Identification of the Genomic Structure of Tumor Suppressor ING1 and Mutation Analysis in Head and Neck Squamous Cell Carcinomas

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Abstract: A candidate tumor suppressor gene, ING1 has been recently cloned and mapped to 13q33-34 region. We characterized the genomic structure of the human ING1 gene and detected somatic mutations of the ING1 gene in head and neck squamous cell carcinomas. 23 out of 34 informative cases (68%) of tumors showed loss of heterozygosity at chromosome 13q33-34, where the ING1 gene is located. By sequence analysis three exons and 2 introns of ING1 were identified. Mutation analysis of ING1 gene showed three missense and three silent changes.

Key Words: Head and neck squamous cell carcinoma, Loss of heterozygosity (LOH), Bacterial artificial chromosome (BAC), SSCP.

Introduction

A candidate tumor suppressor gene, ING1, was mapped on human chromosome 13q34[1)]. Forced overexpression of the ING1 gene led the cells to arrest in the G1 phase of the cell cycle and induced apoptosis in several cell types [1,2)]. Conversely, inhibition of ING1 expression by antisense constructs promoted the transformation of mouse breast epithelial cells and increased the frequency of focus formation with NIH3T3 cells and protected the cells from apoptosis [1,2)]. We characterized the genomic structure of the human ING1 gene and detected somatic mutations in head and neck squamous cell carcinomas.

Materials and Methods

Tissue Samples

Paired normal and tumor samples were obtained from 55 patients with primary HNSCCs at the Department of Otolaryngology, Okayama University Hospital after acquisition of informed consent from each patient. All tissues were frozen in liquid nitrogen immediately after surgery and stored at -80°C until the extraction of DNA and RNA. Histological studies were also performed at the Department of Pathology, and all tumors were confirmed as squamous cell carcinoma.

DNA and RNA Extraction

Genomic DNAs and total RNAs were isolated from frozen tissues as described previously[3)].

Microsatellite Analysis

Primers for amplification of microsatellite markers D13S285, D13S796, D13S278, D13S158, and D13S779 are available through the internet genome database. PCR was carried out as described previously[3)]. 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.

Analysis of the Genomic Structure of the Human ING1 Gene

PCR analysis with ING1-specific primers was used to screen a BAC library, and a positive BAC clone (118N21) was purchased from Genome Systems, Inc. (St. Louis, MO). The BAC DNA was digested with appropriate restriction enzymes, and regions containing ING1 exons were mapped by Southern blotting with ING1 cDNA probes. Some restriction fragments containing ING1 exons were subcloned into pBluescript KS(-) (Stratagene, La Jolla, CA) and sequenced.

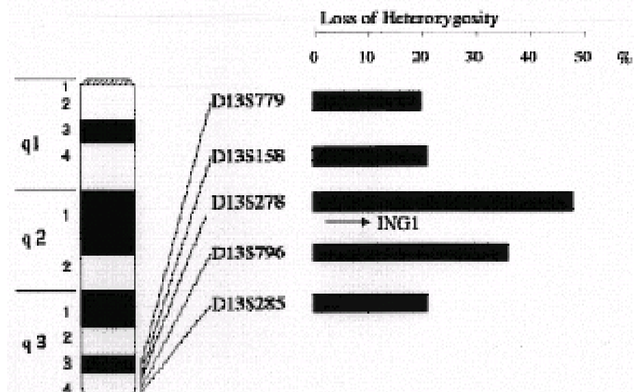
SSCP Analysis

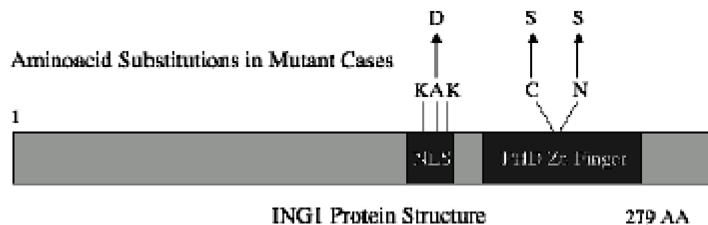
SSCP analysis was performed as described previously[3)].

Results and Discussion

LOH Analysis

Using five microsatellite markers, we showed LOH, at least with one microsatellite marker in 23 out of 34 informative tumors (68%). The most frequent loss (49%) was seen at marker D13S278 (Fig.1). We determined the genomic structure of the ING1 gene by colocalizing the markers and searched for its mutation in HNSCCs.





Genomic Structure of the Human ING1 Gene

We identified and cloned two continuous Xba I fragments (about 8 and 3.5 kb) gene in a BAC clone detected by sequencing analysis, which contains whole coding region of ING1. By sequence analysis, three exons and two introns were identified.

Mutation Analysis

We identified six possible mutations by SSCP analysis. Three samples showed missense mutations with aa change (13%). The other three samples displayed a common alteration without an aa change. Two of the mutations in ING1 gene were located at this zinc finger domain showing its importance (Fig.2). These mutational changes may effect the three dimensional structure of ING1 gene and may abrogate its tumor suppressive function. One mutation was detected at possible nuclear localization signal and may ultimately interfere in the accumulation of ING1 protein in the nucleus.

Our results showed that a considerable subset of head and neck carcinomas harbors inactivating mutations in the ING1 gene accompanied by selective loss of another allele, in accordance with definition of a tumor suppressor gene.

References

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