

Original Article

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

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Abstract: The aim of this study was to evaluate the expression of papillary thyroid carcinoma (PTC)-associated tumor markers in follicular epithelial dysplasia showing PTC-like nuclear alterations (FED) in Hashimoto's thyroiditis (HT) and to explore the relationship between HT and PTC. In this study, 43 PTC, 18 HT with FED and 16 peritumoral benign thyroid tissues were immunohistochemically analyzed for CK19, galectin-3, HBME-1, CD56, claudin-1 and NGAL expression. Our research revealed that in HT, the expression of CK19, galectin-3, HBME-1, claudin-1 and NGAL was focal and limited to FED, while CD56 was strongly positive in FED and most Hürthle cells. The stain intensity of CK19, claudin-1 and NGAL in FED decreased compared with PTC, but were significantly higher than that in peritumoral benign thyroid tissues (all $P < 0.0125$). For galectin-3, HBME-1 and CD56, no statistically significant difference was detected between HT and peritumoral benign thyroid tissues (all $P > 0.05$). In conclusion, In HT, FED might be a precancerous condition closely associated with PTC development as they have overlaps in cytological and immunomarker profiles, indicating that in patients with HT, under prolonged stimuli from chronic inflammation, part of follicular epithelia may show regeneration, hyperplasia, Hürthle cell metaplasia and dysplasia, eventually malignant transformation. Hence, long term follow-up and regular inspection would be necessary for Hashimoto's thyroiditis with FED.

Keywords: Papillary thyroid carcinoma, Hashimoto's thyroiditis, CK19, galectin-3, HBME-1, CD56, claudin-1, NGAL

Introduction

Epidemiological studies have confirmed the correlation between cancer and chronic inflammation. The great examples of this connection were the association between hemochromatosis, viral hepatitis B or C and liver cancer, chronic gastric infection with *Helicobacter pylori* and gastric cancer or MALT, and Crohn's disease or ulcerative colitis and colon cancer [1]. In this regard, chronic inflammatory states are thought to contribute to approximately 20-25% of all human malignancies [1].

Hashimoto's thyroiditis (HT) was also known as chronic lymphocytic or autoimmune thyroiditis. The coexisting of HT with PTC has been noticed for decades. However, there is still no clear evidence whether HT was a risk that can promot-

ed the development of PTC [2]. Recently, the incidence of HT coexisted with thyroid carcinoma, especially with PTC, was rising obviously, and their relationship re-aroused the concern of clinicians and pathologists. We have reviewed 272 histological confirmed HT cases in Tongji Hospital in the last seven years, and find that 25.37% (69) cases were coexistent with thyroid carcinoma, among which all were PTC. From January 2007 through June 2013, the prevalence of PTC and HT coexistence were respectively 21.74%, 16.67%, 16.67%, 18.42%, 27.66%, 28.81% and 31.75%, which showed that HT was associated with an increased risk of developing PTC. Histologically, in addition to eosinophilic variant of follicular epithelium which usually associated with thyroid follicular atrophy or damage, we could also observe follicular epithelium dysplasia (FED) in the form of

Table 1. Properties of primary antibodies used in the study

Antigen	Clone	Source	Dilution	Supplier
Cytokeratin 19	MAB-0056	Mouse mAb	Ready-to-use	Maixin-BIO China
Galectin-3	MAB-0572	Mouse mAb	Ready-to-use	Maixin-BIO China
CD56	MAB-0256	Mouse mAb	Ready-to-use	Maixin-BIO China
HBME-1	GM350504	Mouse mAb	Ready-to-use	Gene Tech, China
Claudin-1	ZA-0365	Rabbit pAb	Ready-to-use	ZSGB-BIO, China
Lipocalin 2 (NGAL)	ab41105	Rabbit pAb	1:200	abcam, UK

Abbreviation: mAb, monoclonal antibody; pAb, polyclonal antibody.

scattered microfollicles lacking of follicular colloid or irregularly shaped follicles in HT tissues, especially in the areas of dense surrounding lymphocytic infiltrate. These follicles were frequently lined by Hürthle cells or cells with lightly stained cytoplasm. Some follicular cells were histologically similar to PTC with enlarged, clear nuclear and grooves. Such lesions were usually small without integrating into a film. In few cases the extension were greater, but still on the background of HT diseases. Whether these alteration implied the earliest or precancerous lesion of PTC has not been a confirm conclusion.

