ORIGINAL ARTICLE

Expression of human telomerase reverse transcriptase (hTERT) in thyroid neoplasms

Alper Sayiner, Dinc Suren

Health Sciences University, Antalya Education and Research Hospital, Department of Pathology, Antalya, Turkey

Summary

Purpose: Distinction of thyroid neoplasms that include papillary carcinoma (PC) and follicular carcinoma (FC) from benign thyroid neoplasms can be performed successfully by histopathologic examination in most of the cases. However, in some cases it may be difficult to distinct PC and FC as well as FC and follicular adenoma (FA) and also FA and the dominant nodule of multinodular goiter (MNG) histopathologically. In this study, we aimed to determine the role of expression of the human telomerase reverse transcriptase (hTERT) in the distinction of thyroid neoplasms and its relation with prognostic factors by immunohistochemical methods.

Methods: This retrospective study included 138 cases histopathologically diagnosed with benign and malignant thyroid neoplasia. Sections obtained from formalin-fixed paraffin-embedded blocks were stained with hTERT antibody. Cases were divided into hTERT-positive and -negative cat-

egories according to hTERT expression score that included percentage and intensity of staining in neoplastic cells.

Results: hTERT expression was negative in 93 (67.4%) and positive in 45 (32.6%) patients. Twenty-three (46.0%) of 50 PC, 12 (36.0%) of 33 FA, 1 (10.0%) of 10 FC, 4 (13.0%) of 31 MNG, 2 (66.0%) of 3 medullary carcinoma (MC) patients were found hTERT (+), showing that the difference between PC and FC was significant (p=0.034). There was also a significant difference between FA and MNG (p=0.030). There was no difference between FA and FC (p=0.117).

Conclusion: The high expression of hTERT can be useful for making a differential diagnosis between PC and FC, and between FA and MNG when histopathological findings are equivocal.

Key words: hTERT, neoplasia, telomerase, thyroid

Introduction

Thyroid cancer accounts for approximately 1% of all malignancies in developed countries with an estimated annual incidence of 122,000 cases worldwide. Age standardized incidence rates per 100,000 population in different parts of the world vary from 0.8 to 5.0 for males and 1.9 to 19.4 for females [1].

Most of the neoplasms seen in thyroid are primary epithelial tumors. Epithelial tumors are divided into three main groups according to the type of cell from which they are derived: follicular cell-derived tumors, C cell-derived tumors and follicular and C cell-derived tumors. More than 95%

of the cases are of follicular cell origin. PC originating from thyroid follicular cells is the most common malignancy. PC and FC are known as "differentiated thyroid carcinomas" [2].

The gold standard in diagnosis of thyroid nodules is pathological evaluation using routine hematoxylin and eosin (H & E) staining. However, morphologic overlap between MNG, FA, FC and between PC and FC is common. In such cases an objective diagnosis based merely on morphological assessment is sometimes impossible. There is no generally accepted immunohistochemical panel to overcome these challenges. Galectin-3,

HBME-1 and cytokeratin-19 immunohistochemicals provide a limited contribution to distinguishing controversial follicular lesions [3,4].

Telomerase is a ribonucleotide polymerase that provides repeating TTAGGG nucleotide sequences at the telomeric ends. Approximately 90% of cancers are said to have increased telomerase activity [5-7]. The catalytic component of telomerase is hTERT. hTERT expression can be evaluated at cellular level and in paraffin blocks with immunohistochemical methods [5-7].

The present study examined the expression level of hTERT immunohistochemically, and aimed to determine the role of the expression of hTERT in the differential diagnosis of certain thyroid neoplasms.

Methods

Selection of patients

This study initially included 150 cases that were histopathologically diagnosed with thyroid neoplasia upon thyroidectomy materials and followed up at Health Sciences University, Antalya Education and Research Hospital between January 2010 and July 2016. The study was approved by the local ethics committee. All cases were re-evaluated by the authors. Tumor histological types and subtypes, tumor diameters, tumor multicentricity, thyroid capsule invasion, lymphovascular invasion and additional thyroid pathologies were recorded.

