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Original

Frequent Deletion of BRG1 Locus at 19p13 Predicts Recurrence and Previous Cancer History in Oral Squamous Cell Carcinomas

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Abstract:Activation of oncogenes and inactivation of tumor suppressor genes (TSGs) is a critical step during carcinogenesis. Inactivation of TSGs occurs through deletion of one allele and mutation in the other allele or decreased mRNA expression. Loss of heterozygosity (LOH) analysis is a sensitive method to detect deletions of specific chromosome regions, which are considered to harbor putative TSGs. By this method we previously demonstrated the frequent deletions of several chromosomal loci and identified candidate TSGs such as ING1, ING3, ING4, Caspase-6 and BRG1 in head and neck cancer. On the other hand, recent researches showed that alterations of chromosomal loci and genes could be used as a predictive marker for the prognosis of the patients, for the behaviour of the tumor and its response to treatments such as chemotherapy and radiotherapy. We recently detected high allelic loss of 19p13 region and identified BRG1 gene as a candidate TSG in 39 oral cancer samples. In the current study, we analyzed the clinicopathological data of the patients and compared with the deletion at BRG1 locus. Our results demonstrated that deletion at BRG1 locus could predict the recurrence, secondary primary cancer, or previous cancer history in oral cancer. Retention of LOH at 19p13 region suggested a high recurrence and secondary primary or previous cancer history. We also detected a higher LOH ratio in cases with smoking and alcohol consumption. The current study suggests that LOH at BRG1 locus could be used as a predictive marker in oral cancer.

Key words: LOH, Tumor suppressor genes, BRG1, 19p13, Prognosis, Survival

Introduction

Oral and oropharyngeal squamous cell carcinoma is the sixth most frequently occurring cancer worldwide, with approximately 400.000 new cases diagnosed each year[1)]. Cancer is believed to be the results of an accumulated, stepwise progression of genetic alterations that produces a clonal population of transformed cells. The collection of genetic and epigenetic alterations of multiple genes and chromosomes lead the development of cancer[2)]. Among these changes, inactivation of the tumor suppressor gene (TSGs) is one of the most critical steps. The deletion of targeted chromosomal region eliminates one allele, while inactivating events (mutation, deletion or promoter hypermethylation) affect the other allele of the concerning TSG[3)]. One of the critical steps for the identification of TSGs is loss of heterozygosity (LOH) analysis. The LOH assay is designed to assess polymorphic chromosomal regions that map close to or within putative or known TSGs. By this method, well known TSGs such as p53, RB1, p16 were cloned, and we previously identified several members of ING family tumor suppressors, including ING1, ING3 and ING4[4-6)]. Prior studies of us and other authors also demonstrated the frequently deleted regions and putative tumor suppressor genes on 7q31, 9p, 4q, 11q, 13q34, 17p, 18q, 19p13 in head and neck cancers[4-11)]. Furthermore, we recently prepared genome-wide deletion mapping of all chromosomes by using 191 microsatellite markers in head and neck squamous cell carcinoma (HNSCC),

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which included several previous and novel hot spots[8].

On the other hand, recent studies compared the relationship of allelic loss at various chromosomal locations and inactivation of TSG with clinicopathological data[12-17]. These studies primarily had two purposes; first, to find a reliable and easily applicable marker which can early diagnose cancer during carcinogenic stage. In such a study, 11 out of 35 premalign lesions followed-up from 1 to 16 years developed cancer and 7 of 11 cases had p53 mutation, suggesting the predictive value of p53 mutation for oral cancer development[18]. Secondly, these molecular analyses would give information about the prognosis and behaviour of the tumor such as metastatic capacity, recurrence or aggressive phenotype, response to chemotherapy or radiotherapy, and survival of the patient. In fact, a lot of studies have been published in the literature, suggesting that the alterations of chromosomal loci and genes could be used as a prognostic or responsive marker for the treatments. Most such studies involved the pathologies of well-known TSGs such as p53, RB, p16/p21/p27 and abnormalities of these loci or genes were detected in patients with poor prognosis[12-23]. However, there have also been some reports mentioning the absence of such prediction[13]. Nevertheless, it is now well known that genetic abnormalities lead to cancer development and these alterations can indicate the prognosis either independently or combined with other alterations.

