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RAS AND p53 EXPRESSION IN HUMAN THYROID CARCINOMA

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ABSTRACT

Objective: To investigate the possible interaction between the ras and p53 genes over-expression in thyroid carcinoma, and whether there is a correlation between the ras and p53 over-expression and clinicopathological criteria. Methods: Eighty patients with thyroid lesions were examined for expression of ras and p53 genes by the labeled streptavidin biotin peroxidase (LSAB) method. Of these patients, 54 were diagnosed (average age: 39.9±15.9 years) with malignant lesions. Of those included in the study, 31 has papillary carcinoma, 13 had follicular carcinoma, 7 had medullary carcinoma, 3 had undifferentiated carcinoma and 19 were stratified to stage I, 28 to stage II, 2 to stage III and 5 to stage IV according to TNM staging system. Twenty-six benign nodular thyroid disorders were studied as control. Results: Positive immunostain results for ras and p53 genes were statistically significant between thyroid carcinomas and benign disorders (90.7% vs 23%, 55.5% vs 30.7%, $P<0.05$). Both p53 and ras overexpressions coexisted in 30 thyroid carcinomas, and of these, 3 died and 5 had recurrences within 4 years. Conclusions: Activation of ras gene and inactivation of p53 gene were cooperatively associated in thyroid tumorigenesis. The concurrent overexpressions of ras and p53 could result in a poor prognosis.

Key words: Thyroid neoplasm, Ras gene, p53 gene, Immunohistochemistry, Prognosis

Both benign and malignant nodular disorder of the thyroid gland may give rise to similar symptomatology. Even though clinical background and pathologic criteria may predict prognosis, there are still patients without these adverse prognosis indicators who develop subsequent local invasion or distant metastasis after surgical intervention and eventually succumb to the disease.[1]

In recent years it has become apparent that malignant transformation is a result of the accumulation of genetic abnormalities, including activation of oncogene and inactivation of tumor suppressor genes.[2] Detection of molecular genetic changes of thyroid carcinoma will allow early and aggressive therapy to improve survival. The present study aims at investigating the possible relationship between the ras and p53 gene expression and the clinical and morphologic criteria in thyroid carcinoma. P21 protein and p53 protein were stained immunohistochemically to look for the possible interaction between these factors.

MATERIALS AND METHODS

Clinical Data

The study group consisted of 80 subjects who underwent surgical intervention from February 1988 to December 1995, 54 were diagnosed with thyroid carcinoma (21 men and 33 women) and 26 with benign nodular thyroid disorders (10 men and 16 women). Benign nodules included 10 follicular adenomas, 10 nodular goiters and 6 Hashimoto's thyroiditis. The patients with malignant lesions ranged in age from 8 to 70 ($\bar{x}$ ±SD: 39.9±15.9) years. Among them, 31 had papillary carcinoma (PTC), 13 had follicular carcinoma (FTC), 7 had medullary carcinoma (MTC) and 3 with undifferentiated carcinoma (UTC). According to TNM staging system, 19 patients were stratified to stage I, 28 to stage II, 2 to stage III and 5 to stage IV.[3] Fifty-four thyroid carcinoma cases had been closely monitored for 2 to 11 years postoperatively.

Immunohistochemical Assay of Ras and p53 Gene Expression

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The surgical resection specimen embedded in paraffin was serially sectioned at 4 μm, and stained immunohistochemicaly with labeled streptavidin biotin peroxidase (LSAB) method according to the protocol of LSAB kit (DAKO, Denmark). The slides were deparaffinized, treated with 3% hydrogen dioxide solution for 5 min at room temperature. After blocking with fetal bovine serum for 10 min, the tissue sections were then incubated at 4℃ overnight with primary antibodies of the mouse anti-human p21 protein monoclonal antibodies (NCC-RAS-001, DAKO) at a dilution of 1: 50, and p53 protein monoclonal antibodies (DO-7, DAKO) at a dilution of 1: 100 respectively. It was then incubated again with goat anti-mouse biotinylated antibodies (secondary antibodies) at 37℃ for 10 min and finally with streptavidin-labeled HRP compound for 10 min. Slides were washed three times at room temperature in phosphate buffer saline (PBS) for 5 min each between above protocols. PBS was used to replace the first antibodies as the negative control. The positive cells were analyzed by coloration with DAB and stained with haematoxylin. According to the criteria by Pilotti S,[4] the results of immunostaining were classified as negative (–) when there was no stain uptake detected; weakly positive (+) when the staining was faint or the positive cells were 30% or less; moderately positive (++) when the positive cells were 31 - 60%; and strongly positive (+++) when the intensely stained cells were greater than 61%.

