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ORIGINAL RESEARCH

Upregulation Of Protein Tyrosine Phosphatase Receptor Type C Associates To The Combination Of Hashimoto's Thyroiditis And Papillary Thyroid Carcinoma And Is Predictive Of A Poor Prognosis

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Yanmeizhi Wu[1]
Jun Han[1]
Kazakova Elena Vladimirovna[1]
Shumei Zhang[2]
Wenhua Lv[2]
Yan Zhang[3]
Esma Jamaspishvili[1]
Jingxue Sun[1]
Qingxiao Fang[1]
Jingjing Meng[1]
Hong Qiao[1]

[1]Department of Endocrinology, No.2 Affiliated Hospital of Harbin Medical University, Harbin, Heilongjiang Province, People's Republic of China; [2]Department of Epigenetics, College of Bioinformatics Science and Technology, Harbin Medical University, Harbin, Heilongjiang Province, People's Republic of China; [3]Department of Bioinformatics and Computational Epigenetics, College of Life and Science and Technology, Harbin Institute of Technology, Harbin, Heilongjiang Province, People's Republic of China

Introduction: PTC is not generally considered a lethal disease, but prone to recurrence as the prognosis. Hashimoto's thyroiditis (HT) is an important factor that affects the prognosis of papillary thyroid carcinoma (PTC). It is crucial to find biomarkers to identify the combination of HT with PTC and to predict the prognosis.

Methods: RNASeq data from the Cancer Genome Atlas (TCGA) database was used to screen differentially expressed genes (DEGs) of PTC with HT via the edgeR package of R software version 3.3.0. Also, the DEGs were applied to the DAVID web-based tool to determine the enrichment of gene functions via Gene Ontology (GO) analysis and to identify associated pathways in the Kyoto Encyclopedia of Genes and Genomes (KEGG) database. By constructing protein interaction networks within Cytoscape software, we screened candidate genes and explored possible relationships with the clinical phenotype of PTC. Finally, additional thyroid tissue samples were collected to verify the results above.

Results: After analyzing the RNA-Seq data of PTC patients from the Cancer Genomic Atlas, 497 differentially expressed PTC genes were found to be associated with HT, of which protein tyrosine phosphatase receptor type C (PTPRC), KIT, and COL1A1 were associated with tumor size and lymph node metastasis ($p < 0.05$). Verification of these results with another 30 thyroid tissues of clinical PTC patients revealed that the expression level of PTPRC in the PTC with HT group was higher than that in the PTC without HT group ($p < 0.05$) and the ROC curve showed a good discrimination (area under the curve = 0.846). However, the correlation with the clinical phenotype was not statistically significant ($p > 0.05$).

Discussion: These data suggest that upregulation of PTPRC enhances the incidence of HT associated with PTC and is also predictive of a poor prognosis.

Keywords: papillary thyroid carcinoma, Hashimoto's thyroiditis, PTPRC, biomarker, prognosis

Introduction

Papillary thyroid carcinoma (PTC) accounts for 85% of all thyroid cancers and is the most common malignancy of the endocrine system with a rapidly increasing incidence rate of 4.5% per year.[1] Hashimoto's thyroiditis (HT), also known as chronic lymphocytic or autoimmune thyroiditis, is the most common inflammatory disease of the thyroid and the most common cause of primary hypothyroidism with an estimated incidence of approximately 0.4%–1.5% and accounts for 20%–25% of

Correspondence: Hong Qiao
Department of Endocrinology, No.2 Affiliated Hospital of Harbin Medical University, No. 148 Baojian Road, Nangang District, Harbin, Heilongjiang Province, People's Republic of China
Tel +86 13945677355
Email qhong0823@163.com

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all thyroid disease.[2] In recent years, the incidence of HT combined with malignant tumors of the thyroid has increased significantly.[3] Pathological series have reported that the incidence of histologically confirmed PTC with HT is 5%–48%,[4] which is almost twice that of other types of thyroid cancer.[5]

There are significant differences between the clinical phenotype and prognosis of PTC with HT versus without, as PTC combined with HT tends to predominantly occur in females with less capsular invasion, extrathyroidal extension,[6] and lymph node (LN) metastasis,[7] as well as a greater risk of recurrence.[8] The combination of HT with PTC is not only a protective factor,[9] but also predictive of a better prognosis.[10,11] Therefore, it is essential to identify biomarkers for PTC associated with HT.

To date, several biomarkers related to PTC have been identified, which include the protein markers cytokeratin 19 and galectin-3,[12] mutations to the B-Raf proto-oncogene,[13] rearrangement of the RET proto-oncogene (RET/PTC),[14] DNA methylation markers, such as the thyroid stimulating hormone receptor,[15] the non-coding microRNAs (miRNAs)146 and 221,[16] and the mRNA marker Sirtuin 6.[17] However, when testing protein markers, the accuracy of a diagnosis can be influenced by the immunohistochemical staining process. In addition, the specificity of the marker cytokeratin 19 decreases in HT patients without PTC because the expression level of this protein is upregulated in this population.[18] The miRNAs have high stability in various tissues, but poor specificity, because the sequences are so similar to one another, while the RET/PTC rearrangement has high specificity in PTC, but low sensitivity.[19] BRAF mutations are more frequent in PTC than in normal tissues, while the prevalence is reportedly much lower when combined with HT,[20] thus it is not an efficient marker of PTC with HT. However, mRNA, a breakthrough we choose in finding new markers, has unique structures of molecules [i.e., 5′ cap structure (m7G) and 3′ poly (**A**) tail] and is convenient for both testing and diagnosis of many diseases.

Cancer is a disease of the microenvironment and immunity, but is different from the immune response. The mechanism of tumorigenesis involves inhibition of the immune response while the immune system is activated to clear residual tumor cells in anti-tumor treatment.[21] Therefore, PTC associated with HT should be regarded as a special state of disease rather than the simple addition of two diseases. Furthermore, Kapan et al[22] found that the accuracy rate of fine needle aspiration biopsy, as a gold standard, was only 50% in PTC patients with HT. Hence, a potential biomarker of PTC with HT should differ from one for PTC alone.

Most previous studies have focused on the correlation between the pathogenesis of PTC versus HT. For example, a prophase study reported that PTC was associated with a high incidence of the BRAF mutation, but such mutations occur less frequently in PTC concomitant with HT.[23] Elevated levels of reactive oxygen species have been found in PTC and the transcription levels of related genes are upregulated in PTC with HT.[24] Together, these findings indicate that PTC with HT is distinctly different from PTC without HT, while major and minor effects are merely manifestations of the numerous differentially expressed genes (DEGs) of PTC with HT. Hence, the aim of the present study was to identify genes mostly related to PTC with HT as biomarkers to improve diagnostic accuracy.