Recently we showed for the first time frequent allelic loss of 19p13 region and identified BRG1 gene as a candidate TSG in oral cancer[7]. Our study demonstrated that the BRG1 locus has been deleted in 57% of the oral cancer samples by using a BRG1 specific microsatellite marker. In the current study, we examined the relationship between the clinicopathological data and deletion of BRG1 gene locus. By this way, we tried to find out the use of BRG1 loss for the prognosis of oral cancer.

Materials and Methods

Patients and samples

Thirty-nine oral squamous cell carcinoma samples obtained at Okayama University Hospital between 1994 and 2001 were included in the study. The samples were previously analyzed for the loss of heterozygosity at 19p13 locus[7] and clinipathological variables of the cases were evaluated in the current study. Patients included 30 men and 9 women with a mean age of 59 years (range, 44-74). The histological diagnosis of all cases was squamous cell carcinoma. The differentiation and diagnosis of the tumor was based on the surgical pathology reports of the hospital. All clinical information was obtained from the patient files, which includes the initial diagnosis, treatment and follow-up data. Informed consent was obtained in advance from the patients participating in the study.

Statistical analysis

To evaluate the correlation between the LOH at BRG1 locus and clinicopathological characteristics of the patients, Fisher's exact probability, Student t test or Pearson's Chi-square tests were used. Survival curves were calculated according to Kaplan-Meier analysis. For comparison of survival between the absence and presence of LOH at BRG1 locus, the log-rank test was used. Overall survival in months was calculated from the day after surgery to the last follow-up examination or death. The duration of disease-free survival (DFS) was determined from the day after surgery to the initial recurrence of the surgically resected cancer, assessed by clinical examination. All statistical manipulations were done using the SPSS version 10 for the Windows software system (SPSS, Inc., Chicago, IL).

Results

Thirty-nine oral cancer samples previously examined for the LOH study were included in the current work. Relationship between LOH status and clinicopathological characteristics was depicted in Table 1. 16 out of 39 (41%) samples showed LOH at the specific BRG1 locus. No difference has been displayed between LOH ratio and gender. However, when the patients were grouped into younger (less than 60 years old) and elder (over 60), higher LOH was detected in elder group though no statistical significance was shown. 13 out of 16 (81%) cases with LOH were elderly patients, while only 3 cases (19%) were belong to younger group (Fig.1).

With regard to smoking status, 12 out of 15 (80%) patients with LOH smoked, whereas only 3 of 15 (20%) cases with LOH had no smoking history. Regarding with alcohol consumption, 11 out of 15 (73%) patients with LOH used alcohol, while 4 cases (27%) with LOH did not use alcohol. In other words, 12 out of 26 (46%) patients with smoking had LOH, while 3 out 12 (25%) cases without smoking had LOH (Table 1). Similarly, 11 out of 23 (48%) patients with alcohol usage demonstrated LOH, whereas 4 out of

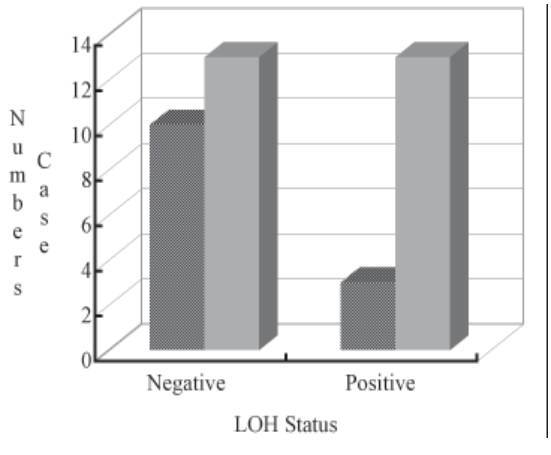

Fig. 1:Graphical demonstration of the relationship between LOH at BRG1 locus and age. 13 out of 16 cases (81%) with LOH were elderly while 13 out of 23 (57%) patients without LOH were over 60 years old. Negative and positive represent the absence and presence of LOH cases, respectively. Gray colums, elderly patients; Oblique lined columns, younger patients.