With high sensitivity and specificity, positive staining panel of Galectin-3, mesothelial antigen HBME-1 and cytokeratin-19 (CK19) have been proved to be the most promising and most frequently used molecular markers in identifying papillary thyroid cancer, including follicular variant [3, 4]. They were first preference in many hospitals and communities for their easy-to-use character [3]. CD56, as a neural cell adhesion molecular [5], was proved to be frequent expressed in normal thyroid follicular cells, whereas, absent in malignant thyroid tumors especially in PTC [6, 7]. Claudin-1, a novel marker for PTC, is identified as a tight junction component belonging to claudin family [8]. Several evidences in gene [9, 10] and protein [7, 11] level showed that the expression of claudin-1 was evaluated in PTC, whereas, it was absent or weak staining on the membrane of normal and benign thyroid lesions. Another new detector for PTC is neutrophil gelatinase-associated lipocalin (NGAL), which involved in cancerogenesis and cancer progression by increasing iron uptake from the extracellular space to inner cells [12, 13]. Barresi's et al. [13, 14] found that NGAL expression appeared to be significantly more frequent in the malignant thy-

roid tumors than in benign ones, the later including follicular adenomas, Hürthle cell adenomas and atypical adenomas. What's more, no NGAL staining was found in the normal thyroid gland, the nodular colloid goiters nor in Hashimoto's thyroiditis.

The present study was designed to evaluate the expression of CK19, galectin-3, HBME-1, CD56, claudin-1 and NGAL in FED, and compare them with PTC and C, so as to investigate the relationship between PTC and FED at protein level, and clarify the biological significance of FED in HT disease.

Materials and methods

Materials

A total of 77 formalin-fixed and paraffin embedded thyroidectomy specimens dating from January 2011 to March 2013 in the Pathology department of Tongji hospital affiliated to Tongji Medical college of Huazhong University of Science and Technology were selected for this experiment, among which 43 cases of PTC (28 were associated with HT) and 16 peritumoral benign thyroid tissues used as control (C) were selected according to the diagnostic criteria of World Health Organization classification of tumor; 18 cases of Hashimoto's thyroiditis with FED were selected using stringent diagnostic criteria shown in the introduction. The selected 43 PTC samples included 3 males and 40 females, ranging from 16 to 63 years old (mean: 39.58±11.052 year).

Immunohistochemistry

Immunohistochemical staining of EnVision detection system was performed on consecutive sections (4 µm thick) from each archival tissue block. Primary antibodies, sources, and dilutions are listed in **Table 1**. According to the manufacture's instruction, all sections were deparaffinized with xylene, rehydrated through a series of descending graded alcohols. Antigen retrieval was performed in 1 mM EDTA PH 8.0 (CK19, galectin-3, CD56, claudin-1) or in citrate buffer PH 6.0 (NGAL) in a pressure cooker for

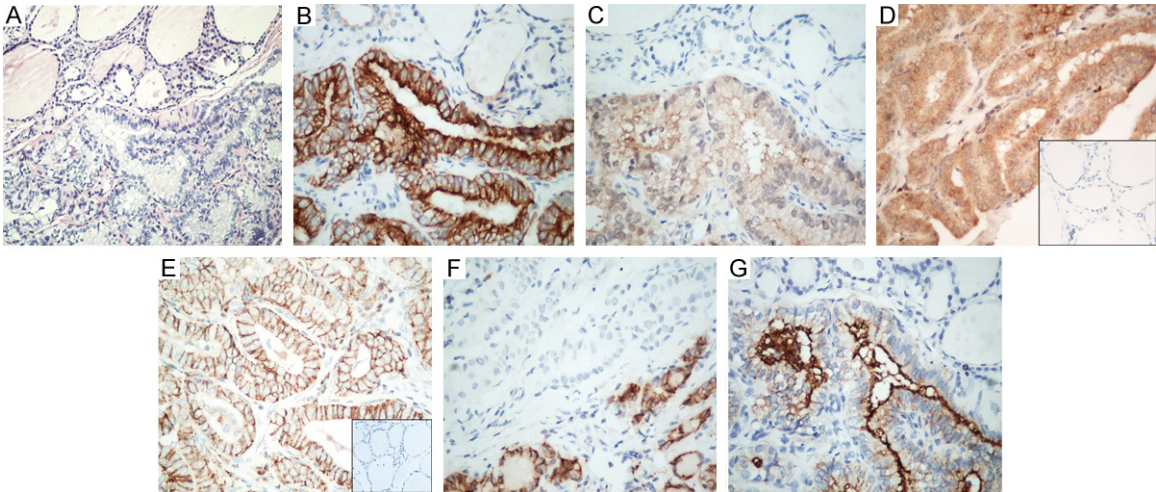


Figure 1. The expression of six thyroid tumor makers in PTC and control (EnVision). A. HE (magnification 200×) for classic PTC and control (upside), PTC showed nuclear features of enlarged, oval, overlapping, clear nuclear and grooves (underside). B. CK19 (magnification 400×) showed strong cytoplasm staining in PTC (underside) and focal weak positive in C (upside). C. Galectin-3 (magnification 400×) showed diffuse but moderate or weak cytoplasm staining in most PTC cases (underside), with occasional nuclear expression, whereas, it was completely negative in control (upside). D. NGAL showed a mixed cytoplasm and nuclear staining pattern, with cytoplasm expressing much stronger in PTC (magnification 400×), whereas, it was negative in control (inset, magnification 200×). E. claudin-1 showed predominantly membrane staining (the lateral membranous surface) in PTC (magnification 400×), while was absent in control (inset, magnification 100×). F. CD56 (magnification 400×) showed strong and complete membrane expression in control (underside), while was absent in PTC (upside). G. HBME-1 (magnification 400×) showed predominantly strong membrane staining pattern (at the luminal side, papillary or the lateral membranous surface) in PTC (underside), whereas absent in C (upside).

