# Fine Deletional Mapping of Chromosome 4q22-35 Region in Oral Cancer

**Yasuo Shinno[1)], Mehmet Gunduz[1)], Esra Gunduz[1)], Li-qun Jia[2)], Kenji Shimizu[1)], Masahiro Yoshiyama[1)], Masayuki Imura[3)], Noriyuki Nagai[1)]**

*1)Okayama University, Graduate School of Medicine, Dentistry and Pharmaceutical Sciences*
*2)China-Japan Friendship Hospital, China*
*3)Japan Institute for Advanced Dentistry, Osaka*

Abstract: We analyzed loss of heterozygosity in detail of the long arm of chromosome 4 in 40 oral cancer by using 16 microsatellite markers based on the recent data from human genome sequence and defined the deletional mapping of the region with putative tumor suppressor genes. Our data revealed two distinct commonly deleted regions around the markers D4S2623 and D4S1644 with an allelic deletion of 44% and 39%, respectively. Additional mapping and use of the markers near one of these hot spots narrowed down the minimally deleted region about 1.5 Mbp around the marker, D4S2623. Caspase 6 is just localized 280 kb distant from the marker, D4S2623. Fine mapping of this region with possible tumor suppressor gene suggest caspase 6 as a putative tumor suppressor gene. Further molecular analysis of caspase 6 should be performed to clarify its role in oral carcinogenesis.

## Introduction

In neoplastic progression, most of the sporadic solid tumors result from a multistep process of accumulated genetic and epigenetic alterations. Among these changes, inactivation of the tumor suppressor genes (TSGs) is one of the most critical steps. In this process, the deleted chromosomal regions eliminated one of the alleles, while inactivating events (mutation, deletion or promoter hypermethylation) affect the other allele of the concerning TSG[1)]. Loss of heterozygosity (LOH) analysis by using polymorphic microsatellite markers is a sensitive method to detect micro-deletions. In most studies on the genetic alterations in cancer of the upper aerodigestive tract, oral squamous cell carcinomas (OSCCs) are included in the more heterogeneous group of head and neck squamous cell carcinomas (HNSCCs).

In head and neck cancer, deletional mapping of this region has not been studied in detail except one study. Pershouse et al.[2)] prepared the deletion mapping of both arm of chromosome 4 by using 27 microsatellite markers and detected 81% deletions and/or alterations associated with the long arm of chromosome 4 in 27 head and neck cancer samples. Karyotype and comparative genomic hybridization studies by using few markers demonstrated the frequent deletion on the long arm of chromosome 4 in head and neck and oral cancer cell lines. We also recently reported frequent deletion on the long arm of chromosome 4 by a genome-wide LOH analysis[3)]. All these studies suggest the existence of tumor suppressor gene(s) in the long arm of chromosome 4. We examined a commonly deleted region of chromosome 4q22-35 in detail by using 16 microsatellite markers from this region and constructed a deletion mapping of the region and putative tumor suppressor gene(s).

## Materials and methods

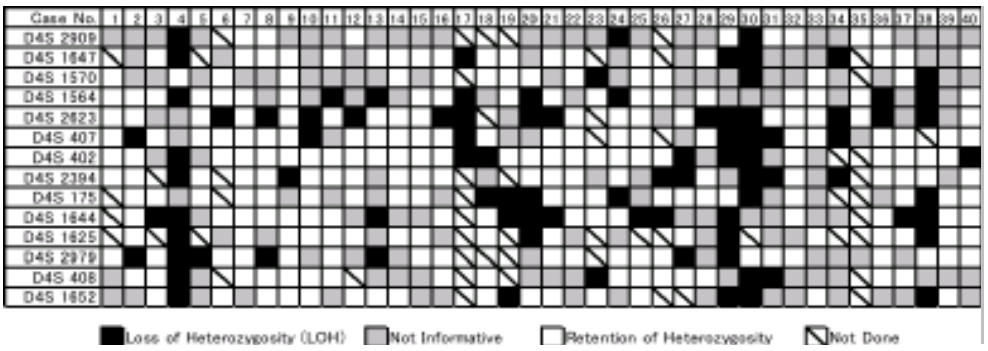
*Tissue Samples.* Paired normal and tumor samples were obtained from 40 patients with primary oral squamous cell carcinoma, Okayama University Hospital after acquisition of informed consent from each patient.

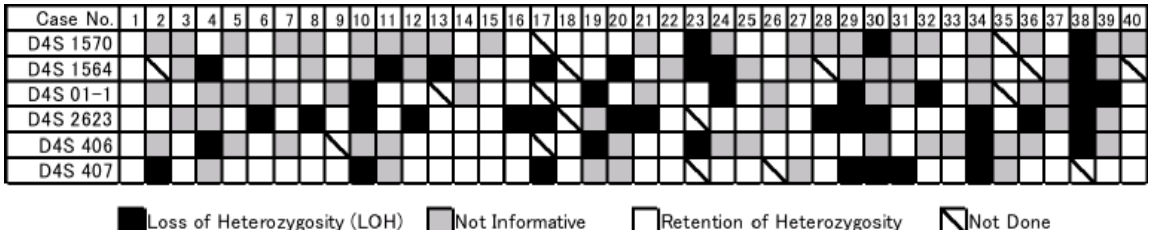
*DNA extractions.* Genomic DNAs were isolated from frozen tissues by SDS/proteinase K treatment, phenol-chloroform extraction, and ethanol precipitation.