Table 1. Relationship between LOH and clinicopathologic characteristics

	LOH (-)	LOH (+)	*p value*
Predictors	*n=23(59%)*	*n=16(41%)*	
Gender*			
male	19	11	0.444
female	4	5	
Age			
younger (<60)	10	3	0.107
elder (>60)	13	13	
Smoking*			
yes	14	12	0.294
no	9	3	
Alcohol consumption*			
yes	12	11	0.319
no	9	4	
Smoking & alcohol together*			
yes	11	11	0.12
no	12	4	
TNM stage***			
early stage(I-II)	10	4	0.197
late stage (III-IV)	12	12	
Tumor stage***			
early T (T1-T2)	13	8	0.688
late T (T3-T4)	10	8	
Nodal stage*			
N(0)	11	5	0.248
N(+)	11	11	
Recurrence or secondary primary tumor*			
yes	17	6	0.037
no	6	9	
Previous cancer history*			
yes	7	0	0.027
no	14	15	
Family cancer history*			
yes	11	7	0.842
no	11	11	
Histological differentiation*			
Well	12	10	0.522
Moderate-poor	11	6	

* Fisher exact test ** Chi-square test
*** according to the International Union Against Cancer 1997 TNM classification system

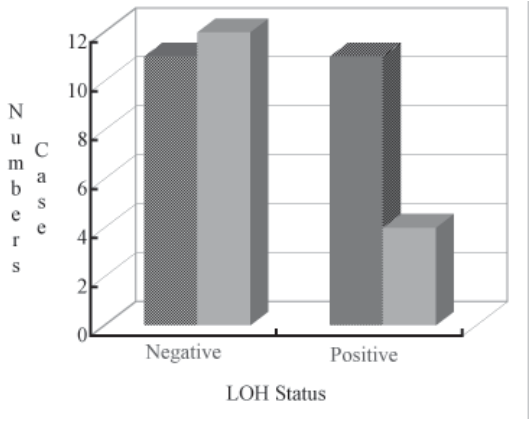

Fig. 2 Graphical demonstration of the relationship between LOH at BRG1 locus and the use of smoking and alcohol. 11 out of 15 cases (73%) with LOH had smoking and alcohol consumption whereas 11 out of 23 (48%) patients without LOH used smoking and alcohol. Negative and positive represent the absence and presence of LOH cases, respectively. Gray colums, patients without smoking and alcohol use; Oblique lined columns, patients with smoking and alcohol use.

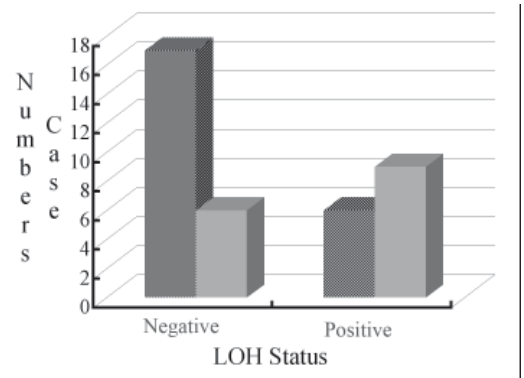

Fig. 3 Graphical demonstration of the relationship between LOH at BRG1 locus and the occurrence of recurrence or secondary primary tumor. 6 out of 23 cases (26%) with recurrence or secondary primary tumor showed LOH while 9 out of 15 (60%) patients without such phenotype had LOH. Negative and positive represent the absence and presence of LOH cases, respectively. Gray colums, patients without recurrence or secondary primary tumor; Oblique lined columns, patients with recurrence or secondary primary tumor.