Table 2. Staining intensity of tumor markers in PTC, controls and FED

Group		n	CK19			Galectin-3			HBME-1		
			0	1+	2+~3+	0	1+	2+~3+	0	1+	2+~3+
PTC	hptc	26	0	2	24	2	14	10	5	10	11
	ptc	17	0	2	15	0	10	7	1	2	14*
C		16	9	5	2	16	0	0	16	0	0
FED		18	0	13	5Δ	11	7	0☆	16	2	0☆
Group		n	CD56			Claudin-1			NGAL		
			0	1+	2+~3+	0	1+	2+~3+	0	1+	2+~3+
PTC	hptc	26	19	5	2	2	6	18	1	5	20
	ptc	17	15	2	0	2	1	14	2	2	13
Control		16	0	0	16	16	0	0	16	0	0
FED		18	1	4	13☆	5	13	0Δ	5	8	5Δ

Abbreviation: No., the number of cases; PTC, all papillary thyroid carcinoma; hptc, papillary thyroid carcinoma coexisted with Hashimoto's thyroiditis; ptc, papillary thyroid carcinoma without Hashimoto's thyroiditis; Control, benign thyroid tissues; FED, follicular epithelial dysplasia showing PTC-like nuclear alterations in Hashimoto's thyroiditis; *compared with hptc, $P = 0.031$; Δcompared with PTC and C, all $P < 0.001$; ☆compared with PTC, all $P < 0.01$.

1.5 minutes at 120°C. Sections did not get the antigen retrieval program with HBME-1. Endogenous peroxidase activity was blocked by

using 3% H_2O_2 for 20 min. They were then incubated with primary antibody for 1 hour at room temperature, followed by a peroxidase-conjugated Polymer (Dako REAL EnVision/HRP, Rabbit/mouse (ENV) reagent of the kit k5007, Denmark) for 30 min. To reveal the immune-staining, the sections were incubated with Dako REAL DAB + Chromogen for 5 min (Dako k5007, Denmark), then counterstaining with hematoxylin, followed by dehydration and mounting. Slides were examined using BX50 optical microscope (Olympus, Japan) with SPOT-II digital imaging software (DIAGNOSTIC instrument, inc. USA). Normal human skin was used as positive control for claudin-1, human colon tissue for lipocalin 2 (NGAL),

gastrointestinal stromal tumor for HBME-1, neuroblastoma tissue for CD56. A classic PTC known to react diffusely and strongly with CK19

Table 3. Expression of six tumor markers in PTC with or without HT

Species	PTC coexisted with HT 26 (%)	PTC without HT 17 (%)	P
CK19 (+)	26 (100)	17 (100)	*
Galectin-3 (+)	24 (92.3)	17 (100)	0.360
HBME-1 (+)	21 (80.8)	16 (94.1)	0.221
CD56 (-)	19 (73.08)	15 (88.2)	0.211
Claudin-1 (+)	24 (92.3)	15 (88.2)	0.521
NGAL (+)	25 (96.2)	15 (88.2)	0.342

and galectin-3 was used as the positive control. PBS was used as negative control instead of primary antibody.

Interpretation of the immunohistochemical staining

The proteins were considered expressed when at least 10% of tumor cells or 10% thyrocytes with PTC-like nuclear alterations were positive according to the data published earlier [3, 11, 14-17]. The immune staining intensity of each thyroid tumor marker was graded as (0) no staining or less than 10% of the cells were faint yellow, (1) more than 10% of the cells were faint yellow, (2) yellow (3) brown yellow.

Statistical analyses

All statistics were analyzed by using SPASS Program Version 18.0, Fisher exact probability test and non-parametric rank sum test (Mann-Whitney test) were used for significance analysis ($P < 0.05$). In addition, the sensitivity (positive ratio) and specificity were assessed as follows: Sensitivity = true positive/true positive + false negative; Specificity = true negative/true negative + false positive.

