CHANGES IN HTERT, VEGF, RET AND P53 EXPRESSIONS IN THYROID CARCINOMA PATIENTS

Lina Yao[1,#], Kun Zhang[1,#], Junsheng Gu[2], Ge Zhao[3], Mengjie Ding[4], Shaoxuan Wu[4], Zhenzhen Yang[4], Mijing Ma[4], Meifeng Yin[4], Wanqiu Yang[5], Linan Zhu[4,*]

[1]No.1 Ward of Internal Medicine, Henan Electric Power Hospital, Zhengzhou, Henan, China, 450000 - [2]Division of Infectious Diseases, The First Affiliated Hospital of Zhengzhou University, Zhengzhou, Henan, China, 450000 - [3]Pediatric Surgery, The East District of the First Affiliated Hospital of Zhengzhou University Zhengzhou, Henan, China, 450000 - [4]Department of Oncology, The First Affiliated Hospital of Zhengzhou University, Zhengzhou, Henan, China, 450052 - [5]The Third Affiliated Hospital of Xinxiang Medical University, Xinxiang, Henan, China, 453003

[#]These authors contributed equally to this work

ABSTRACT

Objective: *To study the expressions of telomerase reverse transcriptase (hTERT), vascular endothelial growth factor (VEGF), Ret and P53, and their relationship to various pathological factors in different pathological types of thyroid carcinoma (TC) patients.*

Method: *Paraffin-embedded pathological specimens of thyroid carcinoma (174) and 40 normal hypothyroid tissue specimens preserved in the Pathology Department from July 2012 to June 2017, were used in the study. The expressions of hTERT, VEGF, Ret and P53 were assayed using immunohistochemical staining in order to analyze their association with pathological types, ages, tumor diameters, lymphatic metastasis and TNM tumor stage of the TC patients.*

Results: *The expressions of hTERT, VEGF, Ret and P53 were 79.89, 78.16, 70.69 and 81.03%, respectively, and significantly higher than the corresponding expressions in normal hypothyroid tissue ($p<0.05$). The positive expressions of hTERT and VEGF were associated with tumor diameter, while VEGF and Ret expressions were related to lymphatic metastasis; hTERT, VEGF, Ret and P53 expressions were associated with TNM tumor stage ($p<0.05$). However, there was no association between the expressions of hTERT, VEGF, Ret and P53, and age and tumor pathology ($p>0.05$).*

Conclusion: *The increased positive expressions of hTERT, VEGF, Ret and P53 in thyroid carcinoma are of clinical significance, and can aid in early diagnosis of thyroid carcinoma.*

Keywords: *Thyroid carcinoma, oncogene, telomerase reverse transcriptase, molecular markers, pathological type.*

DOI: 10.19193/0393-6384_2019_2_168

Received *November 30, 2018;* ***Accepted*** *January 20, 2019*

Introduction

Thyroid carcinoma (TC) is one of malignant tumors frequently seen on the head and neck in the endocrine system. The pathogenesis of TC is still unknown, and there are no preventive or treatment measures so far. Thus, early discovery and diagnosis are important strategies for reducing the mortality of TC patients, and for improving prognosis[(1)]. At present, pathological examination of morphologic features under the microscope is one of main clinical diagnostic measures used. However, arising from the existence of various TC pathological types, single pathological diagnosis is associated with high rates of missed diagnosis and misdiagnosis for TC patients with atypical modality or minimum focus[(2)]. In recent years, the examination indices of some TC-specific markers have been clinically combined to improve the accuracy and sensitivity of early pathological diagnosis. These markers are hTERT, VEGF, Ret, and P53[(3-5)]. In this study, immunohistochemical staining method was used to determine the protein expressions of hTERT, VEGF, Ret and P53 in tissues of TC patients and normal tested tissues so as to investigate their relationship with TC.

