

Mutational analysis of mononucleotide repeats in dual specificity tyrosine phosphatase genes in gastric and colon carcinomas with microsatellite instability

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Song SY, Kang MR, Yoo NJ, Lee SH. Mutational analysis of mononucleotide repeats in dual specificity tyrosine phosphatase genes in gastric and colon carcinomas with microsatellite instability. *APMIS* 2010; 118: 389–93.

Coordinated protein phosphorylation and dephosphorylation are crucial in the regulation of cell signaling, and disruption of the coordination is known to play important roles in cancer development. Recent reports revealed that classical protein tyrosine phosphatase (PTP)-encoded genes are somatically mutated in human colorectal cancer. However, data on dual specificity phosphatase (DPTP) gene mutations in human cancers are lacking. By analyzing a public genomic database, we found that five DPTP genes, *CDC14A*, *MTM1*, *MTMR3*, *SSH1*, and *SSH2*, have mononucleotide repeats in their coding DNA sequences. To see whether these genes are mutated in cancers with microsatellite instability (MSI), we analyzed the mononucleotide repeats in 26 gastric cancers (GC) with MSI (MSI-H), 12 GC with low MSI (MSI-L), 45 GC with stable MSI (MSS), 33 colorectal cancers (CRC) with MSI-H, 14 CRC with MSI-L, and 45 CRC with MSS by single-strand conformation polymorphism (SSCP). We found *CDC14A* and *MTMR3* mutations in five and one cancer (s), respectively. These mutations were detected in MSI-H cancers, but not in MSI-L or MSS cancers. The GC and CRC with MSI-H harbored the mutations in 15% and 6%, respectively. The *CDC14A* and *MTMR3* mutations detected in the GC and CRC were deletion or duplication mutations of one base in the nucleotide repeats that would result in premature stops of the amino acid syntheses. Our data show that frameshift mutations of DPTP genes in MSI-H cancers occur at moderate frequencies. The data suggested that alterations in the *CDC14A* and *MTMR3* genes may play a role in the development of GC and CRC with MSI-H by deregulating phosphatase functions possibly together with mutations of classical PTP genes.

Key words: MSI; mutation; cancer; dual specificity phosphatase.

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Protein phosphorylation, a unique mechanism of protein regulation, participates in many cellular processes, including cellular growth, differentiation and survival (1). Proteins are most

frequently phosphorylated on tyrosine, serine and threonine residues. Protein tyrosine phosphorylation is regulated by protein tyrosine kinases and protein tyrosine phosphatases (PTP) (2–4). Human PTP genes consist of 53 classical PTPs (21 receptor PTPs and 32 non-receptor

PTPs), 33 dual specificity phosphatases (DPTP), and one low molecular weight phosphatase (5).

Activation of protein kinases by protein phosphorylation has been implicated in oncogenic transformation (1). On the contrary, it has been suggested that protein dephosphorylation might contribute to tumor suppression (2, 5), but the exact role of PTPs in cancer pathogenesis remains largely unknown. Recently, analysis of 87 PTP genes in colorectal cancer (CRC) tissues identified mutations in six genes (*PTPRF*, *PTPRG*, *PTPRT*, *PTPN3*, *PTPN13*, and *PTPN14*) (5). Functionally, the *PTPRT* mutants detected in the phosphatase domains possessed reduced phosphatase activity. These data suggest that the PTPs may have characteristics of tumor suppressor genes (5).

