Deletional Mapping of 2q21-37 Region in Oral Cancer

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Abstract: Tumor suppressor genes are defined as genetic elements whose loss or mutational inactivation allows cells to display one or more phenotypes of neoplastic growth. Frequent deletion in a chromosomal region suggests existence of a candidate tumor suppressor gene. We analyzed Ch2q21-37.3 region by using 17 polymorphic microsatellite markers in 39 matched oral normal and cancer tissues. Loss of heterozygosity (LOH) was detected at least one location in 36 of 39 (92%) tumor tissues. High deletions were detected at microsatellite marker locations, D2S2304 (35%), D2S111 (40%), D2S155 (35%), D2S164 (29%), D2S125 (71%) and D2S140 (39%). Three frequently deleted regions at 2q22, 2q35-36 and 2q37.3 were observed. Chromosomal 2q22-37.3 region is highly populated with genes. Several candidate tumor suppressor genes in this region including such as ING5, CASP8, CASP10, PPP1R7 and BOK are located. We are currently analyzing inactivation mutations and mRNA expressions in oral squaomus cell carcinomas.

Key words; oral cancer, chromosome2, loss of heterozygosity

Introduction

The development of cancer is a result of the collection of genetic alterations including multiple genes and chromosomes. Tumor suppressor gene prevents the development of tumor in normal cells. Loss of heterozygosity (LOH) analysis is a sensitive genetic method to detect the microdeletions on chromosomes. The LOH assay is designed to assess polymorphic chromosomal regions that map close to or within putative or known TSGs. Microdeletion analysis showed the involvement of LOH on chromosome 2 alleles in several cancers breast, lung, neuroblastoma, oral squamous cell carcinoma, cervical, thyroid, prostate, leukemia and head and neck cancer. We examined a commonly deleted region of chromosome 2q21-37.3 in detail by using 17 microsatellite markers from this region and constructed a deletion mapping of the region and putative tumor suppressor gene(s).

Materials and Methods

Paired normal and tumor samples were obtained from 39 patients with primary oral squamous cell carcinoma, Okayama University Hospital after acquisition of informed consent from each patient. All tissues were frozen in liquid nitrogen immediately after surgery and stored -80°C until the extraction of DNA. Histological studies were also performed at the department of pathology, and all tumors were confirmed as squamous cell carcinoma. Genomic DNAs were isolated from frozen tissues by SDS/proteinase K treatment, phenol-chloroform extraction, and ethanol precipitation. Primers for amplification of microsatellite markers D2S1328, D2S2304, D2S111, D2S115, D2S116, D2S202, D2S155, D2S1327, D2S164, D2S133, D2S427, D2S206, D2S338, D2S336, D2S125, D2S395 and D2S140 are available through the internet genome database. After amplification, 2-4 µl of the reaction mixture were mixed with 8 µl of loading dye (95% formamide, 20 mM EDTA, 0.05% bromphenol blue, and 0.05% xylene cyanol), heat denatured, chilled on ice, and then electrophoresed through an 8% polyacrylamide gel containing 8 M urea. The DNA bands were visualized by silver staining as described previously. 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 and Discussion

We analyzed chromosome 2 long arm region by PCR LOH assay with 17 polymorphic microsatellite markers in 39 matched oral normal and cancer tissues. LOH was detected on chromosome 2q21-37.3 region at least one location in 36 of 39 (92%) tumor tissues. The resuls of LOH analysis exhibited preferential allelic losses on several locations of chromosome 2q21-37.3 region. The high frequencies of LOH were observed at loci on D2S2304 (%35) at 2q21-24, D2S111 (%40) at 2q24, D2S155 (%35) at 2q35, D2S125 (%71) at 2q37.3 and D2S140 (%39) at 2q37.3 (Figure 1). Three different regions preferentially have been lost. One of these regions included the areas between D2S2304 and D2S111, the other area was between D2S155 and D2S164, and the last area was between D2S125 and D2S140. The representative samples of LOH, informative, MIS and not informative cases on 2q21-37.3 region were shown in figure 2. Since these three regions are distinctly lost, which are supposed to be different hot spots for different candidate tumor suppressor gene.

Further detailed mapping of the markers and genes from this region will identify putative targets in oral cancer. In conclusion, our current data showed the deletional mapping of the long arm of chromosome 2 in detail and discussed the possible tumor suppressor genes in oral cancer. Several candidate tumor suppressor genes in this region including such as ING5, CASP8, CASP10, PPP1R7 and BOK are located. We are currently analyzing inactivation mutations and mRNA expressions in oral squaomus cell carcinomas.

References

1) Piao Z, Lee KS, Kim H, Perucho M, Malkhosyan S. Genes Chromosomes Cancer, 30(2): 113-22, 2001
2) Otsuka T, Kohno T, Mori M, Noguchi M, Hirohashi S, Yokota J. Genes Chromosomes Cancer, 16(2): 113-9, 1996
4) Takita J, Yang HW, Chen YY, Hanada R, Yamamoto K, Teitz T, Kidd V, Hayashi Y. Oncogene, 20(32): 4424-32, 2001
5) Narayan G, Pulido HA, Koul S, Lu XY, Harris CP, Yeh, YA, Vargas H, Posso H, Terry MB, Gissmann L, Schneider A, Mansukhani M, Rao PH, Murty VV. Oncogene, 22(22):3489-99, 2003

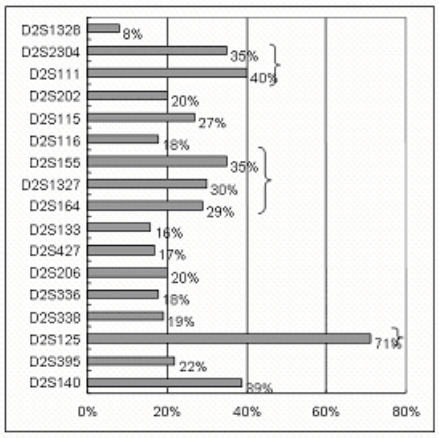

Fig. 1. Frequency of allelic loss on chromosomes 2q at each of the microsatellite marker location.

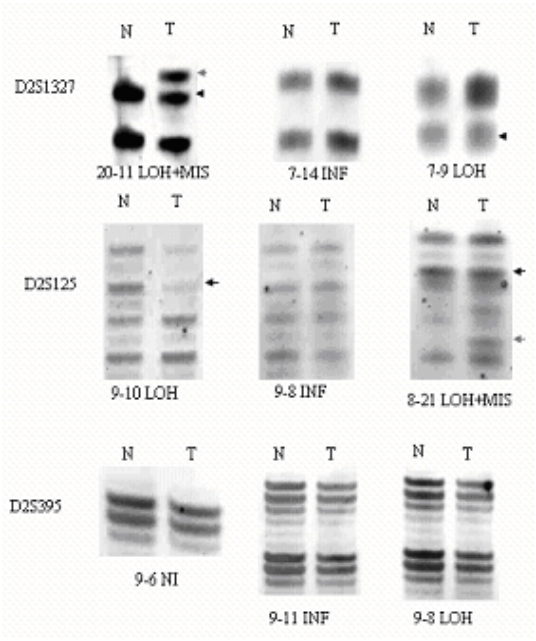

Fig. 2. Primary LOH data from representative oral SCCs. N, normal DNA; T, tumor DNA. Arrowheads show Microsatellite Instability. Arrows show Loss of Heterozygosity