Clinical Data and Methods

Main instruments and reagents

Japan Olympus Company supplied the CX41 inverted optical microscope and OLS4100 laser confocal microscope used in this study. The primary antibodies of hTERT, Ret rabbit anti-human monoclonal antibody, VEGF rabbit anti-human polyclonal antibody and P53 rat anti-human monoclonal antibody were bought from Sigma Company. Hematoxylin staining solution, DAB color kit and immunohistochemistry PV kit were products of Beijing Zhongshan Biological Technology Co., Ltd.

Clinical data of patients

In this study, 174 paraffin-embedded pathological specimens of TC and 40 normal hypothyroid tissue specimens which were preserved at the Pathology Department from July 2012 to June 2017, were selected. The patients for pathological tissues included 91 females and 83 males aged 23-77 years (mean age= 49.42±7.36), with disease course ranging from 3 months to 17 years (mean course= 4.97±5.51 years). There were 138 papillary carcinoma cases, 24 follicle carcinoma cases, 3 medullary carcinoma cases, and 9 cases of undifferentiated carcinoma. All patients received chemotherapy and radioactive therapy.

Immunohistochemical assay

Collection of samples: The paraffin-embedded tissue slices of TC patients were hydrated and sliced. The slices were placed on glass slides, dried for 48 h and subjected to immunohistochemical staining. The immunohistochemical staining was carried out according to the protocol indicated in the kits. The stained pathological slices were observed under 400x microscope for positive expressions of hTERT, VEGF, Ret and P53. Ten visual fields were selected at random to observe the positive expressions.

The expressions were scored 0–5 as follows: 0= zero positive expression; 1= positive expression ≤25%, 2= positive expressions of 25–50%, 3= 50–75% positive expression, and 4= positive expressions >75%. The staining strength of cells was scored 0–3 i.e. 0= negative, 1= weak staining, 2= medium staining, and 3= deep stain. The chromatic index (labeling index, LI) was calculated as the product of percentage expression and staining strength i.e.

LI= (% positive expression) × (staining strength).

The slides were divided into 4 levels based on the total scores i.e. level 1 (0-1), level 2 (2-3), level 3 (4-6) and level 4 (9-12). Levels 1 and 2 indicated low expressions, while levels 2 and 3 showed high expressions.

Statistical Analysis

Measurement data are expressed as mean ± standard deviation (SD) ($\bar{x}\pm s$). Student's t-test was adopted for comparison among groups, while analysis of variance was use for comparison in a group. Enumeration data are expressed as percentage, and were statistically analyzed using χ^2 test and Spearman correlation test. All statistical analyses were done using SPSS version19.0 statistics software. Statistical significance of difference was fixed at $p<0.05$.

Results

Results of pathological examination of patients

The pathological examination results indicated that papillary carcinoma was frequent in 82 T1 patients, 65 T2 patients and 27 T3 patients, as shown in Table 1.

TNM stage	n (cases)	Papillary carcinoma	Follicle carcinoma	Medullary carcinoma	Undifferentiated carcinoma
T_1	82	66	15	1	0
T_2	65	53	7	2	3
T_3	27	19	2	0	6
Total	174	138	24	3	9

Table. 1: Results of pathological examination of TC patients (cases).

Results of immunohistochemical assay

Immunohistochemical assay indicated that the degree of positive staining of hTERT, VEGF, Ret and P53 were 79.89, 78.16, 70.69 and 81.03%, respectively, which were significantly higher than the corresponding expressions in normal hypothyroid tissue ($p<0.05$). In addition, there were no significant differences in the positive expressions of hTERT, VEGF, Ret and P53 in TC tissues among the various pathological subtypes ($p>0.05$). These results are shown in Tables 2 and 3, and in Figures 1-4.

