RESEARCH ARTICLE

Clinicopathologic and Diagnostic Significance of p53 Protein Expression in Papillary Thyroid Carcinoma

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Abstract

Background: p53 protein expression has been detected immunohistochemically in papillary thyroid carcinoma(PTC). We investigated the relations between its expression and clinicopathologic features and its significance as a diagnostic marker. Materials and Methods: We compared and evaluated 93 patients in whom thyroidectomy with lymph node dissection had been performed to treat PTC for clinicopathologic significance and 102 patients with 23 papillary thyroid overt carcinomas (POC), 57 papillary thyroid microcarcinomas(PMC), 5 follicular adenomas (FA), 5 Hashimoto's thyroiditis (HT) and 12 nodular hyperplasias (NH) for significance as a diagnostic marker. Expression of p53 protein was evaluated immunohistochemically in sections of paraffin-embedded tissue. Results: Statistical analysis showed significantly different expression of p53 in PTC versus other benign thyroid lesions (BTL).The diagnostic sensitivity and specificity were 85.0% and 72.7%, respectively. Overexpression of p53 protein was observed in 44 of the 93 PTC cases (47.3%), but no significant correlation between p53 protein overexpression and clinicopathologic features (age, size, multiplicity, lymph node metastasis, extrathyroidal extension and vascular invasion) was noted. Conclusions: p53 is valuable to distinguish PTC from other BTL, but there is no correlation between p53 protein overexpression and clinicopathologic features.

Keywords: Papillary thyroid carcinoma - p53 protein - clinicopathologic significance - diagnostic marker

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Introduction

Papillary thyroid carcinoma (PTC) is the most common cancer of the endocrine system and accounts for about 80% of all tyroid malignancies (Cvejic et al., 2008; Balta et al., 2012). The prognosis of PTC is generally good, but some patients suffer from local recurrence and/or distant metastasis. The known significant poor prognostic factors in PTC are age, male sex, large tumor size, extrathyroidal extension and metastases.

Many prognostic scoring systems such as Tumor, Node. Metastases (TNM) and Metastases, Age, Completeness of resection, local Invasion, tumor Size (MACIS) have been used for more accurate establishment of the prognosis in patients with PTC (Balta et al., 2012; Hamzany et al., 2012). Moreover, in recent years, attention has been focused on potential molecular and cytologic markers of biological behavior (Horie et al., 2001; Zafon et al., 2007; Morita et al., 2008; Kim et al., 2009; Balta et al., 2012; Hamzany et al., 2012).

The diagnosis of PTC is based on architectural features combined with nuclear clearing, overlapping, grooves, and pseudo-inclusions. Accurately distinguishing the follicular variant of PTC from cellular adenomatous nodules may be challenging in the absence of papillary architecture (El Demellawy et al., 2008; Tan et al., 2011).

Less commonly, papillary hyperplastic nodules may be difficult to distinguish from PTC (El Demellawy et al., 2008). Several reports have used ancillary studies, particularly immunohistochemistry and molecular techniques, in an attempt to solve problematic cases. Although some of these techniques are useful, they should be used cautiously as none of the ancillary studies seem to be consistent nor 100% reliable. Hence, none of these markers has solved the diagnostic controversy (Park et al., 1998; Moon et al., 2006; El Demellawy et al., 2008; Tan et al., 2011).

Mutations in the p53 tumor suppressor gene are present in approximately 50% of all human cancers, and they represent the most common genetic changes in malignant cells (Horie et al., 2001; Moon et al., 2006; Zafon et al., 2007; Hansen et al., 2008; Morita et al., 2008; Kim et al., 2009; Parameswaran et al., 2010; Balta et al., 2012).

Several studies have reported frequent occurrence of p53 gene mutations in undifferentiated thyroid cancers. However, prevalence of p53 gene mutations in well-differentiated thyroid carcinoma including PTC has not been established; they ranged 0% to 25% (Horie et al., 2001; Gauchotte et al., 2011). Immunohistochemical detection of p53 protein is thought to be associated with the occurrence of p53 gene mutations (Zafon et al., 2007), but p53 protein expression has been detected

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immunohistochemically in differentiated papillary and follicular thyroid carcinomas irrespective of whether any p53 gene mutations had occurred (Park et al., 1998; Parl et al., 2001; Liu et al., 2002; Jung et al., 2007; Morita et al., 2008).

