Expression and Mutation Analyses of MKK4, a Candidate Tumor Suppressor Gene Encoded by Chromosome 17p in Human Gastric Adenocarcinoma

Kwon-Seok Chae, Sung-Gil Chi

Department of Pathology, College of Medicine, Kyung Hee University, Seoul, Korea

Background Homozygous deletions and somatic mutations of mitogen-activated protein kinase kinase 4 (MKK4), a candidate tumor suppressor gene located at 17p11.2, have been observed in many types of human cancers. To assess the candidacy of MKK4 as a suppressor in gastric tumorigenesis, we characterized the expression and mutation status of MKK4 in 159 gastric tissues including 87 adenocarcinomas, 16 benign tumors, 56 normal gastric tissues, and 6 cell lines.

Methods Expression and mutation status of MKK4 was characterized using quantitative PCR and PCR-SSCP analyses. LOH at chromosome 17p11-12 was determined using three polymorphic STS markers (D17S969, D17S1303, and D17S947). Gene levels of MKK4 and p53 were also measured by quantitative genomic PCR. To explore the possible involvement of aberrant promoter hypermethylation, 6 gastric cell lines were treated with 5-aza-2’-deoxycytidine. Isolation of a processed pseudogene was done by PCR-based cloning and sequencing analyses.

Results Expression study demonstrated that all of 16 benign gastric tumors and 93 malignant carcinomas express easily detectable levels of MKK4 transcripts comparable to normal tissues. None of the carcinomas was recognized as an abnormal expressor and no cancer-specific reduction of MKK4 was identified in 43 matched sets. In addition, expression of MKK4 was not affected by the demethylating agent 5-aza-2’-deoxycytidine, suggesting no aberrant hypermethylation of the MKK4 gene in gastric cancer. Allelotype analysis of the MKK4 locus revealed a significant loss of heterozygosity (LOH) at telomeric markers whereas no evidences for allelic deletion of the MKK4 gene or at centromeric loci were detected. Moreover, any types of mutation leading to amino acid substitutions or frameshifts of MKK4 were found in carcinoma tissues and cell lines whereas a substantial fraction of the same set showed allelic loss or mutations of p53 located at 17p13, suggesting that LOH at telomeric loci or the p53 locus might not extend into the MKK4 gene in this type of cancer. In this study, we also report the identification of a highly conserved MKK4 processed pseudogene, which shares 95% homology with the coding region of functional MKK4 transcript.

Conclusion Our data indicate that MKK4 is not a critical target of genetic alteration at 17p in gastric adenocarcinomas, and argue that MKK4 does not play an important role as a tumor suppressor in gastric tumorigenesis.