13 (31%) cases without alcohol consumption had LOH (Table 1). On the other hand, 11 out of 22 (50%) patients with the use of both smoking and alcohol had LOH, while only 4 out of 16 (25%) patients without smoking and alcohol history showed allelic deletion. Although none of the smoking or alcohol usage alone or both of them gave a statistically significant difference according to LOH results, a tendency has been observed toward an increased LOH ratio in patients with smoking and alcohol use especially when both of them have been consumed (Table 1, Fig.2).

An important and significant data has been revealed when LOH status was compared with recurrence or secondary primer occurrence. 6 out of 15 (40%) cases with LOH had recurrence or secondary primary tumor, while 17 out of 23 (74%) patients without LOH demonstrated recurrence or secondary primary tumor. In other words, 17 out of 23 (74%) cases with recurrence or secondary primary tumor showed no LOH, while 6 out of 15 (40%) cases without such phenotype had no LOH at BRG1 locus (Fig.3). Similarly, an interesting finding was detected in regard to previous cancer history. All of the 15 LOH cases had no previous cancer history, whereas 7 out of 21 (33%) cases without LOH showed previous cancer history (Fig.4). This data gave a significant result in favor of patients with previous cancer history having less LOH. On the other hand, no significant relationship was detected between the LOH status and the presence of family cancer history (Table 1).

We also did not find any significant relationship between the LOH ratio and histological differentiation, tumor thickness (T stage) or TNM stage (early or late). Regarding with the lymph

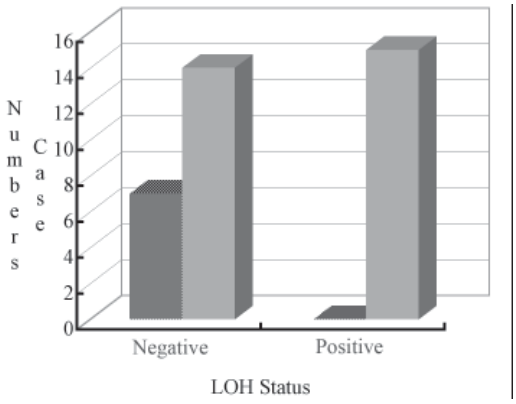

Fig.4 Graphical demonstration of the relationship between LOH at BRG1 locus and the previous cancer history. All of the 7 patients (100%) with previous cancer history did not have LOH whereas 15 out of 29 (52%) cases without previous cancer history showed LOH. Negative and positive represent the absence and presence of LOH cases, respectively. Gray colums, patients without previous cancer history; Oblique lined columns, patients with previous cancer history.

node status, 5 out 16 (31%) cases without lymph node involvement had LOH, while 11 out 22 (50%) cases with positive lymph nodes demonstrated LOH. Although a higher LOH ratio was detected in cases with lymph node involvement, this did not give a significant difference (Table 1).

When we examined the relationship between the survival and the LOH status, no statistically significant difference was detected in either overall or disease-free survival in patients with or without LOH. However, interestingly a longer survival was shown in the patients with LOH as compared to the patients without LOH in terms of disease-free survival. At the time of 100 months (over 8 years), about 50% of the patients with LOH survived, while only 20% of the patients without LOH at the same period survived (Fig.5). However, this difference was minimal at the time of 60 months (about 50% versus 40%).

