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Clinicopathological Characteristics of Medullary Thyroid Carcinoma with the Expression of Thyroglobulin in East – China

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Abstract

Background: Medullary Thyroid Cancer (MTC) is a kind of rare thyroid cancer with high degree of malignancy. Although Thyroglobulin (TG) is rarely expressed in MTC, our previous studies found that there was a certain percentage of TG-positive MTC in clinical practice. We hereby aimed to explore the clinicopathological features of MTC cases with positive expression of TG.

Methods: We conducted a retrospective study from June 2006 to October 2017. All the recruited cases were divided into two groups: TG-positive group and TG-negative group. Demographics, clinical profiles and pathological details were reviewed and following immunohistochemical analyses were conducted for recruited cases.

Results: Of All patients included, 25 were female and 25 were male, with a medium age of 46.7 years old. The proportion of cases with a tumor mass smaller than 1 cm in diameter in the TG-positive group was significantly higher than that in the TG-negative group (46.15% [6/13] *vs.* 5.56% [2/36], P=0.001). The proportion of clinical stage I and stage II MTC cases in the TG-positive group was significantly higher than that in the TG-negative group (53.85% [7/13] *vs.* 11.11% [4/36], P=0.001). In addition, the number of patients with lymph node metastases in the TG-positive group was significantly smaller than that in the TG-negative group (P=0.016). And the TG-positive group had a lower rate of calcium salt deposition than the TG-negative group (7.14% [1/14] *vs.* 36.11% [13/36], P=0.045). The TG-positive groups had also lower rate of tumors with Amyloid deposits than the TG-negative group (50% [7/14] *vs.* 83.3% [30/36], P= 0.016). Immunohistochemical tests showed that TG-positive cells were not only limited to the follicular structures, but also expressed in non-follicular structures such as nests, cords and sheet structures. TG-positive cells showed partially synchronous immune response to CT, CgA, Syn and CEA, which could be differentiated from mixed medullary follicular carcinoma, suggesting that TG positive cells are not the remnants of the thyroid follicular cells. MTC cells showed the ability to express TG and CT, CgA, Syn, CEA simultaneously.

Conclusion: TG-positive MTC cases had significantly smaller size of tumor, lower rate of lymph node metastasis, earlier clinical stages, and better prognosis than TG-negative MTC cases in this study.

Keywords: Medullary thyroid carcinoma; Thyroglobulin; CT; CEA; CgA; Syn

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Introduction

Medullary Thyroid Cancer (MTC) is the third most common thyroid malignancy. It is an independent clinicopathologic entity, with unique incident rate and specialized methods for diagnosis and treatment. It originates from the parafollicular or Calcitonin-producing cells (C cells) and maintains the features of these cells [1,2]. Computed tomography assay is of great significance to the diagnosis of thyroid myelin cancer. However, pathological diagnosis is currently considered to be the gold standard for the diagnosis of thyroid myelin-like cancer, and with the popularization and application of new molecular detection techniques in pathological practice, pathological diagnosis has developed into the era of organic combination of morphological diagnosis and molecular pathology diagnosis [3]. Thyroglobulin (TG) has been used as a marker for thyroid carcinoma for many years due to its high sensitivity and specificity to different thyroid carcinomas. TG is

expressed in more than 95% of papillary and follicular carcinomas, but usually reduced in poorly differentiated carcinoma and absent in anaplastic carcinoma [4-6]. Hales et al discovered TG-positive immunoreaction in MTC that does not produce TG normally [7]. Scholars had different interpretations on it, but the biological significance of the TG in MTC is rarely discussed. There was a report that conducted clinicopathological and prognostic analysis of the MTC with immunoreaction to TG in China [8], but it was speculated that the TG-positive cells were in the remnants of thyroid follicular cells or the atypical follicular carcinoma [9].

In this study, TG-positive cells were not only limited to the follicular structures, but also present in non-follicular structures such as nested, bundle-like and fluke structures as well. Thus, we compared similarities and differences of clinical, histological and immunohistochemical features between the TG-positive and the TG-negative MTC groups, and then discussed possible clinical treatments and prognosis.

