracellular adenosine triphosphate (ATP), ATP directly activates the receptors. Activation of P2X-R by ATP may lead to the release of several kinds of pro-inflammatory cytokines, as well as increasing cell membrane permeability and potentially resulting in apoptosis. A high ATP concentration affects T cell apoptosis by up-regulating and engaging the P2X-R and P2X-R subtypes. Recently, P2X-R expression has been documented in several malignant tumors. In prostate, P2X-R overexpression has been suggested for the early detection of prostate cancer. In contrast, P2X-R expression was decreased in endometrial and uterine epithelial cancers. P2X-R expression was found to be strongly up-regulated in PTC and thyroid cancer cell lines, while it is barely expressed in normal human primary thyrocytes, suggesting its possible involvement with tumorigenesis. Nevertheless, there have been only a few reports evaluating the P2X-R expression in PTC. The clinical or pathological significance of P2X-R expression has not been investigated in PTC coexisting with HT. In the present study, we evaluate the clinicopathological features of PTCs according to coexistence with HT, and investigate a possible prognostic correlation of P2X-R expression with PTC coexisting with HT.

MATERIALS AND METHODS

Patients and tissue samples

This study was conducted using formalin-fixed, paraffin-embedded tissue samples obtained from 170 PTC patients who underwent thyroid surgery at Kangdong Sacred Heart Hospital between January 2006 and December 2007. Among the 170 patients, 84 had available data including the preoperative serum thyroid autoantibodies and pathology reports for HT. HT was diagnosed on the basis of histological findings of diffuse lymphoplasmacytic infiltration with germinal centers, parenchymal atrophy with oncocytic change, and variable amounts of stromal fibrosis throughout the thyroid gland. Lymphovascular invasion was assessed with light microscopy and defined as tumor cells that were present within a vascular space with identification of endothelial lining. After histological review, PTC cases were categorized into two groups, PTC with HT (n = 84) or PTC without HT (n = 86). Clinical information including age, sex, treatment modality, and survival or recurrence was adapted from medical records and radiologic findings and then analyzed. All glass slides from 170 patients with PTC were reviewed by two pathologists for diagnosis confirmation and selection of a representative section for immunohistochemical study. Diagnosis and histologic differentiation were evaluated according to the World Health Organization classification, and tumor staging was based on the American Joint Committee on Cancer updated tumor-node-metastasis cancer staging system. This study was approved by Institutional Ethics Committee of Kangdong Sacred Heart Hospital Seoul, Korea.

Tissue microarray block preparation

After a case review for diagnostic confirmation, a tissue microarray was constructed. The largest definite tumor area was selected for the tissue microarray block. A circle was drawn on the slide around the most representative area. Using the slide as a guide, core samples were obtained from each paraffin-embedded block using a tissue microarray tool (Quick-Ray, Unitma, Seoul, Korea). A punch size 3 mm in diameter was used. Nine cores were embedded in each block in 3 × 3 arrangements. In total, 19 tissue microarray blocks were produced from the 170 tumor samples.

Immunohistochemistry

The 4-µm thick tissue sections were deparaffinized using EZ Prep solution. CC1 standard (pH 8.4 buffer contained Tris/Borate/ethylenediaminetetraacetic acid) was used for antigen retrieval. DAB inhibitor (3% H2O2 endogenous peroxidase) was blocked for 4 minutes at 37°C. Slides were incubated with anti-P2X-R antibodies (1:300, goat IgG, Abcam, Cambridge, UK) for 40 minutes at 37°C, and then incubated with a secondary antibody (Universal HRP Multimer, Ventana Medical Systems, Melbourne, VIC, Australia) for 8 minutes at 37°C. After incubation, slides were stained with the DAB H2O2 substrate for 8 minutes, followed by hematoxylin and bluing reagent counterstaining at 37°C. A reaction buffer (pH 7.6, Tris buffer) was used as a washing solution.

Immunohistochemical evaluation

Both the intensity of immunohistochemical staining and the proportion of stained tumor cells were semi-quantitatively evaluated. The staining intensity was scored as follows: 0, negative; 1, weak; 2, moderate; and 3, strong. Staining proportion was rated according to the percentage of positive cells and scored as follows: 0, less than 10%; 1, 11% to 25%; 2, 26% to 75%; 3, more than 75%. The scores of staining intensity and proportion were multiplied to produce a weighted immunoreactive score (0-6). Cases with a score ≥ 3 were considered high expression and those with a score ≤ 2 were defined as low expression. Two pathologists blinded to the patients’ clinical data interpreted all
immunostained slides, and cases with discrepant scores were re-evaluated to achieve a consensus score.

