

## RESEARCH COMMUNICATION

# P53 Overexpression Impacts on the Prognosis of Laryngeal Squamous Cell Carcinomas

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### Abstract

**Objectives:** To investigate the prognostic implications of p53 expression in the surgical margins of laryngeal squamous cell carcinomas. **Methods:** Thirty one patients with T3-4N0M0 cancers with pathologically negative margins were analyzed by immunohistochemistry (IHC) to detect expression of p53. **Results:** The p53 positive rates by IHC in the surgical margin were 16.1% (5/31). In the p53 positive margin group, the recurrent rate was higher than those without (80% vs 19.2%,  $P = 0.006$ ). Also, the median free of disease period in the p53 positive margin group was shorter than other group (22.2 vs 47.8 months,  $P < 0.0001$ ). **Conclusions:** We found that the overexpression of p53 can serve a prognostic role for both recurrence and disease-specific mortality in head and neck squamous cell carcinoma. p53 expression could stratify patients, in an easy and inexpensive way, according to their risk of local or regional recurrence.

**Keywords:** Laryngeal SCC - P53 gene expression - tumour margins - recurrence - prognosis,

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### Introduction

Surgical resection is the principal treatment for the majority of advanced stage carcinomas of the upper aerodigestive tract and a common choice in treating early lesions as well. The single most important prognostic factor for squamous cell carcinoma of the head and neck (HNSCC) is complete surgical eradication of the neoplasm, because it is generally believed that failure to eradicate the primary tumor is the principal reason of death from this type of cancer (Armstrong et al., 2010).

When gross tumor remains, local recurrence is likely, leading ultimately to death. Similarly, if microscopic cancer is present at a margin of resection, the rate of local recurrence increases significantly and the survival rate declines. Local recurrence occurs in up to half of patients with even microscopically negative surgical margins, and in these patients it is the leading cause of treatment failure. The presence of metastatic squamous cell cancer in cervical lymph nodes also increases the risk of locoregional recurrence and distant metastatic spread and correlates with a 50 percent decrease in survival. The earliest stages of metastasis to the neck can be difficult to recognize by light microscopy. Small foci of metastatic cancer, called micrometastases, are often neglected because of sampling difficulties; a single 5-  $\mu$ m section through a 1-cm lymph node samples only 1/2000 of the

node (Ferlito et al., 2002).

It is important to ascertain new prognostic markers which may help to recognize the biological behavior of the cancer. Many molecular markers have been introduced to find more accurate prognostic factors for determining the accurate treatment plan and to improve overall survival of patients (Roepman et al., 2005). For a long time, it has been known that multiple genetic abnormalities occur during tumorigenesis. Determining which of these changes occur in a particular cancer might correlate with specific clinical and histopathological abnormalities as well as survival, and might help in predicting the risk of tumor development.

Change of the p53 gene is one of the single most commonly reported genetic abnormalities in many cancers, including those arising from the head and neck (Hardisson, 2003). This gene, which is located on chromosome 17p13, plays a critical role in the progression from premalignancy to invasive cancer, and its product controls critical cell functions associated with cell cycle regulation and apoptosis. Based on its properties, it might be expected that the p53 gene is an example for a prognostic marker. In most studies, the inactivation of the p53 gene has been investigated by determining the levels of p53 protein using immunohistochemistry (IHC) on the basis that the half-life of the wild-type protein is too short to permit detection, whereas the mutant protein is stable

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(Bosch et al., 2004).

In head and neck squamous cell carcinoma, several studies have reported that overexpression of p53 correlates with clinicopathologic findings of the tumor, such as lymph node metastases, and disease survival (Waitzberg et al., 2004). However, a number of other studies failed to show a correlation between p53 expression and these clinicopathologic variables (Bosch et al., 2004; Vlachtsis et al., 2005). The degree of heterogeneity in HNSCC in terms of the population of patients, disease site, stage, treatment, and supportive care restricts the suitability of HNSCC for analysis.

Most studies that have evaluated the prognostic significance of p53 have been performed in sets of patients with HNSCC from different sites (oral cavity, pharynx, and larynx). This heterogeneity introduces a bias for a correct interpretation of their results, because it is known that some of the genetic variations implicated in the development of HNSCC do not appear to be distributed equally among the different locations of this area (Rodrigo et al., 2001; Bosch et al., 2004; Freier et al., 2005). In an attempt to shed more light on this subject, we examined samples from 50 patients with squamous cell carcinomas of larynx. We analyzed p53 expression in 31 negative pathological margins for p53 expression using immunohistochemical (IHC) techniques. To avoid bias associated with the selection of the patients, the eligible patients were well balanced by tumor stage, they were treated in the same manner, and all of them had a long-term follow up. In this report, we discuss the prognostic implications of p53 expression in relation to other prognostic indicators and patient outcome.