PTC is not generally considered a lethal disease but more in terms of recurrence as prognosis. Rarely, people die from PTC, but locoregional recurrence is the major clinical problem. The clinical manifestations of PTC with HT are mostly related to the prognosis of PTC patients, so we hope to find out the relationship between the biomarkers and phenotypes, and further predict the prognosis.

Above all, we attempted to identify a biomarker specific to the correlation between the HT and PTC. RNASeq data from the Cancer Genome Atlas (TCGA) database was used to screen DEGs of PTC with HT via the edgeR package of R software version 3.3.0. Also, the DEGs were applied to the DAVID web-based tool to determine the enrichment of gene functions via Gene Ontology (GO) analysis and to identify associated pathways in the Kyoto Encyclopedia of Genes and Genomes (KEGG) database. By constructing protein interaction networks within Cytoscape software, we screened candidate genes and explored possible relationships with the clinical phenotype of PTC. Finally, 30 PTC tissue samples were collected. The results of this study showed that mostly poor prognostic PTC with upregulation of PTPRC was associated with HT. So, PTPRC is an effective biomarker to predict the prognosis of PTC with HT and to chose an appropriate therapeutic regimen to improve the quality of life of these patients.

Materials And Methods

Data Acquisition

The RNASeqV2 gene expression data of human PTC were downloaded from the TCGA database (https://cancergenome.nih.gov/). All the samples used to test the gene expression

level are from human thyroid tissue. Effective samples included both clinical and pathological information, and the expression levels of the whole genome (total of 20,531 genes). The expression levels of genes were measured as the reads per kilobase per million reads. For analysis, there were 50 cases in the healthy control group and 505 cases in the PTC group. All cases in the healthy control group came from healthy people without any disease. Of these, 154 PTC samples were excluded from analysis because of the presence of other diseases, thus 280 cases of PTC without HT and 71 cases of PTC with HT were included for analysis.

Screening Of DEGs

At first, 50 cases in the PTC group were randomly selected to compare with the healthy group. Based on the model of negative binomial distribution and Fisher's exact test (R statistical software version 3.3.0, www.r-project.org/), the edgeR package was used to screen the DEGs. A false positive rate (FDR) of < 0.05 and |log2 FC (fold change)| of > 1 were set as the cut-off points to screen for DEGs. Every step was repeated 100 times and the sum of all the results were taken as Set 1.

For the next step, 71 cases from the PTC without HT group were randomly selected and compared with those from the PTC with HT group. The DEGs were identified with the same method mentioned above. The results are presented as Set 2.

The DEGs of PTC with HT were the intersection of Set 1 and Set 2.

Enrichment Of Biological Pathways And Gene Functions

The DEGs were applied to the DAVID web-based tool (https://david.ncifcrf. gov/tools.jsp) to identify enrichment of GO gene functions and KEGG pathways. According to the FDR value, the KEGG pathways and GO functions were respectively arranged in ascending order with an FDR-corrected probability (p) value of < 0.05 and gene count of > 2 considered as the thresholds. Those KEGG pathways that were significantly correlated to PTC with HT were named as KEGG-Pathways and those genes contained in GO functions that were significantly correlated to PTC with HT were named GO-Genes.

Construction Of A Gene Interaction Network

First, the human protein protein interaction (PPI) network was downloaded from the Human Protein Reference Database (http://www.hprd.org/). Extensible markup language (XML) data of KEGG-Pathways were downloaded from the KEGG database (https://www.genome.jp/kegg/) and a gene interaction network of the KEGG-Pathways was created using the XML package of R.

Next, the PPI and KEGG-Pathways networks were merged as an undirected network (PTC-HT network) using Cytoscape software (www.cytoscape.org/). Then, a PTC-HT sub-network was created, which consisted of DEGs and their first neighbors in the PTC-HT network. DEGs and GO-Genes are marked respectively in the PTC-HT sub-network.

Finally, the topological properties of the PTC-HT sub-network were analyzed and the connectivity was calculated. The degree of centrality of a gene in the network was measured to determine the relative significance. By definition, the degree of centrality is the number of links of one node relative to another. The top 5% of nodes of the degree value were selected as the Hub nodes. The candidate genes were the Hub nodes marked as both DEGs and GO-Genes.

The Analysis Of The Relationship Between The Candidate Genes And Clinical Phenotype

Tumor size (T), LN metastasis (N), and distant metastasis (M) were considered as the clinical phenotype. Complete clinical information, derived from the TCGA database, was classified according to the TNM grading system (eighth edition of the American Joint Committee on Cancer classification of thyroid cancer). Besides, age was also included as a clinical factor. The samples without complete clinical information were excluded from analysis and the remaining PTC cases were randomly assigned to the training set or the validation set at a ratio of 2:1.

For the training set, the Pearson's correlation coefficient, chi-square test, and linear regression analysis were respectively used for univariate and multivariate analysis to identify correlations between the expression levels of the candidate genes and the factors of T, N, M, and age. Then, the significance of the results were verified using the validation set. All statistical analyses were conducted using R software version 3.3.0.

Experimental Verification

Collection Of Clinical Samples

We collected 30 thyroid tissues samples from clinical PTC patients. The study cohort consisted of 30 patients with

papillary thyroid cancer who underwent surgery at the Department of Head and Neck Surgery of the Second Affiliated Hospital of Harbin Medical University from November 2015 to January 2016. All patients underwent total or subtotal thyroidectomy and LN dissection, which included the central LNs. All had complete pathological reports, which included a clear diagnosis, tumor size (T), and LN metastasis (N). Besides, the diagnosis of HT was based on a significant increase in thyroid peroxidase antibody (TPOAb) and thyroglobulin antibody (TGAb). At the same time, we also referred to the diagnosis in the pathological reports. If the diagnosis based on the serological antibodies was not consistent with the pathological diagnosis, the latter was preferred. All patients had normal thyroid function [normal thyroid stimulating hormone (TSH) concentration = 0.35–4.94 μIU/mL]. The informed consent was obtained from all patients and the study, including the consent process was approved by the Ethics Committee of the No. 2 Affiliated Hospital of Harbin Medical University (approval no. KY2016-017).

Detection Of Relatively Expressed Candidate Genes

All cancer tissues were frozen in liquid nitrogen immediately after resection and stored at −80ºC until analysis. Total RNA was extracted from tissues with TRIzol reagent (Invitrogen Corporation, Carlsbad, CA, USA) and then reverse transcribed to generate cDNA using the PrimeScript®1st Strand cDNA Synthesis KIT according to the manufacturer's instructions (Takara Bio, Inc., Shiga, Japan). Quantitative real-time polymerase chain reaction (qRT-PCR) was used to detect the expression levels of the KIT, PTPRC, and COL1A1 genes. Primer pairs were designed using the Primer3 tool. The glyceraldehyde 3-phosphate dehydrogenase (GAPDH) gene was used as an internal reference to normalize sample-to-sample variations. The qRT-PCR was performed in triplicate using SYBR®Premix Ex Taq™ Master Mix (Takara Bio, Inc.) and a 7300 real-time PCR system (Life Technologies, Carlsbad, CA, USA).

