

p53 Tumor Suppressor Gene in Laryngeal Squamous Cell Carcinomas

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Purpose: Mutations in the p53 tumor suppressor gene have been shown to be one of the most common genetic abnormalities in human cancers. Using p53 avidin-biotin- complex immunohistochemical method on fixed, embedded specimens, the frequency of presence of mutant p53 protein was investigated to understand the role of the p53 gene mutations in tumorigenesis of the laryngeal carcinomas.

Methods: Forty formalin fixed, paraffin embedded tumor specimens of squamous cell carcinoma of larynx comprised the patient group and 13 biopsy specimens of benign laryngeal nodule were used as controls. Immunohistochemical staining was applied to all specimens.

Results: Abnormal expression of p53 protein was found in 19 of 40 laryngeal carcinomas (47.5 %). Mutant p53 protein was not found in any of the 13 cases with benign laryngeal nodules. There was no correlation between tumor size, nodal involvement, stage and abnormal expression of the p53 protein. However, a significant correlation was found between tumor grade and p53 expression. In all cases immunostaining of p53 within the malignant tumor cells was confined to the nuclei, and dysplastic epithelial areas also showed positive immunostaining in the nuclei of four cases.

Conclusion: This study may indicate that p53 gene mutation could have a role in laryngeal carcinoma, but the stage at which p53 has its effect on initiation or progression of this disease still remains unclear. Ann Med Sci 2001;10:55-58

Key words: p53 gene, laryngeal carcinoma

The p53 gene alterations are the most frequently encountered genetic abnormalities in human neoplasms, and their roles in the development of human cancers have been demonstrated in a wide range of tumors, including colon, breast, lung cancers and hematopoietic neoplasms^{1,2}. Tumor suppressor gene products can suppress the function and/or expression of other genes involved in cell growth and proliferation. Only one locus of the gene mutation is sufficient to completely inactivate the suppressor function in the case of p53 tumor suppressor gene and even functional inactivation may be responsible for the loss of tumor suppressor function².

The p53 gene product was initially identified

as a host cell protein bound to the large T antigen which is a dominant transforming oncogene of simian virus 40, so p53 gene was first suggested as an oncogene. It is known that wild-type p53 acts as a tumor suppressor gene, and suppresses the cell transformation, while the mutant alleles act as oncogene and favor cell growth. Inactivation of the p53 gene may occur as a consequence of deletions, or by binding to viral oncoproteins or to host proteins².

The normal p53 protein has a very short half life (6-20 minutes), so the detection of the p53 protein usually implies a mutation, because mutant forms have longer half lives of up to six hours. The aim of this study is to ascertain the frequency of abnormal p53 overexpression in human laryngeal squamous cell carcinoma (LSCC) and to explore any correlation between

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this overexpression and the clinical parameters, such as histologic grade, stage, tumor size, and nodal involvement.

Materials and Methods

Forty formalin-fixed, paraffin-embedded tumor specimens of LSCC were obtained from patients (39 male, one female) whose tumors were resected at the Çukurova University Department of Otolaryngology between 1994-1996. Specimens containing not only carcinomas but also adjacent normal tissue and premalignant lesions were selected for this study. No radiation or other preoperative therapy was mentioned in the clinical history of the patients, and all of them were heavy smokers. Thirteen biopsy specimens of benign laryngeal nodule (9 males, 4 females) were used as controls. The mean age of patients with LSCC and laryngeal nodules was 57 (range 30 to 75) and 26 (range 10 to 40) years respectively. The patients with LSCC were staged according to American Joint Committee Staging System and the majority were Stage III and IV (87.5 %).

All specimens and histologic slides were reviewed to identify normal, dysplastic, and tumor areas, and to determine the type and grading of the tumors³. Grading of the tumors was made as follows: Tumors displaying prickly cells, orderly stratification and heavy keratinization with keratin pearls are classified as well differentiated; tumors showing prickly cells, some stratification with few or no keratin pearls are classified as moderately differentiated; and tumors with nuclear pleomorphism and hyperchromasia as well as many and atypical mitoses are classified as poorly differentiated tumors³. After identification of premalignant and carcinoma areas, immunohistochemical staining was applied. Avidin-biotin-complex method was applied (DO-7 Novocastra k-32; Novocastra, UK) to paraffin-embedded sections. Brown precipitates seen within the nucleus confirmed the presence of the p53 protein. The intensity and pattern of p53 immunostaining were tabulated as follow: positive if more than 10 % of tumor nuclei were intensely stained and negative, if less than 10 % of tumor nuclei were faintly stained or tumor nuclei were faintly stained or tumor nuclei were unstained⁴. The normal and premalignant lesions adjacent to tumors are also investigated for the presence of the p53 protein.

Fisher's exact test was used to compare the cancer and control groups, and *chi-square test* was used to evaluate differentiation, tumor size, nodal involvement, and tumor stage in statistical analyses.

Results

Samples from 19 of 40 (47.5 %) patients with LSCC demonstrated positive nuclear staining for p53. In contrast, there was no detectable staining in any of the 13 non-malignant laryngeal nodules which was statistically significant ($p=0.001$). In four cases (10 %), some of the dysplastic cells adjacent to the tumor tissue were also stained. The pattern of immunoreactivity was exclusively nuclear, and there was no reaction in normal epithelium or stromal cells. Variations were observed in the percentage of reactive cells as well as in the intensity of staining between nuclei of the same tumor (Figure 1).

