

# Diagnostic significance of CK19, RET, galectin-3 and HBME-1 expression for papillary thyroid carcinoma

Xiaoli Zhu,<sup>1</sup> Tuanqi Sun,<sup>2</sup> Hongfen Lu,<sup>1</sup> Xiaoyan Zhou,<sup>1</sup> Yongming Lu,<sup>1</sup> Xu Cai,<sup>1</sup> Xiongzen Zhu<sup>1</sup>

<sup>1</sup>Department of Pathology, Cancer Hospital, Fudan University, Shanghai, China

<sup>2</sup>Department of Head and Neck Surgery, Cancer Hospital, Fudan University, Shanghai, China

## Correspondence to

Xiongzen Zhu, Department of Pathology, Cancer Hospital, Fudan University, 270 Dongan Road, Shanghai 200032, China; [xiongzenzhu@yahoo.com.cn](mailto:xiongzenzhu@yahoo.com.cn)

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## ABSTRACT

**Aims** To evaluate CK19, RET, galectin-3 and HBME-1 expression in papillary thyroid carcinoma (PTC) and to evaluate their diagnostic significance.

**Methods** 155 PTC specimens and 83 other diseased-thyroid specimens were collected. Immunohistochemistry for CK19, RET, galectin-3 and HBME-1 was performed.

**Results** The 155 PTC cases were classified into eight variants according to the WHO classification, including 74 cases of classic PTC, 40 cases of papillary microcarcinoma, and rare variants. CK19, RET, galectin-3 and HBME-1 expression was 87.1% (135/155), 71.0% (110/155), 91.6% (142/155), and 95.5% (148/155), respectively, for the PTC group; expression of all these markers was much higher than that in the control group ( $p < 0.05$ ). However, the expression of these markers did not differ among the variants ( $p > 0.05$ ). The expression of these markers, particularly CK19 and RET, was diffuse and strong in the papillary structure of PTC, but weak and focal in the papilla of tissue with benign disease. The expression of CK19 in follicular PTC was significantly higher than in follicular thyroid carcinoma (FTC) ( $p < 0.05$ ).

**Conclusions** CK19, RET, galectin-3 and HBME-1 expression in PTC was higher than that in benign disease cases, but these were not specific markers for PTC. In summary, combining markers can increase the reliability and differential diagnosis of PTC. It is also worth noting that CK19 was very useful not only for the differentiation of benign and malignant papillary structure but also for the differential diagnosis of follicular PTC and FTC.

Papillary thyroid carcinoma (PTC) is a common malignancy affecting the endocrine system. According to the World Health Organization (WHO) classification, PTC can be classified into 15 variants based on their histological structure, cell type, tumour size, shape and matrix.<sup>1</sup> The clinical manifestations, pathology, treatment and prognosis differ among the variants.<sup>1 2</sup> Therefore, it is important to specifically identify the variants in order to achieve effective diagnosis, treatment and better prognostic prediction. However, it is often difficult to differentiate follicular PTC from follicular carcinoma and papillary structure in PTC with benign papillary hyperplasia of the thyroid gland based on their morphology. There are several methods for differentiating between these disease variants. Morphological examination of paraffin sections and immunohistochemical staining is the most routine method for diagnosing PTC. In this study, we identified four immunohistochemical markers, CK19, RET, galectin-3 and HBME-1, for

evaluating PTC and other malignant and benign thyroid diseases. Furthermore, we assessed their significance in the diagnosis and differential diagnosis of PTC.

## MATERIALS AND METHODS

### Specimens

A total of 238 thyroid samples collected by the Pathology Department in the Cancer Hospital of Fudan University between 1991 and 2004 were used in this study. The PTC group consisted of 155 PTC cases, and the control group contained 83 cases (11 cases of follicular thyroid carcinoma (FTC), 10 cases of medullary thyroid carcinoma (MTC), 4 cases of undifferentiated thyroid carcinoma (UTC), 21 cases of follicular adenoma (FA), 20 cases of Hashimoto thyroiditis (HT), and 17 cases of nodular goitre (NG)). Informed consent was obtained from all patients that donated their specimens, and all experiments were reviewed by the hospital's ethics committee prior to our study.

