Frameshift Mutation of *MARS* Gene Encoding an Aminoacyl-tRNA Synthetase in Gastric and Colorectal Carcinomas with Microsatellite Instability

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To the editor,

Gene translation is one of the most fundamental biologic processes for cells. Aminoacyl-tRNA synthetases (ARSs) are enzymes that charge tRNAs with their cognate amino acids during the translation.¹ Of the ARSs, nine ARSs form macromolecular complexes with ARS-interacting multifunctional proteins (AIMP1, 2 and 3). Many of the complex-forming ARSs and AIMP play roles not only in gene translation, but also in other biological processes such as apoptosis, angiogenesis and inflammation.¹ It has been known that *AIMP* genes may act as tumor suppressor genes.² Although non-translational roles of the complex-forming ARSs are known, their roles in cancer biology remain unknown.

Microsatellite instability (MSI) is defined by length alterations in repeated DNA sequences, and 10-30% of colorectal cancer (CRC) and gastric cancer (GC) are classified as MSI-positive cancers.³ Many tumor suppressor genes in cancers with MSI harbor frameshift mutations at the repeats in coding sequences.³ By analyzing public database, we found a T9 repeat in exon 3 of *methionyl-tRNA synthetase* (*MARS*) gene (nucleotides 204-212) that had not been analyzed for the mutations in cancers. To see whether the T9 is mutated in GC and CRC with MSI, we analyzed them by polymerase chain reaction (PCR)-based single strand conformation polymorphism (SSCP). We analyzed 30 high-MSI (MSI-H) and 15 low-MSI (MSI-L) GC, and 40 MSI-H and nine MSI-L CRC.

Malignant cells and normal cells were selectively procured from hematoxylin and eosin-stained slides using a 30G1/2 hypodermic needle by microdissection as de-

scribed previously.⁴ Tumor and normal DNA were amplified with by PCR using a primer pair that could amplify the T9 in exon 3 (product size 118 bps). Radioisotope (³²P]dCTP) was incorporated into the PCR for detection by SSCP. After SSCP, mobility shifts compared to wild-type bands were analyzed by visual inspection. Direct DNA sequencing was performed in the cancers with mobility shifts in the SSCP. We repeated the experiments twice to ensure the specificity of the results.

PCR-SSCP analysis identified aberrant bands in two of the GC with MSI-H (2/30; 6.7%) and one of the CRC with MSI-H (1/40; 2.5%), but not in those with MSI-L. DNA from normal tissue showed no shifts in SSCP, indicating the mutations had arisen somatically. Direct DNA sequencing of the cancers with the aberrant bands led to identification of a recurrent *MARS* mutation in the T9 repeat sequences (Fig. 1). The mutation was c.212delT, which would result in premature stops of the amino acid

Fig. 1. Frameshift mutation of *MARS* in a gastric carcinoma with MSI. Direct DNA sequencing analysis of *MARS* exon 3 from a gastric adenocarcinoma with MSI-H shows a heterozygous T deletion in tumor tissue as compare to normal tissue.

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synthesis (p.Leu71CysfsX33).

Human MARS exists as a component of the multi-ARS macromolecular complex, but this protein can dissociate from the complex and move to nucleus.⁵ After entering the nucleus, MARS plays a role in biogenesis of rRNA in nucleoli.⁵ Human MARS (about 900 amino acids long) contains N-terminal 267 amino acids and C-terminal 40 amino acids that are involved in protein-protein interactions. The intervening sequences are for its catalytic functions. Also, there are two nuclear localization signals (NLSs) (amino acids 897-900 and 724-730).⁵ The frame-shift mutation of MARS identified in this study would lead to premature stops of amino acid syntheses in MARS protein (p.Leu71CysfsX33) and would abolish most of the domains of MARS (the catalytic domain, the NLSs and parts of the protein interaction domains). The MARS mutation may alter the structure of MARS, and might inactivate the functions of MARS. However, the sequencing data of the mutation (heterozygous mutation) suggest that the second allele may be intact. It is possible that the catalytic function of MARS for the protein translation that is essential for viability of cells may be preserved by the intact second allele. The mutant allele may alter the non-catalytic functions of MARS and might play a role in cancer pathogenesis. However, to date, the background data on non-catalytic functions of MARS is not enough to interpret the role of the mutant MARS in cancers. In this study, we for the first time identified an ARS-encoding gene mutation in human cancers. To our knowledge, there has been no report on functions of MARS protein

related to MSI. To address consequences of such down-regulations in cancer development (especially related to cancers with MSI-H), functional studies on the mutated genes and their products should be further performed. Also, to see whether mutation of MARS gene is a common feature of cancers, the gene status should be further analyzed in other cancers.

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