LETTER TO THE EDITOR

Gut and Liver, Vol. 4, No. 2, June 2010, pp. 292-293

Mutational Analysis of *ASPP1* and *ASPP2* Genes, a p53-related Gene, in Gastric and Cololorectal Cancers with Microsatellite Instability

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To the editor,

In most human cancers, functions of p53 tumor suppressor are inactivated by many mechanisms, most commonly by somatic mutation.[1] Alternatively, p53 is inactivated by genes whose protein products interact with p53.[1] Apoptosis-stimulating protein of p53-1 (ASPP1) and -2 (ASPP2) promote p53-mediated apoptosis by enhancing its transactivation function.[2] *ASPP2*$^{+/-}$ mice suffer from an increased incidence of tumorsm,[3] suggesting it as a haploinsufficient tumor suppressor gene. However, neither of *ASPP1* or *ASPP2* mutation has been reported in cancers.

Microsatellite instability (MSI) is characterized by length alterations in repeated DNA sequences, and 10-30% of colorectal cancer (CRC) and gastric cancer (GC) are categorized as MSI-positive cancers.[4] In a public database, we found an A7 repeat in exon 8 of *ASPP1* and an A7 repeat in exon 6 of *ASPP2*. To see whether these A7 are mutated in GC and CRC with MSI, we analyzed them by polymerase chain reaction (PCR)-based single-strand conformation polymorphism (SSCP) as described previously.[5] We analyzed 32 high-MSI (MSI-H) and 13 low-MSI (MSI-L) GC, and 38 MSI-H and 15 MSI-L CRC. We used two primer pairs that amplified each A7 of *ASPP1* and the *ASPP2*. Tumor and normal DNA from microdisscted cells were amplified by the PCR. Radioisotope ([^{32}P]dCTP) was incorporated into the PCR for detection by SSCP. Direct DNA sequencing was performed in the cancers with mobility shifts in the SSCP.

The PCR-SSCP analysis of *ASPP2* identified aberrant bands in one GC with MSI-H (3.1%) and two CRC with MSI-H (5.3%), but none in those with MSI-L (Fig. 1). Normal DNA of the patients showed no shifts, indicating the mutations had risen somatically. Direct DNA sequencing identified an identical *ASPP2* deletion mutation in the A7 repeat (c.576delA), which would result in a premature stop (p.Val193fsX1) and resembled a typical loss-of -function mutation. For *ASPP1*, there was no aberrantly migrating SSCP band in the cancer tissues.

As a possible mechanism for p53 inactivation, we ana-

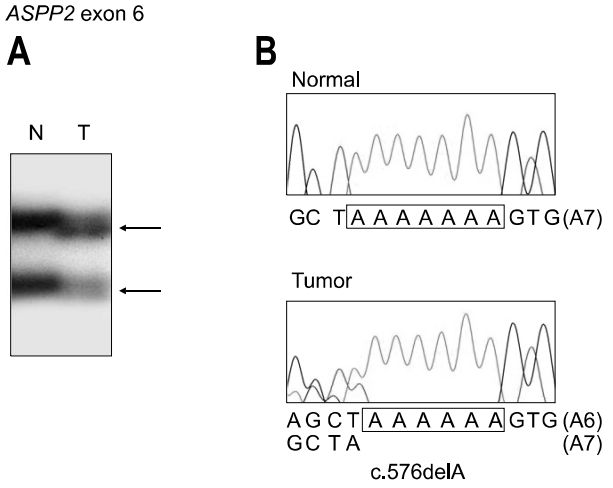

Fig. 1. Mutation of *ASPP2* in a gastric carcinoma with high-microsatellite instability. (A) PCR product of *ASPP2* from a colon carcinoma shows aberrant bands (arrows in lane T) as compared to single-strand conformation polymorphism from normal tissue (lane N) of the same patient. (B) Direct DNA sequence analysis shows a heterozygous A deletion within the A7 in tumor tissue compared to normal tissue.

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Received on January 18, 2010. Accepted on January 29, 2010.
DOI: 10.5009/gnl.2010.4.2.292

lyzed *ASPP1* and *ASPP2* mutations in their repeat sequences and found some of the cancers with MSI-H harbored *ASPP2* frameshift mutation. In colon, tumors with MSI have frameshift mutations in specific target genes, and fewer mutations are found in *K-RAS* and p53.[4] Although the incidence of the *ASPP2* mutation is not high, our data indicate that the *ASPP2* mutation could partially explain a p53-inactivating mechanism in GC and CRC with MSI.

Four ankylin repeats (amino acids 930-1047) and an SH3 domain (amino acids 1064-1114) of *ASPP2* are essential for the interaction of *ASPP2* with p53.[2,3,6] The frameshift mutation identified in this study (p.Val193fsX1) would remove ankylin repeats and SH3 domain, and pro-apoptotic function of *ASPP2* may be decreased in the affected cancers. Our data suggest that decreased p53 functions by the *ASPP2* mutation might possibly contribute to pathogenesis in MSI-H cancers. Whether inactivation of *ASPP* genes further contributes to GC and CRC development in addition to the frameshift mutation of *ASPP2*, mutational analysis of entire coding region of *ASPP1* and *ASPP2* genes is required in future studies.

ACKNOWLEDGEMENTS

This study was supported by a grant from Education, Science and Technology of Korea (2009-007-1498).

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