Results

Expression of CK19, galectin-3, HBME-1, CD56, claudin-1 and NGAL in PTC and peritumoral benign thyroid tissues

CK19, Galectin-3 and NGAL expressed mainly in the cytoplasm of follicular epithelium, at the same time, Galectin-3 and NGAL also showed occasional nuclear expression (**Figure 1B-D**). Claudin-1 expressed mainly in the membrane (**Figure 1E**) with occasional cytoplasm weak positive. CD56 often showed strong and complete membrane expression in benign thyroid

follicular epithelium (**Figure 1F**). The expression of HBME-1 is mainly on membrane, which is at the luminal side, papillary or the lateral membranous surface, with or without the cytoplasm and the content of glandular cavity staining positive (**Figure 1G**). In most PTC cases, CK19, galectin-3, HBME-1, claudin-1 and NGAL all showed a strong and diffuse staining pattern, while in peritumoral benign thyroid tissues (control), Galectin-3, HBME-1, claudin-1 and NGAL were all negative and CK19 showed a focal weak staining pattern (**Figure 1B-E, 1G**). CD56 showed moderate or strong and diffuse expression in all controls, while absent in the majority of PTC tissues (**Figure 1F**). Fisher exact probability test and Mann-Whitney test showed that the differences for the positive ratio and staining intensity of CK19, Galectin-3, HBME-1, CD56, claudin-1 and NGAL between PTC and control were all statistically significant (all $P < 0.001$).

Expression of CK19, galectin-3, HBME-1, CD56, claudin-1 and NGAL in PTC associated with HT and PTC alone

The staining intensity of the above six thyroid tumor markers between PTC associated with HT and PTC without HT were summarized in **Table 2**. In 57.7% (15/26) PTC-associated with HT, the staining intensity of HBME-1 was often negative or weak positive, while 82.35% (14/17) PTC without HT tissues were frequently moderate or strong and diffuse positive with HBME-1. Mann-Whitney test detected that the difference was statistically significant ($P = 0.031$). But the difference in terms of the staining intensity of the remaining five thyroid tumor markers between PTC associated with HT and PTC without HT were not statistically significant (all $P > 0.05$). As shown in **Table 3**, in 26 cases of PTC coexisted with HT, the sensitivity of CK19, galectin-3, HBME-1, CD56, claudin-1 and NGAL were 100% (26/26), 92.3% (24/26), 80.8% (21/26), 73.08% (19/26), 92.3% (24/26) and 96.2% (25/26), respectively. While among 17 PTC without HT, the sensitivity of CK19, galectin-3, HBME-1, claudin-1 and NGAL were 100% (17/17), 100% (17/17), 94.1% (16/17), 88.2% (15/17), 88.2% (15/17), 88.2% (15/17), respectively. Fisher exact probability test showed no statistically significant difference for the expression of the above six indicators between PTC associated with HT and PTC without HT (the value of P were all > 0.05).