About 10–30% of CRC and gastric carcinomas (GC) are categorized into MSI (microsatellite instability)-positive cancers (6). Many cancer-associated genes have been found to harbor mutations at mono- or dinucleotide repeats in the coding sequences in cancers with MSI (7–9). Recently, Korff *et al.* analyzed coding sequences of the classic PTP genes in CRC and found that six PTP genes harbored frameshift mutations in the mononucleotide repeat sequences (10). By analyzing DPTP gene sequences in the public database (<http://genome.cse.ucsc.edu/>), we found five genes (*CDC14A*, *MTM1*, *MTMR3*, *SSH1*, and *SSH2*) have mononucleotide repeats in the coding sequences (*CDC14A* exon 5 (C7), *MTM1* exon 10 (A7), *MTMR3* exon 16 (C7), *MTMR3* exon 17 (C7), *SSH1* exon 15 (C7), *SSH2* exon 14 (C7), and *SSH2* exon 15 (C7)) that could be highly

affected by loss or mutations of a defective repair system (6). Any frameshift mutations in the coding sequences would result in a production of truncated proteins and the activity of the truncated PTP proteins would be inactivated in the cancer cells by the frameshift mutations. The aim of this study was to examine whether the DPTP gene mutations in the repeats occur in MSI-positive GC and CRC.

MATERIALS AND METHODS

Tissue samples and microdissection

Methacarn-fixed tissues of 83 GC and 92 CRC were used for this study. All patients with cancers were Koreans. All of the cancers were sporadic cases and there was no germline mutation in the DNA repair genes. The cancers consisted of 26 GC with high MSI (MSI-H), 12 GC with low MSI (MSI-L), 45 GC with stable MSI (MSS), 33 CRC with MSI-H, 14 CRC with MSI-L, and 45 CRC with MSS according to the NCI criteria (11). The pathologic features of the cancers with MSI are summarized in Table 1. 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 by microdissection, as described previously (12, 13). DNA extraction was performed by a modified single-step DNA extraction method by proteinase K treatment, as described previously (12, 13).

PCR-SSCP and DNA sequencing

Genomic DNA from the microdissected cells was isolated, and amplified by polymerase chain reaction (PCR) with specific primer pairs that are specific to

Table 1. Summary of pathologic features of cancers

		No. of gastric carcinomas				No. of colorectal carcinomas	
		MSI-H	MSI-L			MSI-H	MSI-L
TNM	I	15	5	TNM	I	5	2
	II	5	4		II	11	4
	III	5	2		III	14	7
	IV	1	1		IV	3	1
Lauren's subtype	Diffuse	12	6	Location (colon)	Cecum	6	0
	Intestinal	10	5		Ascending	19	2
	Mixed	4	1		Transverse	6	2
EGC vs AGC	EGC	2	1		Descending and sigmoid	2	4
	AGC	24	11		Rectum	0	6

MSI, microsatellite instability; TNM, tumor, lymph nodes and metastasis; EGC, early gastric cancer; AGC, advanced gastric cancer.

CDC14A exon 5, *MTM1* exon 10, *MTMR3* exon 16, *MTMR3* exon 17, *SSH1* exon 15, *SSH2* exon 14, and *SSH2* exon 15. Each PCR reaction was performed under standard conditions with 8 μ L of reaction mixture. Radioisotope (32 P)dCTP) was incorporated into PCR products for detection by SSCP autoradiogram. PCR conditions were as follows: initial denaturation for 1 min at 94 °C followed by 30 cycles of denaturation for 40 s at 94 °C, annealing for 40 s at 47–60 °C, and extension for 40 s at 72 °C. Final extension was continued for 5 min at 72 °C. After amplification, PCR products were denatured for 5 min at 95 °C in a 1:1 dilution of sample buffer containing 98% formamide/5 mmol/L NaOH and were loaded onto an SSCP gel (FMC Mutation Detection Enhancement system; Intermountain Scientific, Kaysville, UT, USA) with 10% glycerol. Then, the PCR products were electrophoresed overnight at 8 W at room temperature. After electrophoresis, the gels were transferred onto 3 mm Whatman paper and dried, and autoradiography was performed. Mobility shifts on the SSCP were determined by visual inspection. Direct DNA sequencing reactions were performed among the cancers with the mobility shifts in the SSCP. Sequencing of the PCR products was carried out using a capillary automatic sequencer (ABI Prism Genetic Analyzer; Applied Biosystems, Foster City, CA, USA). We repeated the experiments twice, including PCR, SSCP and DNA sequencing analysis to ensure the specificity of the results.

