Abstract  The purpose of the present study was to investigate the clinical usefulness of the detection of serum p53 antibodies (p53 Abs) in patients with oral squamous cell carcinoma (SCC). Preoperative values of p53 Abs were measured by enzyme-linked immunosorbent assay in 113 patients with primary oral SCC and seropositive patients were reevaluated postoperatively. The positivity rate of p53 Abs was 16%, and the 5-year survival rate of patients positive for p53 Abs was significantly lower than that of patients negative for p53 Abs (56.2% vs. 80.7%; \( P = 0.018 \)). The preoperative presence of p53 Abs was found to be an independent prognostic factor in a multivariate analysis (\( P = 0.028 \), hazards ratio = 3.34), and its positivity was significantly related to secondary cervical lymph node metastases (\( P = 0.029 \)). Six of nine patients who remained seropositive for p53 Abs through the disease course and the one with seropositive reversion from temporary negative status developed treatment failure. Therefore, the detection of p53 Abs in the serum of patients with SCC may be a useful prognostic marker.

Key words  p53 \cdot p53 antibodies \cdot Serum \cdot Prognosis \cdot Oral squamous cell carcinoma

Introduction

At present the only clinical tumor marker used for the evaluation of oral squamous cell carcinoma (SCC) is SCC antigen (Ag).\(^1\) Although the frequency of serum positivity for SCC Ag increases with advancement of the clinical stage or the extent of the disease, it is seldom elevated at the early disease stage,\(^2\) whereas it is elevated in several benign lesions, including pulmonary infection and renal dysfunction.\(^3\) SCC Ag is reported to be too insensitive to use as a postoperative monitoring marker to detect early relapse of the disease;\(^4\) hence, additional tumor markers are being investigated.

Alteration in the \( p53 \) tumor suppressor gene is the most common genetic change in human cancers.\(^5\) This alteration leads to an accumulation of mutant p53 protein in the nucleus of tumor cells with a half-life of several hours, compared with 20 min for wild-type p53 protein, and it can induce a specific humoral response in cancer patients, namely, the production of serum p53 antibodies (Abs).\(^7\) Recently, a quick, simple method to detect serum p53 Abs with the use of an enzyme-linked immunosorbent assay (ELISA) kit has been established.\(^8\) Such antibodies are predominantly associated with \( p53 \) gene missense mutations, and p53 accumulation in the tumor and consequently serological analysis of p53 Abs might be suitable as an indirect procedure to diagnose p53 alteration. The presence of serum p53 Abs in patients with premalignant or malignant oral lesions has been reported,\(^9\) but there is little information about clinicopathological and prognostic significance of preoperative p53 Ab status in patients with oral cancer.\(^10\) Changes in the values of serum p53 Abs during postoperative follow-up of patients with oral SCC have been little reported.\(^\text{11}\)

The aim of the present study was to investigate the usefulness of the detection of p53 Abs as a tumor marker to assist in the determination of the prognosis for oral SCC.
Materials and methods

Patient clinical information and collection of blood samples

A total of 113 previously untreated patients with primary oral SCC who underwent treatment between January 1996 and June 2003 at Hokkaido University Dental Hospital, Sapporo, Japan, were enrolled in this study. The present protocol was approved by the Ethics Committee of Hokkaido University. The median follow-up time for survivors was 51 months (range, 7–104 months). The patients consisted of 70 men and 43 women, with a mean age of 64 years (range, 28–91 years). The tumor sites were the tongue in 54 patients, the gingiva in 32, the cheek in 16, the floor of the mouth in nine, and the hard palate in two patients. All tumors were classified according to the TNM system of the International Union Against Cancer (UICC).15 Eighteen tumors were stage I, 43 were stage II, 20 were stage III, and 32 were stage IV. Serum samples were collected from all 113 patients at routine blood sampling, prior to any treatment after diagnosis. Whole blood was centrifuged at 800 g for 15 min, and the supernatant was stored at −80°C until analysis. Clinical outcomes of 102 patients who were followed for more than 36 months after surgery were evaluated. When possible, postoperative titration of p53 Abs was re-evaluated every 3 months in the preoperatively seropositive patients.