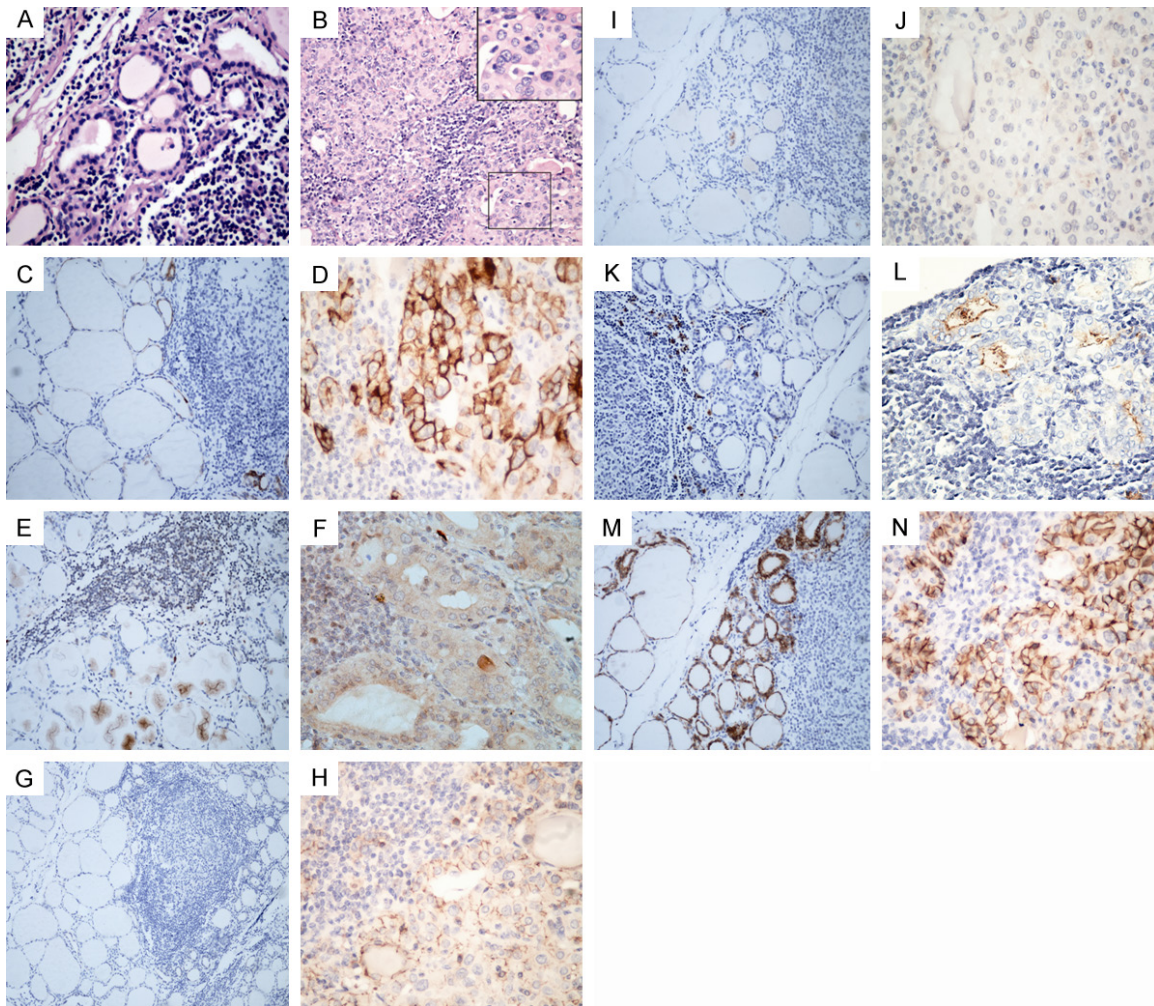


Figure 2. The expression of six thyroid tumor markers in FED and Hürthle cells (EnVision). A. HE sections of Hürthle cells in HT with abundant eosinophilic cytoplasm, hyperchromatic, mild nuclear atypia (enlarged, round and rare grooves), but maintain the normal lobular architecture (magnification 400×). B. HE section of FED with moderate cytological atypia (enlarged, oval, crowded, irregularity, clear nuclei and grooves) and follicular architectural distortion (lacking of follicular colloid) in areas of severe inflammation (magnification 200×; inset, 400×). C, D. CK19 showed focal weak positive in Hürthle cells (magnification 200×), and scattered strong positive in FED tissues (magnification 400×). E, G, I, K. NGAL, Claudin-1, Galectin-3 and HBME-1 were all completely negative in Hürthle cells (magnification 200×, 100×, 200×, 200×). F. In FED, NGAL expressed mainly on the cytoplasm, with occasionally nuclear expression (magnification 400×); H. Claudin-1 showed weak membrane staining pattern in FED (magnification 400×). J. Galectin-3 was nearly negative in FED (magnification 400×). L. In FED, HBME-1 stain was focal and limited to thyrocytes with PTC-like nuclear alterations (magnification 400×). M, N. CD56 showed strong and complete staining pattern in both Hürthle cells and FED tissues (magnification 200×, 400×).

Expression of CK19, galectin-3, HBME-1, CD56, claudin-1 and NGAL in HT with FED

On routine microscopic examination, HT showed follicular regenerative activity in the form of numerous small follicles, frequently lined by Hürthle cells. Hürthle cells had abundant eosinophilic cytoplasm, hyperchromatic, mild to moderate nuclear atypia (mildly enlarged with round shape and rare grooves) but still main-

tain normal lobular architecture (**Figure 2A**). FED (**Figure 2B**) usually presented in areas of severe inflammation with moderate cytological atypia (enlarged, oval, crowded, irregularity, clear nucleus and grooves) and irregularly shaped follicles. Some may have small follicular structure, but lack of follicular colloid. Such lesions were insufficient to be diagnosis as PTC morphologically for the presence of dense surrounding lymphocytic infiltrate, absence of

sclerosis (stromal desmoplasia), infiltrative growth, papillary architecture, and intranuclear pseudoinclusions. The expression of PTC associated proteins in FED was noted frequently in scattered thyrocytes showing PTC-like nuclear alterations.

As shown in **Table 2**, 18 cases of HT all showed scattered CK19 expression in FED, but 13 cases were weak positive and the remaining 5 were moderate staining (**Figure 2D**). In Hürthle cells (**Figure 2C**), CK19 showed focal and weak staining pattern as it has been in control group. Furthermore, most positive cells were closely adjacent to lymphoid aggregates and displaying severe eosinophilic change. In 72.2% (13/18) NGAL positive FED cases, 8 was focal weak positive (**Figure 2F**), 4 were moderate, only 1 was strong positive. While Hürthle cells with eosinophilic change alone were negative with NGAL (**Figure 2E**). Mann-whitney U test showed the different in terms of staining intensity of CK19 and NGAL between FED and PTC were statistically significant (both $P < 0.001$), the same for FED and control ($P_{ck19} = 0.004$, $P_{ngal} < 0.001$). In conclusion, the expression of CK19 and NGAL in FED decreased when compared with PTC, but still significantly higher than that in the control.

Claudin-1 all showed focal and weak expression in FED tissues (**Figure 2H**). In Hürthle cells with eosinophilic change alone, claudin-1 was absent (**Figure 2G**). The difference in terms of staining intensity between FED and PTC, and between FED and C were both statistically significant (both $P < 0.001$). In short, the expression level of claudin-1 in FED was intermediate between PTC and the control.