Except the tumor grade, no significant correlation was found between p53 protein expression and tumor size, nodal involvement, and tumor stage (Table 1).

Table 1. p53 positivity in relation to the TNM, stage and grade of the lesions.

Study Group	No.Pts	p53 (+)	p53 (-)	p
T1	1	0	1	0.13
T2	7	3	4	
T3	18	6	12	
T4	14	10	4	
N0	24	13	11	0.45
N1	12	5	7	
N2	4	1	3	
Stage I	1	0	1	0.39
Stage II	4	2	2	
Stage III	20	7	13	
Stage IV	15	10	5	
Grade I (well diff)*	8	1	7	0.02
Grade II (moderately diff)*29	15	15	14	
Grade III (undiff)**	3	3	0	

*diff: differentiated

**undiff: undifferentiated

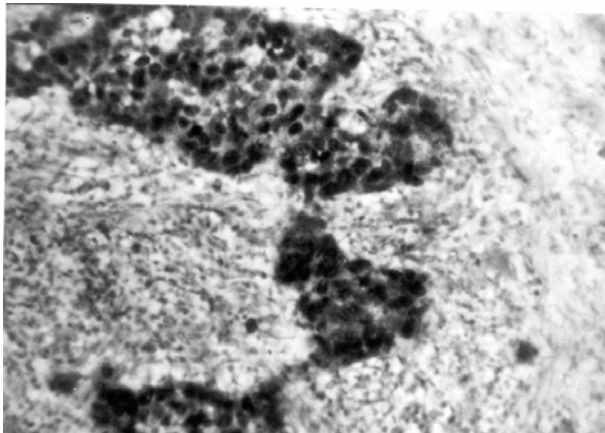


Figure 1. Immunohistochemical detection of p53 protein in laryngeal squamous cell carcinoma, demonstrating nuclear staining (X 600).

Discussion

The role of p53 gene in the development of human cancers has been demonstrated in a wide variety of tumors. Similarly, mutation or increased expression of the p53 protein in head and neck squamous cell carcinomas has been reported in many studies². A number of reports have considered the role of p53 gene to be a late event in the development of colonic cancer⁵, breast cancer⁶, and lung cancer⁷; however Chibia et al⁸ considered that mutations in the p53 gene occurred frequently in the early stages of non-small cell lung cancers. It is feasible that the p53 protein may have a number of roles in the development of head and neck cancer. This hypothesis is based on the observations relating to the data on p53 over-expression in dysplastic/carcinoma insitu lesions of larynx^{9,10}. However results of end stage head and neck cancer patients indicating that p53 expression correlates with very poor prognosis, suggest that p53 has a role in the late stages of the disease process¹¹. These studies show that the stage at which p53 gene has its effect on the initiation or progression of the disease remains unclear in LSCC.

In the present study we have investigated the tumor material, by immunohistochemistry, for the expression of p53 protein. Although this technique may underestimate the frequency of p53 mutations, false positivity due to infrequent non-mutational stabilization of the protein has also been observed in the literature¹². We identified p53 protein expression in 47.5 % of LSCC and 10 % of adjacent dysplastic tissues, but did not detect p53 protein in any of the benign laryngeal nodules as expected. The incidence of p53 protein expression in LSCC was reported as 60 – 78.5 % in several studies (Table 2)^{9,10,13,14,15}.

Our finding, 47.5 % positive staining, was found to be slightly lower than what have been reported in the literature. This could result from many different factors, including the use of different antibodies and different techniques for detection, differences in evaluation for positivity, heterogeneity of tumor for p53 expression, increased rate of degradation of p53 protein, or complete loss of p53 alleles^{9,16,17}.

Table 2. Comparison of p53 expression in squamous cell carcinomas of the larynx.

	No of Tumors Analysed	% Expressing p53
Maestro et al., 1992	58	60
Dolcetti et al., 1992	30	78.5
Anwar et al., 1993	43	65
Gorgoulis et al., 1994	40	77.5
Dursun et al., 1995	51	62.8
Present Series	40	47.5

There was not any correlation between p53 expression and tumor size, nodal involvement and stage of LSCC in this study. This result was supported with other studies in the literature where no correlation was found between p53 immunoreactivity and these parameters^{9,13,15}. However, in our study significant correlation was found between p53 expression and histological grading of the tumors, which was not found in previous studies^{9,10,13,14,15}. The continued basis for grading squamous cell carcinoma has been its presumed value in predicting biologic behavior. However recently no significant correlation between histopathologic grading scales and prognosis for laryngeal cancer has been demonstrated¹⁸. This significant correlation between p53 expression and histological grading may be coincidental or may indicate that p53 gene could have a role in the tumor behavior, but this theory needs to be supported with larger series.

In 4 of 19 patients (21 %) with positive staining with p53, expression of this protein was detected in the dysplastic epithelium adjacent to the tumor. Dolcetti et al¹³ undertook an interesting study on p53 expression in LSCC where they identified dysplastic areas and/or carcinoma in situ in conjunction with invasive carcinomas from the same patients. In the dysplastic and/or carcinoma in situ lesions that were found to be p53 positive staining, the corresponding invasive lesions were also positive staining. However in the negative staining tumors, the dysplastic regions were also found to be negative, but there was no correlation between p53 positivity and the prognosis of the disease.

Conclusion

P53 abnormalities occur at a very high frequency in LSCC. The p53 tumor suppressor gene may have a role in the development of LSCC, but the stage at which p53 has its effect on initiation or progression of this disease still remains unclear.

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