High Loss of Heterozygosity of 9p24 Region and Identification of BRM as A Candidate Tumor Suppressor Gene in Head and Neck Cancer

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Abstract: Biochemical and genetic studies has identified several ATP-dependent multiprotein complexes that are involved in the remodeling of chromatin during gene activation. The SWI/SNF complex is one of these molecules. Molecular pathological changes of several members of SWI/SNF complex, especially human brahma (hBRM) and brahma-related gene 1 (BRG1) were reported to be involved in carcinogenesis of human cancer. hBRM can bind and cooperate with hypophosphorylated RB protein in repressing E2F1 transcriptional activation in transient transfection studies. We analyzed the LOH of the short arm of chromosome 9 in 64 head and neck squamous cell carcinomas by using 13 highly polymorphic microsatellite markers and found two deletion hot spots at 9p21 and 9p24. P16 tumor suppressor gene is likely to be a target for the deletion of 9p21 region. When the map of 9p24 region was redefined, a possible tumor suppressor gene hBRM was identified. Therefore we prepared an hBRM specific microsatellite marker and found 67% deletion of this gene at 9p24 region. In RT-PCR analysis about 60% of tumor samples demonstrated reduced mRNA expression as compared to matched normal samples.

Key words: SWI/SNF, BRM, Oral Cancer, 9P24, LOH, Alternative Splicing.

Introduction

Mutations that potentiate the activities of proto-oncogenes create the oncogenes that force the growth of tumor cells. Conversely genetic lesions that inactivate suppressor genes liberate the cell from constraints imposed by these genes, yielding the unconstrained growth of cancer cell. Loss of heterozygosity (LOH) analysis by using polymorphic microsatellite markers is a sensitive molecular method to detect micro deletions in some chromosome regions, which are considered to harbor putative tumor suppressor genes. We analyzed the LOH of the short arm of chromosome 9 in 64 matched head and neck squamous cell carcinomas and normal tissues and found allelic deletion about 67% of cases at 9p24 region.

Materials and Methods

Tissue Samples.

Paired normal and tumor samples were obtained from 64 patients with primary oral squamous cell carcinoma from Okayama University Hospital after acquisition of informed consent from each patient.

DNA and RNA extractions.

Genomic DNAs were isolated from frozen tissues by SDS/proteinase K treatment, phenol-chloroform extraction, and ethanol precipitation. Total RNAs were prepared by using a modified acid guanidium phenol chloroform method (ISOGEN; Nippon Gene Co., Tokyo, Japan).

Microsatellite Analysis.

Primers for amplification of microsatellite markers D9S1161, D9S118, D9S1748, D9S925, D9S285, D9S254, D9S269, D9S921, D9S286, D9S199, GATA62F03, and D9S1813 are available through the internet genome database. We also designed and used a BRM specific microsatelite marker.

LOH and mRNA expression analysis was performed as described previously.

Results and Discussion

To examine allelic loss in the BRM locus, we selected 6 microsatellites markers on chromosome 9p24, which covered a relatively wide chromosomal area including the BRM gene. We first analyzed genomic DNAs from 64 pairs of matched oral squamous cell carcinomas and normal tissues for losses at microsatellite markers D9S1161, D9S118, D9S1748, D9S925, D9S285, D9S254, D9S269, D9S921, D9S286, D9S199, GATA62F03, and D9S1813. This initial microsatellite analysis showed highest LOH at the marker GATA62F03. we more specifically examined the LOH status of BRM gene by designing a microsatellite marker specific for this gene. This BRM specific microsatellite marker demonstrated the highest LOH (Fig.1, 2).

We also examined expression level of BRM mRNA in tumor samples comparing with the paired normal tissue. In 64 available matched RNA samples, the expression levels of BRM mRNAs were compared by quantitative RT-PCR using glyceraldehyde phosphate dehydrogenase (GAPDH) mRNA as a control. PCR primers were designed to encompass the exon-intron junctions on the cDNA in order to eliminate the potential contamination of genomic DNA. Previous allelotype analysis showed frequent LOH at the long arm of chromosome 9 at various cancers including renal carcinoma, cervical cancers and lung cancers. In the present study we have demonstrated that 67% of head and neck cancers showed BRM gene allelic deletion at its chromosomal location

9p24. Twenty nine out of 48 (60%) tumor tissues available for RNA extraction showed decreased expression of mRNA as compared with that of each paired normal tissue. In 12 samples similar mRNA expression levels of BRM were detected in normal and tumor tissues. Frequent allelic loss and decreased mRNA expression of BRM suggest that it may function as a tumor suppressor in head and neck cancer.

References

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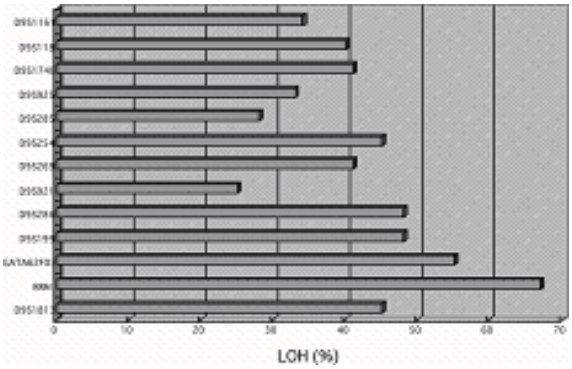

Figure 1. Total results of LOH. BRM specific marker showed the highest LOH

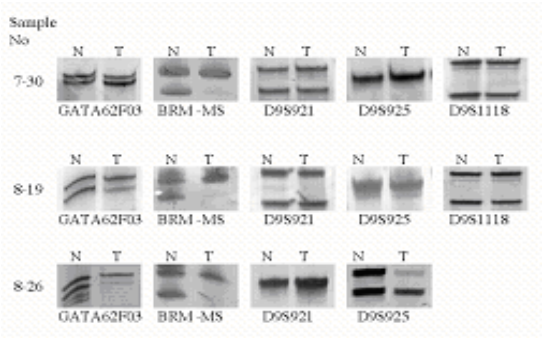

Figure 2. Representative examples of LOH.