The expression of galectin-3 and HBME-1 was also focal and weak in FED tissues (**Figure 2J, 2L**), while in Hürthle cells and controls, both of them were completely absent (**Figure 2I, 2K**). Compared with the three indicators described earlier, both the positive ration and staining intensity of galectin-3 and HBME-1 were low, and the positive ratio was 38.9% (7/18) and 11.1% (2/18), respectively, which were the lowest in this research. The difference in terms of staining intensity between FED and the control was not statistically significant (both $P > 0.05$), but were statistically significant between PTC and FED (both $P < 0.001$).

The positive ration of CD56 in FED was 94.4% (17/18), among which 13 (72.2%) cases were moderate to strong positive (**Figure 2N**). Only one was negative and 4 was weak positive. The difference in terms of staining intensity had no statistical significance between FED and control group ($P = 0.251$), while were statistically significant between FED and PTC ($P < 0.001$). Meanwhile, CD56 also showed strong and complete staining pattern in Hürthle cells with eosinophilic change alone (**Figure 2M**).

In conclusion, in HT tissues, FED shared similar immune phenotype with PTC, but not completely. The stain intensity of CK19, claudin-1 and NGAL decreased compared with PTC, but it was still significantly higher than in C. Although galectin-3 and HBME-1 expression in FED had no significant difference as contrasted with C, a minority of thyrocytes with PTC-like nuclear alteration still showed clearly galectin-3 and HBME-1 staining, while in controls and Hürthle cells, Galectin-3 and HBME-1 were completely negative. Only in very few atypical cells with PTC-like nuclear alteration, the expression of CD56 decreased.

Discussion

Recently, epidemiological studies have confirmed the correlation between cancer and chronic inflammation, in particular for the gastrointestinal tract, where the link between chronic active inflammation and the risk of digestive carcinoma onset is now well-established [18]. HT, induced by multiple genetic and environmental factors, was featured with complex autoimmune disorder [19] and was first described by Hakaru Hashimoto in 1912 [2, 20]. So far, up to about 2% of general population was plagued by this disease, which was predominantly female with gender prevalence ratios of 5 to 10:1 [20] and the incidence still increasing sharply. PTC is the most prevalent form of thyroid cancer comprising approximately 80% of thyroid epithelial malignancies [2]. The controversial relationship between PTC and HT had gone on debate for more than 60 years [21]. But some concordant epidemiological data truly revealed that HT was frequently associated with thyroid carcinoma, particularly with PTC.

Plenty of epidemiological survey reported that the occurrence of HT in PTC varied from 5 to

85% [22], it was more frequently observed in PTCs than in benign thyroid diseases and other carcinomas. Although population-based FNAB studies report no significant relationship between these two diseases, studies on thyroidectomy specimens report a positive link [23]. In addition, other studies recovered a genetic link between HT and PTC involving the PI3K/Akt pathway and RET/PTC gene rearrangement [18]. There are also several similarities between HT and PTC at histological level, such as proliferating nodules and PTC-like nuclear alterations.

Prasad et al. [17] found that PTC-associated proteins (galectin 3, CITED1, cytokeratin 19 (CK19), HBME1 and fibronectin 1) were focal expressed in thyrocytes with PTC-like nuclear alterations in HT. Consistently, HT also showed focal or scattered expression with CK19, Galectin-3, HBME-1, CD56, claudin-1 and NGAL in follicular epithelial dysplasia showing PTC-like nuclear alterations in our research. Among 18 FED cases, although the positive ratio of CK19, NGAL, claudin-1, galectin-3, HBME-1 were 100% (18/18), 72.2% (13/18), 72.2% (13/18), 38.9% (7/18), 11.1% (2/18), respectively, the stain intensity were weak and local contrast with PTC. The over-expression of Galectin-3 has been demonstrated to be necessary for maintaining the transformed phenotype in PTC cell lines, a phenotype associated with malignant transformation and progression toward metastatic potential [24]. HBME1, galectin-3, claudin-1 and NGAL were not expressed in cells other than PTC and FED. Their expression in thyrocytes is abnormal and appears to be associated with neoplastic change. The expression of CD56 was closely related to the differentiation of thyroid epithelium [25]. In present study, diffuse strong CD56 staining was seen in 94.4% (17/18) of FED. As compared with PTC and controls, only very few cells in FED were negative with CD56 suggesting that malignant transformation has not yet been fully established in FED tissues. In summary, we could propose that follicular epithelial dysplasia with PTC-like nuclear alterations may possibly be a precursor of PTC, but not sufficient for the diagnosis of PTC.