Statistical analysis

Results are expressed as mean ± standard deviation or frequencies and proportions where appropriate. Comparisons between groups were performed using the Student’s t-tests for continuous data. Differences in the frequency of single variables were tested using the χ² test. Univariate and multivariate analyses were used to estimate the influence of P2X₇R expression on clinicopathological parameters. SPSS ver. 18 (SPSS Inc., Chicago, IL, USA) was used for all statistical analyses, and a p < .05 was considered statistically significant.

RESULTS

Clinicopathological features

Patients consisted of 18 men and 152 women with an age range of 26 to 76 years (mean, 47.5 ± 11.7 years). The 170 cases consisted of 150 conventional PTC (88.2%) and 20 variants (11.8%), including follicular variants (n = 16), oncocytic variants (n = 3), and a diffuse sclerosing variant (n = 1). Extrathyroid extension and lymphovascular invasion were identified in 45.9% (78/170) and 48.8% (83/170) of cases, respectively. In addition, 64 patients (37.6%) had multifocality. Lymph node metastasis was identified in 41.8% (71/170) of cases. The clinical and pathological characteristics according to the presence of HT are summarized in Table 1. PTC patients with HT were more likely to be women (p = .01), with less lymphovascular invasion (p < .001) and extrathyroid extension (p < .001). Less lymph node metastasis (p < .001) was evident among the PTC with HT group compared to the PTC without HT group. There were no statistical differences in terms of age, histological variant, tumor size, or tumor multifocality between the PTC with HT and PTC without HT groups. Of the 84 patients with HT, only one patient (1.2%) had recurrence during a mean follow-up of 64.3 ± 11.1 months, whereas six (7%) patients without HT had recurrence during a mean follow-up of 64.8 ± 8.6 months. Lower recurrence (p = .026) was noted in the PTC with HT group, however, this observation is not mentioned in the table because of the low number of patients. Two patients expired during the follow-up period, but their cause of death was not clear; hence, they were excluded.

P2X₇R expression in PTC

The 170 patients consisted of 90 having high P2X₇R expres-

| Characteristic                  | PTC with HT (n = 84) | PTC without HT (n = 86) | p-value |
|--------------------------------|----------------------|-------------------------|---------|
| Age at diagnosis (yr)          |                      |                         |         |
| <45                            | 47.1 ± 11.6          | 48.8 ± 12.2             | .33     |
| ≥45                            | 30 (35.7)            | 22 (25.6)               |         |
| Gender                         |                      |                         | .01     |
| Male                           | 4 (4.7)              | 14 (16.3)               |         |
| Female                         | 54 (64.3)            | 64 (74.4)               |         |
| Histological variant           |                      |                         | .14     |
| Conventional                   | 13 (15.4)            | 7 (8.1)                 |         |
| Variants                       |                      |                         | .84     |
| Yes                            | 31 (36.9)            | 33 (38.4)               |         |
| No                             | 53 (63.1)            | 53 (61.6)               |         |
| Tumor size (cm)                |                      |                         | .11     |
| <2                             | 76 (90.4)            | 70 (81.4)               |         |
| 2-4                            | 6 (7.1)              | 15 (17.4)               |         |
| >4                             | 2 (2.6)              | 1 (1.2)                 |         |
| Lymphovascular invasion        |                      |                         | <.001   |
| Yes                            | 24 (28.6)            | 59 (68.6)               |         |
| No                             | 60 (71.4)            | 27 (31.4)               |         |
| Extrathyroid extension         |                      |                         | <.001   |
| Yes                            | 23 (27.4)            | 55 (64)                 |         |
| No                             | 61 (72.6)            | 31 (36)                 |         |
| Lymph node metastasis          |                      |                         | <.001   |
| Yes                            | 20 (23.8)            | 51 (59.3)               |         |
| No                             | 64 (76.2)            | 35 (40.7)               |         |

Values are presented as mean ± standard deviation or number (%). PTC, papillary thyroid carcinoma; HT, Hashimoto’s thyroiditis.

Table 1. Clinicopathological features of PTC according to presence of HT

P2X₇R expression and extrathyroid extension (OR, 3.16; 95% CI, 1.31 to 7.6; p = .01).
Expression of P2X7R in PTC with or without HT

High P2X7R expression was significantly associated with an absence of HT (OR, 5.43; 95% CI, 2.45 to 12; p < .001). In the group with coexisting HT, P2X7R expression was significantly higher in patients with tumor multifocality (p = .05), lymphovascular invasion (p < .001), and extrathyroid extension (p < .001) (Table 4). For the PTC without HT group, P2X7R expression was significantly higher in women (p < .001) and the tumor multifocality group (p < .001) (Table 5).