**Materials and Methods**

This study comprises a review of fifty consecutive cases of laryngeal epidermoid carcinoma (T3-4N0M0) diagnosed at the pathology department of Imam Khomeini hospital between March of 2004 and September of 2008. All patients underwent total laryngectomy without neck dissection. All patients received standard adjuvant treatment as required, including postoperative radiation therapy. Two pathologists reexamined all specimens in a blinded fashion with standard light microscopy. In histopathologic examination using conventional hematoxylin-eosin staining techniques, there were positive margins in nineteen cases. The margins of samples (negative in histopathologic examination) were sent to immunohistochemistry to check for expression of antibody p53. IHC for P53 protein expression was assessed on 4-m serial sections of formalin-fixed paraffin embedded HNSCC. Breast carcinoma served as positive control, and squamous epithelium of the tonsil served as a negative control for p53. Slides were prepared with antigenic recovery through irradiation in micro-wave oven, blocking endogenous peroxidase, incubation with primary antibody p53 (Dako), reaction of the avidin-biotin-streptavidin complex + peroxidase kit (Dako®) and counterstaining.

At least 10 fields were examined under high power, and only nuclear staining was considered. P53 protein

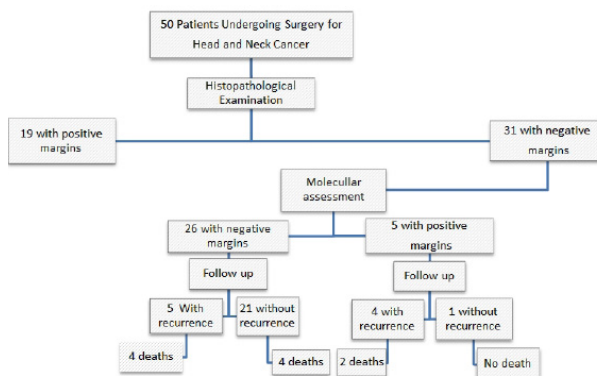
over-expression was scored as positive if greater than or equal to 5% of cells with nuclear staining were measured. This was based on criteria from our service, as there is no consensus in the literature as to the minimum thresholds to consider results as positive. In terms of molecular assessment, our patients were divided into two groups: patients with positive margins (N=5) and patients with negative margins (N=26). These patients (N=31) were followed up on a 6-monthly basis.

All statistical analyses were performed using the SPSS statistical software package (version 11.5; SPSS Inc., Chicago, Ill). The chi-square test or the Fisher exact test, as appropriate, was used to correlate p53 expression with the incidence of recurrences. Survival curves were calculated using the Kaplan-Meier product-limit estimate. Differences between survival times were analyzed by the log-rank method. A P value < 0.05 was considered statistically significant.

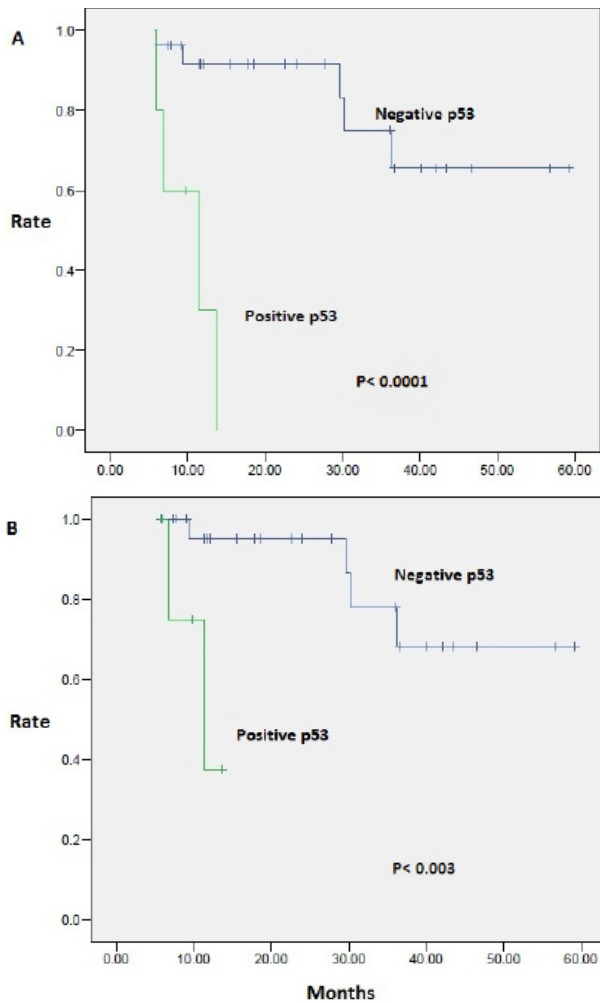
**Results**

Thirty one patients were followed up on a 6-monthly basis and the minimum, maximum, and mean follow-up times were 12, 56 and 23± 1.5 months, respectively. All patients were male and smokers. None of them consumed alcohol. Their ages of cases were between 47 and 79 and the mean age was 62±14 years. Five patients (15.6%) had supraglottic tumors, 3 (9.4%) had glottic and 24 (75%) had transglottic tumors. Most of the patients had advanced-stage squamous cell carcinoma of the larynx, as is typical in a tertiary referral center. In 5 of the 31 patients (16.1 percent, CI 95%: 5.4 - 33.7%), the amplified p53 region from at least one surgical margin hybridized to the tumor-specific probe, demonstrating the presence of neoplastic cells containing mutations.

