Transcriptional Silencing of a Putative Tumor Suppressor RASSF1 in Human Gastric Adenocarcinoma

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Background Homozygous deletion and loss of heterozygosity at chromosome 3p21 has been frequently observed in various types of human malignancies including lung and ovarian cancers. Recently, a putative tumor suppressor gene RASSF1 has been identified at 3p21. RASSF1 encodes a RAS association domain (RA) and its carboxy terminus shows high homology to mouse RAS effector protein Nore1 and to the homologous rat protein Maxp1. To explore the possible implication of RASSF1 alteration in gastric carcinogenesis, we characterized expression and mutation status of three major isoforms of RASSF1, A, B and C in 121 gastric tissues and cell lines.

Methods Expression and mutation status of RASSF1 was explored by quantitative PCR and PCR-SSCP analyses. To define the possible involvement of aberrant promoter hypermethylation, 6 gastric carcinoma cell lines were treated with 5-aza-2’-deoxycytidine and expression levels of RASSF1 mRNA were examined by RT-PCR.

Results Among 11 gastric carcinoma cell lines we examined, 6 cell lines (SNU-1, SNU-5, SNU-719, AGS, MKN28, and MKN74) expressed no or extremely low levels of of RASSF1 A isoform transcripts. RASSF1 B expression was detected in 6 cell lines and 5 of the 6 were nonexpressors of A isoform, suggesting the reciprocal relationship in expression of the two isoforms. In contrast to A and B isoform, RASSF1 C mRNA was identified in all cell lines. To define the molecular basis for loss of RASSF1 expression, deletion and mutation analyses of the gene were performed using quantitative PCR and PCR-SSCP. However, allelic loss or any types of mutations leading to amino acid change were recognized. Interestingly, all of the nonexpressor cell lines showed reexpression of RASSF1 by treatment with the demethylating agent 5-aza-deoxycytidine, indicating that epigenetic transcriptional silencing by aberrant hypermethylation might be a main cause for expression loss. Furthermore, methylation-specific PCR and sequencing analysis for the CpG-rich region of the RASSF1 promoter demonstrated that all nonexpressor cell lines carry complete methylation of this region whereas that the SNU-5 cells which showed low level of expression showed partial methylation. Likely cell lines, approximately 54.0%(47/87) and 43.7%(38/87) of primary carcinomas showed expression loss of RASSF1 A and B isoform, respectively. Loss of expression was strongly associated with stage and grade but not with histologic types of tumors. No evidences for allelic deletion or sequence alterations of the gene were detected in primary carcinoma tissues.

Conclusion Our observation of frequent RASSF1 inactivation suggests that RASSF1 alteration might contribute to the malignant progression of gastric cancers.