The qRT-PCR analysis was performed as follows. A 10-μL aliquot of the PCR reaction mixture was prepared for each reaction, which included 5 μL of SYBR Premix Ex Taq II (2x), 0.2 μL of ROX Reference Dye (50x), 2 μL of each primer (2.0 μM), 1 μL of the template, and 1.8 μL of DNase-free water. The reaction mixture was incubated in the wells of a 96-well plate at 95°C for 1 min, followed by 40 cycles at 95°C for 5 s and 60°C for 30 s. Dissociation curve analysis of the PCR products was performed at the final stage of 60°C to 95°C. All reactions were performed in triplicate. The relative expression levels of KIT, PTPRC and COL1A1 were normalized to that of GAPDH (ΔCt) and fold changes among the samples were calculated using the 2-ΔΔCt method.

Statistical Analysis

The edgeR package was used to screen the DEGs. A false positive rate (FDR) of < 0.05 and |log2 FC (fold change)| of > 1 were set as the cut-off points. Chi-square test and linear regression analysis were respectively used for univariate and multivariate analysis to identify correlations between the expression levels of the candidate genes and the factors of T, N, M, and age.

The independent sample*T*-test was used to identify difference in the expression levels of the candidate genes between the groups of PTC without and with HT. Pearson's correlation coefficient was used to explore the correlation between the expression levels of the candidate genes and the tumor size. *T*-test was used to identify difference in the expression levels of the candidate genes between the groups of PTC with (N0) and without LN metastasis (N1).

R software version 3.3.0 was used for all statistical analyses and a probability (p) value of < 0.05 was considered statistically significant.

Results

Screening And Functional Analysis Of DEGs

Use of the edgeR package (R3.3.0) identified 497 DEGs (334 upregulated and 163 downregulated genes) with RNA-Seq in PTC patients from the TCGA database.

After applying the DEGs in DAVID, 53 GO functions were enriched, which included immunity, thyroid, and protein tyrosine kinase (related to abnormal proliferation of cancer cells) among others (Figure 1A–C). Besides, seven KEGG-Pathways were also enriched (Figure 1D).

Establishment And Analysis Of An Integrated PTC-HT Network

The PPI network, consisting of 13,368 proteins with 80,977 interactions, was downloaded from the Human Protein Reference Database. Analysis of the KEGG-Pathways with the XML package in R identified interactions among pairs of genes. A PTC-HT network of the interactive pairs of KEGG-Pathways and PPI was created

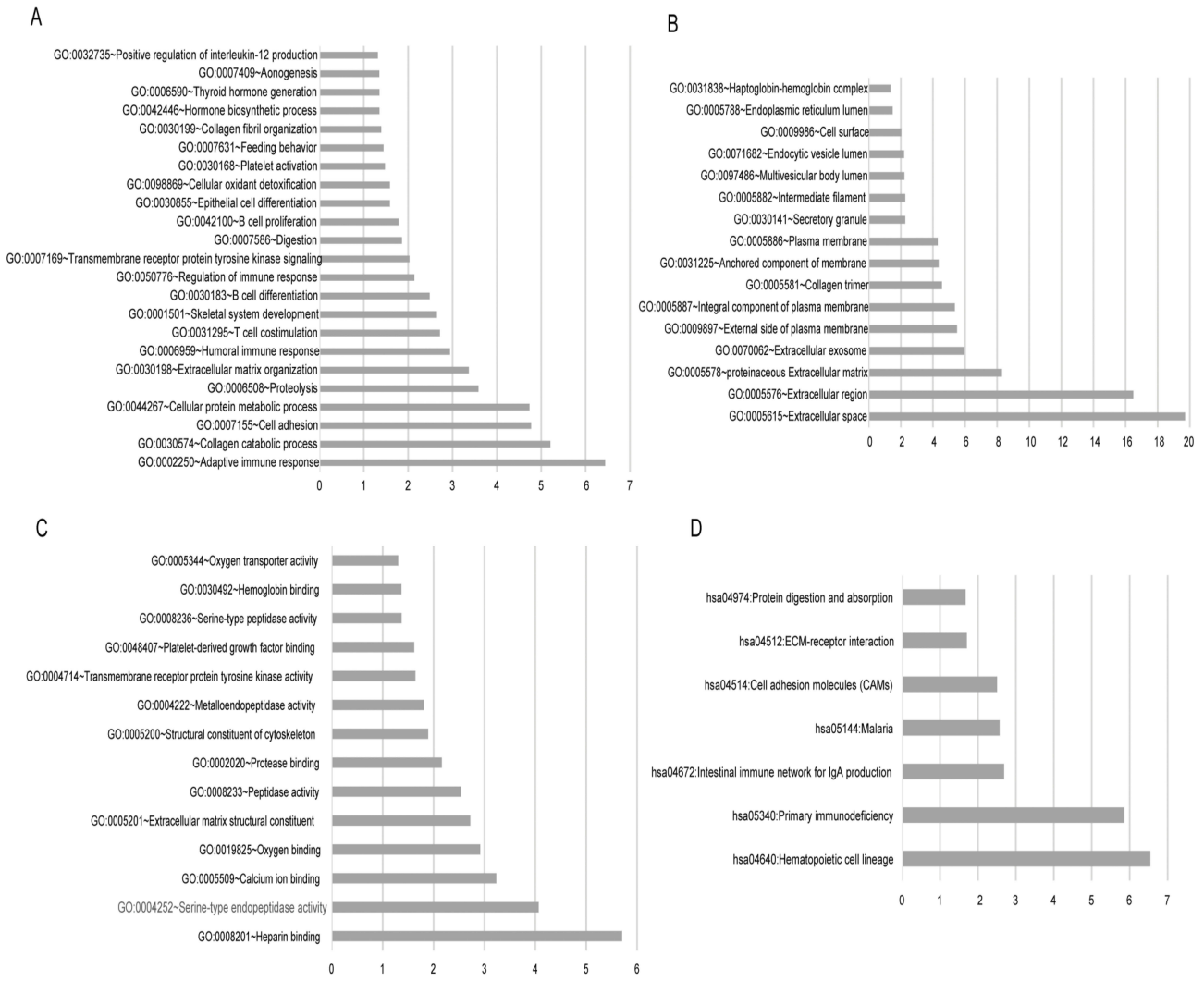

Figure 1 GO enrichment terms and KEGG pathway analysis of DEGs using the DAVID web-based tool.
Notes: The statistically significant enriched GO terms in (**A**) Biological Process, (**B**) Cellular Component, and (**C**) Molecular Function. (**D**) All statistically significant enriched KEGG-Pathways. The FDR corrected p values are displayed on a -log10 scale.

with Cytoscape software. Then, the DEGs and their first neighbors were chosen from the PTC-HT network to create a PTC-HT sub-network (Figure 2), which consisted of 1,778 nodes and 11,721 edges. After analyzing the PTC-HT sub-network, 88 Hub nodes were defined, which included the three candidate genes (PTPRC, COL1A1, and KIT).