Expression of hTERT were analyzed via immunohistochemistry. Due to technical reasons, 12 cases in which the immunohistochemical expression was not eligible for evaluation were excluded. As a result, 138 cases of thyroid neoplasias were enrolled into the present study. The distrubution of patients were as follows; 33 FA, 31 MNG, 50 PC, 10 FC, 3 MC, 3 undifferentiated carcinomas (UDC), 2 poorly differentiated carcinomas (PDC), 4 well differentiated tumors of uncertain malignant potential (WDT-UMP) and 2 follicular tumors of uncertain malignant potential (FT-UMP).

Information about the patient age and gender, type of surgery and tumor localization was obtained from patient files.

Tissue preparation and immunohistochemical staining

Resected tissue samples obtained just after thyroidectomy were immediately fixed in 10% formaldehyde and embedded in paraffin. Then 4μm thick sections were obtained from paraffin blocks and were stained with H & E for initial assessment. Cross-sections of 4μm thickness prepared for immunohisto-
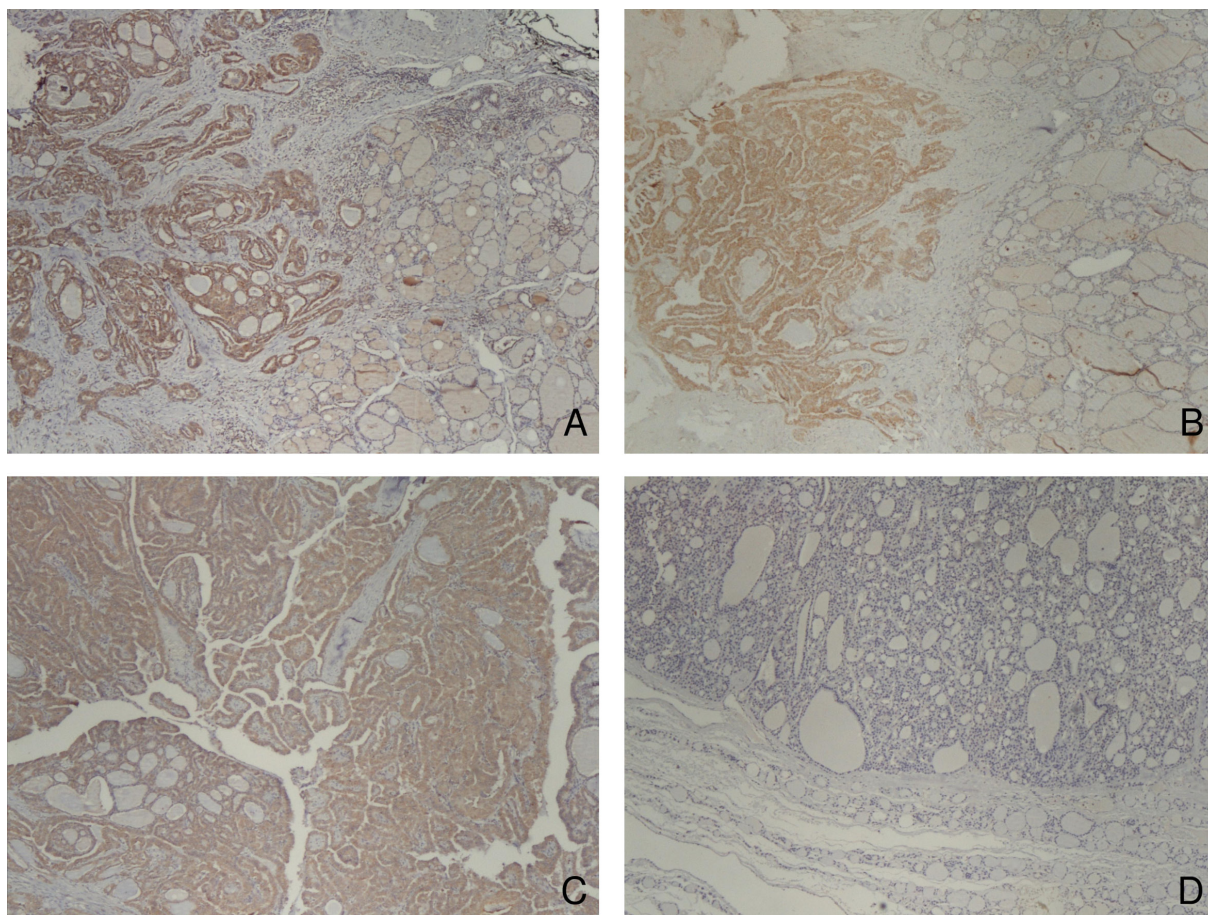