Statistical Analysis

The difference between groups was tested by χ^2 test. The impact of ras and p53 expression on survival and recurrent rate was tested by log-rank test. *P* value less than 0.05 was considered significant.

RESULTS

The p53 positive staining was dark brown, and was distributed in the nucleus and cytoplasm (Figure 1), but p21 protein positive grains were almost confined to the cytoplasm(Figure 2). Figure 3 showed that the positive expressing rate of p53 and ras protein were significantly higher in malignant tissues than in benign lesions (χ^2 test, $P<0.05$). Both p53 and ras overexpressions coexisted in 30 specimens of 54 thyroid carcinomas, and positive relation of p53 and ras overexpressions was found ($\gamma=0.35$, $P<0.05$). However, there was no apparent correlation ($P>0.05$) among the p53 and ras overexpressions in thyroid carcinomas among the histological subtypes (Figure 4) and clinicopathological staging (Figure 5).

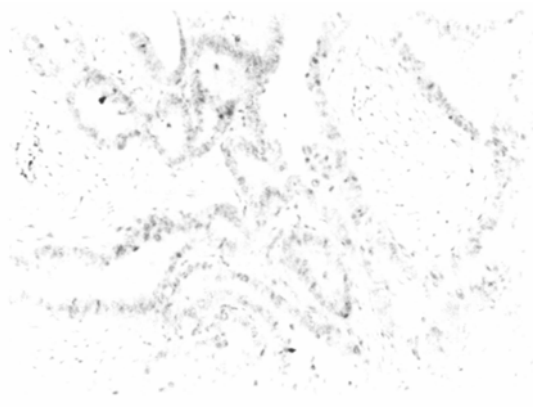

Fig. 1. The positive stain of p53 in papillary thyroid cancer (LSAB; original magnification × 100)

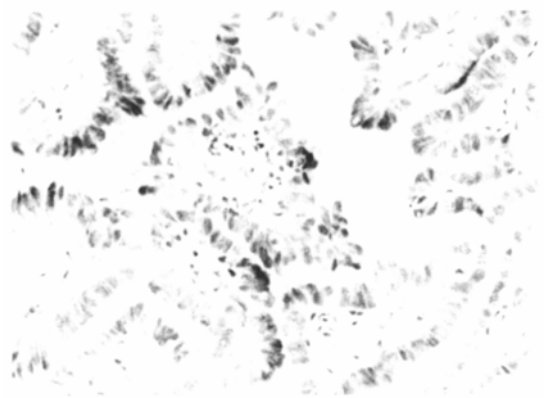

Fig. 2. The positive stain of p21 in papillary thyroid cancer (LSAB; original magnification × 400)

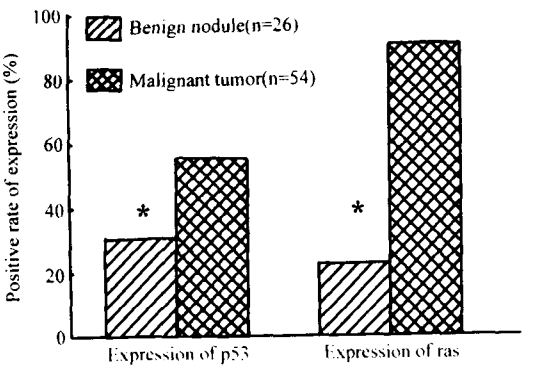

Fig.3. The positive expressing rate of p53 and ras protein were significantly higher in malignant tissues than in benign lesions (χ^2 test, $^*P<0.05$ vs malignant tumor).