Correlations Between The Expression Levels Of The Candidate Genes And The PTC Phenotype

A total of 278 PTC cases with complete clinical information were divided allocated to the training set (n = 183) or the validation set (n = 95).

For the training set, univariate analysis was used to identify the correlation between the candidate genes and the PTC phenotype [ie, tumor size (T), LN metastasis (N), distant metastasis (M), and age. The results showed that three candidate genes were associated with tumor size ($p < 0.05$) and LN metastasis ($p < 0.05$), but less so with distant metastasis and age ($p > 0.05$). The expression level of PTPRC was negatively correlated to tumor size, but a positive factor to LN metastasis (OR=1.89, 95% CI:1.02–3.56).

The results of the multivariate analysis showed that the expression levels of the candidate genes were correlated to tumor size ($p < 0.05$) and LN metastasis ($p < 0.05$) when other factors, including age, were removed (Table 1).

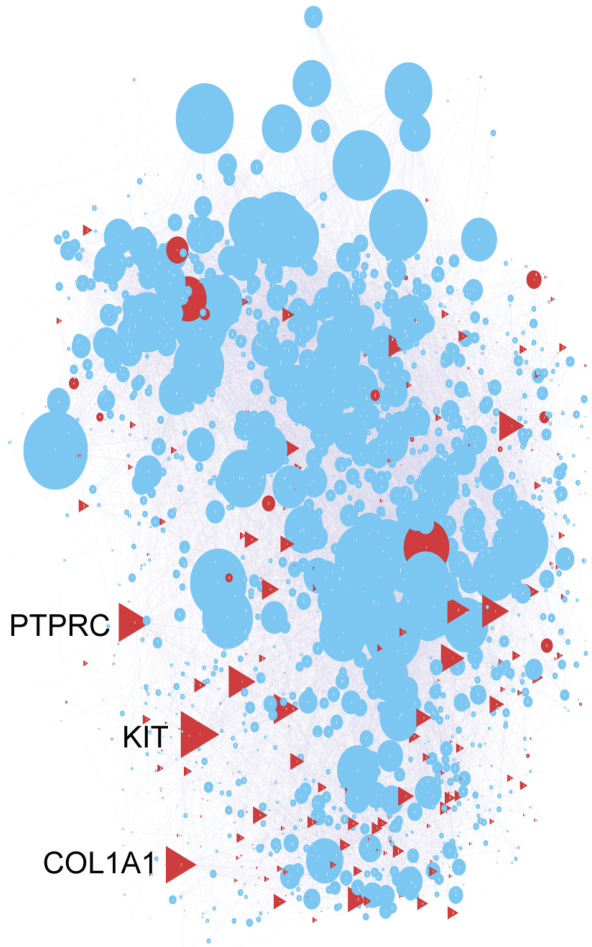

Figure 2 Visualization of the PTC-HT sub-network using Cytoscape software. **Notes:** The sub-network is shown above. The node size is proportional to the degree. DEGs are shown in red and the nodes in blue are the first neighbors in the network. Nodes denoted by triangles, regardless of color, are the GO-Genes. Candidate genes are those that are both hub nodes, GO-genes and DEGs genes simultaneously. So, the three largest red triangles (nodes) indicate the candidate genes (KIT, PTPRC, and COL1A1).

Differences In The Expression Levels Of PTPRC, COL1A1, And KIT Between The Groups

To verify the results, an additional 30 PTC tissue samples were collected by the same method described above. All patients were women. There was no significant difference in the mean age of the 26 patients in the PTC without HT group and the four cases in the PTC with HT group (45.5 ± 10.38 vs 43.1 ± 3.41 years, respectively, $p > 0.05$). Clinical information is shown in Table 2.

PTPRC expression was greater in the PTC with HT group than the PTC without HT group ($p = 0.007$). The differences in COL1A1 and KIT levels were not statistically significant (Figure 3A). In addition, the results of the receiver operating characteristic (ROC) curve analysis illustrated that PTPRC can efficiently discriminate between PTC with vs without HT [area under the curve (AUC) = 0.846; Figure 3B).

Correlation Between The Expression Levels Of The Candidate Genes And The PTC Phenotype

According to the Pearson's correlation analysis, there was no significant correlation between the expression level of PTPRC and the tumor size ($p = 0.079$). Similar results were found for COL1A1 and KIT.

There was no significant difference in the occurrence of LN metastasis between the N1 and N0 groups (14 vs 16 cases, respectively, $p > 0.05$; Figure 4A). Also, expression of COL1A1 and KIT were not correlated to LN metastasis.

Table 2 Sample Summary

Characteristics	PTC Without HT	PTC With HT
Age at diagnosis (years)	45.5 ± 10.38	43.1 ± 3.41
TSH level (μIU/mL)	1.79 ± 1.00	2.15 ± 1.44
TPOAb level (μIU/mL)	29.5 ± 86.38	173.8 ± 347.42
TGAb level (μIU/mL)	24.0 ± 89.71	63.1 ± 52.90
Tumor size (cm)	1.02 ± 0.44	1.00 ± 0.36
Lymph Node status		
N0	14	2
N1	12	2
Total	26	4

Notes: N0 means PTC with LN metastasis and N1 means PTC without LN metastasis. Data are expressed as mean ± standard deviation.

Table 1 Analysis Of The Training Set

	P value Of Univariate Analysis				P value Of Multivariate Analysis	
	T (PCC)	N (OR)	M	Age	T~exp	N~exp
KIT	0.001 (−0.240)	0.041 (0.59)	0.447	0.977	0.042	0.042
PTPRC	0.028 (−0.162)	0.011 (1.89)	0.120	0.583	0.002	0.003
COL1A1	0.050 (0.145)	0.000 (1.35)	0.447	0.050	0.005	0.005

Abbreviations: PCC, Pearson Correlation Coefficient; OR, odds ratio.

