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LETTERS TO THE EDITOR

Somatic mutation of *EXO1* gene in gastric and colorectal cancers with microsatellite instability

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To the Editor,

Both endogenous (e.g. metabolism) and exogenous (e.g. ultraviolet light) factors continuously cause DNA damage in cells. To maintain genomic stability, DNA damage is repaired by a series of events, including cell cycle arrest and activation of DNA damage repair signaling [1]. In the case of large amount of DNA damage, however, the cells eventually undergo apoptosis. DNA repair requires nucleases to process different DNA intermediate structures and to maintain genomic stability. *Exonuclease-1* (*EXO1*) gene encodes a protein with 5' to 3' exonuclease activity and 5'-flap exonuclease activity [2]. EXO1 protein plays important roles in DNA repair pathways, including post-replication repair, mismatch repair and mitotic recombination [2]. Errors in DNA repair and replication result in accumulation of mutations, which contribute to development of cancers. Many proteins involved in DNA repair signaling are altered in tumors, for example mutations of *MLH1* and *MSH2* are associated with colorectal cancer development [3].

Microsatellite instability (MSI), a type of genomic instability caused by errors in mismatch repair, is characterized by length alterations in simple repeated mono- or dinucleotide DNA sequences. About 10–30% of colorectal cancer (CRC) and gastric cancer (GC) are categorized into MSI-positive cancers [3]. By analyzing a public database (http://genome.ucsc.edu/), we found that there are two mononucleotide repeats in the coding exons of *EXO1* gene (T7 in exon 12 and A7 in exon 13) that could be potential mutation targets in cancers with MSI. However, to date, the mutation status of these repeats in *EXO1* in cancers with MSI has not been reported.

To see whether the mononucleotide repeats are somatically mutated in GC and CRC, we analyzed the two exons by polymerase chain reaction (PCR)-based single strand conformation polymorphism (SSCP) assay. The cancers consisted of 28 GC with high MSI (MSI-H), 12 GC with low MSI (MSI-L), 32 CRC with MSI-H and 12 CRC with MSI-L. All of the cancers were sporadic cases and there was no germline mutation in the DNA repair genes. 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 [4]. 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 [4].

Overall, the PCR-SSCP analysis identified aberrant bands in seven of the 84 GC and CRC analyzed. None of the corresponding normal samples showed evidence of mutations by SSCP, indicating the aberrant bands had risen somatically. Direct DNA sequencing analysis of the cancers with the aberrant bands in the SSCP led to identification of six frameshift mutations in the exon 12 and one in the exon 13 (Table I). All of the mutations were detected in the cancers with MSI-H (12%; 7/60) (Table I), but not in those with MSI-L (0%; 0/24). The mutations were found in and two of 28 gastric cancers with MSI-H (7%) and five of 32 CRC with MSH-H (16%). All of the mutations would result in premature stops of the amino acid synthesis

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Table I. Summary of *EXO1* mutations in gastric and colorectal cancers.

Location	Repeats (wild type)	Repeats (Mutation)	Incidence in MSI-H cancers (%)	Nucleotide change (predicted amino acid change)
Exon 12	T7	T8	Gastric: 1/30 (3) Colorectal: 2/33 (6)	c.1522dupT (p.Cys508LeufsX7)
Exon 12	T7	T6	Gastric: 1/30 (3.3) Colorectal: 2/33 (6)	c.1522delT (p.Cys508AlafsX13)
Exon 13	A7	A8	Gastric: 0/30 (0) Colorectal: 1/33 (3)	c.2190dupA (p.Asp731ArgfsX13)

(Table I). We carefully reviewed the clinicopathologic data of the patient (age, sex, histologic grade, stage, and metastasis), but there was no significant association of the *EXO1* mutations with them. There was not any correlation between histological features of the tumors and the presence of *EXO1* mutations, either. To confirm the mutation data, we repeated the PCR-SSCP twice.

Despite earlier intensive works that discovered many frameshift mutations in cancers with MSI, it appears that many frameshift mutations in the cancers still remain to be discovered. In this study, we found that *EXO1* gene harbored somatic frameshift mutations within the mononucleotide repeats in the coding sequences. These mutations were found in the cancers with MSI-H, but not in those with MSS, indicating that association of the mutations with MSI-H is specific. Alteration of DNA damage repair has long been recognized as an important step in cancer development [1]. For example, inactivation of EXO1 in mice results in DNA mismatch repair defect and increases cancer susceptibility [5]. Also, depletion of EXO1 in mouse and human cells inactivates DNA damage-induced apoptosis [6]. The *EXO1* mutations identified in our study would lead to premature stops of amino acid syntheses in EXO1 protein and hence resembles a typical loss-of-function mutation, which might inactivate DNA damage repair and apoptosis in affected cancer cells and contribute to development of GC and CRC with MSI-H. To confirm the roles of the *EXO1* frameshift mutations, further functional studies should be performed.

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