Three of 54 thyroid malignant patients succumbed postoperative at 6, 9 and 13 months, respectively. All 3 patients, 2 UTC and 1 MTC, were stratified to stage IV and detected ras and p53 concurrent overexpressions. Among 51 surviving patients with

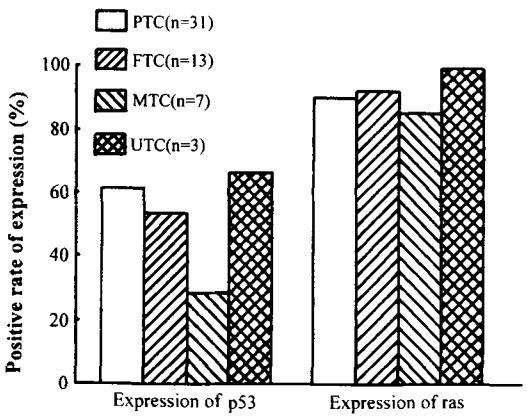

Fig. 4. There was no apparent correlation ($P>0.05$) in the p53 and ras overexpressions in thyroid carcinomas among the histological subtypes. PTC=papillary carcinoma; FTC= follicular carcinoma; MTC= medullary carcinoma, UTC= undifferentiated carcinoma.

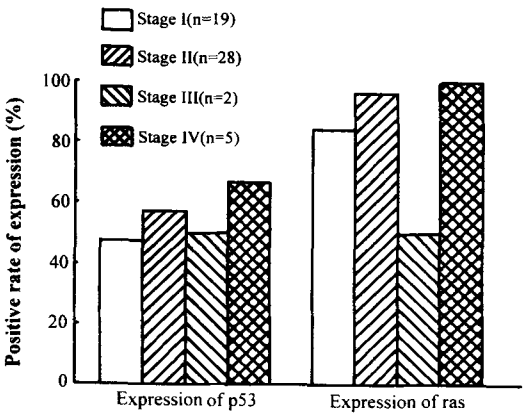

Fig. 5. No apparent correlation was found ($P>0.05$) in the p53 and ras overexpressions in thyroid carcinomas among clinicopathological staging.

thyroid carcinoma, 6 further surgical resections were performed for tumor recurrence postoperatively from 7 to 46 (20± 25.8) months. Of 6 recurrent patients, 4 were stratified to stage II, 1 to stage III and 1 to stage IV after the first operation. Moreover, all 6 recurrent patients were found to have ras and 83% (5/6) with p53 overexpressions. As shown in Table 1, the mortality and recurrence rate were significantly higher in p53 and ras positive than in negative group ($P<0.05$).

Table 1. the correlation between p53 and p21 expression and mortality and recurrence rates

Variables	P53		P53	
	Positive	Negative	Positive	Negative
Mortality(%)	10	0*	6.1	0*
Recurrent rate(%)	16.7	4.2*	12.2	0*

*$P<0.05$ vs positive group

DISCUSSION

Activating mutation in ras, gene, which encodes a G-protein-like signal transduction protein have been reported in malignant thyroid neoplasm.[5] Our study showed that the ras overexpression was highly prevalent in thyroid malignances. On the other hand, mutation of p53 gene seems to be the most common genetic alteration in various kinds of human malignant tumor.[2] The present experiments observed 55.5% p53-positive cases in thyroid cancer, and seems basically consistent with other studies.[4-6]

Although the role of oncogenes and tumor suppressor genes in thyroid carcinogenesis is the subject of intense investigation, few studies have addressed the question of their possible interactions. Therefore, we studied the concurrent expression of the oncogene ras and tumor suppressor gene p53 in thyroid carcinomas. In this series, of the 54 thyroid cancer cases studied, 30 had concurrent overexpressions of ras and p53 genes. However, Salvatore recently reported that 2 of 56 thyroid tumors had associated ras and p53 mutations.[7]

We subsequently found that the patients with coexisting ras and p53 overexpressions had a higher mortality and recurrence rate. Among 30 patients, who were detected with concurrent overexpressions of ras and p53 genes, 3 died within 13 months and 5 had recurrences in less than 4 years. It strongly suggests that coexistent aberrations of oncogene ras and tumor suppressor gene p53 were associated with the development of thyroid cancer. Samilar results were found in pancreatic cancer by intensively studying interactions between aberrations of oncogene ras and tumor suppressor gene p53. The coexistent aberrations could be used as diagnostic tumor marker.[8]

In summary, the experimental results indicate that the cooperative association between ras and p53 overexpressions could play a role in thyroid tumorigenesis. In addition, our findings suggest that concurrent overexpression of ras and p53 could carry a poor prognosis.

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