Expression and Mutation Analyses of p53R2, a Newly Identified p53 Target Gene, in Human Gastric Adenocarcinoma

Do-Sun Byun, Byung-Kyu Ryu, Sung-Gil Chi

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

Background p53R2, a recently identified p53 target gene located at 8q23.1, encodes a protein with striking similarity to a small subunit (R2) of ribonucleotide reductase. p53R2 has been shown to be involved in the p53-dependent cell cycle checkpoint pathway after genotoxic stresses through DNA repair activity. To explore the p53R2’s candidacy for a suppressor in gastric tumorigenesis, we examined expression and mutational status of p53R2 in 102 gastric tissues and 6 cell lines.

Methods Expression and mutation status of p53R2 was characterized using quantitative PCR and PCR-SSCP analyses. LOH of the p53R2 gene was determined using a biallelic polymorphism within 5’ untranslated region of the p53R2 gene. Gene levels of p53R2 was also measured by quantitative genomic PCR. Effects of p53R2 expression on cell cycle progression was analyzed using [3H]thymidine incorporation and flow cytometric analyses. DNA repair capability of p53R2 was explored by host cell reactivation (CAT) assay.

Results Expression of p53R2 transcript was observed in all gastric tissues and cell lines we examined. After exposure to DNA damage-inducing agents such as adriamycin or etoposide, p53R2 transcription was clearly induced in cell lines by a p53-dependent manner. Host cell reactivation assay showed that disruption of p53R2 slightly reduces the repair capability of the cells for cisplatin-induced DNA damage. Cell cycle analysis using [3H]thymidine incorporation and flow cytometry demonstrated that blocking of p53R2 expression by transfection of antisense p53R2 expression vector does not cause significant G1 or G2 cell cycle arrest of wild-type p53-carrying gastric cancer cells. Expression analysis revealed that none of 50 primary carcinomas including 30 matched sets express abnormally low levels of p53R2 transcripts. Quantitative genomic PCR analysis also indicated the absence of allelic deletion of the p53R2 gene in carcinomas. In addition, mutational analysis of the entire coding region of the p53R2 transcript in 56 carcinoma tissues and cell lines failed to detect any types of mutation, whereas 19(33.9%) of the same set showed p53 alterations. While 14(73.7%) of these mutant p53-carrying tumors showed low or nearly undetectable levels of p21Waf1 mRNA, no association of p53R2 expression with the mutational status of p53 or expression level of p21Waf1 was recognized.

Conclusion Collectively, our data suggest that p53R2 might function as a p53 target gene and participate in a p53-mediated DNA repair signaling pathway, but its mutational alteration is an infrequent event in gastric tumorigenesis.