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Loss of Heterozygosity and Microsatellite Instability on the Long Arm of Chromosome 2 in Human Oral Squamous Cell Carcinoma

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ランニングタイトル： Loss of heterozygosity on chromosome 2q in oral cancer

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原稿の分類・・・ Short Communication

Abstract

Frequent allelic imbalances, including loss of heterozygosity (LOH) and microsatellite instability (MSI), have been found on the long arm of chromosome 2 (2q) in several types of human cancer. This study was designed to identify the tumor suppressor locus (or loci) associated with oral squamous cell carcinoma (SCC) on 2q. In order to understand the details of genetic alterations on chromosome 2, we performed polymerase chain reaction analysis of microsatellite polymorphisms corresponding to 10 loci on this chromosome. We identified a novel tumor suppressor locus in this region in primary oral SCCs. To further determine the role of 2q deletions in oral cavity carcinogenesis, 19 oral SCCs (19 sets of primary and corresponding normal tissues) were examined for allelic imbalances (LOH or MSI) on 2q using 10 microsatellite markers. Among these 19 patients, 11 (57.9%) showed LOH at one or more loci. Deletion mapping of these tumors revealed four discrete, commonly deleted regions on the chromosome arm. Furthermore, we detected MSI in 4 of those tested cases (21.1%). We compared our results with the clinicopathologic features. A number of sites displaying LOH on 2q could be detected in early stage lesions, and

the frequencies of LOH tended to be higher in later clinical stages, but no statistical correlation was observed. Our results suggest that allelic imbalances on 2q are involved in the development of oral SCC and that at least one or more putative tumor suppressor genes contributing to the pathogenesis of this disease are present on 2q.

Key words: Chromosome 2, Oral squamous cell carcinoma, loss of heterozygosity, Microsatellite instability, Tumor suppressor gene

INTRODUCTION

The activation of oncogenes and inactivation of tumor suppressor genes are considered to play important roles in multistep carcinogenesis in humans[1)]. Losses of alleles on a specific chromosome suggest the presence of a tumor suppressor gene. Such allelic losses have been detected as a loss of heterozygosity (LOH) and microsatellite instability (MSI)[2-3)], demonstrating their usefulness for the mapping of DNA regions in which an unknown tumor suppressor gene may be present[4)].

In this study, assuming that a novel tumor suppressor specific to oral squamous cell carcinoma is present on chromosome 2, we prepared a detailed deletion map of 2q by a comparison of tumor and normal tissue DNAs in individual patients with oral squamous cell carcinoma, and investigated the following items: 1) Evaluation of LOH and MSI, and identification of the locus of a novel unknown tumor suppressor gene specific to oral squamous cell carcinoma, and 2) association with clinicopathological characteristics.

MATERIALS AND METHODS

The subjects were 19 patients diagnosed with oral Squamous cell carcinoma at the Oral Maxillofacial surgery Department of Tokyo Dental College Chiba Hospital from whom sample specimens could be obtained. The primary tumor and corresponding normal tissues were used (Table 1). From the tumor and normal tissues, DNA was extracted and purified by phenol-chloroform extraction, and washed and concentrated by ethanol precipitation. Each DNA sample was adjusted to 50 ng/μl, and used as a template for the polymerase chain reaction (PCR) to amplify the DNA.

PCR microsatellite analysis was performed using markers of 10 microsatellite regions on the long arm of chromosome 2 (Table 2). In statistical analysis, the significance of the relationship between the frequencies of LOH and MSI and the clinicopathological characteristics was analyzed using Fisher's direct probability method.

RESULTS

LOH was noted at least at one gene locus in 11 of the 19 patients (57.9%). MSI was noted in 4 of the 19 patients (21.1%). Deletion maps of the 19 patients were prepared, and one common deficient region was identified on this chromosome (Fig. 1). The gene locus with a high frequency of LOH was D2S206 in the 2q36 region (33.3%).

The relationship between the clinicopathological factors and the presence of LOH and MSI was investigated. LOH was more frequently noted in females, and MSI was noted only in males. Regarding the location, LOH was frequently detected in the gingiva, and MSI was noted only in the tongue. On comparison of the T and stage classifications, LOH was frequently detected in early-stage cases (Table3).

The correlation between the imbalance of alleles (LOH and MSI) and the clinicopathological factors was analyzed by Fisher's direct probability method, but no significant correlation was noted.

DISCUSSION

Deletion maps of the 19 patients were prepared, and one region with a high frequency of LOH, which may be a common deficient region on 2q, was identified at D2S206 in the 2q36 region. In this region, a deficient region common to granular cell tumor has recently been identified by Watson et al.[5], suggesting that a tumor suppressor gene related to oral squamous cell carcinoma and granular cell tumor is present in this region. Since no gene in the 2q36 region has been cloned, the identification of the region considered to be a common deficient region in the 2q36 region strongly suggested that a novel unknown tumor suppressor gene is present.

LOH was frequently detected in the gingiva in relatively early cancer, suggesting that inactivation of the tumor suppressor gene occurred in an early step, and the frequency of inactivation may vary depending on the location. Extensive analysis of an increased number of patients, and large-scale gene analysis with frequent and careful course observation of oral cancer patients are necessary for the realization of an optimal treatment for individual patients.