### Reagents

The antibodies included: CK19 (mouse monoclonal anti-human antibody clone RCK108; 1:50; Dako Corporation, Carpinteria, California, USA); HBME-1 (mouse monoclonal anti-human antibody; 1:100; Dako Corporation); RET (mouse polyclonal anti-human antibody clone C-19; 1:100; Santa Cruz Biotechnology, Santa Cruz, California, USA); and galectin-3 (mouse monoclonal anti-human antibody clone: 9C4; 1:50; Beijing Zhong Shan Biotechnology, Beijing, China).

### Methods

Sections were generated from the tissue specimens and stained with (H&E). The morphology was microscopically examined and a diagnosis was made. The WHO classification system was used to classify the PTC specimens.<sup>1</sup> The antibodies were evaluated using the dextran/peroxidase technique (Dako Envision). A visualisation reagent was applied for 30 min, which was followed by incubation with a substrate-chromogen solution (3,3-diaminobenzidine). The colour was developed for 10 min. The slides were counterstained by immersion in a haematoxylin bath for 5 min. Specimens were incubated with Tris-buffered saline (TBS) instead of the primary antibodies as a negative control and corresponding positive samples were used as a positive control. The appearance of brown particles in the cytoplasm for CK19, RET and galectin-3, and in the membrane or cytoplasm for HBME-1 represented a positive staining result.

Negative staining was defined when none or <5% of the cells were positively stained; 1+ staining was defined when 5–25% of the cells positively stained; 2+ staining was defined when 25–50% of the cells positively stained; and 3+ staining was defined when 50–100% of the cells positively stained. Furthermore, 1+ was considered weakly positive, 2+ was moderately positive and 3+ was strongly positive. Statistical analysis was performed using SPSS V.11.5. The  $\chi^2$  test and Fisher exact test were used for comparison of the immunohistochemistry results between the PTC and control groups.

## RESULTS

### Clinical and morphological features of PTC

There were 155 cases in the PTC group, which included 36 men and 119 women (1:3.3), with an average age of 46 years (range 18–84 years). In 68.4% of these cases, the age was  $\geq$ 45 years. There was no difference regarding the localisation on either side of the body (1:1). In addition, 29% (45/155) of the cases were accompanied by HT; 9.7% (15/155) were accompanied by FA or NG; 12.9% (20/155) exhibited local invasion (angioinvasion, and direct extension into the perithyroidal fat, skeletal muscle, oesophagus, larynx and trachea); 51.6% (80/155) had lymph node metastasis (LNM); and 3.2% (5/155) had distant metastasis.

According to the WHO classification, 155 patients were diagnosed with eight PTC variants, including 74 cases of typical PTC (47.7%), 40 cases of papillary microcarcinoma (25.8%), 7 cases of the follicular variant (FVPTC) (4.5%), 9 cases of the oncocytic variant (5.8%), 8 cases of the diffuse sclerosing variant (5.2%), 9 cases of the tall-cell variant (5.8%), 7 cases of the solid variant (4.5%), and 1 case of the clear-cell variant (0.6%).

### Immunohistochemistry results

The expression of CK19, RET, galectin-3 and HBME-1 was 87.1% (135/155), 71.0% (110/155), 91.6% (142/155), and 95.5% (148/155) for the PTC group, respectively. The expression of CK19, RET, galectin-3 and HBME-1 was diffuse and strong in the PTC cases but negative in the normal thyroid follicles surrounding the tumour. The expression in the control group specimens was low and focally weak. The expression of CK19,

RET, galectin-3 and HBME-1 was weak in the cytoplasm of cells with ground-glass nuclei in HT patients and in the papillary structure in NG and FA specimens (figure 1).

The expression of CK19, galectin-3 and HBME-1 among the PTC variants was not significantly different ( $p>0.05$ ) (table 1).

As table 2 shows, the expression of CK19 and galectin-3 in the PTC group was significantly higher than that in the control group ( $p<0.05$ ). The expression of CK19 in FVPTC was significantly higher than that in FTC ( $p<0.05$ ). The expression of galectin-3 in FVPTC and FTC did not differ, but the expression was higher than that in FA ( $p<0.05$ ) (table 2).

