Frequent Deletion and Down-Regulation of ING3 in Head and Neck Cancer

Beyhan Cengiz [1), Mehmet Gunduz[1), Esra Gunduz[1), Deng Chunfu[2), Mamoru Ohuchida[1),
Goichi Kinoshita[3), Kenji Shimizu[1) and Noriyuki Nagai[1)

[1) *Department of Oral Pathology and Medicine, Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University*
[2) *China Medical University, China*
[3) *Japan Institute for Advanced Dentistry, Osaka*

Abstract: Loss of heterozygosity (LOH) has been frequently detected at chromosome 7q31 region in human head and neck squamous cell carcinomas (HNSCC) and many other cancers, suggesting the existence of the tumor suppressor genes (TSG). However, the targeted gene was yet identified. We analyzed LOH at 7q31 region in 49 HNSCC by using 6 polymorphic microsatellite markers and found allelic deletion in 48% of the informative cases. We detected two preferentially deleted locations one around D7S643 and the other around D7S486. When we redefined the map of 7q31 region according to the contiguous sequences, an ING1 like gene, ING3, which we named it due to homology to ING1, was found in the proximity of D7S643. ING3 protein harbors a PHD zinc finger domain highly homologous among ING family proteins, in which we previously found mutations in a related gene, ING1. As only one missense mutation of the ING3 gene was found in HNSCC, we examined the mRNA expression levels. RT-PCR analysis demonstrated decreased or no expression of ING3 mRNA in 45 % of primary tumors as compared with that of mathced normal samples. All these findings suggest a possibility that ING3 functions as a TSG in a subset of HNSCC.

Key words; LOH, ING1, ING3, Tumor suppressor gene, Head and neck cancer

Introduction

Tumor Suppressor Genes are defined as genetic elements whose loss or mutational inactivation allows cells to acquire neoplastic growth. Cytogenetic studies have shown frequent chromosome 7 abnormalities in human head and neck carcinomas. However, the candidate gene was not identified in this region. In this study, we examined the 7q22-31 region by using a set of highly polymorphic microsatellite markers to find out allelic loss in HNSCC. In light of LOH analysis, we redefined the map of chromosome 7q31 region and examined some genes on this region by analyzing mutation and mRNA expression. The results suggested that the ING3 gene is a likely candidate for one of the TSGs at 7q31 region.

Materials and Methods

Tissue samples: Paired normal and tumor samples were obtained from 49 patients with primary HNSCC operated at Okayama University Hospital.

Microsatellite Analysis: Six highly polymorphic primers, D7S480, D7S643, D7S522, D7S486, D7S525 and D7S1799 were used for PCR amplification and LOH analysis.

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 ISOGEN.

Quantification of the RT-PCR products: ING3 mRNA expression in tumor and in normal tissues was examined by the duplex RT-PCR method. PCR products were separated through 2% agarose gel and stained with ethidium bromide. The intensity of ethidium bromide staining of each band was measured by a CCD image sensor and anlyzed by a computer program for band quantification. Luciferase Assay was used for promoter activity and PCR-SSCP analysis was performed for mutation detection.

Results and Discussion

We examined LOH using six microsatellite markers on the chromosome 7q22-31 region in paired normal and HNSCC DNAs. Overall, 48% (22/46) samples showed LOH at least one marker on 7q31. We detected two distinct regions of deletion. Markers D7S643 and D7S486 showed the highest incidence of LOH with a value of 35%, while flanking markers demonstrated lower frequency (Figure 1). We analyzed the promoter for the ING3 gene, the 5' flanking 1.5kb region including 5'noncoding region of exon1 was subcloned in sense or antisense orientation. Luciferase construct in sence orientation showed about 213-fold increase in luciferase plasmid, while activity of luciferase construct in antisense orientation was not detectable. Analysis of a candidate tumor suppressor gene, ING3, at 7q31 demonstrated reduced

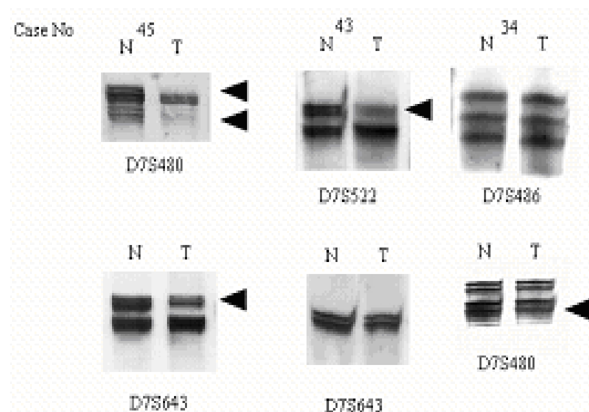

Fig. 1. Primary LOH data from four representative HNSCC. Allelic losses are shown by arrows.

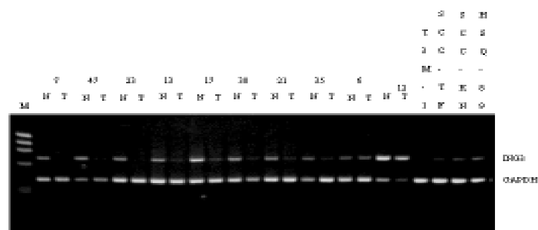

Fig. 2. ING3 RT-PCR analysis. 45% of primary tumors showed decreased or no expression as compared with that of matched normal samples.

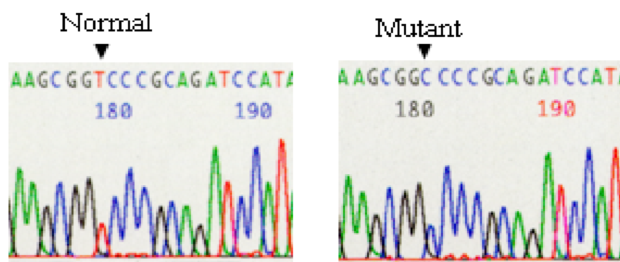

Fig. 3. Sequences of missense mutation. Sample 41 demonstrated a nucleotide change from GAC to CAC at codon 20 with an amino acid substitution from aspartic acid to glycine.

mRNA expression in 45 % of tumors and one tumor specific mutation (Figure 2 and Figure 3). Frequent allelic loss, down-regulation and high homology of ING3 with ING1 suggested that ING3 might also function as a tumor suppressor in head and neck cancer.

References

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