Figure 1. A: Papillary carcinoma classical variant, 3 (+) staining in 100% of tumor cells (hTERT, x40); **B:** Papillary microcarcinoma 3 (+) staining in tumor cells (hTERT, x40); **C:** Papillary carcinoma oncocytic variant, 2(+) staining in tumor cells (hTERT, x40); **D:** Multinodular goiter, negative staining (hTERT, x40).

chemical staining were deparaffinized in oven at 60°C for 2 hrs. Afterwards, they were kept in xylene for 30 min, and in gradient ethanol for 30 min (70% ethanol for 10 min, 96% ethanol for 10 min, 100% ethanol for 10 min) and washed with tap water. Next, the tissue sections were heated in a 10% citrate buffer solution (#RE7113; Leica Microsystems, Inc., Milton Keynes, UK) in the microwave at 800 W for 10 min and then at 400 W for an additional 10 min. Sections were brought out of the microwave and allowed to cool at room temperature for 30 min. Endogenous peroxidase activity was blocked by incubation in 3% hydrogen peroxide for 10 min. Sections were washed with phosphate buffered saline (PBS) for 2 min. Then, sections were incubated with primary antibodies against hTERT (#ab150; dilution 1:50; Abcam, Lab Vision, Cambridge, MA, USA) for 60 min. Afterwards, they were treated with secondary antibody (Biotinylated Goat-anti-rabbit Immunoglobulin secondary antibody; #BP-9100; ready to use; Vector Laboratories, Burlingame, CA, USA) for 20 min at 30°C and washed with PBS for 5 min. Sections were then incubated with conjugated peroxidase (#RE7110-K; Novocastra; Leica Microsystems Inc., USA) for 20 min and then washed with PBS for 5 min, and were kept in chromogenic 3,3'-diaminobenzidine for 5 min. Sections were washed under tap water and counterstained with hematoxylin. Then, the tissue samples were dehydrated, dried and covered with Entellan®.

Microscopic examination of hematoxylin & eosin-stained sections

In all cases, tumor type, tumor diameters, presence of multicentricity, presence of thyroid capsule invasion, and presence of lymphovascular invasion were registered.

Evaluation of immunohistochemically stained sections

Positive immunohistochemical staining of hTERT in the nuclei of lymphocytes were used as a positive internal control, whereas the primary antibodies were omitted for the negative controls. Because cytoplasmic staining was seen in neoplastic thyrocytes, a scoring was performed based on this staining pattern. According to this scoring system, weak staining was considered as 1+, moderate staining was considered as 2+ and strong staining was considered as 3+. The rate of staining of neoplastic cells was calculated as percentage. After determination of the intensity and percentage of stained neoplastic cells weak (below 50%) or no staining of neoplastic cells were considered hTERT (–), while medium and high intensity stainings and any staining over 50% of neoplastic cells were accepted as hTERT (+). The entire sections were examined by the authors who were blinded to all clinicopathological information. Examples of immunohistochemical staining are presented in Figure 1.

Statistics

Statistical analyses were performed by using SPSS software version 24.0 for Windows (SPSS, Inc., Chicago, IL, USA). Descriptive analyses were presented as mean ± standard deviation. Cross tables were made and the scores of hTERT expression level in benign and malignant neoplasms were identified. Differences between groups were analyzed using the x^2 and Student t-test. $P<0.05$ was considered to indicate statistically significant difference. Correlation analyses were done with Pearson correlation test, values of 0,90-1,00 were evaluated as very high and values of 0.79-0.89 were evaluated as high correlation.