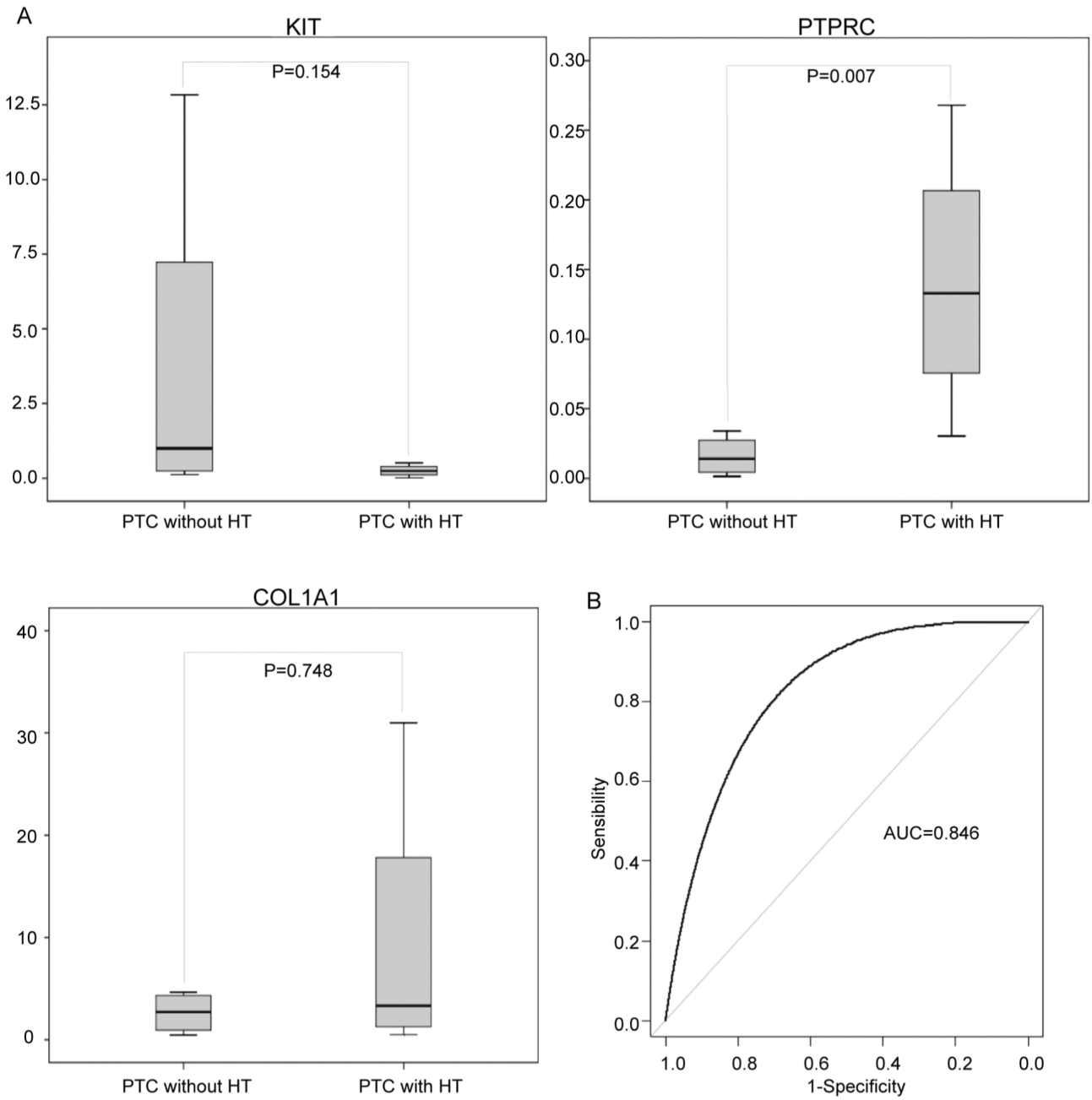

Figure 3 (**A**) Differences in the expression levels of the three candidate genes between two groups. (**B**) The ROC curve of PTPRC.

Discussion

PTC with HT is of great difference from the simple addition of two diseases. Inflammatory and infectious diseases may be strongly associated with tumor formation. Tumor cells secrete several molecules, which promote the growth of cancer cells and metastasis, and can cause genetic alterations in proliferating cells.[25] The thyroid gland is affected by autoimmune attacks more than any other organ. HT is the most common thyroidal autoimmune disease and its pathogenesis is based on a cellular immune response against thyroid autoantigens, with the contribution of genetic and environmental factors.[26] For instance, BRAF, which is closely associated with the presence and development of PTC, may induce the activation of genes coding for molecules involved in the immune response, is an important role in the first steps of lymphocytic infiltration in HT.[27,28]

Conversely, the immune response directed against thyroid cancer might be important for the prevention of metastasis and disease recurrence.[29] Despite the long survival, the recurrence rate in PTC patients is high in patients with

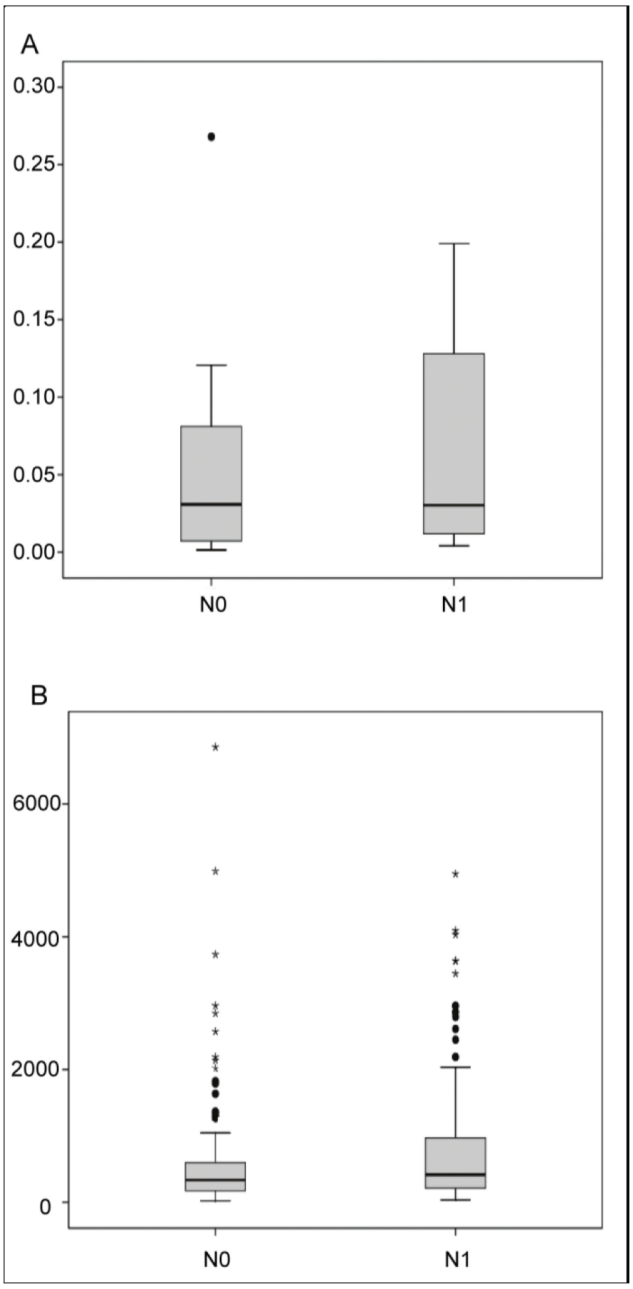

Figure 4 (**A**) Comparison of PTPRC levels between the N0 and N1 groups. (**B**) PTPRC levels between the N0 and N1 groups based on the data from the TCGA database. *represents some outliers. When we define the distance between three quarters and one quarter of the data as standard, *is more than three times of the standard.

and without LN metastasis (38.5%–58.8%, and 32%, respectively).[30] Like most lethal cancers, death from PTC is from metastatic spread rather than invasion by the primary tumor. Therefore, it is of particular importance to quickly identify those patients with aggressive disease, so that treatment can be started before metastatic spread. In our view, PTC with HT is different from PTC alone by its better prognosis, but affecting the lymph node metastasis and recurrence in PTC patients, thus specific biomarkers are needed. The results of this study demonstrated a difference in the expression level of at least on gene.