There are some studies about immunohistochemical detection of p53 protein showing significant and independent prognostic indicator in differentiated thyroid carcinomas (Hosal et al., 1997; Park et al., 1998; Horie et al., 2001; Omar et al., 2004; Zafon et al., 2007; Morita et al., 2008; Kim et al., 2009; Balta et al., 2012). We investigated the relations between p53 protein expression and the clinicopathologic features and its significance as a diagnostic marker in PTC.

Materials and Methods

Study approval was obtained from the Institutional Review Board at Kangnam Sacred Heart Hospital of Hallym University Medical Center. 93 patients in whom thyroidectomy with lymph node dissection had been performed to treat PTC from April 2010 to June 2011 for clinicopathologic significance and 102 patients who underwent thyroid surgery from June 2009 to July 2010 (including four cases of follicular adenoma in 2003) for significance as a diagnostic marker at Kangnam Sacred Heart Hospital of Hallym University Medical Center were selected for this study.

All cases had hematoxylin and eosin (H&E) stained slides and paraffin blocks for immunohistochemical staining available for review. The H&E slides were reviewed by two authors independently and the diagnosis was agreed upon using well-established histopathological criteria. We followed the same histological criteria as those proposed by WHO (Dellis et al., 2004) for the diagnosis of PTC. Adenomas were defined as completely encapsulated follicular tumors with homogeneous architecture and morphology, lacking nuclear features of PTC, and without capsular and vascular invasion. We independently reviewed the H&E-stained sections and interpreted immunohistochemical staining results. A consensus regarding controversial cases was reached at a multi-headed microscope.

Immunohistochemistry was performed on 4 μm-thick sections using a standard technique (streptavidin-biotin-peroxidase technique) with appropriate positive and negative controls. The primary antibody was anti-p53 mouse monoclonal antibody (clone DO-7; Dako, 1:3000 dilution). Expression of p53 protein was evaluated by score (intensity+positive cell proportion). The intensity was graded into 0, 1+, 2+ and 3+ by eyeball movement. And the positive cell proportion was semiquantitavely evaluated according to the estimated percentage of positive tumor cells: no positive cells; 0, staining of <10% of the cells; 1, staining in 10-33% of the cells; 2, staining in 33-66% of the cells; 3, staining in >66% of the cells; 4.

A score of 4+ or, <4+ was considered negative, and scores of more than 4+ were considered as positive for clinicopathologic significance study and scores of more than 3+ were considered as positive for significance as a diagnostic marker study .

Statistical analysis

The statistical analysis was performed with SPSS ver. 15.0 (SPSS Inc., Chicago, IL, USA). Fisher's exact test was used to compare frequencies between the groups. The sensitivity, specificity, and accuracy of the markers for diagnosing of PTC were compared. The p values<0.05 were considered significant.

Results

The selected cases for clinicopathologic significance consisted of 93 PTCs [including 25 papillary thyroid overt carcinomas (POC) and 68 papillary thyroid mocrocarcinomas (PMC)]. Among the 68 PMC, three cases of follicular variant were found and the remaining 65 PMC and 25 POC were the classic type.

The relation between p53 protein overexpression and clinicopathologic features is shown in Table 1. p53 positive cases were 44 cases (47.3%) and p53 negative case were 49 cases. The average age of p53 positive cases was 48.7 and the average age of p53 negative cases was 48.9 and and the average tumor size of p53 positive cases was 0.925 and the average tumor size of p53 negative cases was 0.928. In multiplicity of the PTCs, 18 cases of p53 positive cases showed multiplicity and 22 cases of p53 negative cases showed multiplicity.

Extrathyroidal extension was present in 25 cases of p53 positive cases and in 22 cases of p53 negative cases. Three cases of p53 positive cases and four cases of p53 negative cases showed vascular invasion and lymph node metastasis was present in 11 cases of p53 positive cases and 16 cases of p53 negative cases. All parameters (age, tumor size, multiplicity, extrathyroidal extension, vascular invasion, lymph node metastasis and mean number of metastatic lymph node) revealed no significant correlation with p53 protein overexpression in statistical analysis.

The relation between lymph node metastasis and clinicopathologic features is shown in Table 2. Lymph node metastasis was present in 27 cases out of 93 cases and showed significant statistical correlation with sex, tumor size and extrathyroidal extension. But other clinicopathologic features (age, multiplicity and vascular invasion) revealed no significant correlation with lymph node metastasis in statistical analysis.