Patients and Methods

Patients

A total of 210 cases of MTC were surgically and pathologically diagnosed in Zhejiang Cancer Hospital from June 2006 to October 2017, from which only 50 cases were included in the retrospective statistical analyses, excluding 160 cases that do not meet the diagnostic criteria (Figure 1).

The inclusion criteria were as follows: (1) WHO performance status of I to II; (2) serum calcitonin (CT) level ≥ 500 pg/Ml; (3) Pathological diagnosis met MTC standards; (4) The patient had a serum TG test. The exclusion criteria were as follows: (1) There was no pathological tissue examination or the clinical data were incomplete; (2) the patient had significant cardiac, hematopoietic, hepatic, or renal dysfunction or received chemotherapy and/or radiation therapy within 4 weeks before random assignment.

All patients provided written informed consents. This study protocol was consistent with the ethical guidelines of the Declaration of Helsinki and was approved by the Institution of Anji Hospital, Zhejiang University (IRB number: 2018AN0381).

Demographics, levels of serum CT, TG and other biomedical indicators, pathological diagnostics and clinical data of all the recruited patients were collected. The clinical manifestations, TNM stages [9,10], pathological characteristics and prognosis were summarized. Pathological analysis was carried out by Hematoxylin-Eosin (HE) pathological staining and immunologic tissue chemistry.

Immunohistochemistry

Formalin-Fixed Paraffin-Embedded (FFPE) tissue sections were stained with HE for histologic examination. Immunostaining was performed to the FFPE sections using antibodies against thyroglobulin (TG, 1:600, DAKO), Calcitonin (CT, 1:150, DAKO), Chromogranin A (CgA, 1:600, DAKO), Synaptophysin (Syn, 1:150, DAKO), Thyroid Transcription Factor-1(TTF-1, 1:1500, DAKO), Ki-67(1:1000, DAKO) and Carcinoembryonic Antigen (CEA, 1:600, DAKO) using an indirect immunoperoxidase technique. All antibodies were from DAKO (Denmark). For antibody staining, sections were heated for antigen retrieval for 5 min at 120°C in 0.01 M citrate buffer pH 9.0. The immunohistochemical method was an indirect method for peroxidase labeling. The positive staining of TG, CT, CgA and Syn was localized in the cytoplasm, the positive staining of CEA was located in the capsule, and the positive staining of TTF-1and Ki-67 was located in the nucleus. Brown indicated positive, while no staining indicated negative.

Statistical methods

Values are presented as Mean ± SEM. Statistical analyses were conducted using Graph Prism software (SAS Institute). Statistical comparisons between groups were analyzed for significance by two-tailed t test or one-way ANOVA with Chi-square test. Results were considered significant at P values of <0.05.

Results

Baseline demographics, tumor features and clinical features

A total of 50 patients were included, among which were 25 females and 25 males, with a mean age at 50.5 years. The TG-positive group (10 females and 4 males) accounted for 71.43%, and the TG-negative group (15 females and 21 males) accounted for 41.67%. The medium age of TG-positive group was 52 years, while the medium age of the TG-negative group was 48 years. However, there was no statistical significance between two groups in gender or age. Among all cases, 8 had a tumor smaller than 1 cm in diameter, 41 larger than 1 cm in diameter, and 1 with unknown tumor size. Among cases with a tumor smaller than 1 cm, 6 were TG-positive, accounting for 46.15% of all TG-positive cases, and only 2 were TG-negative, accounting for 5.56% of all TG-negative cases (P=0.001). Among all cases, 11 were diagnosed with Stage I or II MTC, and 38 with Stage III or higher. Among all Stage I and II MTC cases, 7 were TG-positive, accounting for 53.8% of 13 TG-positive cases, and 4 were TG-negative, accounting for only 11.1% of all TG-negative cases (P=0.001). The results showed significant differences between the TG-positive and the TG-negative groups in tumor size and MTC staging. However, there was no significant difference between the two groups in mass number, the range of the affected thyroid gland and the level of calcitonin (Table 1).