Moreover, prediction of the combination of HT is of great importance for treatment of PTC. The guidelines of the National Ccomprehensive Cancer Network and the American Thyroid Association suggest thyroxine replacement therapy after thyroidectomy in order to inhibit TSH production, as TSH plays an important role in disease recurrence by promoting the proliferation of PTC cells. HT is regarded as a destructive tissue-specific autoimmune disease with widespread lymphocyte infiltration, fibrosis, and parenchymal atrophy of the thyroid tissue. The disease usually leads to hypothyroidism, which is characterized by deficits in T3 and T4, and elevated TSH levels. When associated with HT, PTC patients may have a higher level of TSH caused by HT. An inadequate treatment for PTC patients with HT may lead to a greater risk of recurrence due to uncontrolled TSH production. Therefore, it is crucial to identify biomarkers of PTC associated with HT in order to improve disease prevention and treatment.

In addition, lymph node dissection and radioactivity therapy for PTC patients have been controversial. These are all closely related to lymph node metastasis and recurrence in PTC patients.

Analysis of data from the TCGA database in regard to the function and interaction of genes demonstrated that the expression levels of KIT, COL1A1, and PTPRC were all significantly higher in the PTC with HT group than in the PTC without HT group. Among these genes, only PTPRC was found to be common in both clinical PTC patients and the TCGA database. ROC curve analysis was also shown to have good discriminatory power, as shown in Figure 3B. AUC is an evaluation index to measure the quality of binary classification model, and its value range from 0 to 1. The closer the value is to 1, the better the discrimination is. When AUC=0.5, it equals random. Thus, it shows a great discrimination as AUC = 0.846 in our study.

Each of the candidate genes had an equal impact on the combination of HT with PTC, but PTPRC was the most reliable. In other words, patients with upregulated expression levels of PTPRC are more prone to have a combination HT with PTC and a greater probability of changes to thyroid function. There was no significant difference between the two groups in the expression levels of KIT and COL1A1, but both were highly expressed in the PTC with HT group, which was similar to that of the database (Figure 3A). So, a larger sample size should be used in further studies.

According to the database, the expression level of PTPRC was negatively correlated with tumor size (p = 1.26e-10, PCC = −0.14), but positively correlated with LN metastasis (p = 0.01159, OR = 1.89), which was confirmed by both univariate and multivariate analyses. Notably, age is considered to be strongly associated with PTC[31] and is even an independent factor of LN metastasis.[32] In multivariate analysis, the expression level of PTPRC was still significant when age was removed, suggesting that PTPRC is an effective biomarker to predict LN metastasis in patients with PTC. As known, metastatic lymph node significantly correlated with worse survival, macrometastasis, and extracapsular spread.[33] Some molecular biomarkers like DEGs are closely related to the tumor stage (T), lymph node metastasis (N) of PTC, which could reflect behavior of tumor progression.[34] Therefore, we can deduce that PTPRC may be related to the poor prognosis of PTC patients.

The extent of lymph node dissection in patients with PTC has always been a controversial issue in clinical practice. We would also like to use research to provide better reasons and evidence. In this study, we only found a correlation between up-regulation of PTPRC gene expression and lymph node metastasis. But there is no reasonable inference about the choice of lymph node dissection. In the next research, we will pay more attention to these issues.

The role of thyroid antibodies should be clarified. For the diagnosis of HT, serological antibodies and pathological diagnosis were combined. Studies have shown that TPOAb levels in PTC patients are elevated,[35] which may lead to false positive results when using only thyroid antibodies for the diagnosis of HT. Therefore, if serological antibody analysis is not consistent with the pathological diagnosis, we prefer the latter. On the other hand, TPOAb positivity without diffuse lymphatic invasion is not related to tumor invasiveness.[36] So, antibodies were not discussed and excluded as factors in this study of prognostic factors associated with PTC.

Unfortunately, similar differences were found in the verification group, but without statistical significance (Figure 4B). Cerutti[37] reported that PTPRC was a potential marker of LN metastasis in PTC patients as the expression level of PTPRC in LNs was significantly higher than in the healthy thyroid and primary tumor tissue. So, a larger sample size is needed for further confirmation. Our results revealed that PTC with upregulated PTPRC levels is more likely to suggest a smaller tumor size, but a poorer prognosis. Remarkably, since the association of HT is beneficial for the prognosis of PTC[11] and upregulation PTPRC is harmful, PTC patients with HT and upregulated PTPRC may have a different prognosis due to the co-existence of both diseases.

PTPRC is a member of the PTP family. PTP is closely related to the JAK/STAT pathway. The STAT and CXCL families are both cancer-related pathways. For example, the single nucleotide polymorphism of C77G with PTPRC is associated with the uneasily infiltrated tumor types of ovarian cancer.[38] PTPRC in metastatic LN is predictive of favorable survival of patients with non-small cell lung carcinoma.[39] PTPRC is connected with molecular death of PTC owing to cytotoxicity.[40] Moreover, PTPRC, which is also known as CD45, has been shown to be an essential regulator of T and B cell antigen receptor signaling, and closely related to many autoimmune diseases, such as chronic colitis[41] and type 1 diabetes.[42] Fetal cell microchimerism with CD45 has been found in both HT and PTC, but seldom in normal thyroid tissue.[43]

Via the David tool, we annotated the functions of PTPRC, such as B cell proliferation, as it is an integral component of the plasma membrane. PTPRC, which might recognize memory cells, was found mostly in PTC, and correlated well with the expression of human leukocyte antigen – antigen D related, which is highly expressed on the surfaces of tumor-infiltrating lymphocytes in papillary carcinoma. These findings imply the presence of an active, but variable, immune response.[44] Moreover, overexpression of PTPRC at the mRNA level indicates that LN metastasis is likely to be present.[45] These findings imply that PTPRC may be a good biomarker for PTC with HT. However, the sample size of the present study was small, thus further extended studies are needed to rectify the shortages of the current research.