Enzyme immunoassay for serum p53 antibodies

Serum p53 Abs were assessed by ELISA with an ANTI-P53 ELISA II Kit (Pharmacell, Paris, France). In brief, the samples were added to the wells of a microtiter plate coated with recombinant wild-type human p53 or a control protein and were incubated for 1 h at 20–25°C. The plates were washed four times with a wash solution. Peroxidase-conjugated goat antihuman immunoglobulin G-binding anti-p53 Ab was added to each well, and the plate was incubated for 1 h at 20–25°C. The washing procedure was repeated, and then the substrate 3, 3′,5,5′-tetramethylbenzidine was added, followed by a 10-min incubation at 20–25°C. After addition of a stop solution (2 N H2SO4), the color reaction was measured immediately by absorption at 450 nm with a photospectrometer (model 680, BioRad, Hercules, CA, USA). A calibration curve was constructed from the specific signals of standards and from the levels of Abs indicated on the standard vials. The calibration curve was a linear regression curve that intersected the x axis at 0. Levels of anti-p53 Abs were then determined from the calibration curve. The cut-off value was determined as 1.3 U/ml, as previously reported by Shimada et al.5 Seven of the 153 healthy control donors were positive for p53 Abs (specificity, 95.5%) according to the study.

Enzyme immunoassay for squamous cell carcinoma antigen

SCC Ag in serum was tested with a radioimmunoassay kit (SCC-RIABEAD, Dainabot, Tokyo, Japan). The cut-off concentration of 1.5 ng/ml was in accordance with the manufacturer’s instructions.

Statistical analyses

The χ-squared test and Fisher’s exact test were used to determine significance levels between the two groups. The Kaplan-Meier method was used to generate survival curves, which were evaluated by the log-rank test. The clinicopathological variables associated with cause-specific survival were evaluated first in a univariate analysis and then in a multivariate analysis using the Cox proportional hazards model. All P values less than 0.05 were considered statistically significant.

Results

Eighteen (16%) of the 113 patients with primary oral SCC were positive for serum p53 Abs. This positivity rate was significantly higher (P = 0.002) than that of healthy control donors (control data previously reported). Table 1 shows the clinicopathological factors in oral SCC patients with and without p53 Abs in the serum. There was no association of p53 Abs with any of these parameters (age, sex, primary tumor site, stage, TN classification, World Health Organization histological grade, or mode of invasion). Table 2 shows clinical outcomes according to p53 Ab status. A significant difference was observed between p53 Ab status and secondary cervical lymph node metastases (P = 0.029); secondary cervical lymph node metastases were present in four of ten (40%) patients positive for p53 Abs, but in only seven of 69 (10%) patients negative for p53 Abs. There was a significant relationship between the p53 Ab status and the cause-specific cumulative survival rate (P = 0.018); 5-year survival rates were 56.2% and 80.7% in p53 Ab-positive and negative patients, respectively (Fig. 1). Locoregional failure and distant metastases without locoregional failure were unrelated to p53 Ab status.

We evaluated the effects of a number of prognostic variables on overall survival. Tumor stage, nodal involvement, and p53 Ab status were found to be significant prognostic factors in a univariate analysis (Table 3). Multivariate analysis using the Cox proportional hazards model showed the presence of p53 Abs to be an independent prognostic factor (P = 0.020, hazard ratio = 3.509), as was nodal involvement (P = 0.039, Table 4).

Of the 18 patients positive for serum p53 Abs preoperatively, we were able to follow periodically 12 patients postoperatively; 11 of these patients underwent surgery alone, and one patient had preoperative radiotherapy (Fig. 2). Three of the 12 seropositive patients became seronegative after addition of a stop solution (2 N H2SO4), the supernatant was stored at −80°C until analysis. Clinical outcomes of 102 patients who were followed for more than 36 months after surgery were evaluated. When possible, postoperative titration of p53 Abs was re-evaluated every 3 months in the preoperatively seropositive patients.

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