Results

Clinicopathological characteristics

A total of 138 patients, 106 of whom (76.8%) were female and 32 (23.2%) male were included in the study. Histopathological evaluation revealed PC in 50 (36.2%) cases, FA in 33 (23.9%) cases, MNG in 31 (22.5%) cases, FC in 10 (7.2%) cases, UDC in 3 (2.2%) cases, PDC in 2 (1.4%) cases, WDT-UMP in 4 (2.9%) cases, FT-UMP in 2 (1.4%) cases and MC in 3 (2.2%) cases (Table 1).

Of the 50 PC cases, 19 (38.0%) were microcarcinoma variant, 14 (28.0%) classic variant, 10 (20.0%) follicular variant, 6 (12%) oncocytic variant and 1 (2.0%) was tall cell variant. Of the 10 FC cases, 5 (50.0%) were minimally invasive FC and 5 (50.0%) were widely invasive FC. Of the 33 FA cases, 28 (85.0%) were classic variant, 4 (12.0%) oncocytic variant and 1 (3.0%) was clear cell variant (Table 2).

Sixty-eight (49.3%) of the patient tumors were malignant, 64 (46.4%) were benign, 6 (4.3%) were of uncertain malignant potential, while FA and MNG were evaluated as benign neoplasms, FT-UMP and WDT-UMP were accepted as tumors with uncertain malignant potential and others as malignant.

Multicentricity was detected in 31 patients (22.5%), thyroid capsule invasion was detected in 24 (17.4%), and lymphovascular invasion was detected in 21 patients (15.2%).

Table 1. Histopathological distribution of the cases

	PC	FA	FC	MNG	WDT-UMP	FT-UMP	PDC	UC	MTC	TOTAL
n	50	33	10	31	4	2	2	3	3	138
(%)	(36.2)	(23.9)	(7.2)	(22.5)	(2.9)	(1.4)	(1.4)	(2.2)	(2.2)	(100.0)

For abbreviations see text

Immunohistochemical study findings

Immunohistochemical hTERT expression was negative in 93 (67.4%) patients and positive in 45 (32.6%). Table 3 demonstrates hTERT expression of the cases.

When the tumors were divided into malignant, benign and of uncertain malignant potential groups, hTERT was positive in 27 (40.0%) of 68 cases in the malignant group, positive in 16 (25.0%) of 64 cases in the benign group and positive in 2 (33.0%) of 6 cases in the uncertain malignant potential group. There was no difference in the hTERT expression between the malignant and benign, malignant and UMP and benign and UMP groups (p=0.073, p=0.773, p=0.661, respectively) .

The difference between PC and MNG, between PC and FC and between FA and MNG were significant (p=0.002, p=0.034, p=0.030, respectively). No difference was found between FA and FC (p=0.117). There was no difference between FC and MNG, between FT-UMP and FC, between FA and FT-UMP, between WDT-UMP and PC, FA between FC, FT-UMP and MNG, between WDT-UMP and MNG, and between MTC and PC (p=0.813, p=0.198, p=0.708, p=0.426, p=0.664, p=0.166, p=0.529, respectively). Positivity in MC was significantly higher than in FC (p=0.043).

Table 2. Distribution of PC, FC and FA cases by subtypes

Tumor subtypes	n (%)
PC	50 (100.0)
Papillary microcarcinoma	19 (38.0)
Classic variant	14 (28.0)
Follicular variant	10 (20.0)
Oncocytic variant	6 (12.0)
Tall cell variant	1 (2.0)
FC	10 (100.0)
Minimally invasive FC	5 (50.0)
Widely invasive FC	5 (50.0)
FA	33 (100.0)
Classic variant	28 (85.0)
Oncocytic variant	4 (12.0)
Clear cell variant	1 (3.0)

For abbreviations see text

hTERT staining showed no significant difference between groups of tumors with diameters between ≤1 and 1.1-4 cm, between 1.1-4 and >4 cm and between ≤1 and >4 cm groups (p=0.734, p=0.695, p=0.946, respectively).

There was no significant relationship between hTERT staining and presence of lymphovascular invasion, and multicentricity, age, gender, and tumor diameters (p=0.424, p=0.604, p=0.407, p=0.540, p=0.057 respectively).