Pathologic characteristics between the TG-positive and the TG-negative groups

The pathological diagnosis of MTC remains the gold standard. The structure of MTC was diverse, with both groups showing follicular, adenoid, nested, organoid, island, trabecular, bundle-like, and fluke structures. The Amyloid deposits and neuroendocrine-type tumors are major characteristics of MTC (Figure 2A, 2B).

In this study, 50% of the TG-positive cases had Amyloid deposits in tumor tissues, compared to 83.33% in the TG-negative group (P=0.016). The neuroendocrine tumor structure was present in

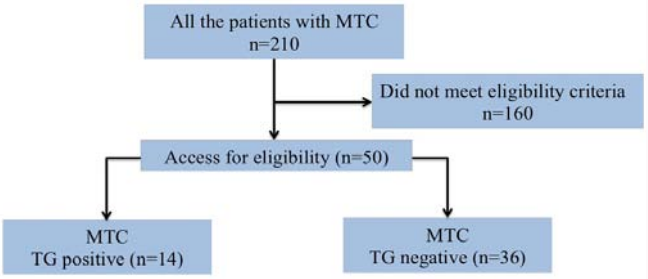

Figure 1: Schematic accounting for all Medullary Thyroid Cancer (MTC) patients included in this study. Patients were admitted from June 2006 to October 2017. High exclusion rate was largely because of a lack of confirmation of Thyroglobulin (TG).

Table 1: Comparison of clinical features of TG-positive MTC and TG-negative MTC.

Total	TG+ (n=14)	TG-(n=36)	χ^2	P
Sex				
M	4	21	3.571	0.06
F	10	15		
Age				
>50	8	17	0.397	0.53
≤ 50	6	19		
Tumor size				
≤ 1 cm	6	2	11.52	0
>1 cm	7	34		
The range of thyroid tissue involvement				
Unilateral	13	25	3.029	0.08
Bilateral	1	11		
Mass number				
1	10	22	1.054	0.31
>1	3	14		
Calcitonin level				
>2000	1	11	1.419	0.23
<2000	6 (7 untested)	18 (7 untested)		
Clinical stage				
≤ II	7	4	10.02	0
> II	6	32		

In 14 cases of TG-positive MTC, the tumor size and mass number was unknown in 1 case, so the clinical level also lack for that case

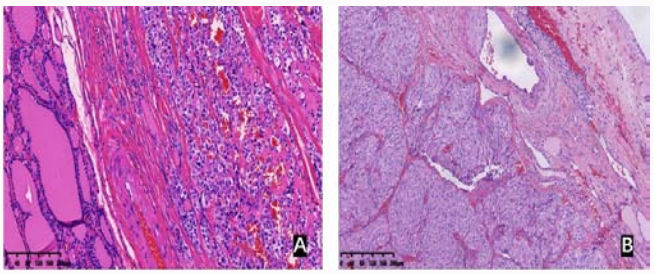

Figure 2: Histological characteristics of major MTCs (x100).

78.57% and 88.89% of the TG-positive group and the TG-negative group, respectively. And calcium salt deposition was less merged in the carcinoma tissue of the TG-positive group than of the TG-negative group (7.14% *vs.* 36.11%, P=0.041). In addition, the rate of lymph node metastasis in the TG-positive group was significantly lower than that in the TG-negative group (50% *vs.* 83.3%, P=0.016). The proportion of the thyroid capsule invasion and internal dissemination of thyroid was lower in the TG-positive group, but there was no statistical significance (0% *vs.* 19.44%, P=0.070). Furthermore, both groups showed similar characteristics of the vascular tumor thrombus (14.29% *vs.* 16.67%, P=0.837), nerve invasion (14.29% *vs.* 11.11%, P=0.756), distant metastasis (0% *vs.* 2.78%, P=0.529), cancer nodule (28.58% *vs.* 30.56%, P=0.891), nodular goiter (64.29% *vs.* 36.11%, P=0.072), follicular structure (85.72% *vs.* 77.781%, P=0.529), neuroendocrine tumor structure (78.57% *vs.* 88.89%, P=0.345) and cell morphology (78.57% *vs.* 91.67%, P=0.200) (Table 2).