Group	n(case)	hTERT	VEGF	Ret	P53
Normal tissue	40	8 (20.0)	7 (17.5)	5 (12.5)	9 (22.5)
TC tissue	174	139 (79.89)*	136 (78.16)*	123 (70.69)*	141 (81.03)*

Table. 2: Changes in hTERT, VEGF, Ret and P53 expressions in TC tissues and normal tissues (n, %).
**p<0.05, compared with normal tissue.*

Group	n	hTERT		VEGF		Ret		P53	
		+	-	+	-		+	-	+
Papillary carcinoma	138	111 (80.43)	27 (19.57)	104 (75.36)	34 (24.64)	98 (71.01)	40 (28.99)	116 (84.06)	22 (15.94)
Follicle carcinoma	24	19 (79.17)	5 (20.83)	21 (87.5)	3 (12.5)	16 (66.67)	8 (33.33)	15 (62.5)	9 (37.5)
Medullary carcinoma	3	3 (100)	0	3 (100)	0	3 (100)	0	3 (100)	0
Undifferentiated carcinoma	9	6 (66.67)	3 (33.33)	8 (88.89)	1 (11.11)	6 (66.67)	3 (33.33)	7 (77.78)	2 (22.22)
Total		139 (79.89)	35 (20.11)	136 (78.16)	38 (21.84)	123 (70.69)	51 (29.31)	141 (81.03)	33 (18.97)

Table. 3: Immunohistochemical results of TC patients in each pathological subtype (n, %).

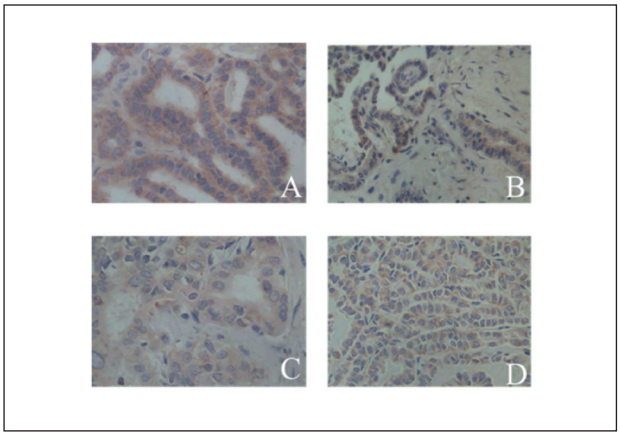

Figure 1: hTERT, VEGF, Ret and P53 expressions in thyroid carcinoma tissue.
A: hTERT positive expression in thyroid carcinoma tissue (×400); B: VEGF positive expression in thyroid carcinoma tissue (×400); C: Ret positive expression in thyroid carcinoma tissue (×400); D: P53 positive expression in thyroid carcinoma tissue (×400).

Relationship between pathological parameters and the expressions of hTERT, VEGF, Ret and P53

The results of analysis indicated that hTERT, VEGF, Ret and P53 expressions were not related to age. However, the expressions of hTERT and VEGF were related to tumor diameter: the larger the tumor diameter, the higher the positive expressions of hTERT and VEGF ($p<0.05$). Moreover, VEGF and Ret expressions were associated with lymphatic metastasis: TC tissue with lymphatic metastasis had higher VEGF and Ret positive expression rates than non-translated tissues ($p<0.05$). The expressions of hTERT, VEGF, Ret and P53 were related to TNM tumor stage: the higher the TC tumor stage, the higher the positive expressions of hTERT, VEGF, Ret and P53 ($p<0.05$). These results are shown in Table 4.