RESULTS

Genomic DNA isolated from normal and tumor tissues of the 38 GC and 47 CRC with MSI, and 45 GC and 45 CRC with MSS were analyzed for detection of frameshift mutations of *CDC14A*, *MTM1*, *MTMR3*, *SSH1*, and *SSH2* by PCR-SSCP analysis. On the SSCP, we observed aberrant bands in five cases of *CDC14A* exon 5 and one case of *MTMR3* exon 17 (Fig. 1; Table 2). There was no aberrant band in the SSCP of the other three genes analyzed. DNA from normal tissues from the same patients showed no evidence of the mutation in SSCP, indicating that mutations had arisen somatically (Fig. 1A). DNA sequence analysis of the aberrantly migrating bands led to the identification of *CDC14A* frameshift mutations in five cancers and *MTMR3* frameshift mutation in one cancer (Fig. 1; Table 2). All of the mutations were deletion or duplication mutations of one base in the repeats that would result in premature stops of the amino acid synthesis (Table 2).

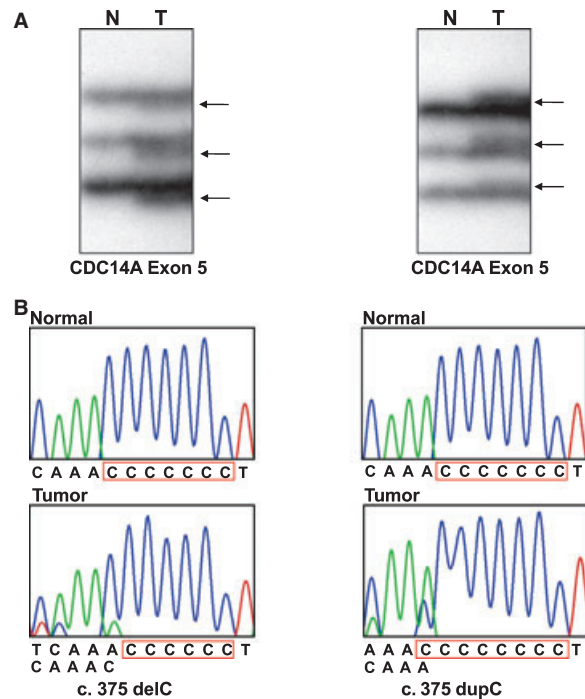


Fig. 1. Representative SSCP and DNA sequencing analysis of *CDC14A*. SSCP (A) and DNA sequencing analysis (B) of *CDC14A* from tumor (lane T) and normal tissues (lane N). (A) In the SSCP, the arrows (lane T) indicate aberrant bands compared with the SSCP from normal tissues (N). (B) Direct DNA sequence analysis shows heterozygous deletion or duplication of a nucleotide in tumor tissue DNA as compared with normal.

All of the mutations were found in the GC or CRC with MSI-H (Table 2), but not in those with MSI-L or MSS. There was a statistical difference between the frequencies of the mutations in the carcinomas with MSI-H (6/59) and non-MSI-H [MSI-L (0/36) and MSS (0/90)] (Fisher's exact test, $p = 0.001$). Of the 26 GC with MSI-H, four cancers harbored one mutation each (4/26; 15%), while of the 33 CRC with MSI-H, two cancers harbored one mutation each (2/33; 6%). There was no significant difference in the mutation frequencies between the GC with MSI-H and CRC with MSI-H (Fisher's exact test, $p > 0.05$). We reviewed the clinicopathologic data of the patient (age, sex, histologic grade, stage, and metastasis), but there was no significant association of mutations with them. There was not any correlation between histologic features of the tumors and the presence of mutations, either.