Immunohistochemical tests showed that TG positive cells were not only limited to the follicular structures, but also present in non-follicular structures such as nested, bundle-like and fluke structures (Figure 3A, 3B).

They revealed partially synchronous immune response to TG as well as to CT, CgA, Syn and CEA. Compared with the TG-negative group, the rate of positive Ki-67 (>1%) in the TG-positive group was lower (60% *vs.* 82.35%) (Figures 4A-4F).

Table 2: Comparison of histological characteristics of TG-positive MTC and TG-negative MTC.

	TG+ (n=14)	TG- (n=36)	χ^2	P
Follicular structure				
Yes	12	28	0.397	0.529
No	2	8		
Neuroendocrine tumor structure				
Yes	11	32	0.891	0.345
No	3	4		
Cell morphological characteristics				
Mainly polygonal cells	11	33	1.637	0.2
Mainly spindle cells	3	3		
Amyloid substance				
Yes	7	30	5.821	0.016
No	7	6		
Calcium salt deposition				
Yes	1	13	4.196	0.041
No	13	23		
Nodular goiter				
Yes	9	13	3.247	0.072
No	5	23		
Thyroid capsular invasion				
Yes	5	20	1.587	0.207
No	9	16		
Internal disseminatian of thyroid				
Yes	0	7	3.165	0.07
No	14	29		
Vascular tumor thrombus				
Yes	2	6	0.043	0.837
No	12	30		
Nerve invasion				
Yes	2	4	0.096	0.756
No	12	32		
Cancer nodule				
Yes	4	11	0.019	0.891
No	10	25		
Lymph node metastasis				
Yes	7	30	5.821	0.016
No	7	6		
Distant metastasis				
Yes	0	1	0.397	0.529
No	14	35		

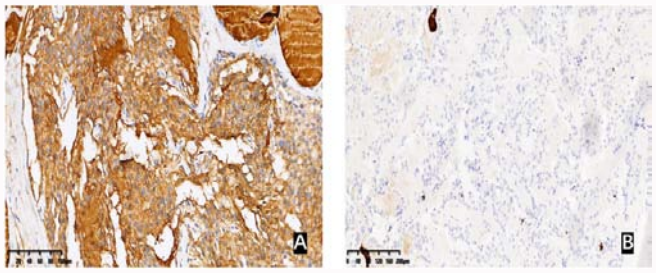

Figure 3: A) The immunohistochemical tests showed that TG was positive expression in MTC (x100); B: The immunohistochemical tests showed that TG was negative expression in MTC (x100).

There was no significant difference in the positive rate of CT, CgA, Syn, CEA and TTF-1 between the two groups (Table 3).

Prognostic analysis

Follow-up was ended on 60 months. The mean follow-up was 52 months (range: 48 to 60 months). During the follow-up, there was only one case of death in each group. One of the patients from the TG-positive group died of tumor recurrence 3 years after the operation, whereas one of the patients from the TG-negative group died of bone metastases 5 years after the operation. There was no significant difference in survival rate between two groups.

Discussion

Medullary Thyroid Carcinoma (MTC) is a malignant tumor with neuroendocrine characteristics, categorized as an APUD system tumor. Although accounting for about 4% to 10% of all thyroid cancer incidences, MTC is responsible for about 13.4% of all the thyroid cancer-related deaths [11]. Therefore, it is necessary to develop a more specialized and sensitive prognosis metric for MTC in clinical practice.

MTC can synthesize and secrete CT, CgA, Syn, CEA and so on. TG is a protein precursor of triiodothyronine and thyroxine, and is produced exclusively by thyroid follicular cells, both benign and malignant. There were a few researchers proposed that TG, as a mature product of terminal secretion appeared in MTC, suggests low malignancy and slow invasion behavior of the tumor, TG-positive MTC has a better prognosis than TG-negative MTC [11,12]. However, the study on expression of TG in MTC is rarely reported worldwide.