Although a recent study by Nasr et al. [26] suggested that HBME1+ and CK19+ atypical cell clusters, found in HT, could not be considered to be preneoplastic as they did not harbor the

BRAF mutation, several studies conducted by Muzza et al [27], Kang et al [28] and Sargent et al [29]. on a large series of PTCs with or without thyroiditis, consistently showed that the RET/PTC rearrangement was more often present in PTCs associated with autoimmunity, whereas the BRAFV600E mutation was encountered more frequently in PTCs alone. In our research, the immune phenotype between PTC with or without HT was basically the same except for HBME-1, which implied that the expression spectrum of most tumor markers between them were concordant. Thus, they may be etiologically related. In our research, 57.7% (15/26) PTC-associated with HT were negative or weak positive with HBME-1, while 82.35% (14/17) PTC without HT tissues were moderate to strong and diffuse positive with HBME-1. Mann-Whitney test detected that the difference was statistically significant. Whether it was responsible for the long recurrence-free survival in PTC coexisted with HT patients need further investigations.

Tumor formation is a progressive process that multiple gene and protein alteration were involved, resulted in a continuous morphological and cytological change from normal tissue to a fully established neoplasm. According to our experimental observation, we believed that FED was an intermediate stage between benign Hashimoto's thyroiditis and PTC, a condition sharing part of genetic and morphological features with PTC [30]. In HT tissues, thyroid follicles showed variably atrophy, regeneration, hyperplasia and Hürthle cell metaplasia, until the presence of focal or scattered PTC-like nuclear atypical, particularly in areas with intense lymphoma infiltration and lymphoid follicles formation, which implied that these alteration in thyroid follicular epithelium may be a progressive spectrum on the background of autoimmune inflammatory disorder. The expression of several PTC-associated proteins were focal or scattered and mainly in small clusters of thyrocytes with PTC-like nuclear alteration, suggesting that focal molecular transformation occurred on a background of atrophy, regeneration and Hürthle cell metaplasia in atypical follicular epithelium with PTC-like nuclear alteration [17].

In Hashimoto's thyroiditis, FED might be a precancerous lesion associated with PTC development. The overlaps between them in cytological, immunomarker profiles and genetic fea-

tures further supported this assumption, indicated that in patients with HT, under prolonged stimuli from chronic inflammation, part of follicular epithelium may show regeneration, hyperplasia, Hürthle cell metaplasia and dysplasia, eventually malignant transformation. Hence, long-term follow-up and regular inspection would be necessary for patients with Hashimoto's thyroiditis.

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Declaration of conflict of interest

None.

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References

- [1] Hussain SP, Harris CC. Inflammation and cancer: an ancient link with novel potentials. *Int J Cancer* 2007; 121: 2373-80.
- [2] Lee JH, Kim Y, Choi JW, Kim YS. The association between papillary thyroid carcinoma and histologically proven Hashimoto's thyroiditis: a meta-analysis. *Eur J Endocrinol* 2013; 168: 343-49.
- [3] Paunovic I, Isic T, Havelka M, Tatic S, Cvejic D, Savin S. Combined immunohistochemistry for thyroid peroxidase, galectin-3, CK19 and HBME-1 in differential diagnosis of thyroid tumors. *Apmis* 2012; 120: 368-79.
- [4] de Matos PS, Ferreira AP, de Oliveira FF, Assumpcao LV, Metzke K, Ward LS. Usefulness of HBME-1, cytokeratin 19 and galectin-3 immunostaining in the diagnosis of thyroid malignancy. *Histopathology* 2005; 47: 391-401.
- [5] Lanier LL, Testi R, Bindl J, Phillips JH. Identity of Leu-19 (CD56) leukocyte differentiation antigen and neural cell adhesion molecule. *J Exp Med* 1989; 169: 2233-38.
- [6] El DD, Nasr AL, Babay S, Alowami S. Diagnostic utility of CD56 immunohistochemistry in papillary carcinoma of the thyroid. *Pathol Res Pract* 2009; 205: 303-09.
- [7] Abd EAR, Shash LS. Potential diagnostic utility of CD56 and claudin-1 in papillary thyroid carcinoma and solitary follicular thyroid nodules. *J Egypt Natl Canc Inst* 2012; 24: 175-84.
- [8] Furuse M, Fujita K, Hiiiragi T, Fujimoto K, Tsukita S. Claudin-1 and -2: novel integral membrane proteins localizing at tight junctions with no sequence similarity to occludin. *J Cell Biol* 1998; 141: 1539-50.
- [9] Fluge Ø, Bruland O, Akslen LA, Lillehaug JR, Varhaug JE. Gene expression in poorly differentiated papillary thyroid carcinomas. *Thyroid* 2006; 16: 161-75.
- [10] Hucz J, Kowalska M, Jarzab M, Wiench M. [Gene expression of metalloproteinase 11, claudin 1 and selected adhesion related genes in papillary thyroid cancer]. *Endokrynol Pol* 2006; 57 Suppl A: 18-25.
- [11] Németh J, Németh Z, Tátrai P, Péter I, Somorácz Á, Szász AM, Kiss A, Schaff Z. High expression of claudin-1 protein in papillary thyroid tumor and its regional lymph node metastasis. *Pathol Oncol Res* 2010; 16: 19-27.
- [12] Yang J, Goetz D, Li JY, Wang W, Mori K, Setlik D, Du T, Erdjument-Bromage H, Tempst P, Strong R, Barasch J. An iron delivery pathway mediated by a lipocalin. *Mol Cell* 2002; 10: 1045-56.
- [13] Barresi V, Leni A, Tuccari G, Barresi G. Neutrophil gelatinase-associated lipocalin (NGAL) immunohistochemical expression in follicular cell-derived thyroid tumors: a novel diagnostic tool? *Histol Histopathol* 2012; 27: 329-36.
- [14] Barresi V, Vitarelli E, Reggiani BL, Tuccari G, Barresi G. Diagnostic value of neutrophil gelatinase-associated lipocalin (NGAL) immunorexpression in follicular-patterned lesions of the thyroid gland. *Virchows Arch* 2012; 460: 319-25.
- [15] Park WY, Jeong SM, Lee JH, Kang HJ, Sin DH, Choi KU, Park DY, Huh GY, Sol MY, Lee CH. Diagnostic value of decreased expression of CD56 protein in papillary carcinoma of the thyroid gland. *Basic and Applied Pathology* 2009; 2: 63-68.
- [16] Mataraci EA, Ozguven BY, Kabukcuoglu F. Expression of cytokeratin 19, HBME-1 and galectin-3 in neoplastic and nonneoplastic thyroid lesions. *Pol J Pathol* 2012; 63: 58-64.
- [17] Prasad ML, Huang Y, Pellegata NS, de la Chapelle A, Kloos RT. Hashimoto's thyroiditis with papillary thyroid carcinoma (PTC)-like nuclear alterations express molecular markers of PTC. *Histopathology* 2004; 45: 39-46.
- [18] Bozec A, Lassalle S, Hofman V, Ilie M, Santini J, Hofman P. The thyroid gland: a crossroad in inflammation-induced carcinoma? An ongoing debate with new therapeutic potential. *Curr Med Chem* 2010; 17: 3449-61.