Unfortunately, this study lacks pure HT patients as control group. This is because the data of HT patients are not available in TCGA database. Moreover, no HT patients were willing to donate their thyroid tissue when collecting samples. So we have no chance to set up HT control group. However, this is indeed an issue that has an impact, including potential bias, on the results of this study. Therefore, we record this as one of the inadequacies of this study. It reminds us to continue learning and try to better solve this problem in further study.

Conclusion

In conclusion, the expression level of PTPRC is an effective biomarker to predict the prognosis of HT combined

with PTC. Upregulation of PTPRC contributes to a poor prognosis of the combination of HT with PTC.

Abbreviations

AUC, Area under curve; COL1A1, Collagen type I alpha 1 chain; DEGs, Differentially expressed genes; FC, Fold change; FDR, False positive rate; GAPDH, Glyceraldehyde-3-phosphate dehydrogenase; GO, Gene ontology; HT, Hashimoto's thyroiditis; KEGG, Kyoto encyclopedia of genes and genomes; KIT, Kit proto-oncogene receptor tyrosine kinase; OR, Odds ratio; PCC, Pearson correlation coefficient; PPI, Protein protein interaction; PTC, Papillary thyroid carcinoma; PTPRC, Protein tyrosine phosphatase, receptor type C; ROC curve, Receiver operating characteristic curve; QRT-PCR, Quantitative real time polymerase chain reaction; TCGA, the Cancer genomic atlas; TSH, Thyroid stimulating hormone.

Ethics Approval And Informed Consent

The informed consent was obtained from all patients and the study, including the consent process was approved by the Ethics Committee of the No. 2 Affiliated Hospital of Harbin Medical University (approval no. KY2016-017). The patient consent was written informed consent, and that this was conducted in accordance with the Declaration of Helsinki.

Data Availability

The datasets used in this study are available from the corresponding author on reasonable request.

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Author Contributions

YW designed the experiments, analyzed and interpreted the patient data, and was a major contributor to writing the manuscript. JH and EJ downloaded and organized the data from the TCGA database. KEV, JS, and QF collected the thyroid tissues of PTC patients and registered all clinical information. SZ, WL, JM and YZ gave some advice about the selection of statistical methods and assisted in the interpretation of the results. HQ, the corresponding author, instructed the experimental design and offered some useful suggestions during the review process. All authors contributed to data analysis, drafting or revising the article, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

Disclosure

The authors report no conflicts of interest in this work.

References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2015. *CA-Cancer J Clin*. 2015;65:5. doi:10.3322/caac.21294
2. Jankovic B, Le KT, Hershman JM. Clinical review: hashimoto's thyroiditis and papillary thyroid carcinoma: is there a correlation? *J Clin Endocr Metab*. 2013;98:474–482. doi:10.1210/jc.2012-2978
3. Konturek A, Barczyński M, Wierzchowski W, et al. Coexistence of papillary thyroid cancer with Hashimoto thyroiditis. *Langenbeck Arch Surg*. 2013;398:389. doi:10.1007/s00423-012-1021-x
4. Kim KW, Park YJ, Kim EH, et al. Elevated risk of papillary thyroid cancer in Korean patients with Hashimoto's thyroiditis. *Head Neck*. 2011;33:691–695. doi:10.1002/hed.21518
5. Cipolla C, Sandonato L, Graceffa G, et al. Hashimoto thyroiditis coexistent with papillary thyroid carcinoma. *Am Surgeon*. 2005;71:874–878.
6. Baser H, Ozdemir D, Cuhaci N, et al. Hashimoto's thyroiditis does not affect ultrasonographical, cytological, and histopathological features in patients with papillary thyroid carcinoma. *Endocr Pathol*. 2015;26:356–364. doi:10.1007/s12022-015-9401-8
7. Zhu Y, Zheng K, Zhang H, et al. The clinicopathologic differences of central lymph node metastasis in predicting lateral lymph node metastasis and prognosis in papillary thyroid cancer associated with or without Hashimoto's thyroiditis. *Tumor Biol*. 2016;37:8037–8045. doi:10.1007/s13277-015-4706-2
8. Nam HY, Lee HY, Park GC. Impact of co-existent thyroiditis on clinical outcome in papillary thyroid carcinoma with high preoperative serum antithyroglobulin antibody: a retrospective cohort study. *Clin Otolaryngol*. 2016;41:358–364. doi:10.1111/coa.12520
9. Zeng R, Jin L, Chen E, et al. Potential relationship between Hashimoto's thyroiditis and BRAFV600E mutation status in papillary thyroid cancer. *Head Neck*. 2015;38(Suppl 1):E1019. doi:10.1002/hed.24126
10. Kwak HY, Chae BJ, Eom YH, et al. Does papillary thyroid carcinoma have a better prognosis with or without Hashimoto thyroiditis? *Int J Clin Oncol*. 2015;20:463–473. doi:10.1007/s10147-014-0754-7
11. Song E, Jeon MJ, Park S, et al. Influence of coexistent Hashimoto's thyroiditis on the extent of cervical lymph node dissection and prognosis in papillary thyroid carcinoma. *Clin Endocr*. 2017;88:123–128. doi:10.1111/cen.13475
12. Arcolia V, Journe F, Renaud F, et al. Combination of galectin-3, CK19 and HBME-1 immunostaining improves the diagnosis of thyroid cancer. *Oncol Lett*. 2017;14:4183. doi:10.3892/ol.2017.6719
13. Li X, Abdel-Mageed AB, Kandil E. BRAF mutation in papillary thyroid carcinoma. *Int J Clin Exp Med*. 2012;5:310–315.
14. Zhang X, Su X, Chen WC, et al. RET/PTC rearrangement affects multifocal formation of papillary thyroid carcinoma. *Chn J Otorhinolaryngol Head Neck Surg*. 2017;52:435.
15. Kartal K, Onder S, Kosemehmetoglu K, et al. Methylation status of TSHr in well-differentiated thyroid cancer by using cytologic material. *BMC Cancer*. 2015;15:1–6. doi:10.1186/1471-2407-15-1
16. Rosignolo F, Sponziello M, Giacomelli L, et al. Identification of thyroid-associated serum microRNA profiles and their potential use in thyroid cancer follow-up. *J Endocr Soc*. 2017;1:3–13. doi:10.1210/js.2016-1032