The selected cases for signigicance as a diagnostic marker consisted of 102 cases composed of 23 POC, 57 PMC, 5 FA, 5HT and 12NH.

p53 protein overexpression and a comparison of the results between groups is presented in Table 3. p53 protein overexpression was present in 68 (85%) out of 80 PTC cases. p53 expression was also positive in 20 of 23 in POC cases (87.0%) and 48 of 57 PMC cases (84.2%). No statistical difference was observed between the POC and PMC (p=1.0000). p53 protein overexpression was present in 1 of 5 in FA cases (20.0%), 3 of 12 NH cases (25.0%) and 2 of 5 HT cases (40.0%). Using Fisher's exact test, p53 distinguished PTC from FA, NH, and HT (Table 3) with statistical significance. p53 was 85.0% sensitive and

Table 1. Relation between p53 Protein Overexpression and Clinicopathologic Features

Parameter		p53 (+)	p53 (-)	p value
No. of cases		44	49	
Age		48.70±12.12	48.94±9.91	0.910
Tumor size(mm)		0.925±0.523	0.928±0.667	0.977
Multiplicity	Present	18	22	0.834
	Absent	26	27	
Extrathyroidal extension				
	Present	25	22	0.301
	Absent	19	27	
Vascular invasion				
	Present	3	4	
	Absent	41	45	1.000
Lymph node metastasis				
	Present	11	16	
	Absent	33	33	0.495
Mean No. of metastatic lymph node		1.00±2.28	1.16±2.82	0.761

Table 2. Relation between Lymph Node Metastasis and Clinicopathologic Features

Parameter		LN metastasis (+)	LN metastasis (-)	p value
No. of cases		27	66	
Age		48.0±9.6	49.2±11.5	0.643
Sex	Male	9	6	0.01
	Female	18	60	
Tumor size (mm)		1.23±0.59	0.80±0.55	0.001
Multiplicity	Present	15	12	0.166
	Absent	12	41	
Extrathyroidal extension				
	Present	20	27	0.006
	Absent	7	39	
Vascular invasion				
	Present	4	3	0.187
	Absent	23	63	

*LN, lymph node

Table 3.

p53	Positive	Negative	Total	Comparing	p value
POC	20	3	23	With PMC	1
PMC	48	9	57		
PTC	68	12	80	With FA+NH+HT	<0.0001
FA	1	4	5	With PTC	0.004
NH	3	9	12	With PTC	<0.0001
HT	2	3	5	With PTC	0.0365
Total	74	28	102		

*POC; papillary thyroid overt carcinomas; PMC; papillary thyroid microcarcinomas; PTC; papillary thyroid carcinomas; FA; follicular adenomas; NH; nodular hyperplasias; HT; Hashmoto's thyroiditis

Table 4. Differentiating PTC from Other Thyroid Benign Lesions

	Sensitivity	Specificity	PPV	NPV	Accuracy
p53(+)	85.00%	72.73%	91.89%	57.14%	82.35%

*PTC: papillary thyroid catcinomas; PPV: positive predictive value; NPV: negative predictive value

72.7% specific for distinguishing PTC from other BTL (Table 4).

Discussion

p53 is a tumor suppressor gene that codes for a multifunctional DNA-binding protein involved in cell cycle arrest, DNA repair, differentiation, and apoptosis. Mutation in the p53 tumor suppressor gene could be seen in about 50% of the human cancers and is one of the most frequently seen alterations in the malignant cells and the majority of the mutations are in the exons 5-8 (Horie et al., 2001; Park et al., 2002; Morita et al., 2008; Hansen et al., 2008; Parameswaran et al., 2010; Balta et al., 2012). In the thyroid gland, p53 mutations have been shown in 40-62% of undifferentiated carcinomas and 0-25% in well-differentiated carcinomas (Morita et al., 2008; Parameswaran et al., 2010). The prognostic significance of p53 mutation in PTC is controversial. Immunohistochemical (IHC) detection of p53 protein is thought to be attributable to the presence of a p53 gene mutation in up to 95% of PTC cases (Zafon et al., 2007). Wild-type protein is undetectable due to its short half-life, but mutated forms show greater stability and a longer half-life. Thus, it was once thought that only mutated forms can be detected by immunochemistry. However, it has been recently demonstrated that p53 overexpression is not always due to p53 mutations. Indeed, the overexpression of wild-type protein might result from unidentified factors and could be considered as a protective mechanism in human tumours (Park et al., 2001; Liu et al., 2002; Zafon et al., 2007). p53 mutations can cause expression of abnormal proteins or result in complete absence of p53 expresion (Liu et al., 2002). It has been reported that 11-59% of PTC overexpresses p53 protein (Park et al., 1998; Horie et al., 2001; Morita et al., 2008). In the present study, p53 protein overexpression was observed in 47.3% of the PTC. Although several studies have reported finding that detection of p53 protein was a significant and independent prognostic indicator in differentiated thyroid carcinoma (Hosal et al., 1997; Horie et al., 2001; Omar et al., 2004; Morita et al., 2008; Kim et al., 2009; Balta et al., 2012), the association has been a matter of controversy (Park et al., 1998; Kalidag et al., 2007; Jung et al., 2007; Zafon et al., 2007; Cvejic et al., 2008; Hamzany et al., 2012).

