A Frameshift Mutation of the Pro-Apoptotic VDAC1 Gene in Cancers with Microsatellite Instability

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Dear editor.

Apoptosis is a fundamental biochemical cell-death pathway that plays important roles in various physiological and pathological processes during fetal development and in adult tissues.¹ In the intrinsic pathway, a number of death stimuli engage the apoptotic machinery by causing release of cytochrome c from mitochondria, which in turn induces recruitment of caspase-9 into an apoptosome.¹ Activated caspase-9 initiates a proteolytic cascade by activating effector caspases that cleave key molecules to induce apoptosis. As inactivation of pro-apoptotic genes through genetic alterations have been reported in many cancers, they are considered tumor suppressor genes.^{2,3}

Voltage-dependent anion channel 1 (VDAC1) is located in the outer mitochondrial membrane (OMM), and is involved in controlling metabolic cross-talk between mitochondria and the cytosol.⁴ In addition, VDAC1 plays a key role during intrinsic apoptosis. VDAC1 constitutes an OMM channel that mediates the release of a number of apoptogenic molecules, including cytochrome c, second mitochondria-derived activator of caspase (Smac), HTRA serine peptidase 2 (HTRA2), apoptosis-inducing factor (AIF), and endonuclease G (EndoG) from mitochondria to the cytosol by OMM permeabilization.⁴ VDAC1-deficient mitochondria from a mutant cell did not exhibit a Bax/Bak-induced loss in membrane potential of OMM and in cytochrome c release.⁵ Since VDAC1 plays an important role in apoptosis signaling, it could be hypothesized that VDAC1 gene is inactivated through somatic mutation in human cancers. To date, however, the data on the mutation status of VDAC1 in human cancers is lacking.

By analyzing the public database (http://genome.cse.ucsc. edu/), we found an A8 repeat in exon 6 of VDAC1 gene (nucleotides 325-332) that had not been analyzed for mutations in cancers. Microsatellite instability (MSI) is defined by

length alterations in repeated DNA sequences, and 10% to 30% of colorectal cancer (CRC) and gastric cancer (GC) are classified as MSI-positive cancers.⁶ To see whether the A8 repeat is mutated in GC and CRC with MSI, we performed polymerase chain reaction (PCR)-based single strand conformation polymorphism (SSCP). We analyzed 30 GC with high-MSI (MSI-H) and 10 GC with stable MSI (MSS), 37 CRC with MSI-H, and 14 CRC with MSS according to the NCI criteria.⁷ The GC consisted of 21 diffuse-type and 19 intestinal-type adenocarcinomas by Lauren's classification. The TNM stages of the GC were 14 stage I, 16 stage II, 8 stage III, and 2 stage IV, while those of the CRC were 8 stage I, 18 stage II, 22 stage III, and 3 stage IV. Male to female ratios of gastric and colorectal cancers were 23:17 and 30:21, respectively. All of the specimens were surgically removed by gastrectomy and colectomy. Approval for this study was obtained from The Catholic University of Korea, College of Medicine's Institutional Review Board.

Malignant cells and normal cells were selectively procured from hematoxylin and eosin-stained slides using a 30G1/2 hypodermic needle by microdissection as described previously.^{2,3} This microdissection method was proven to procure tumor cells with nearly being devoid of normal cell contamination.^{2,3} DNA from tumor and normal cells were amplified by PCR using a primer pair that could amplify the A8 (product size 128 bps). Radioisotope ([32P]dCTP) was incorporated into the PCR for detection by SSCP. After SSCP, mobility shifts compared to wildtype 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 one of the GC with MSI-H (1/30, 3.3%) and one of the CRC with MSI-H (1/37, 2.7%), but not in those with MSS. DNA from normal tis-

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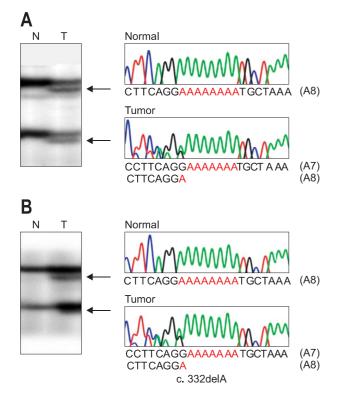


Fig. 1. Frameshift mutations of *VDAC1* in a gastric cancer and a colonic cancer with microsatellite instability. Direct DNA sequencing analyses of *VDAC1* exon 6 from a gastric adenocarcinoma (A) and a colonic adenocarcinoma (B) with MSI-H show heterozygous A deletion in tumor tissues compared with normal tissues.

sue showed no shifts in SSCP, indicating the mutations had risen somatically. Direct DNA sequencing of the cancers with the aberrant bands led to identification of a recurrent VDAC1 mutation in the A8 repeat sequence (Fig. 1). This mutation, c.332delA, leads to a premature stop codon, resulting in truncation of the amino acid synthesis (p.Asn111MetfsX34). The SSCP and DNA sequencing patterns indicate that the mutation was heterozygous (Fig. 1). There was no significant difference of the mutations with respect to the clinicopathologic features of the GC and CRC (Fisher's exact test, p>0.05).

Earlier studies showed that VDAC1 exists as oligomers of varying sizes in the cells.^{4,8} Mader *et al.*⁸ demonstrated that a VDAC1 mutant bound with wild-type VDAC1 displayed a dominant-negative effect in the intrinsic apoptosis pathway. The frameshift mutation of *VDAC1* identified in this study would lead to a premature stop of amino acid synthesis in VDAC1 protein (p.Asn111MetfsX34) and would remove about 60% length of C-terminal VDAC1 protein. In this case, it is possible that the hemizygously mutated VDAC1 may bind with normal VDAC1 to construct a structurally abnormal oligomer. As a possible mechanism of apoptosis inactivation in human cancers, we analyzed *VDAC1* mutation in GC and CRC with MSI, and we identified for the first time a frameshift mutation in human cancer. To our knowledge, there has been no report on functions of

VDAC1 protein related to MSI. To address consequences of such down-regulations in cancer development (especially related to cancers with MSI-H), additional functional studies on the mutated gene should be performed. Although we identified somatic mutations of *VDAC1* in both GC and CRC, the incidence was not high. To see whether other mechanisms of *VDAC1* inactivation are present in these cancers, characterization of additional somatic alterations in *VDAC1* should be undertaken. Antibody activity against frameshift neopeptides provides a promising tool for diagnostic application in MSI cancer patients. It might be clinically significant to analyze the immunogenic behavior of the VDAC1 neopeptides in future studies.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

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