Discussion

HNSCC is a worldwide common cancer. Despite aggressive and multiple treatment methods including chemotherapy, radiotherapy and surgery, limited improvement in terms of survival has been obtained. Locoregional recurrence, lymph node and distant metastasis, and/or secondary primary tumors are the major reasons for the treatment failure. Moreover, the behaviour of the tumors in different locations and even in the same location is quite different. Some of the tumors may have a better character with good chemoradiotherapy response, less metastasis and invasion. On the other hand, some others may show highly aggressive phenotype with early metastasis and poor prognosis. So far, the prognostic factors that are routinely considered when deciding the treatment are host and tumor factors such as TNM staging, site of the primary tumor, the presence or absence of nodal and distant metastasis, and pathological grading of differentiation. However, the behaviour of tumor is highly variable. Therefore, new predictive markers should be identified both for the diagnosis and treatment strategies. Recent developments in human genetics and molecular biology have improved our understanding of the biological basis of tumor development, progression and metastasis. These studies included the molecules that are altered during development or progression of the tumor such as deletions at the TSG loci, amplification of the oncogene locations, and various mutations of these and DNA repair genes.

p53 gene, one of the most studied marker, was reported to be a predictive and prognostic factor in HNSCC. In one of these reports, p53 mutation at the margin that was surgically and histopathologically clean revealed much more recurrence as compared to samples without p53 alteration[20,23]. In another report,

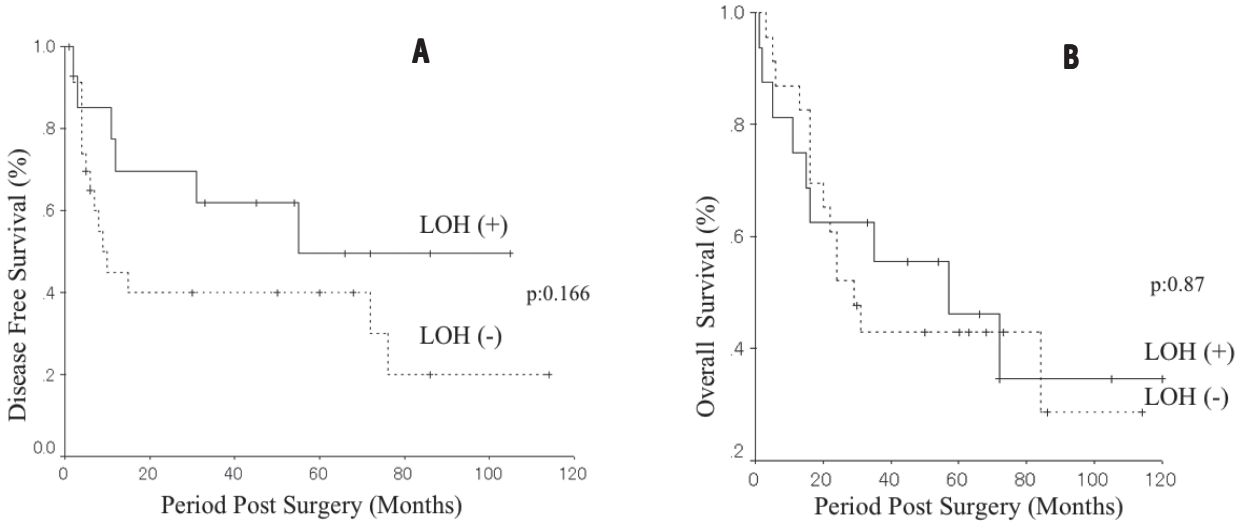

Fig. 5 Kaplan-Meier survival curves for LOH status at BRG1 locus. A. Disease-free survival analysis. B. Overall survival analysis. No significant difference either in overall survival (p: 0.87) or disease-free survival (p: 0.166) was detected.

detection of p53 mutation status predicted tumor response to chemotherapeutical agents[22]. Similarly, allelic deletion at 8p21 and 9p21 regions correlated with recurrence and bad prognosis in HNSCC[19,21]. Furthermore, the relationship between clinicopathological factors such as stage, differentiation, metastasis, recurrence or survival and LOH at various loci or alteration of different genes such as p16, p53, FHIT, CyclinD1 has been reported in HNSCC[12-23].