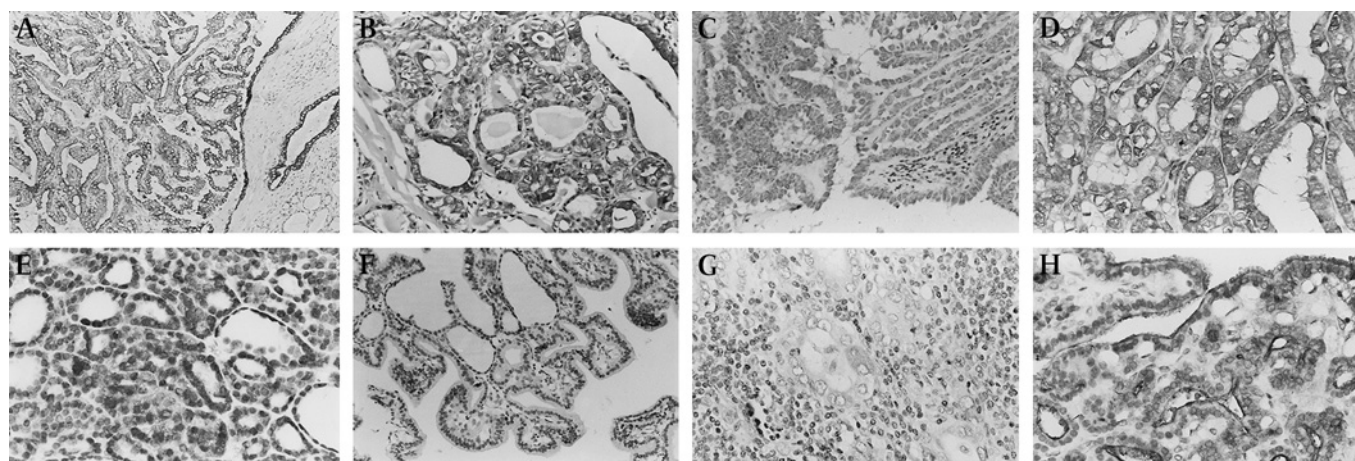
The expression of RET in the PTC group was significantly higher than that in the FTC, FA and NG groups but was not different from that in the HT group. The expression of CK19, RET and galectin-3 in the HT group was significantly higher than that in the FA and NG groups ( $p<0.05$ ). The expression of HBME-1 in the PTC group was significantly higher than that in the MTC, FA, HT and NG groups ( $p<0.001$ ) but was not different from the FTC group. The expression of HBME-1 in FTC specimens was significantly higher than that for NG ( $p=0.024$ ) specimens but was not different from that in FA and HT specimens ( $p>0.05$ ).

Statistical analysis determined that the expression of CK19, RET, galectin-3 and HBME-1 was not related to gender, age, lymphocytic thyroiditis, local invasion or distant metastasis ( $p>0.05$ ). The expression of CK19, RET and HBME-1 was not related to lymph node metastasis (LNM); however, galectin-3 expression in PTC with LNM was significantly higher than that in the cases without LNM ( $p=0.006$ ).

The expression of three or more immunohistochemical markers was 100% for the PTC group, which was significantly higher than that for FTC patients (36.4%) and patients with benign disease (0–10.3%;  $p<0.05$ ).

## DISCUSSION

PTC is the most common form of thyroid cancer as it accounts for 75–80% of thyroid malignancies.<sup>3</sup> According to the WHO classification, there are 15 variants with different clinical manifestations, pathologies, treatments and prognosis. Therefore, it is important to specifically identify the variant in order to obtain



**Figure 1** The expression of CK19, RET, galectin-3 and HBME-1 in papillary thyroid carcinoma (PTC) and the control group. (A) CK19 was diffuse and strong cytoplasmic staining in PTC, but negative in normal thyroid follicles around the tumour. (B) The intensive positive of CK19 in a follicular variant (FVPTC) case. (C) A typical PTC showing strong cytoplasmic staining of RET. (D) One FVPTC case showing strong cytoplasmic staining of galectin-3. (E) Follicular thyroid carcinoma showing strong cytoplasmic staining of galectin-3. (F) Follicular adenoma (FA) showing negative staining of galectin-3. (G) CK19 weak staining in the cytoplasm of cells with ground-glass nuclei in Hashimoto thyroiditis. (H) HBME-1 strong positive in papillary structure in FA.

