LETTER TO THE EDITOR

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Mutational Analysis of *MITOSTATIN*, a Candidate Tumor-Suppressor Gene, at a Mononucleotide Repeat in Gastric and Colorectal Carcinoma

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To the editor,

Human *MITOSTATIN* gene encodes a mitochondrial protein, which promotes apoptosis and inhibits cell growth.[1] The *MITOSTATIN* gene is somatically mutated in cancer cell lines (gastric, prostate and pancreas cell lines) and is down-regulated in bladder and breast cancer tissues.[1] These data suggest that *MITOSTATIN* gene may be a tumor suppressor gene, inactivation of which plays an important role in cancer development. Although somatic mutations of *MITOSTATIN* gene have been identified in cancer cell lines, its mutation status has not yet been studied in cancer tissues. Frameshift mutations of genes containing mononucleotide repeats are features of gastric (GC) and colorectal carcinomas (CRC) with microsatellite instability (MSI).[2] Many cancer-associated genes, such as *TGFBR2*, *BAX*, *IGFR2* and *TCF4*, have been found to harbor mutations at mono- or dinucleotide repeats in the coding sequences in these cancers with MSI.[2] There is a polyadenine tract (A8) in the exon 5 of the *MITOSTATIN* gene, but the frameshift mutation at this site is not known.

To see whether the mononucleotide repeat A8 in the exon 5 of *MITOSTATIN* gene is mutated in GC and CRC, we analyzed the exon 5 by polymerase chain reaction (PCR)-based single strand conformation polymorphism (SSCP) assay. For the mutation analysis, methacarn-fixed tissues of 42 GC and 51 CRC with MSI were used in this study. These cancers consisted of 31 GC with high MSI (MSI-H), 11 GC with low MSI (MSI-L), 37 CRC with MSI-H and 14 CRC with MSI-L. Malignant cells and normal cells from the same patients were selectively procured from hematoxylin and eosin-stained slides using a 30G1/2 hypodermic needle affixed to a micromanipulator.[3] Genomic DNA each from tumor cells and corresponding normal cells were amplified with a primer pair by PCR. Radioisotope ([^{32}P]dCTP) was incorporated into the PCR products for detection by SSCP autoradiogram. After SSCP, migration of the PCR products on the SSCP was analyzed by visual inspection. Direct DNA sequencing reactions were performed in the cancers with the mobility shifts in the SSCP. Other procedures of the PCR and SSCP were described in our previous studies.[3,4]

On the SSCP, all of the PCR products from the cancers were clearly seen. The PCR-SSCP analysis identified aberrant bands in one (3.2%) of the 31 GC with MSI-H, but not in the other cancers. DNA from normal tissues from the same patients showed no evidence of the mutation in SSCP, indicating the mutations had risen somatically. A direct DNA sequence analysis of the GC with the aberrant SSCP bands led to identification of a *MITOSTATIN* deletion mutation within the A8 repeat sequences (Fig. 1). The mutation was c.522delA, which would result in premature stops of the amino acid synthesis (p.Lys174AsnfsX26). We repeated the experiments twice, including PCR, SSCP and DNA sequencing analysis to ensure the specificity of the results, and found that the data were consistent.

Failure of apoptosis allows survival of cells and plays an important role in development of cancers.[3] As a mechanism of apoptosis resistance in cancer cells, somatic mutations of pro-apoptosis genes have been reported in many human cancers, and many of the mutations were

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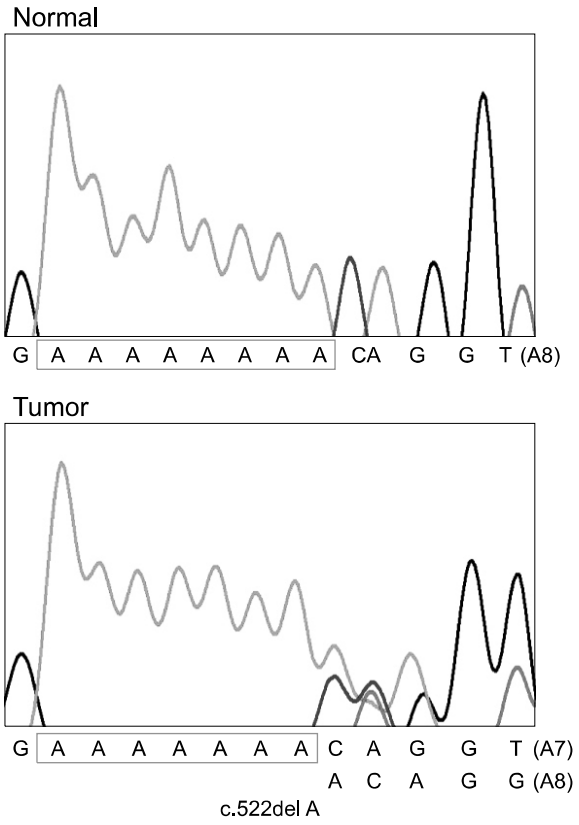

Fig. 1. Frameshift mutation of *MITOSTATIN* in a gastric carcinoma with microsatellite instability. Direct DNA sequence analysis of *MITOSTATIN* exon 5 from a gastric adenocarcinoma shows a heterozygous A deletion in tumor tissue relative to normal tissue.

proven to inactivate cell death.[3,5] Because the main tumor suppressor function of MITOSTATIN appears to depend on apoptosis activity, we analyzed frameshift mutation of *MITOSTATIN* gene at a mononucleotide repeat that may inactivate the apoptosis. However, we detected only one *MITOSTATIN* mutation in the cancers with MSI, indicating that the mutation at the repeat is rare in GC and CRC with MSI. In the earlier study[1] that first disclosed tumor suppressor functions of *MITOSTATIN*, the authors found that loss of expression and mutation of the gene in solid tumors and cell lines, respectively. Our and the earlier data[1] suggest that *MITOSTATIN* may be inactivated by various mechanisms. To see whether loss of function of MITOSTATIN is a feature of human cancers, the status of *MITOSTATIN* gene should be further analyzed at various levels in many cancer types.

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