# Monitoring of Circulating Tumor-associated DNA as a Prognostic Tool for Oral Carcinoma

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Abstract:The objective of our study was to assess the possibility of prognostication and monitoring of oral squamous cell carcinoma by microsatellite blood assay. DNA from normal and tumorous tissues and serum DNA obtained at three time points (preoperatively, postoperatively, and 4 weeks postoperatively) from 64 patients with oral squamous cell carcinoma was examined at nine microsatellite loci. Thirty-eight (59%) DNA samples from tumorous tissues and 52% from serum showed allelic imbalances in at least one locus. Patterns of allelic imbalances in serum DNA were matched to those detected in tumor DNA. Of them, allelic imbalances were frequently detected preoperatively (44%, 28/64), and postoperatively (20%, 13/64). Moreover, among 12 cases with allelic imbalances during the postoperative period, six had no evidence of an allelic imbalance 4 weeks postoperatively, and they had no recurrence and were disease free. In contrast, six patients with allelic imbalance-positive DNA 4 weeks postoperatively have died with distant metastasis within 44 weeks. Thus, our results suggest that the assessment of microsatellite status in serum DNA could be a useful predictive tool to monitor disease prognosis.

Keywords: oral carcinoma; allelic imbalance; circulating tumor DNA, prognosis

## Introduction

Despite its clinical importance, at present there is no predictive procedure related to the recurrence, metastasis, or both. It is widely accepted that the accumulation of genetic damage is a major cause of the development of human malignancies. Thus, DNA can provide one of the most direct sources for potential markers. Because sample materials for diagnosis should be easily accessible by a minimally invasive procedure, there has been much interest in the potential use of nucleic acid markers in the blood of patients with cancer. Allelic imbalances (AIs) appearing as loss of heterozygosity (LOH) or as microsatellite instability (MSI) have been detected in the circulating DNA of patients with a variety of malignancies. Nawroz et al[1)] first demonstrated that AIs could be detected in plasma/serum DNA of head and neck SCC, suggesting that circulating tumor-associated DNA in the blood of patients with oral SCC can be a key determinant in predicting tumor recurrences or metastasis.

In the present study, we evaluated the microsatellite status of serum-isolated DNA preoperatively, postoperatively, and 4 weeks postoperatively from patients with oral SCC using nine microsatellite markers recently reported to be frequent loci showing AIs in primary oral SCCs.

## Materials and Methods

Patients:

Sixty four patients who underwent primary oral SCC resection at the Division of Dentistry and Oral-Maxillofacial Surgery, Chiba University Hospital were selected to participate in the present study. No patients underwent a blood transfusion. Informed consent was obtained from all patients and the patients' families, and our protocol was reviewed and approved by the institutional review board of Chiba University.

*DNA Isolation and Microsatellite Analysis*

Peripheral blood (5 ml) samples were collected preoperatively, postoperatively, and 4 weeks postoperatively. Blood samples were centrifuged in EDTA tubes for 10 min at 3,000 g to obtain the serum from the supernatant. Tumor tissues were obtained at the time of surgical resection. The peripheral lymphocytes of each patient were used as a source of normal DNA. DNA was extracted from tissue, sera, and lymphocytes using a QIAamp Blood and Tissue Kit (Qiagen, Hilden, Germany) as recommended by the manufacturer. Nine microsatellite markers on seven chromosome arms were selected for high polymorphism, small size of amplified fragment, and location at sites frequently undergoing AIs in oral SCC. The following microsatellite markers purchased from Research Genetics were selected based on previous reports[2-7)]: D5S178 (5q21), D9S104 (9p21), IFNA (9p22), D11S910 (11q23), D11S1356 (11q25), D13S273 (13q14-21), TP53 (17p13), D18S46 (18q21), and D22S274 (22q13). Polymerase chain reaction (PCR) amplification and assessment of AIs were performed according to these previous papers. Significant differences were calculated by Fisher's exact test.

## Results

Using a panel of nine microsatellite markers, we checked for the presence of tumor-associated DNA in serum by comparing the markers with each normal control in the 64 patients with oral SCC. Of all patients with AIs (n=38), 5 patients had only one AI in tumor tissue DNA without AI in serum obtained at any time point. Therefore, it was possible to detect at least one AI in serum DNA in 87% (33/38) of the patients with AI in tumor DNA, suggesting the presence of circulating tumor DNA. All AI patterns in sera were matched to those detected in tumor DNA from the

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identical patients, indicating that the presence of AI in serum was associated with the presence of AI in paired tumor. Free-circulating tumor-associated DNA was detected preoperatively (44%, 28/64) and postoperatively (20%, 13/64). Among the patients with AI preoperatively (n=28), 20 (71%) had no evidence of AI in sera postoperatively, they have had no recurrence or distant metastasis, and are disease free. In contrast to AI-free patients, two patients with AI-positive DNA 4 weeks postoperatively died with distant metastasis within 44 weeks after surgery. The most frequently altered chromosome in serum DNA was at the IFNA locus on 9p21 (40%, 15/38).

## Disucussion

In the current study, we evaluated the hypothesis that detecting circulating tumor-associated DNA using tumor-specific AIs might have clinical use in oral SCC, and our data on oral SCC suggest that the detection of free-circulating tumor-associated DNA during the postoperative period may be of prognostic value. Since we selected serum samples at different time points with high polymorphic markers, the high prevalence of AIs could be detected in this study. The most frequent marker with AI was the IFNA locus at 9p21, where p16 and p15 tumor suppressor genes frequently mutated, deleted, and methylated in various types of human cancers are in close proximity. In this context, the presence of a chromosomal deletion at the 9p21 locus has been reported to be linked to local, regional, or distant recurrence in head and neck SCCs[8].

It is of interest to note that while most AI-positive patients (84%, 32/38) had lost the tumor-associated DNA in their serum by 4 weeks postoperatively, AI in the serum DNA still was detected in six patients 4 weeks after surgery, and those patients had a poor prognosis with distant metastasis. Furthermore, the AI was detected not only in tumor DNA but also in serum at all time points. At present, we have no exact explanation for why these patients still had AIs even at the last time point examined. However, the proposed mechanisms may include: 1) differences in activation of their tumor cells; 2) differences in the number of tumor cells; and 3) decline of their autoimmune systems. Thus, we propose that the microsatellite blood assay should be considered as a clinically important monitoring tool for assessing patient response to adjuvant therapy and in the surveillance of patients who are clinically disease-free for the earliest signs of recurrence or distant metastasis.

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