During follow up, 9 patients developed recurrence. At follow-up, 4 of 5 patients (80 percent, CI 95%: 28.3 - 99.4%) with positive margins by molecular analysis had biopsy-proved recurrences of carcinoma (Figure 1). All four recurrences occurred by the 14th month and the median follow-up in the 5 patients was 24 months. However, only 5 of 26 patients (19.2 percent, CI 95%: 6.5 - 39.3%) whose surgical margins were negative by the same technique had recurrent disease (P < 0.006). The median free of disease period in these 5 patients was



**Figure 1. Molecular Analysis and Histopathological Assessment of the Surgical Margins of 50 Patients with Laryngeal Squamous Cell Carcinoma**



**Figure 2. Kaplan-Meier Recurrence-specific Curves (A) and Death-specific (due to recurrence) Curves (B) for Patients with p53 Expression in Tumor Margins**

22.2 months. The median follow-up in 26 patients was 47.8 months (range, 39 to 56). The odds ratio 16.8 was estimated for recurrence. Also, the patients with positive margins were involved recurrence earlier than the patients with negative margins ( $p < 0.0001$ ) (Figure 2A).

Ten patients died from their primary tumor in the follow-up period (32.2%). Six deaths (60%) caused by the tumor. Four patients who died from causes that were not related to the index tumor were excluded from all analyses that involved the length of survival. In the positive margins, there were 2 deaths (7 and 12 months) for disease-specific mortality. In the negative margins, there were 8 deaths (mean 42.6 months) for all the cause mortality and 4 deaths for disease-specific mortality (mean 35.3 months). As the same of recurrence, the patients with positive margins deceased earlier than the patients with negative margins ( $p < 0.003$ ) (Figure 2B).

**Discussion**

p53 changes occur early in head and neck carcinogenesis and can be detected in premalignant lesions. It is observed that p53 mutations are present before and maintained in the metastasis. Homann et al reported the association between mucosal p53 overexpression and an increased incidence of second primary cancer (Homann et al.,

2001). One of the most important prognostic factors in HNSCC is complete surgical resection of the tumor and the presence of microscopic residual tumor cell in tumor margin increases the rate of local recurrence, it is suggested that the identification of p53 alterations could be of use in follow-up HNSCC patients.

We have demonstrated by the molecular detection of tumor-specific p53 mutations that 16.1 percent (5 of 31) of our patients with squamous-cell carcinoma of the head and neck who underwent cancer resections presumed to be complete actually had positive surgical margins.

We found lower rate of p53 mutation in surgical margins than previous studies. Another researches reported the p53 mutations in 42-60% of head and neck cancers (Brennan et al., 1995; Rodrigues et al., 2008). There are several possible explanations for the observed dichotomy in the results, including differences in methodology and cutoff points used to define p53 overexpression. The cut point for p53 of  $\geq 5\%$  was based on our analysis of p53 alterations in Laryngeal cancer. Another possible explanation is our selected patient population. We know that there is relationship between tobacco and alcohol consumption and the prevalence and pattern of p53 mutations (relative risk, 3.5) (Cabanillas et al., 2007). Neither of our patients consumed alcohol. Also, it may be due to interpretive errors in immunohistochemical technique by the pathologist.

The p53 protein is involved in the maintenance of the cellular integrity after DNA damage. Correlation between the occurrence of p53 alteration and poor prognosis in HNSCC patients is still a matter of debate (Bosch et al., 2004; Almadori et al., 2005). We found that the overexpression of p53 can serve a prognostic role for both recurrence and disease-specific mortality in HNSCC (Blons et al., 2003). The eighty percent of our patients with positive p53 in margins recurrences of carcinoma as compared with 19.2% of the patients with negative margins. Similarly to our study, Brennan et al. showed that 38% of patients with positive for p53 mutations in margins had recurred locally as compared with none of the 12 patients with negative margins (Brennan et al., 1995).

Nine of 31 patients (29%) had recurrence. This unexpected incidence of treatment failure can be due to high stage SCC (T3 or T4) in our patients.

In conclusion, the findings presented in this paper suggested that overexpression of p53 in the margins of tumor could be a gross predictor of clinical outcome. The assessment of p53 mutation estimates the extension of the disease more accurate than clinical TNM. The p53 expression could stratify patients, in an easy and inexpensive way, according to their risk of local or regional recurrence (Cabanillas et al., 2007). The immunohistochemistry is simple to perform and evaluate. It also is cheap, fast, and can be used readily on the same small biopsies that are used to diagnose head and neck tumors.

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