Table 3: Comparison of immunohistochemistry of TG-positive MTC and TG-negative MTC.

	TG+MTC (n=14)	TG-MTC (n=36)	χ^2	*P*
CT				
Positive	13	35	2.55	0.11
Negative	1	0 (1 untested)		
CgA				
Positive	13	36		
Negative	0 (1 untested)	0		
Syn				
Positive	12	33		
Negative	0 (2 untested)	0 (3 untested)		
CEA				
Positive	8	25	0.66	0.42
Negative	1 (5 untested)	1 (10 untested)		
TTF1				
Positive	12	28	0.01	0.94
Negative	2	5 (3 untested)		
Ki-67				
≤ 1%	2	3	1.1	0.29
>1%	3 (9 untested)	14 (19 untested)		

In this study, we found that there were more females than males in 14 cases of TG-positive MTC, and the average age of onset was

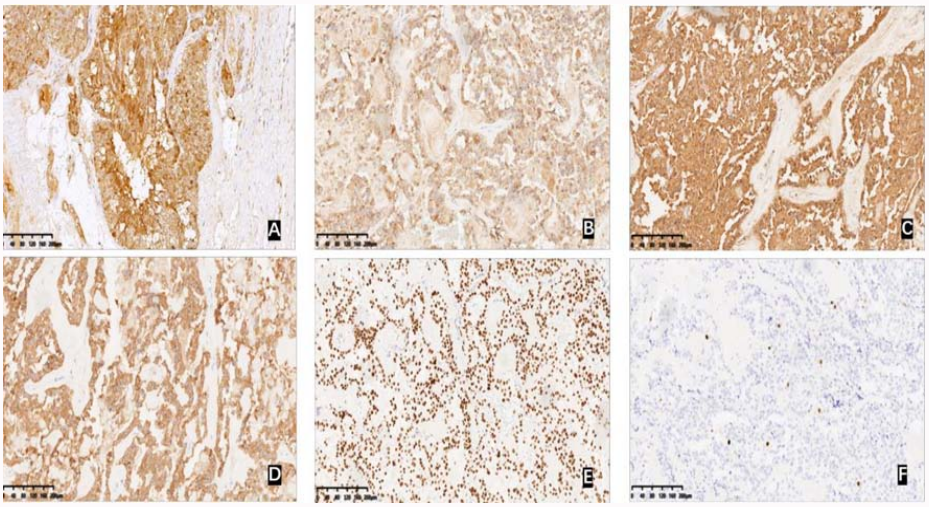

Figure 4: A) The immunohistochemical tests of CT, which was strongly positive expression in cancer-cytoplasm of MTC (x100); B: The immunohistochemical tests of CgA, which was weakly positive expression in cancer- cytoplasm of MTC (x100); C: The immunohistochemical tests of CEA, which was strongly positive expression in cancer-cytoplasm of MTC (x100); D: The immunohistochemical tests of Syn, which was strongly positive expression in cancer-cytoplasm of MTC (x100); E: The immunohistochemical tests of TTF-1, which was strongly positive expression in cancer-nucleus of MTC (x100); F: Ki-67 The immunohistochemical tests of ki-67, which was sporadic positive expression in cancer-nucleus of MTC (x100).

46.7 years old, which is in agreement with the Tumors of Endocrine Organs, 2006 WHO in which the average age of onset of sporadic cases was 50 years old, with slightly higher incidence in females. We also found, 46.15% of cases in the TG-positive group have a tumor smaller than 1 cm in diameter, much higher than 5.56% in the TG-negative group. The number of cases with a tumor size more than 1 cm was 0 in TG-positive MTC group and 7 in the TG-negative group. It is widely believed that the survival time of the MTC patients is shorter and the prognosis is poor when the tumor is larger than 4 cm in diameter. The proportion of late stage MTC (stage III or higher) in the TG-positive group was lower than that in the TG-negative group (46.15% *vs.* 88.89%, P=0.001), which may indicate that TG positive MTC has better prognosis. According to some reports [7], both a primary tumor diameter more than 4 cm and thyroid capsular invasion, which are both indicators of Stage III MTC, have an effect on the prognosis of MTC patients.