Table 2. Summary of frameshift mutations of *CDC14A* and *MTMR3*

Gene (GenBank accession no.)	Location	Repeats (wild type)	Repeats (mutation)	Incidence in MSI-H cancers (%)	Nucleotide change (predicted amino acid change)
<i>CDC14A</i> (NM_003672)	Exon 5	C7	C6	Gastric: 3/26 (11.5) Colorectal: 1/33 (3.0)	c.375delC (p.Tyr126IlefsX64)
		C7	C8	Gastric: 0/26 (0) Colorectal: 1/33 (3.0)	c.375dupC (p.Tyr126LeufsX35)
<i>MTMR3</i> (NM_153050)	Exon 17	C7	C6	Gastric: 1/26 (3.8) Colorectal: 0/33 (0)	c.2349delC (p.Arg784GlyfsX15)

MSI, microsatellite instability.

DISCUSSION

It is generally believed that tyrosine kinases possess oncogenic functions, and that PTPs possess tumor suppressor functions. Frequent mutations of classical PTP genes in CRC with MSI (5) and without MSI (10) led us to analyze mutations of the DPTP genes in GC and CRC. Because mononucleotide repeats are frequent targets for somatic mutation in GC and CRC with MSI, we focused our analysis on the repeats in *CDC14A*, *MTM1*, *MTMR*, *SSH1*, and *SSH2* genes that have mononucleotide repeats. Our study detected frameshift mutations in the genes of *CDC14A* (11.5% of MSI-H GC; 6.1% of MSI-H CRC) and *MTMR* (3.8% of MSI-H GC). These mutations were found in the cancers with MSI-H, but not in those with MSI-L or MSS, indicating that the association of mutations with MSI-H is specific. Our study shows that frameshift mutations in the repeat sequences of PTP genes occur not only in the classical PTP genes (10), but also in the DPTP genes. Together, these data suggest that the mutations of both classical PTP and DPTP genes are features of GC and CRC with MSI.

DPTP is a subclass of the PTP gene superfamily, which functions for dephosphorylating both phosphothreonine and phosphotyrosine residues within the same proteins. The DPTP targets many kinases, including mitogen-activated protein (MAP) kinases and cyclin-dependent kinase (CDK) (14–16), which promote cell proliferation and survival. Thus, it is plausible that the inactivation of DPTP functions might provide conditions that are essential for carcinogenesis. Human *CDC14A* is highly similar to *Saccharomyces cerevisiae* Cdc14, a PTP involved in the exit of cell mitosis and initiation of DNA replication (17, 18). This protein has been shown to interact with, and dephosphorylate

tumor suppressor p53, and is thought to regulate the function of p53 (19). Moreover, protein expression of *CDC14A* is down-regulated in many cancer cell lines, and treatment of demethylating agent induces its expression (20). Down-regulation of endogenous *CDC14A* induces mitotic defects, including impaired centrosome separation and failure to undergo productive cytokinesis (21), suggesting that *CDC14A* might be involved in promoting genetic instability and subsequent tumorigenesis. The *CDC14A* frameshift mutations identified in this study would lead to premature stops of amino acid synthesis in the *CDC14A* protein and hence resemble a typical loss-of-function mutation. The mutated *CDC14A* may inactivate its cell cycle-related and p53-related functions, and might contribute to the pathogenesis of affected MSI-H cancers. *MTMR3* protein that is structurally similar to myotubularin binds to phosphoinositide lipids, and hydrolyzes phosphatidylinositol(3)-phosphate and phosphatidylinositol(3,5)-biphosphate (22). However, to date, there is little knowledge on cancer-related functions of *MTMR3*. The significance of the *MTMR3* mutation remains to be clarified in future studies.

One of the main topics in cancer research is to find novel mutations that might possibly contribute to cancer pathogenesis. To date, there has been no report on somatic mutations of the DPTP genes in human cancers. In the present study, we found that both GC and CRC with MSI-H harbored *CDC14A* and *MTMR3* gene mutations that may inactivate functions of the genes. Although the mutations detected in this study were not common, they might contribute to the development of affected MSI-H cancers together with other common mutations in cancers with MSI, such as those of *TGFBR2*, *BAX*, *APC* and *IGFR2* genes.

This study was supported by a grant from Education, Science and Technology of Korea (R01-2008-000-10014-0).

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