**DISCUSSION**

In the present study, we found that PTC with HT correlated with more favorable biological characteristics than PTC without HT. In PTC, the absence of HT was associated with high frequencies of female patients, extrathyroid extension, lymph node metastasis, lymphovascular invasion, and frequent recurrences. Similarly, the presence of autoimmune thyroiditis in thyroid cancer has been correlated with good prognosis. Recent meta-analysis demonstrated that PTCs with coexisting HT are strongly associated with female patients, tumor multifocality, the absence of extrathyroidal extension, absence of lymph node metastasis, and high recurrence-free survival rates. Kim et al. also suggested that PTC coexisting with HT may protect against

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**Table 2. P2X7R expression in papillary thyroid cancer**

| Characteristic               | P2X7R low expression (n=80) | P2X7R high expression (n=90) | p-value |
|-----------------------------|-----------------------------|-----------------------------|---------|
| Age at diagnosis (yr)       | 47.5 ± 12.2                 | 48.4 ± 11.7                 | .13     |
| <45                         | 29 (36.2)                   | 23 (25.6)                   |         |
| ≥45                         | 51 (63.8)                   | 67 (74.4)                   |         |
| Gender                      |                             |                             | .08     |
| Male                        | 5 (6.2)                     | 13 (14.4)                   |         |
| Female                      | 75 (93.8)                   | 77 (85.6)                   |         |
| Histological variant        |                             |                             | .78     |
| Variants                    | 10 (12.5)                   | 10 (11.1)                   |         |
| Conventional                | 70 (87.5)                   | 80 (88.9)                   |         |
| Tumor multifocality         |                             |                             | .12     |
| Yes                         | 35 (43.8)                   | 29 (32.2)                   |         |
| No                          | 45 (56.2)                   | 61 (67.8)                   |         |
| Tumor size (cm)             |                             |                             | .55     |
| <2                          | 70 (87.5)                   | 76 (84.4)                   |         |
| 2-4                         | 8 (10)                      | 13 (14.4)                   |         |
| >4                          | 2 (2.5)                     | 1 (1.2)                     |         |
| Lymphovascular invasion     |                             |                             | <.001   |
| Yes                         | 23 (28.8)                   | 60 (66.7)                   |         |
| No                          | 57 (71.2)                   | 30 (33.3)                   |         |
| Extrathyroid extension      |                             |                             | <.001   |
| Yes                         | 20 (25)                     | 58 (64.4)                   |         |
| No                          | 60 (75)                     | 32 (35.6)                   |         |
| Lymph node metastasis       |                             |                             | <.001   |
| Yes                         | 21 (26.2)                   | 50 (55.6)                   |         |
| No                          | 59 (73.8)                   | 40 (44.4)                   |         |
| Hashimoto’s thyroiditis     |                             |                             | <.001   |
| Yes                         | 60 (75)                     | 24 (26.7)                   |         |
| No                          | 20 (25)                     | 66 (73.3)                   |         |

**Table 3. Multivariate analysis of factors potentially affecting the expression of P2X7R**

| Factor                     | Odds ratio | 95% Confidence interval | p-value |
|----------------------------|------------|-------------------------|---------|
| Age                        | 1.42       | 0.63-3.21               | .4      |
| Gender                     | 0.75       | 0.2-2.8                 | .67     |
| Multifocality              | 1.15       | 0.31-3.99               | .87     |
| Lymphovascular invasion    | 1.31       | 0.48-3.61               | .59     |
| Lymph node metastasis      | 1.81       | 0.71-4.6                | .21     |
| Extrathyroid extension     | 3.16       | 1.31-7.6                | .01     |
| Hashimoto’s thyroiditis    | 5.43       | 2.45-12                 | <.001   |