17. Qu N, Hu JQ, Liu L, et al. SIRT6 is upregulated and associated with cancer aggressiveness in papillary thyroid cancer via BRAF/ERK/Mcl1 pathway. *Int J Oncol*. 2017;50:1683. doi:10.3892/ijo.2016.3762
18. Ma H, Yan J, Zhang C, et al. Expression of papillary thyroid carcinoma-associated molecular markers and their significance in follicular epithelial dysplasia with papillary thyroid carcinoma-like nuclear alterations in Hashimoto's thyroiditis. *Int J Clin Exp Pathol*. 2014;7:7999–8007.
19. Krane JF, Cibas ES, Alexander EK, et al. Molecular analysis of residual ThinPrep material from thyroid FNAs increases diagnostic sensitivity. *Cancer Cytopathol*. 2015;123:356–361. doi:10.1002/cncy.21546
20. Ronger S, Yuan-Ping H, Jun H, et al. Association between BRAF V600E, mutation and the american college of radiology thyroid imaging, reporting and data system in solitary papillary thyroid carcinoma. *Acad Radiol*. 2018;26(2):154–160. S1076633218302496.
21. Prendergast GC, Jaffee EM. Cancer immunologists and cancer biologists: why we didn't talk then but need to now. *Cancer Res*. 2007;67:3500–3504. doi:10.1158/0008-5472.CAN-06-4626
22. Kapan M, Onder A, Girgin S, et al. The reliability of fine-needle aspiration biopsy in terms of malignancy in patients with Hashimoto thyroiditis. *Int Surg*. 2015;100:249. doi:10.9738/INTSURG-D-13-00209.1
23. Kim SK, Woo J-W, Lee JH, et al. Chronic lymphocytic thyroiditis and BRAF V600E in papillary thyroid carcinoma. *Endocr Relat Cancer*. 2016;23:27–34. doi:10.1530/ERC-15-0408
24. Yi JW, Park JY, Sung JY, et al. Genomic evidence of reactive oxygen species elevation in papillary thyroid carcinoma with Hashimoto thyroiditis. *Endocr J*. 2015;62:857. doi:10.1507/endocrj.EJ14-0369
25. Colotta F, Allavena P, Sica A, et al. Cancer-related inflammation, the seventh hallmark of cancer: links to genetic instability. *Carcinogenesis*. 2009;30:1073–1081. doi:10.1093/carcin/bgp127
26. Boi F, Pani F, Mariotti S. Thyroid autoimmunity and thyroid cancer: review focused on cytological studies. *Eur Thyroid J*. 2017;6:178. doi:10.1159/000468928
27. Rotondi M, Chiovato L, Romagnani S, et al. Role of chemokines in endocrine autoimmune diseases. *Endocr Rev*. 2007;28:492–520. doi:10.1210/er.2006-0044
28. Antonelli A, Rotondi M, Fallahi P, et al. Iodine-131 given for therapeutic purposes modulates differently interferon-γ-inducible α-chemokine CXCL10 serum levels in patients with active Graves' disease or toxic nodular goiter. *J Clin Endocrinol Metab*. 2007;92:1485–1490. doi:10.1210/jc.2006-1571
29. Neshandar AI, Siahkali AS, Shafie B, et al. Prognostic value of basal serum thyroglobulin levels, but not basal antithyroglobulin antibody (TgAb) levels, in patients with differentiated thyroid cancer. *Mol Imag Rad Ther*. 2014;23:54–59.
30. Jensen K, Patel A, Hoperia V, et al. Dynamic changes in E-cadherin gene promoter methylation during metastatic progression in papillary thyroid cancer. *Exp Ther Med*. 2010;1:457–462. doi:10.3892/etm_00000071
31. Liang J, Zeng W, Fang F, et al. Clinical analysis of Hashimoto thyroiditis coexistent with papillary thyroid cancer in 1392 patients. *Acta Otorhinolaryngol*. 2017;37:393–400.
32. Shi Y, Su C, Hu H, et al. Serum MMP-2 as a potential predictive marker for papillary thyroid carcinoma. *PLoS One*. 2018;13:e0198896. doi:10.1371/journal.pone.0198896
33. Pyo JS, Sohn JH, Chang K, et al. Prognostic role of metastatic lymph node ratio in papillary thyroid carcinoma. *J Pathol Transl Med*. 2018;52:331–338. doi:10.4132/jptm.2018.08.07
34. Cai WY, Chen X, Chen LP, et al. Role of differentially expressed genes and long non-coding RNAs in papillary thyroid carcinoma diagnosis, progression, and prognosis. *J Cell Biochem*. 2018. doi:10.1002/jcb.26836
35. Qin J, Yu Z, Guan H, et al. High thyroglobulin antibody levels increase the risk of differentiated thyroid carcinoma. *Dis Markers*. 2015;2015:648670. doi:10.1155/2015/105358
36. Lee YK, Park KH, Park SH, et al. Association between diffuse lymphocytic infiltration and papillary thyroid cancer aggressiveness according to the presence of thyroid peroxidase antibody and BRAF V600E mutation. *Head Neck*. 2018;40:2271–2279. doi:10.1002/hed.25327
37. Cerutti JM, Oler G, Michaluart P Jr, et al. Molecular profiling of matched samples identifies biomarkers of papillary thyroid carcinoma lymph node metastasis. *Cancer Res*. 2007;67:7885–7892. doi:10.1158/0008-5472.CAN-06-4771
38. Landskron J, Kraggerud SM, Wik E, et al. C77G in PTPRC (CD45) is no risk allele for ovarian cancer, but associated with less aggressive disease. *PLoS One*. 2017;12:e0182030. doi:10.1371/journal.pone.0182030
39. Kilvaer TK, Paulsen EE, Khanehkenari MR, et al. The presence of intraepithelial CD45RO+ cells in resected lymph nodes with metastases from NSCLC patients is an independent predictor of disease-specific survival. *Br J Cancer*. 2016;114:1145–1151. doi:10.1038/bjc.2016.92
40. Denning K, Smyth P, Cahill S, et al. ret/PTC-1 expression alters the immunoprofile of thyroid follicular cells. *Mol Cancer*. 2008;7(1):44. doi:10.1186/1476-4598-7-44
41. Schieffer KM, Choi CS, Emrich S, et al. RNA-seq implicates deregulation of the immune system in the pathogenesis of diverticulitis. *Am J Physiol Gastrl*. 2017;313:G277.
42. Yang J, Wen X, Xu H, et al. Antigen-specific T cell analysis reveals that active immune responses to β Cell antigens are focused on a unique set of epitopes. *J Immunol*. 2017;199:91. doi:10.4049/jimmunol.1601570
43. Cirello V, Recalcati MP, Muzza M, et al. Fetal cell microchimerism in papillary thyroid cancer: a possible role in tumor damage and tissue repair. *Cancer Res*. 2008;68:8482–8488. doi:10.1158/0008-5472.CAN-08-0672
44. Lindhorst E, Schumm-Draeger PM, Bojunga J, et al. Differences in tumor cell proliferation, HLA DR expression and lymphocytic infiltration in various types of thyroid carcinoma. *Exp Clin Endocrinol Diabetes*. 2002;110:27–31. doi:10.1055/s-2002-19991
45. Riggins G, Cerutti J Protein markers for the detection of thyroid cancer metastasis: US, US 8394580 B2. 2013.