*Microsatellite Analysis.* Primers for amplification of microsatellite markers ware D4S2909, D4S1647, D4S1570, D4S1564, D4S2623, D4S406, D4S407, D4S402, D4S2394, D4S175, D4S1644, D4S1625, D4S2979, D4S408, D4S1652 and D4S01. After amplification, the reaction mixtures were electrophoresed through an 8% polyacrylamide gel containing 8 M urea. The DNA bands were visualized by silver staining. LOH was scored if one of the heterozygous alleles showed at least 50% reduced intensity in tumor DNA as compared with the corresponding normal DNA.

## Results

We this time examined LOH using 16 microsatellite markers on the chromosome 4q22-35 region in 40 paired normal and oral






cancer DNAs. Overall, 77 % (32/40) samples showed LOH at least one marker on 4q22-35. **F**ig.1 summarizes the results of LOH analyses. This ratio is higher than most of the other reports possibly due to selection of the markers, which include a wide chromosomal area with multiple targeted areas. Eight tumors (samples 4, 17, 20, 29, 30, 31, 34 and 38) displayed a large deletion that included most polymorphic markers tested. In the other 24 cases, a partial deletion was found, providing information about the areas of preferential loss. We detected two distinct regions of deletion. Markers D4S2623 and D4S1644 showed the highest incidence of LOH with the values of 44% and 39%, respectively, while flanking markers demonstrated lower frequency. In the current study, we focused the more centromeric and frequently lost area around D4S2623. We examined and did deletional mapping in detail by using additional 2 markers (D4S01-1 and D4S407) within this region (Fig.2). Further LOH analysis clearly demonstrated about 1.5 Mbp minimally deleted region around the marker D4S2623. In particular, four cases (case 8, 12, 16 and 28) showed distinct LOH in the limited region only at the location of D4S2623.

## Discussion

The functional loss of tumor suppressor genes is closely associated with the initiation and/or progression of human cancer[4]. Microdeletion analysis demonstrated the involvement of LOH on chromosome 4 alleles in various carcinomas. Although karyotype and comparative genomic hybridization studies by using few markers demonstrated the frequent deletion on the long arm of chromosome 4 in head and neck precancerous and cancerous lesions and oral cancer cell lines, deletional mapping of this region has not been studied in detail. All these and our studies suggest the existence of tumor suppressor gene(s) in the long arm of chromosome 4. Therefore we this time examined the region of chromosome 4q22-35 in detail based on the recent mapping information of human genome project by using 16 microsatellite markers from this region and defined a deletion mapping of the region and putative tumor suppressor gene(s). Our data interestingly revealed two independent commonly deleted regions with the highest percentage of deletion at the markers D4S2623 (4q25) and D4S1644 (4q31). This time, we focused the locus of D4S2623 since it was the marker with highest LOH. Further usage of additional markers at the region narrowed down the minimally deleted region to about 1.5 Mbp around the marker D4S2623. The putative tumor suppressor gene in this region was most likely to be around the marker of D4S2623 because 4 samples showed only LOH of this marker with keeping of the flanking genomic regions. This area is heavily populated with genes of known and unknown. The most likely as a candidate tumor suppressor gene in the region is caspase-6. Caspase-6 is just localized 280 kbp from the marker D4S2623 and is already known to be an important molecule in apoptotic pathway[5]. Somatic mutations and loss of heterozygosity of several members of caspase family including caspase 3, 5, 7, 8, 9, 10 were recently reported in various human cancer. Considering these reports and our current data, caspase-6 is a highly possible candidate tumor suppressor gene in the region and further studies including mutation status and mRNA expression of caspase-6 should be performed.

In conclusion, our current data showed the deletional mapping of the long arm of chromosome 4 in detail and discussed the possible tumor suppressor genes in oral cancer. Next should be the molecular analysis of the candidate genes such as caspase-6 from the region.

## References

1) Knudson A.G. Jr: Mutation and cancer: statistical study of retinoblastoma. Proc Natl Acad Sci U S A 68:820-823, 1971.
2) Beder L.B., Gunduz M., Ouchida M., Fukushima K., Gunduz E., Ito S., Sakai A,. Nagai N., Nishizaki K., Shimizu K.: Genome-wide analyses on loss of heterozygosity in head and neck squamous cell carcinomas. Lab Invest 83:99-105, 2003.
3) Pershouse M.A., El-Naggar A.K., Hurr K., Lin H., Yung W.K., Steck P.A.: Deletion mapping of chromosome 4 in head and neck squamous cell carcinoma. Oncogene 14:369-373, 1997.
4) Weinberg R.A.: Tumor suppressor genes. Science 254:1138-1146, 1991.
5) Fernandes-Alnemri T., Litwack G., Alnemri E.S.: Mch2, a new member of the apoptotic Ced-3/Ice cystein protease gene family. Cancer Res 55:2737-2742, 1995.