Hosal et al. (1997) reported that p53 protein overexpression was present in cases with extrathyroidal extension and metastases (Hosal et al., 1997). In one study by Morita et al. (2008) significant correlation between p53 protein expression in the primary tumor and large tumor size, the presence of lymph node metastasis, and the mean number of lymph node metastases was present (Morita et al., 2008). Horie et al. (2001) also said that overexpression of p53 protein significantly correlated with large tumor size and the presence of capsular invasion (Horie et al., 2001). But several studies (Park et al., 1998; Kalidag et al., 2007; Jung et al., 2007; Zafon et al., 2007; Cvejic et al., 2008; Hamzany et al., 2012) found no association between p53 positivity and clinicopathologic data. In this study all parameters (age, tumor size, multiplicity, extrathyroidal extension, vascular invasion, lymph node metastasis and mean number of metastatic lymph node) revealed no significant correlation with p53 protein overexpression in statistical analysis. Lymph node metastasis was present in 27 cases out of 93 cases and showed significant statistical correlation with sex, tumor size and extrathyroidal extension. But other clinicopathologic features (age, multiplicity and vascular invasion) revealed no significant correlation with lymph node metastasis in statistical analysis.

The diagnosis of PTC is based on architectural features combined with nuclear clearing, overlapping, grooves, and pseudo-inclusions. Benign and malignant thyroid nodules in the approach to discrimination is one of the major problems encountered in surgical pathology. The differential diagnosis of the lesions in follicular morphology such as NH, FA, follicular carcinomas (FC) and PTC can be quite difficult at times. Several reports have used ancillary studies, particularly immunohistochemistry and molecular techniques, in an attempt to solve problematic cases. Although some of these techniques are useful, they should be used cautiously as none of the ancillary studies seem to be consistent nor 100% reliable. Hence, none of these markers has solved the diagnostic controversy. We aimed to evaluate the difference of the expression of p53 among the PTC, FA, NH and HT. Alterations in the p53 gene and p53 protein expression has been repeatedly reported in human thyroid tumors (Nasir et al., 2004).

However in the thyroid gland, p53 mutations have been shown in 40-62% of undifferentiated carcinomas and only 5-10% in other carcinomas (Nasir et al., 2004; Parameswaran et al., 2010). The mutations of p53 or increased expression of p53 protein is associated with the progression from differentiated to anaplastic carcinoma. Thus, presence of p53 mutations is a late event in thyroid cancer progression (Nasir et al., 2004; Parameswaran et al., 2010). Balta et al. (2012) stated in their study that the difference of p53 protein expression in PTC compared with control group (benign lesions) were statistically significant. In a study by Tan et al. (2011), 35.3% of p53 protein expression in PTC and no expression in FC and FA. Nasir et al. (2004) reported that 90% of cases of FC exhibited a strong nuclear p53 expression, but p53 staining was weakly expressed in only 15% of the cases of FA. And in one study (Omar et al., 2004) p53 protein expression was more evenly distributed among the FA, NH and PTC. In this study p53 protein overexpression distinguished PTC from FA, NH, and HT with statistical significance. p53 was 85.0% sensitive and 72.7% specific for distinguishing PTC from other BTL. No p53 protein expression was detected among normal thyroid tissue.

In conclusion, p53 is valuable to distinguish PTC from other BTL, but there is no correlation between p53 protein overexpression and clinicopathologic features.

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