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ORIGINAL RESEARCH

Evaluation of Epithelial Dysplasia at the Surgical Margins in Patients with Early Tongue Carcinoma — Immunohistochemical Study of Recurrence of Epithelial Dysplasia

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Abstract

Objective: *The diagnosis of epithelial dysplasia at the surgical margins in oral cancer plays an important role in its prognosis and requires careful evaluation. Epithelial dysplasia is usually asymptomatic, but often associated with recurrence and malignant transformation.*

Patients and Methods: *The patients included 9 men and 5 women (mean age, 54.6 years; range, 31 to 76 years) with early tongue carcinoma and tumour-cell-"free" surgical margins. Immunohistochemical analysis of p53 and Ki-67 expression was performed in order to evaluate epithelial dysplasia and pathological changes.*

Results: *None of the patients had cancer recurrence, but 2 showed recurrence of epithelial dysplasia. The resection margins at initial surgery and at recurrence were positive for p53 and Ki-67 in both these patients.*

Conclusions: *Epithelial dysplasia, with positive staining for p53 and Ki-67, has the potential for morphological changes, with possible recurrence of epithelial dysplasia or malignant transformation. Such patients will require careful follow-up for local recurrence. Examination of surgical margins for these markers is useful for the early detection of pathological changes.*

Key words: *Ki-67 antigen, Precancerous conditions, Recurrence, Tongue neoplasms, Tumor suppressor protein p53*

Introduction

The accurate diagnosis and treatment of premalignant oral lesions and epithelial dysplasia in the periphery is an important factor in improving oral cancer cure rates and prognosis. Diagnostic criteria for epithelial dysplasia differ between medical centres, and differential diagnosis from early cancer is often difficult. Epithelial dysplasia is often asymptomatic, but recurrence and malignant transformation are common. The diagnosis of epithelial dysplasia and early carcinoma requires uniform evaluation criteria and the prognosis of these oral lesions requires careful evaluation of the surgical margins in each patient. The immunohistochemistries of p53, a tumour suppressor gene, and Ki-67, a marker of cell proliferation, have been used to evaluate premalignant and malignant oral lesions,[1-4] but few studies have investigated the clinical usefulness of these tumour markers in the surgical margins of resected lesions.[5-7]

To establish prognostic factors for epithelial dysplasia and early invasive cancer, we performed immunohistochemical staining for p53 and Ki-67 in epithelial dysplasia of surgical margins from patients undergoing surgery at our hospital for early tongue cancer. Few, if any, studies have compared the histopathology findings of initially resected lesions and recurrence. In this report, we describe findings in recurrent epithelial dysplasia after tongue surgery.

Patients and Methods

Patients

This study included 14 patients with T1 and early T2 tongue cancer, diagnosed and treated at our hospital between April 1998 and March 2005, with histological findings of

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carcinoma (negative) and dysplasia (positive) at the surgical margins. There were 9 men and 5 women, with a mean age of 54.6 years (range, 31 to 76 years). Surgery consisted of tumour excision after preoperative biopsy or total excision and biopsy after preoperative cytology. Vital staining with iodine was done in all patients, and the surgical margins were about 5 mm from unstained areas. If intraoperative rapid histological examination showed severe dysplasia, further resection was performed. Serial sections were prepared by bread-loaf step sectioning.[8] The surgical margins were examined in all patients. The tumour type in all cases was squamous cell carcinoma (N0). Tumour invasion was limited to the lamina propria mucosa, without invasion into the muscularis propria. The study was performed in accordance with the guidelines of the ethics committee at our hospital.

Immunohistochemistry

Serial sections of each specimen were stained by the labelled streptavidin-biotin (LSAB) method using standard procedures. Namely, 5 μm thin sections were deparaffinised with xylene and then treated with 0.3% methanolic peroxide for 15 minutes to block endogenous peroxidase. For antigen retrieval, the specimens were immersed in 0.01 M citrate buffer and irradiated with microwave energy (750 Watts) for 5 minutes. This was performed twice. Following washing with 0.01 M phosphate-buffered solution, the specimens were reacted with p53 (DO-7; DAKO, Japan; diluted 1:200) and Ki-67 (MIB-1; Immunotech, France; diluted 1:200) as primary antibodies for 60 minutes at room temperature. The slides were incubated with secondary antibody and peroxidase-labeled streptavidin using an LSAB2 Kit (Dako, Japan) and visualised with 3,3'-diaminobenzidine.

Epithelium of the surgical margins was examined under 200 times magnification, with observation of the basal, prickle, and granular cell layers for positive findings. The findings were judged p53- and Ki-67-positive if at least 20 of 500 cells were positive.[3,4]

Results

Of the 14 patients in this study, surgical margins were positive for p53 and Ki-67 in 5 and 7 patients, respectively. None of the patients had recurrent cancer, but 2 had recurrent epithelial dysplasia. The other patients are doing well and being followed-up on a regular basis (Table 1).

Epithelial dysplasia in the surgical margins, graded in accordance with the 1997 World Health Organization (WHO) diagnostic criteria, was mild in 4 patients, moderate in 8, and severe in 2 patients. In this study, no correlation was found between being positive for p53 or Ki-67 and the severity of dysplasia based on the WHO criteria.

Case Presentations

Patient No. 1

Initial Lesion

Histopathology findings: no tumour cells were present in the surgical margins, but drop-shaped rete ridges consistent with moderate dysplasia were observed (Figure 1a). Slightly central to the margin, immunostaining for p53 was positive in cells from the basal cell layer to just below the granular cell layer (Figure 1b). Scattered Ki-67 positive cells were found in the rete ridges (Figure 1c).

Recurrent Lesion, 3 Years 2 Months Postoperatively

1) Intraoral findings: a 6-mm area of leukoplakia was found in the mucosal transition between the tongue and floor of mouth, corresponding to the initial posterior resection margin (Figure 2). Because of a suspicion of tumour recurrence, total excision and biopsy were performed.

2) Histopathology findings: hypertropic epithelium and hyperparakeratosis were present in the entire surgical margin,

Table 1. *Results of follow-up examination of surgical margins in patients.*

Case no.	Age (years)/ gender	Margin dysplasia	p53 positivity	Ki-67 positivity	Follow-up period	Clinical course
1	76/female	Moderate	Positive	Positive	8 years 1month	Recurrent dysplasia (postoperative, 3 years and 5 years)
2	63/male	Mild	Negative	Negative	7 years 9 months	
3	54/male	Moderate	Negative	Negative	7 years 1 month	
4	47/female	Moderate	Negative	Positive	6 years 3 months	
5	52/male	Mild	Negative	Negative	5 years 1 month	
6	75/female	Moderate	Positive	Negative	4 years 8 months	
7	51/female	Moderate	Positive	Negative	4 years 6 months	
8	50/male	Severe	Negative	Positive	4 years 5 months	
9	53/male	Mild	Negative	Negative	4 years 2 months	
10	37/male	Moderate	Positive	Positive	2 years 6 months	Recurrent dysplasia (postoperative, 1 year 10 months)
11	57/male	Moderate	Positive	Negative	2 years 4 months	
12	46/female	Mild	Negative	Positive	2 years 3 months	
13	73/male	Severe	Negative	Positive	1 years 7 months	
14	31/male	Moderate	Negative	Positive	1 years 2 months	