Item	N (case)	hTERT			VEGF			Ret			P53		
		(+)	χ^2	*p*	(+)	χ^2	*p*	(+)	χ^2	*p*	(+)	χ^2	*P*
Age													
<45	86	67 (77.91)	0.167	0.092	66 (76.74)	0.086	0.137	61 (70.93)	0.054	0.251	67 (77.91)	0.286	0.054
≥45	88	72 (81.82)			70 (79.55)			62 (70.45)			74 (84.09)		
Tumor diameter (cm)													
<1.5	101	69 (68.32)	4.379	0.028	66 (65.35)	5.335	0.019	56 (55.45)	0.264	0.057	78 (77.23)	0.275	0.056
≥1.5	73	70 (95.89)			70 (95.89)			67 (91.78)			63 (86.3)		
Lymphatic metastasis													
Metastasis	99	82 (82.83)	1.012	0.050	87 (87.88)	3.769	0.038	82 (82.83)	3.664	0.038	80 (80.81)	0.162	0.131
Non-metastasis	75	57 (76.0)			49 (65.33)			41 (54.67)			61 (81.33)		
TNM stage													
T_1													
T_2	86	67 (77.91)	0.167	0.092	66 (76.74)	0.086	0.137	61 (70.93)	0.054	0.251	67 (77.91)	0.286	0.054
T_3	88	72 (81.82)			70 (79.55)			62 (70.45)			74 (84.09)		

Table. 4: Relationship between hTERT, VEGF, Ret and P53 expressions and various clinical pathological factors (n, %).

Discussion

In China, thyroid carcinoma (TC) is one of malignant tumors frequently seen on the head and neck in endocrine system, and incidence increasing year by year, especially in coastal regions[6]. Some epidemiological investigations have indicated that the incidence of hypothyroid tuberosity is about 5% in regions with improved iodine supply. The disease develops slowly, and combined with the absence of any obvious clinical indications at the early stages, the possibility of missed diagnosis is relatively high. Indeed, the degree of diagnosis from pathological examination under the microscope is only 20–30%, but autopsy shows that the incidence of hypothyroid tuberosity is above 65%, which implies low accuracy of diagnosis[7].

Thyroid cancer occurs in stages including oncogene mutation, growth factor control and

tumor tissue clone[8]. It is one of the malignant tumors with very high incidence, but its pathological classification is relatively complicated[9]. Therefore, there are wide differences in treatment schemes and prognosis. In recent years, due to advancements in ultrasonic techniques and wide application of CDFI, the degree of detection of TC has greatly improved[10]. However, it is more difficult to detect malignant tumor[11]. Within the period 2012-2017, TC patients treated in our hospital were mostly at T_1 stage, and were mainly of papillary carcinoma, while patients with follicle carcinoma, medullary carcinoma and undifferentiated carcinoma were relatively lower in number. Arising from this, it was assumed that iodine deficiency had been greatly reversed especially after the energetic promotion of use of iodized salt in China. In 2009, the American Thyroid Association advised that, besides the fine-needle aspiration biopsy used to judge the features of hypothyroid tuberosity, it was necessary to check hypothyroid molecular markers to assist in the diagnosis.

It is known that hTERT is a ribonucleoprotein which is positively expressed (and has high sensitivity) in most TC cancer cells[12]. In this study, hTERT positive expression (79.89%) was significantly higher in TC than in normal galactophore tissue, and was closely related to tumor diameter and TNM tumor stage. This indicates that hTERT is related to the attack and proliferation of tumor cells but not clearly related to tumor metastasis. Vascular endothelial growth factor (VEGF) is one of the most important specific vascular endothelial growth factors which have been identified so far. It is involved in the growth and metastasis of tumor cells. In this study, positive expression of VEGF was 78.16%, but it was as high as 88.89% in undifferentiated carcinoma, and higher than the degree of positive expression in normal galactophore tissue. Moreover, it was related to tumor diameter, lymphatic metastasis and TNM tumor stage. This implies that VEGF is associated with blood supply and growth condition of tumor cells. Ret gene activation is an early event in TC and a prerequisite for the combination of YI015 and PLC-γ[13]. In this study, Ret gene rearrangement was in 70.69% of TC patients, but Ret expression was related only to lymphatic metastasis and TNM tumor stage. The P53 gene is an anti-oncogene and it reduces cell growth and inhibits proliferation of tumor cells. Mutation in P53 gene is closely related to normal physiological disturbance and has typical oncogene activity. In this study, the positive expression of P53 gene was 81.03%, and was significantly higher than the corresponding expression in normal galactophore tissue. It was associated with all pathological factors tested except TNM tumor stage.