Pathological diagnosis is the gold standard. Microscopically, the tissues of the MTC are mainly composed of polygonal cells and spindle cells. The typical medullary carcinoma can be either well-defined or directly infiltrating the surrounding thyroid tissue. Nested, bundle-like and fluke structures can be found arranged in the tumor [3]. There are more or less fibrous septums full of vessels between tumor cell nests. Amyloid structure can be found in the intercellular space of the tumor and interstitial cells, which is composed of eosinophilic and amorphous substance produced by cancer cells through a variety of methods. In this study, the rate of the Amyloid substance deposition in tumor of the TG-positive group was significantly lower than that of the TG-negative group (50% *vs.* 83.33%, P=0.001). According to the histological structure, MTC can be divided into several histological types, which are often mixed. The diagnosis of MTC is mainly according to a variety of permutations composed of spindle cells and epithelioid cells, and combined with immunohistochemical expression of CT, CgA, Syn, CEA and TTF-1. Both TG-positive MTC and TG-negative MTC have a variety of structures: follicular structures also appear in the fluke type; glandular-like and follicular-like structures are mixed in nesting type; most of them have neuroendocrine structures. The proportion of follicular structures in two groups was similar, which indicates that the tumor cells of follicular structure may not necessarily express TG. The TG-positive cells are not necessarily the remaining thyroid follicular cells or the atypical follicular carcinoma.

Xu Lai et al. reported that the 15-year survival rate of MTC with nodular goiter was significantly higher than that of MTC without nodular goiter, indicating that the prognosis of MTC with nodular goiter was excellent [8]. The proportion of nodular goiter in the TG-positive group was higher than that of the TG-negative group (64.3% *vs.* 36.1%), although there was no statistical difference, which could be due to small sample size and insufficient follow-up time in this study. The main route of MTC metastasis is regional lymph node metastasis. With the increase of tumor volume, the rate of lymph node metastasis is increased gradually. The most common sites of metastasis are tracheoesophageal groove lymph nodes and cervical lymph nodes, followed by upper mediastinal lymph nodes. Research showed that cervical lymph node metastasis is an important prognostic factor for MTC [13,14]. However, a multifactor analysis revealed that cervical lymph node metastasis might not be an independent risk factor for prognosis.

The rate of lymph node metastasis in the TG-positive group was significantly lower than that in the TG-negative group (50% *vs.* 83.33%, P=0.016). One patient in the TG-positive group and 5 patients in the TG-negative group had upper mediastinal lymph node metastases. Upper mediastinal lymph node metastasis is a characteristic of MTC. The upper mediastinal lymph node metastasis may spread from VI region lymph nodes along recurrent laryngeal nerve or from the pretracheal or paratracheal lymph nodes through lymph circulation. And the upper mediastinal lymph node metastasis may be an important cause of lung metastasis. No pulmonary metastases were found in patients of the two groups in this study.

The invasion of the thyroid capsular and vessel is related to a decreased survival rate of MTC patients. The TG-positive group had lower rate of capsular invasion (35.71% *vs.* 55.56%) and vascular invasion (14.29% *vs.* 16.67%) than the TG-negative group, but there was no significant difference. Tumor with vascular invasion is prone to distant metastasis. The most common distant metastatic sites are lung, liver, bone and brain. In this study, one case of the TG-negative group had lumbar and sacral metastases, but no distant metastases were found in the TG-positive group.