## Original article

**Table 1** The immunohistochemistry results of papillary thyroid carcinoma (PTC) variants

	CK19					RET					Galectin-3					HBME-1				
Variants	n	—	+	++	+++ %	—	+	++	+++ %	—	+	++	+++ %	—	—	++	+++ %			
Typical	74	12	11	17	34 83.8	17	12	12	33 77.0	5	4	4	61 93.2%	2	5	8	59 97.3%			
Follicular	7	1	1	0	5 85.7	3	1	1	2 57.1	0	0	1	6 100%	0	1	1	5 100%			
Oncocytic	9	0	1	1	7 100	3	0	2	4 66.7	1	1	0	7 88.9%	0	0	1	8 100%			
Diffuse sclerosing	8	0	0	2	6 100	4	1	2	1 50.0	0	0	0	8 100%	1	0	0	7 87.5%			
Tall cell	9	0	0	1	8 100	4	2	1	2 55.6	0	0	0	9 100%	0	0	1	8 100%			
Solid	7	2	1	0	4 71.4	2	0	2	3 71.4	0	0	0	7 100%	0	0	0	7 100%			
Clear cell	1	0	0	0	1 100	0	0	1	0 100	0	0	0	1 100%	0	0	0	1 100%			
Microcarcinoma	40	5	6	7	22 87.5	12	4	5	19 70.0	7	4	1	28 82.5%	4	0	3	33 90.0%			
Total	155	20	20	28	87 87.1	45	20	26	64 71.0	13	9	6	127 91.6%	7	6	14	128 95.5%			

The expression of CK19, galectin-3 and HBME-1 among PTC variants was not significantly different ( $p>0.05$ ).

an accurate diagnosis, treatment and prognostic prediction. Our immunohistochemistry results determined that the expression of CK19, RET, galectin-3 and HBME-1 did not differ among the variants, suggesting that these markers are not valuable for the differential diagnosis of PTC variants. The diagnosis of PTC variants mainly relies on their specific morphological features.

Follicular variant of PTC (FVPTC) is one of the PTC variants. However, the morphology of FVPTC is often difficult to differentiate from follicular thyroid carcinoma (FTC), which is another common malignant follicular-derived tumour. The clinical, biological and histological features of FVPTC are different from FTC.<sup>4–5</sup> Similar to previous studies,<sup>6–7</sup> our study determined that the diffuse and intense expression of CK19 and RET in follicular PTC is more valuable for differentiating it from FTC. However, galectin-3 and HBME-1 expression are not useful for differentiating FVPTC from FTC. Although galectin-3 expression is not useful for the differential diagnosis of FVPTC and FTC, it is useful in differentiating FA. Nakamura *et al* reported that HBME-1, galectin-3 and CK19 expression or a panel of HBME-1, CITED1 and galectin-3 expression was usually the most effective in distinguishing FA from FVPTC.<sup>8</sup> In our study, any of our four markers were useful in differentiating FVPTC from FA.

The basic histological structure of PTC is a papilla with a central fibrovascular core, in addition to the follicular variant of PTC. However, the nodular or diffuse papillary structure is also commonly observed in NG, FA and other benign diseases; therefore, it is not easy to differentiate this structure from the papillary structure of PTC. Some studies have reported that the papillary structure is a degenerative process in NG, but a hyperplastic process in thyroid adenoma.<sup>3–5</sup> The markers of cell proliferation can be expressed in the papillary structures of benign tumours, which suggest that the papilla is formed by cell activation and proliferation.<sup>9–10</sup> Our study determined that the

expression of CK19, RET, galectin-3 and HBME-1 in PTC was higher than that in FA and NG. The four markers are intensely and diffusely expressed in the papilla of PTC but not expressed inside the normal follicles surrounding the PTC tumour, and only focal or weak expression is observed in the papillary structures of FA and NG. This indicates that CK19, RET, galectin-3 and HBME-1 are valuable markers in the differential diagnosis of benign and malignant papillary tumours.