Discussion

The gold standard in diagnosis of thyroid nodules is pathological evaluation using routine H & E staining. However, morphologic overlap between MNG, FA, FC and between PC and FC is common. In such cases an objective consistent diagnosis based merely on morphological assesment is sometimes impossible [4].

There is no generally accepted immunohistochemical panel to overcome these challenges. Immunohistochemical analyses with Galectin-3, HBME-1 and cytokeratin-19 provide a limited contribution in the differentiation of controversial neoplasms [2,3].

Telomerase is a ribonucleotide polymerase that provides repeating TTAGGG nucleotide sequences at the telomeric ends, thereby ensuring the continuity of these regions. The enzyme contains a protein portion (TERT) containing reverse transcriptase activity and a RNA template (TERC) required for telomere recovery [5].

The relevance of telomerase activity to how many times a cell divides will lead to a role in immortal cell arrays such as cancer cells. By attaching telomeric repeats to chromosomal DNA ends, immortal cancer cells develop. Approximately 90% of the cancers are said to bear increased telomerase activity [5-7].

The regulation of telomerase activity is based on two main components. Since the catalytic component of telomerase is hTERT and the RNA component is hTERC, hTERT has become an important gene in cancer and tumorigenesis research [8,9].

Table 3. hTERT expression by neoplasia type

	PC	FA	FC	MNG	WDT-UMP	FT-UMP	PDC	UC	MTC	TOTAL
hTERT(+)	23	12	1	4	1	1	0	1	2	45
n (%)	(46.0)	(36.0)	(10.0)	(13.0)	(25.0)	(50.0)	(0.0)	(33.0)	(67.0)	(32.6)
hTERT(-)	27	21	9	27	3	1	2	2	1	93
n (%)	(54.0)	(64.0)	(90.0)	(87.0)	(75.0)	(50.0)	(100.0)	(67.0)	(33.0)	(67.4)
Total	50	33	10	31	4	2	2	3	3	138

For abbreviations see text

There are many studies related to telomerase activity and hTERT. As a result of these studies it has been shown that telomerase is active in 90% of human cancers and that hTERT expression correlates with telomerase activity [5-10].

There are few studies examining the immunohistochemical expression of hTERT in various tumors and very few of them examining hTERT in thyroid neoplasms. Wang et al. found 23/36 FC and 14/36 FA had high immunohistochemical hTERT expression (medium-strong immunoreactivity). Other cases were poorly or negatively stained and the difference between FA and FC was statistically significant in their study [11]. Sugishita et al. studied 14 FC, 47 FA, 5 Hurthle cell carcinoma and 12 Hurthle cell adenomas. hTERT was evaluated with immunohistochemistry and 86% of FC cases were positive whereas only 49% of FA cases were positive. hTERT expression was observed in all of Hurthle cell adenomas and carcinomas [12]. In our study, hTERT positivity was 36% for FA and 10% for FC, without statistical difference between the two conditions. Our results were not compatible with those studies [11,12].

In those two studies only the percentage of staining was taken into account. In our study we did not make a positive-negative evaluation according to the percentage of staining. However, when we evaluated the results, there was a correlation between the percentage of staining and the intensity of staining, and the staining intensity did not exceed 1 when stained below 50%. This means that the hTERT staining result is determined using only a 50% limit value. In other words, even if we divide our values into hTERT (+) or (-) by taking only the percentage of 50%, there will be no difference compared to our current scoring system.

We found a significant increase of hTERT expression at PC in contrast to FC. The same is true at FA in contrast to MNG.

These findings lead us to think hTERT expression can be useful for making differential diagnosis between PC and FC, and between FA and MNG.

Conclusion

Distinction of thyroid neoplasms can be successfully performed by histopathological examination in most of the cases, however, in some cases it may be difficult to make exact diagnosis when histopathological findings are ambiguous or overlapped. Our findings suggest that hTERT expression can be useful for making differential diagnosis between PC and FC, and between FA and MNG.

Conflict of interests

The authors declare no conflict of interests.

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