- [19] Baretić M. [100 years of Hashimoto thyroiditis, still an intriguing disease]. *Acta Med Croatica* 2011; 65: 453-57.
- [20] Ahmed R, Al-Shaikh S, Akhtar M. Hashimoto thyroiditis: a century later. *Adv Anat Pathol* 2012; 19: 181-86.
- [21] Dailey ME, Lindsay S, Skahen R. Relation of thyroid neoplasms to Hashimoto disease of the thyroid gland. *AMA Arch Surg* 1955; 70: 291-97.
- [22] Okayasu I, Fujiwara M, Hara Y, Tanaka Y, Rose NR. Association of chronic lymphocytic thyroiditis and thyroid papillary carcinoma. A study of surgical cases among Japanese, and white and African Americans. *Cancer* 1995; 76: 2312-18.
- [23] Jankovic B, Le KT, Hershman JM. Clinical Review: Hashimoto's thyroiditis and papillary thyroid carcinoma: is there a correlation? *J Clin Endocrinol Metab* 2013; 98: 474-82.
- [24] Coli A, Bigotti G, Zucchetti F, Negro F, Massi G. Galectin-3, a marker of well-differentiated thyroid carcinoma, is expressed in thyroid nodules with cytological atypia. *Histopathology* 2002; 40: 80-87.
- [25] Migita K, Eguchi K, Kawakami A, Ida H, Fukuda T, Kurata A, Ishikawa N, Ito K, Nagataki S. Detection of Leu-19 (CD56) antigen on human thyroid epithelial cells by an immunohistochemical method. *Immunology* 1991; 72: 246-49.
- [26] Nasr MR, Mukhopadhyay S, Zhang S, Katzenstein AL. Absence of the BRAF mutation in HBME1+ and CK19+ atypical cell clusters in Hashimoto thyroiditis: supportive evidence against preneoplastic change. *Am J Clin Pathol* 2009; 132: 906-12.
- [27] Muzza M, Degl'Innocenti D, Colombo C, Perrino M, Ravasi E, Rossi S, Cirello V. The tight relationship between papillary thyroid cancer, autoimmunity and inflammation: clinical and molecular studies. *Clin Endocrinol (Oxf)* 2010; 72: 702-08.
- [28] Kang DY, Kim KH, Kim JM, Kim SH, Kim JY, Baik HW, Kim YS. High prevalence of RET, RAS, and ERK expression in Hashimoto's thyroiditis and in papillary thyroid carcinoma in the Korean population. *Thyroid* 2007; 17: 1031-38.
- [29] Sargent R, LiVolsi V, Murphy J, Mantha G, Hunt JL. BRAF mutation is unusual in chronic lymphocytic thyroiditis-associated papillary thyroid carcinomas and absent in non-neoplastic nuclear atypia of thyroiditis. *Endocr Pathol* 2006; 17: 235-41.
- [30] Arif S, Blanes A, Diaz-Cano SJ. Hashimoto's thyroiditis shares features with early papillary thyroid carcinoma. *Histopathology* 2002; 41: 357-62.