In the present, most of studies on MTC refer to CT and CEA, including serum level and immunohistochemical expression in tissue cells. Calcitonin is a sensitive and specific marker of MTC, and it is also an important indicator for preoperative diagnosis and postoperative follow-up. The high level of serum CEA>50% is reported to be high level in serum. The expression level of CEA is synchronous with CT [15]. Previous study discovered that the combination of CT and CEA could increase the diagnostic sensitivity of MTC to 95.7% [16]. There are lot similarities between MTC and other thyroid tumors in the morphological characteristics; therefore, the diagnosis of medullary cancer by optical microscopy alone has certain risks and limitations. Immunohistochemical staining is an important means of examination for auxiliary diagnosis. The commonly used markers include CT, CEA, CgA, Syn and TTF-1. A large majority of MTC tumor cells express CT. In this study, almost all cases of MTC expressed CT, except for one TG-positive case that was CT-negative, and one TG-negative case whose CT was unmeasured. In the TG-positive group, CT was mostly weakly or partially positive; indicating that CT may also be an important indicator of MTC prognosis, which supported the findings of our previous article in 2017, but the prognosis in MTC was still unclear [17]. In this study, except for one CEA-negative case in each group, all other cases were positive for CEA. Positive immunoreactions to several neuroendocrine substances including CgA and Syn were detected in both TG-positive and TG-negative groups, which confirmed that MTC is endocrine-derived.

TTF-1 can be used as a marker of thyroid neuroendocrine carcinomas, such as medullary carcinoma. In this study, 2 cases in the TG-positive group and 5 cases in the TG-negative group showed negative results for TTF-1 staining, whereas the other cases were positive for TTF-1. TTF-1 was a more sensitive marker of thyroid carcinoma than other markers [17,18]. It is expressed in more than 90% of thyroid carcinomas, with the exception of anaplastic carcinoma in which its sensitivity is close to zero.

Few studies have focused on the expression of TG in MTC. Hales et al., for the first time, proposed that TG is expressed in the MTC. Some researchers had conducted clinicopathological and prognostic analysis of MTC and found immunoreaction to TG [19], which is consistent with the findings in this study that immunohistochemical TG-positive cells was not only limited to the follicular structures, but also present in non-follicular structures such as nested, bundle-like

and fluke structures, with partially synchronous immune response to CT, CgA, Syn and CEA as well, whereas the normal thyroid tissue wrapped around MTC did not show corresponding positive reactions.

MTC often invades the surrounding tissues and causes lymph node metastases. Therefore, early operation is the key to the treatment. Other therapeutic options including different extent of thyroidectomy, radiotherapy and chemotherapy schemes [20-22]; however, few of them are effective in curing MTC. In recent years, great progress has been made in molecular targeted therapy. The 5 years survival rate of MTC is about 60% to 75%; with early diagnosis and standardized treatment, the survival rate can reach 90%. This study found that, compared with TG-negative MTC, TG-positive MTC was significantly smaller in tumor size, had a lower rate of lymph node metastasis, and was inclined to be categorized into earlier clinical stages, which indicated better prognosis. There was one death 3 years after operation in the TG-positive group and one death five years after operation in the TG-negative group. There was no significant difference between two groups in survival rate, which may be due to the limited follow-up time. From our study, it can be speculated that TG can be an indicator of the prognosis of MTC and provide a theoretical basis for molecular targeted therapy.

There are some limitations in our study, including the limited number of patients and region, which cannot represent all MTC cases globally. Furthermore, only a few patients got genetic tests due to financial limitations, so we did not carry out the analysis of molecular genetic characteristics. Nevertheless, so far there has been a very limited research on TG expression in MTC. This study retrospectively analyzed a number of MTC cases with TG expression and found a relationship between pathological morphological characteristics and clinical prognosis of TG-positive and TG-negative MTC. Further studies with larger sample size and extended follow-up time combined with molecular biology indicators are required in the future to fully understand the biological significance of TG expression in MTC, and improve prognosis and clinical evaluation of treatment of MTC.

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Availability of Data and Material

All data generated or analyzed during this study are included in this published article.

Authors' Contribution

JS performed the majority of experiments and drafted the paper. SY and JJ helped with experiments. HY and ZZ analyzed the data and drafted part of the paper. HX and QZ conceived the study, supervised the experiments and edited the manuscript.

Ethics Approval and Consent to Participate

This study protocol was consistent with the ethical guidelines of the Declaration of Helsinki and was approved by the Institution of Anji Hospital, Zhejiang University (IRB number: 2018AN0381).

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