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Table 1 :CASE

CASE	Gender	Age	Site	T	N	Stage	Differentiation	Mode of invasion	pN	prognose (duration)
1	M	47	Tongue	1	0	*I*	Well	3	+	Alive (53 months)
2	M	61	Tongue	2	2a	*IV*	Well	4C	-	Alive (44 months)
3	M	77	oral floor	2	1	*III*	Mod.	4C	+	Alive (30 months)
4	F	69	Gingiva	4	2b	*IV*	Well	3	+	Alive (29 months)
5	M	66	palate	1	0	*I*	Well	2	/	Alive (45 months)
6	M	74	Tongue	2	1	*III*	Well	3	-	Alive (31 months)
7	M	61	Tongue	2	0	*II*	Well	3	/	Alive (47 months)
8	M	36	Gingiva	2	0	*II*	Mod.	4C	/	Alive (48 months)
9	M	76	palate	1	0	*I*	Mod.	4C	+	Died (39 months)
10	M	39	Tongue	1	0	*I*	Well	4C	+	Died (28 months)
11	M	46	Tongue	1	0	*I*	Poor.	4C	+	Died (11 months)
12	M	51	Tongue	2	1	*III*	Mod.	3	-	Alive (43 months)
13	M	64	oral floor	4	2c	*IV*	Poor.	3	+	Alive (49 months)
14	M	59	Gingiva	2	1	*III*	Well	3	-	Alive (51 months)
15	M	60	Tongue	1	0	*I*	Well	3	/	Alive (33 months)
16	M	79	Tongue	1	1	*III*	Poor.	3	-	Alive (29 months)
17	F	80	Gingiva	2	0	*II*	Well	4C	+	Died (21 months)
18	F	68	Gingiva	2	2b	*IV*	Mod.	3	+	Died (10 months)
19	F	55	Gingiva	1	0	*I*	Well	3	-	Alive (50 months)

M: Male, F: Female. Well: well differentiated, mod: moderately differentiated, poor: poorly differentiated

Table 2：Microsatellite markers

Markers	Locations	Size of PCR products (bp)	Sequence of primers
D2S436	2q11.1-14	192	5/-GATATGGGAGCAACATGAGC-3/ 5/-GGAATCAACTTTCAGTATAAACCC-3 /
D2S1328	2q14-21	157	5/-GTGGCTTTGGAGGAACACTA-3/ 5/-TGGCACATGTACACCAGAAC-3 /
D2S111	2q23-33	126-140	5/-TTTTCTTTTTTGCAGTTTATCC-3/ 5/-CACTTCAGTGCCTTCTTGAGA-3 /
D2S202	2q32	265	5/-AAGGCAGATCCAAGTACTCA-3/ 5/-CATAAGCAACTGATTAGAACC-3 /
D2S1327	2q32-35	162	5/-TGACCAGGGGAAGATACTGA-3/ 5/-TGAATTGAATAATAACACTCTGTGC-3 /
D2S116	2q33	134-150	5/-CAATCTCCACAAGTTGCTCA-3/ 5/-GGGATAGATAATTTAGGAGTGGG-3 /
D2S155	2q35	163-171	5/-ACAGAAAACATACGTGTGTG-3/ 5/-CGGAACCTAGCAAAACTAC-3 /
D2S164	2q35	265-303	5/-GTCCTAACAGGCCACAGACC-3/ 5/-GCTGGCAGTATCACATGACA-3 /
D2S133	2q36	283-301	5/-CAGGAATCCAAGACAGACAG-3/ 5/-CAGATAGTAACTGTATATCAAGGGG-3 /
D2S206	2q36	123-151	5/-TTAAAAATTAAGTAGGCTTTTGGTT-3/ 5/-GTCCTCATGTGTTTATGCTGT-3 /

Table 3: Frequencies of LOH and MSI (%) in relation to clinicopathological factors

		LOH (+)	LOH (%)	MSI (+)	MSI (%)
Gender	Male	8/15	53.3%	4/15	26.7%
	Female	3/4	75%	0/4	0%
Site	Tongue	4/9	44.4%	4/9	44.4%
	Gingiva	5/6	83.3%	0/6	0%
	other	2/4	50%	0/4	0%
T classification	T1	5/8	62.5%	2/8	25%
	T2	6/9	66.7%	2/9	22.2%
	T4	0/2	0%	0/2	0%
TNM stage	I	5/7	71.4%	1/7	14.3%
	II	2/3	66.7%	1/3	33.3%
	III	2/5	40%	2/5	40%
	IV	2/4	50%	0/4	0%
Differentiation	well	7/11	63.6%	2/11	18.2%
	mod.	4/5	80%	1/5	20%
	poor.	0/3	0%	1/3	33.3%
pN	(+)	5/9	55.6%	0/9	0%
	(-)	4/6	66.7%	2/6	33.3%
Mode of invasion	2	1/1	100%	0/1	0%
	3	5/11	45.5%	4/11	36.4%
	4C	5/7	71.4%	0/7	0%
Prognosis	Alive	7/14	50%	4/14	28.6%
	Died	4/5	80%	0/5	0%

Fig. 1: Deletion mapping of the long arm of chromosome 2