**Fig. 1.** Immunohistochemical expression of P2X7 receptor (P2X7R) in papillary thyroid carcinomas (PTCs). (A) Low expression of P2X7R; PTC with Hashimoto’s thyroiditis (HT). (B) High expression of P2X7R; PTC without HT.
central lymph node metastasis. Huang et al. reported that coexisting HT with either PTC or follicular thyroid carcinoma is linked with improved clinical stage and favorable prognosis. The mechanisms by which cancer cells may be destroyed by autoimmunity have been suggested. Lymphocytic infiltrates in thyroid cancer contain cytotoxic T lymphocytes, and Fas-mediated apoptosis is the major mechanism by which cytotoxic T lymphocytes cause target cell lysis. In addition, interleukin-1, secreted by infiltrating lymphocytes, inhibits human thyroid carcinoma cell growth. Only three articles have been published on the possible link between thyroid cancer and P2X receptor expression. In vitro study has shown that thyroid papillary carcinoma cell lines express high levels of P2X-R. Gu et al. suggested that P2X-R expression is associated with lymph node metastasis in PTCs. In their logistic regression analysis, P2X-R expression, tumor size, and capsular invasion are predictors for lymph node metastasis, suggesting that P2X-R expression may predict the aggressiveness of PTC. However, these studies have not demonstrated the association between P2X-R expression and PTC with HT.

In the present study, PTC with HT correlates with good prognostic factors. PTC with high P2X-R expression showed significantly higher frequencies of lymphovascular invasion, extrathyroid extension, lymph node metastasis, and absence of HT. In the multivariate analysis, high P2X-R expression was independently associated with the absence of HT and the presence of extrathyroid extension. Our results suggested that P2X-R expression in PTC correlates with poor prognostic factors.

In the PTC with HT group, the expression of P2X-R was significantly higher in females than in males and those with tumor multifocality. These results may imply that a different mechanism of P2X-R expression may be involved according to coexistence of HT. Recently, Beynon et al. reported that activated memory T-cells primed by interferon-β suppress the activation of monocytes by inhibiting P2X-R-mediated signaling, indicating that P2X-R expression in HT may be associated with activated T lymphocytes of HT.

In conclusion, the occurrence of PTC in HT individuals may

| Table 4. P2X-R expression in papillary thyroid cancer with Hashimoto’s thyroiditis |
|-----------------|-----------------|-----------------|-----------------|
| Characteristic   | P2X-R (scores 0-2) | P2X-R (scores 3-9) | p-value |
| Age at diagnosis (yr) | h=60 | h=24 |
| <45             | 25  | 5  |
| ≥45             | 35  | 19 |
| Gender          |     |     | .33 |
| Male            | 2   | 2  |
| Female          | 58  | 22 |
| Histological variant |     |     | .85 |
| Variants        | 9   | 4  |
| Conventional    | 51  | 20 |
| Tumor multifocality |     |     | .05 |
| Yes             | 26  | 5  |
| No              | 34  | 19 |
| Tumor size (cm) |     |     | .17 |
| <2              | 52  | 24 |
| 2-4             | 6   | 0  |
| >4              | 2   | 0  |
| Lymphovascular invasion |     |     | <.01 |
| Yes             | 12  | 12 |
| No              | 48  | 12 |
| Extrathyroid extension |     |     | <.01 |
| Yes             | 11  | 12 |
| No              | 49  | 12 |
| Lymph node metastasis |     |     | .47 |
| Yes             | 13  | 7  |
| No              | 47  | 17 |

P2X-R, P2X receptor.

| Table 5. P2X-R expression in papillary thyroid cancer without Hashimoto’s thyroiditis |
|-----------------|-----------------|-----------------|-----------------|
| Characteristic   | P2X-R (scores 0-2) | P2X-R (scores 3-9) | p-value |
| Age at diagnosis (yr) | h=20 | h=66 | <.01 |
| <45             | 19  | 11 |
| ≥45             | 1   | 55 |
| Gender          |     |     | .74 |
| Male            | 3   | 12 |
| Female          | 17  | 54 |
| Histological variant |     |     | .55 |
| Variants        | 1   | 6  |
| Conventional    | 19  | 60 |
| Tumor multifocality |     |     | <.01 |
| Yes             | 0   | 34 |
| No              | 20  | 32 |
| Tumor size (cm) |     |     | .84 |
| <2              | 16  | 53 |
| 2-4             | 4   | 12 |
| >4              | 0   | 1  |
| Lymphovascular invasion |     |     | .48 |
| Yes             | 5   | 22 |
| No              | 15  | 44 |
| Extrathyroid extension |     |     | .13 |
| Yes             | 10  | 21 |
| No              | 10  | 45 |
| Lymph node metastasis |     |     | .94 |
| Yes             | 8   | 27 |
| No              | 12  | 39 |

P2X-R, P2X receptor.
predict a favorable tumor behavior such as less tumor multifocality, lymphovascular invasion, and extrathyroid extension, compared to those having PTC without HT. P2X-R expression in PTC was correlated with poor prognostic factors and the absence of HT.

Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

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