Methylation Analysis and mRNA Expression Status of ING1 Splicing Variants in Head and Neck Carcinomas

Sathi Gul San Ara1), Mehmet Gunduz1), Beyhan Cengiz1), Esra Gunduz1), Mahmood Javed2), Lu Zhengfu3), Toshiyuki Matsuba4) and Noriyuki Nagai1)

1) Okayama University, Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Department of Oral Pathology and Medicine, Okayama, Japan
2) Bangladesh Dental College, Department of Oral Pathology, Dhaka, Bangladesh
3) China Medical University, China
4) Japan Institute for Advanced Dentistry, Osaka

Abstract: We previously reported the characterization of the genomic structure of the human ING1 gene and found tumor specific missense mutations. We also demonstrated that four mRNA variants were transcribed from three different promoter regions. In this study, we examined the mRNA expression of two major splicing variants of ING1 gene in 40 matched samples of normal and tumor tissues from head and neck cancers by RT-PCR. One of the splicing variant, p24, showed decreased and increased expression in 51% and 27% of the samples, respectively, while another major variant, p33, demonstrated low and high mRNA expression in 20% and 35% of the samples, as compared to normal controls. To elucidate the silencing mechanism of the ING1 gene, we examined the methylation status of each splicing variant. Our results suggest that alternative variants of ING1 gene may have different role in the carcinogenic pathway of head and neck cancers.

Introduction
Tumor suppressor genes are defined as genetic elements whose loss or mutational inactivation allows cells to display one or more phenotypes of neoplastic growth1). We previously characterized the genomic structure of human tumor suppressor gene ING1. We also demonstrated for the first time tumor specific mutations and 4 different mRNA splicing variants from three different promoter regions. The locus of ING1 was mapped to chromosome 13q33-342). Epigenetic modification such as methylation of CpG islands in the promoter region is another major mechanism for inactivation of tumor suppressor genes. Therefore, we examined mRNA expression and methylation status of the two major variants of ING1 gene in head and neck squamous cell carcinomas.

Materials and Methods

Tissue samples
Paired normal and tumor samples were obtained from 40 patients.

DNA and RNA extractions
Genomic DNAs and RNAs were isolated from frozen tissues as described previously3).

mRNA expression analysis
RT-PCR for mRNA expression analysis

Methylation analysis
Restriction endonuclease based methylation analysis (PCR after cutting with MspI and Hpa II)

Results and Discussion
Increased, decreased or no change of mRNA expression of p33ING1b were found in 35, 20 and 45% of the tumor samples, respectively. 51% of the tumor samples showed decreased expression of p24ING1c, while increased or no change of the same splicing variant was detected in 27 and 22% of the samples, respectively (Figure 1). To elucidate the silencing mechanism of the ING1 gene, we examined the methylation status of splicing variants p24ING1c and p33ING1b by restriction endonucleases based method using MspI cut and HpaII cut (Fig.2). After digestion with specific endonucleases PCR products for the methylated p33ING1b only band can be seen in the samples with HpaII, which can not digest methylated sites. Negative and positive controls define normal DNA treated in the same condition with or without enzyme for each reaction. P24ING1c did not display any methylation in the same samples although its expression was decreased in half of the tumor samples. 19 out of 39 (49%) tumor samples showed methylation for the p33ING1b variant by using restriction endonucleases based method. p24INGc did not display any methylation in the same samples although its expression was decreased in half of the tumor samples. p33ING1b mRNA expression was absent or decreased in 7 of 19 methylated tumor samples as compared to their normal counterparts, while expression of p33ING1b in the rest of the 12 samples did not change, suggesting that the methylation ratio of each tumor is different from very low levels through intensive methylation. Mouse corresponding variants of p33ING1b and p24ING1c suggested the opposite effect of these two products of the same gene as the first one functions as oncogene while the latter is a tumor suppressor gene. Increased expression of p33ING1b but decreased expression of p24ING1c in some samples suggested that the two major human variants of ING1 gene may show both similar and different characteristics and the balance between these splicing variants may be important for carcinogenesis. It is also likely that p33ING1b can compensate the function of ING1 gene in the absence of p24ING1c variant. Our result suggested that alternative variants of ING1 gene might have different role in the carcinogenic pathway of head and neck cancers.
References

Fig. 1. Representative examples of mRNA expression of two major splicing variants of ING1 gene. For the p33ING1b, the samples 5-22, 6-8, 7-3 showed decreased or no expression while samples 7-40 and 5-44 demonstrated increased expression. The expression of p33ING1b in the samples 6-9 and 6-12 did not change. Likewise, for the p24ING1c, the samples 5-22, 7-25 and 7-3 showed decreased or no expression whereas the samples 7-40 and 6-10 displayed increased or same expression.

Fig. 2. Representative examples of PCR products for the methylated p33ING1b. After digestion with specific endonucleases, only bands can be seen in the samples cut with HpaII, which can not digest methylated sites. Negative and positive controls define normal DNA treated in the same conditions with or without enzyme for each reaction, respectively.