This time we analyzed the clinicopathological data from 39 patients. No statistically significant relationship was found except the status of recurrence, secondary tumor occurrence or previous cancer history. Regarding with the gender and age of the patients, more LOH was detected in the elderly (>60 years old) as compared to younger patients. Half of the elderly patients had LOH while only 23% of the younger showed allelic loss at BRG1 locus. On the other hand, more recurrence or secondary tumor was detected in patients without LOH. 74% of the patients with recurrence or secondary tumor had no LOH. Moreover, all of the 7 patients who were previously diagnosed with cancer did not show LOH at BRG1 locus. It has been thought that cancer displays a more aggressive course in younger patients[24]. However, the reason behind this observation is unknown. Cancer occurs as a result of accumulated stepwise genetic alterations[2]. Several chromosomal loci are deleted early during carcinogenic process. Le et al.[16] reported that a stepwise allelic loss occurred involving 9p21, 3p, 17p13, 13q21, 14q24, 8p23, 4q26-28 in order during carcinogenic process from hyperplasia through dysplasia, carcinoma in situ and invasive cancer. So far there has been no report about the stage of the loss of BRG1 locus at 19p13. Considering higher LOH ratio in elderly patients without a history of previous cancer and a more favorable phenotype during the management of tumor, LOH at 19p13 is likely to be a late event. Early deletions at one allele of 9p or 17p13 and possible early alterations of major TSGs such p53, RB1 or p16 and/or inherited genetic predisposition[25] or polymorphic alterations related with the activity of genes[26,27] could be responsible for aggressive tumor phenotype or secondary cancer development in younger patients. Those patients are likely to be cancer prone due to alterations of the TSGs or genetic predisposition for a long time.

Regarding with smoking and alcohol consumption, a near significant relationship was detected with the LOH in the current study. Although the percentage of LOH in patients who used either smoking or alcohol was about 1.5 to 2 times more as compared to those without such habit, it did not give a statistically significant result. This relationship between LOH and smoking or alcohol was more evident when both were used. Half of the patients with use of both smoking and alcohol showed LOH while only one fourth of the cases without such consumption had allelic loss at this locus. The role of smoking in the allelic loss in various cancers has already been reported in several papers including head and neck cancers[28-30]. The use of smoking with alcohol is likely to have an additive effect on the transformation of normal epithelial cells as shown in the current study. Carcinogenic effect of smoking on TSG has also been known. Brennan et al.[31] reported that 58% and 33% of patients with the use of smoking and alcohol together or smoking only, respectively, demonstrated p53 mutations as compared to 17% mutation in the cases without such use.

We could not find any significant relationship between LOH at BRG1 locus and histological differentiation or tumor stage. However, there was a tendency in advanced tumors with higher LOH ratio. Half of the late stage cancers showed LOH while it was only 28% in early stage tumors. Similar finding was detected between LOH status and lymph node metastasis. Though not significant, a tendency towards higher LOH in clinically lymph node positive patients (50%) as compared to N0 cases (31%) was revealed.

An interesting finding was found when a survival analysis was performed. Although statistically not significant, a worse prognosis was shown in cases without LOH in Kaplan-Meier Analysis in terms of disease-free survival. At the time of 100 months after surgical treatment, about 50% of the patients with LOH survived whereas only 20% of the cases without LOH were alive. However, at the time of 60 months after operation, this difference was less evident with about 50% and 40% survival, respectively. On the other hand, no difference at all was detected in terms of overall survival in both groups. It is possible that the death over 60 months could be due to the reasons other than primary tumor related. Finally, it may be concluded that LOH at 19p13 did not give prediction for the disease-free or overall survival in oral cancer.

In conclusion, our data analyzed for the first time the relationship between clinicopathological characteristics and LOH at BRG1 locus. A significantly higher LOH was detected in patients with recurrence, secondary primary tumor or previous cancer history. A near significant relationship was obtained in terms of age with higher LOH in the elderly and the combined use of smoking and alcohol. Further studies in a large sample population including various sites of LOH and alteration of different genes would give better predictive marker detection in oral cancer and contribute to better management of this cancer type.

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