Many studies have shown that PTC is often accompanied by HT, suggesting a link between these two diseases.<sup>11–13</sup> Larson *et al* indicated that the risk of thyroid cancer in HT patients was three times higher than that in patients without HT.<sup>14</sup> Prasad *et al* reported that the positive expression of immunological protein markers for PTC in the local PTC-like changes in the tissue of HT patients suggests an early stage of PTC or a focal premalignant transformation period.<sup>15</sup> In our study, the expression of the four markers in PTC accompanied by HT (45 cases) did not differ from PTC without HT (data not shown). CK19, galectin-3 and HBME-1 expression in PTC was higher than that observed in HT, but the expression of RET in PTC did not differ from HT. In HT patients, CK19, RET, galectin-3 and HBME-1 were only expressed in the cytoplasm of cells with ground-glass nuclei, suggesting the possibility of cellular transformation from HT to PTC. A follow-up for these patients is necessary, especially in cases with positive expression of all four markers even if a diagnosis of PTC has been excluded.

In this study, more than three immunohistochemical markers were simultaneously positive in 100% of PTC cases and in 0–30% of patients with other types of disease. Therefore, using a combination of these four markers is beneficial for the differential diagnosis of PTC. CK19 was very useful not only for the differentiation of benign and malignant papillary structure but also for the differential diagnosis of follicular PTC and FTC. Therefore, we suggest using a panel of markers, including CK19,

**Table 2** Immunohistochemistry results in papillary thyroid carcinoma (PTC) and other thyroid lesions

CK19*						RET†					Galectin-3*					HBME-1‡					
Lesions	n	-	+	++	+++	%	-	+	++	+++	%	-	+	++	+++	%	-	+	++	+++	%
PTC	155	20	20	28	87	87.1	45	21	25	64	71.0	13	9	6	127	91.6%	7	6	14	128	95.5%
FTC	11	8	2	1	0	27.3	7	4	0	0	36.4	4	1	2	4	63.6%	2	1	2	6	81.8%
MTC	10	6	3	1	0	40.0	5	4	1	0	50.0	7	2	1	0	30.0%	4	1	2	3	60.0%
UTC	4	3	1	0	0	25.0	4	0	0	0	0	2	2	0	0	50.0%	2	1	0	1	50.0%
FA	21	18	3	0	0	14.3	18	2	0	1	14.3	18	2	1	0	14.3%	10	4	3	4	52.4%
HT	20	10	10	0	0	50.0	9	6	3	2	55.0	7	10	3	0	65.0%	10	9	1	0	50.0%
NG	17	15	2	0	0	11.8	14	3	0	0	17.6	13	4	0	0	23.5%	11	6	0	0	35.3%

\*The positive rates of CK19 and galectin-3 in PTC were significantly higher than in the control group ( $p<0.05$ ). The expression of CK19 in follicular PTC was significantly higher than in FTC ( $p<0.05$ ).

†The positive rate of RET in the PTC group was not different from HT.

‡The positive rate of HBME-1 in PTC was significantly higher than in MTC, FA, HT or NG ( $p<0.001$ ), but was not different from FTC.

FA, follicular adenoma; FTC, follicular thyroid carcinoma; HT, Hashimoto thyroiditis; MTC, medullary thyroid carcinoma; NG, nodular goitre; UTC, undifferentiated thyroid carcinoma.



## Take-home messages

- Immunohistochemistry detection is not valuable for the differential diagnosis of papillary thyroid carcinoma (PTC) variants. The diagnosis of PTC variants mainly relies on their specific morphological features.
- The expression of CK19, RET, galectin-3 and HBME-1 is diffuse and strong in the PTC cases but low and focally weak in the benign papillary structure.
- The morphology of follicular PTC is often difficult to differentiate from follicular thyroid carcinoma (FTC). In this study, the diffuse and intense expression of CK19 and RET in follicular PTC was more valuable for differentiating it from FTC.
- Using a panel of markers, including CK19, is beneficial for the differential diagnosis of PTC. If CK19 and two or three other markers are strongly expressed, then PTC can be diagnosed with certainty.

to diagnose PTC. If CK19 and two or three other markers are strongly expressed, then PTC can be diagnosed with certainty.

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**Competing interests** None.

**Patient consent** Obtained.

**Ethics approval** This study was conducted with the approval of the Ethics Committee of the Cancer Hospital of Fu Dan University.

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