Conclusion

The positive expressions of the tumor-related genes hTERT, VEGF, Ret and P53 are increased in thyroid carcinoma tissue and play important roles in incidence and development of thyroid carcinoma. Thus, the expressions of hTERT, VEGF, Ret and P53 can provide experimental reference for early diagnosis of thyroid carcinoma. These findings deserve application in clinical diagnosis of thyroid carcinoma.

References

1) Iglesias ML, Schmidt A, Ghuzlan AA, Lacroix L, Vathaire F, et al. Radiation exposure and thyroid cancer: a review. Arch Endocrinol Metab 2017; 61: 180-187.
2) Vuong HG, Duong UN, Altibi AM, Ngo HT, Pham TQ, et al. A meta-analysis of prognostic roles of molecular markers in papillary thyroid carcinoma. Endocr Connect 2017; 6: 8-17.
3) Penna GC, Vaisman F, Vaisman M, Sobrinho-Simões M, Soares P. Molecular Markers Involved in Tumorigenesis of Thyroid Carcinoma: Focus on Aggressive Histotypes. Cytogenet Genome Res 2016; 150: 194-207.
4) Lee SE, Hwang TS, Choi YL. Molecular Profiling of Papillary Thyroid Carcinoma in Korea with a High Prevalence of BRAF^{V600E} Mutation. Thyroid 2017; 27: 802-810.
5) Penna GC, Vaisman F, Vaisman M. Molecular Markers Involved in Tumorigenesis of Thyroid Carcinoma: Focus on Aggressive Histotypes. Cytogenet Genome Res 2016; 150: 194-207.
6) Morandi L, Righi A, Maletta F. Somatic mutation profiling of hobnail variant of papillary thyroid carcinoma. Endocr Relat Cancer 2017; 24: 107-117.
7) Smith N, Nucera C. Personalized therapy in patients with anaplastic thyroid cancer: targeting genetic and epigenetic alterations. J Clin Endocrinol Metab 2015; 100: 35-42.
8) Nagar S, Ahmed S, Peeples C. Evaluation of genetic biomarkers for distinguishing benign from malignant thyroid neoplasms. Am J Surg 2014; 207: 596-601.
9) Asa SL. The evolution of differentiated thyroid cancer. Pathology 2017; 49: 229-237.
10) Liu Z, Cai J, Yu Y, Fang H, Si Y, et al. Tumor Abnormal Protein as a Novel Biomarker in Papillary Thyroid Carcinoma. Clin Lab 2017; 63: 479-485.

11) Philchenkov AA, Balcer-Kubiczek EK. Molecular markers of apoptosis in cancer patients exposed to ionizing radiation: the post-Chornobyl view. Exp Oncol 2016; 38: 224-237.
12) Goulart APS, Gonçalves MAG, DA-Silva VD. Evaluation of Telomerase (hTert), Ki67 and p16ink4a expressions in low and high-grade cervical intraepithelial lesions. Rev Col Bras Cir 2017; 44: 131-139.
13) Cameselleteijeiro JM, Peteirogonzález D, Caneirogómez J, Sánchez-Ares M, Abdulkader Iet al. Cribriform-morular variant of thyroid carcinoma: a neoplasm with distinctive phenotype associated with the activation of the WNT/β-catenin pathway. Mod Pathol 2018; 31: 1168-1179.

Acknowledgement
This study was supported by Xiamen Science and Technology Fund/Research on pDC quantity and function in hepatic tissue of HBeAg positive chronic hepatitis B patient/3502Z20144028.

Corresponding Author:
Linan Zhu
Email: ev1183@163.com
(China)