

# Mutational Analysis of *TTK* Gene in Gastric and Colorectal Cancers with Microsatellite Instability

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## Purpose

The *TTK* gene plays a crucial role in regulation of the mitotic checkpoint. The *TTK* gene has an A9 mononucleotide repeat in the coding sequences, which harbors mutations in gastric (GC) and colorectal cancers (CRC) with microsatellite instability (MSI). However, there are three more repeats (the A7s) in the coding sequences that have not been analyzed. The aim of this study was to explore whether the three A7s as well as the A9 are altered in GC and CRC, and to find any association of *TTK* mutation with clinocopathologic characteristics of GC and CRC.

## Materials and Methods

We analyzed exon 5 (A7 and A7) and exon 22 (A9 and A7) which have repeat sequences in 30 GC with high MSI (MSI-H), 15 GC with low MSI (MSI-L), 35 CRC with MSI-H, and 15 CRC with MSI-L, by single-strand conformation polymorphism (SSCP) and DNA sequencing assays.

## Results

Overall, we detected 23 frameshift mutations in the repeat sequences of *TTK* in the GC with MSI-H (11/30; 36.7%) and the CRC with MSI-H (12/35; 34.3%), but not in the cancers with MSI-L. The mutations were observed in both A9 and A7 of exon 22, but in neither of the two A7s of exon 5. The mutations consisted of c.2560delA, c.2560dupA, c.2571delA and c.[2560delA (+)2571delA]. All of the mutations were frameshift mutations and would result in premature stops of *TTK* protein synthesis. There was no significant difference in clinopathologic parameters of the cancers with the mutations.

## Conclusion

Our data indicate that frameshift mutations of *TTK* are common in both GC and CRC with MSI-H, and that the mutations occur not only in the A9 repeat but also in the A7 repeat. The data suggest that frameshift mutations of *TTK* might alter cell cycle control in the affected cells and contribute to pathogenesis of cancers with MSI-H.

## Key words

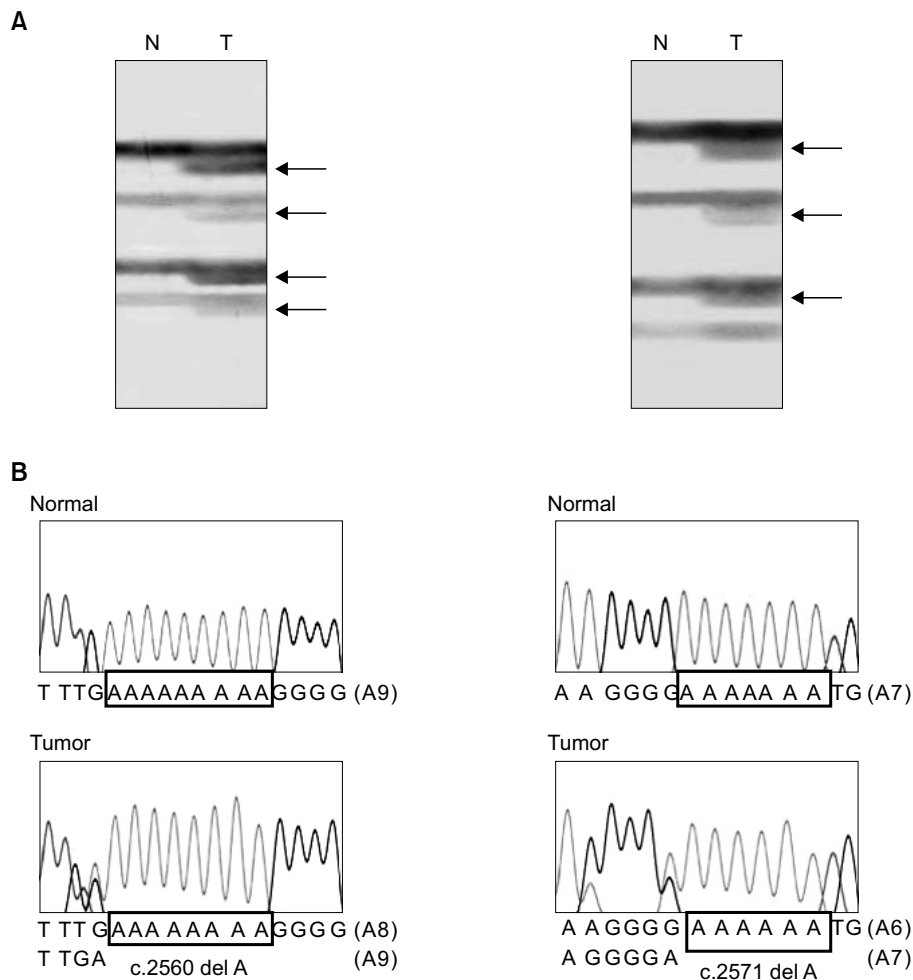
*TTK*, Mutation, Microsatellite instability, Gastric neoplasms, Colorectal neoplasms

## Introduction

Defective repair of mismatched bases in DNA results in widespread microsatellite instability (MSI), which is characterized by length alterations in simple repeated mono- or dinucleotide DNA sequences (1-3). MSI occurs in 10~30% of colorectal (CRC), gastric (GC) and endometrial cancers (1-3). Many cancer-associated genes such as *TGFBR2*, *BAX*, *IGFR2* and *TCF4* have been found to harbor mutations at the repeats with MSI (4-6). Also, many other genes implicated in cell cycle control and DNA damage signaling/ repair

pathways are frequently mutated in the repeat sequences in cancers with MSI (7-10).

Although earlier works report many frameshift mutations in the repeats in cancers with MSI, it appears that more mutations still remain to be discovered. *TTK*, also known as Mps1, is a dual-specific protein kinase that phosphorylates tyrosine and serine/threonine residues (11). *TTK* plays a role in the mitotic checkpoint; inhibition of *TTK* causes a pleiotropic phenotype resulting from the combination of severe mitotic abnormalities and failures in centrosome duplication (12-15). In exon 22 of *TTK*, there is a polyadenine repeat (A9). Earlier studies detected frameshift mutations in the A9 in GC and CRC with



**Fig. 1.** Representative SSCP and DNA sequencing of *TTK* mutations. SSCP (A) and DNA sequencing analysis (B) of *TTK* from tumor (Lane T) and normal tissues (Lane N) DNA. (A) In the SSCPs, the arrows (Lane T) indicate aberrant bands compared to the SSCP from normal tissues (N). (B) Direct DNA sequencing analyses of *TTK* show heterozygous deletions of one nucleotide in tumor tissue as compare to normal tissues.

microsatellite instability (16,17). However, there are additional mononucleotide repeats in exon 5 (two A7s) and exon 22 (one A7) of the *TTK* gene that have not been analyzed. The previous studies discovered *TTK* mutation in GC and CRC, but they did not disclose any clinicopathologic characteristics of GC and CRC associated with the *TTK* mutations. The aim of this study was to see whether *TTK* frameshift mutations occur in the three A7s as well as in the A9 in GC and CRC with MSI. In addition, we analyzed the clinicopathologic data of the cancers to determine possible relationships to the *TTK* mutations.

## Materials and Methods

Methacarn-fixed tissues of 45 GC and 48 CRC with MSI were used for this study. The cancers consisted of 30 GC with high MSI (MSI-H), 15 GC with low MSI (MSI-L), 33 CRC with MSI-H and 15

CRC with MSI-L according to the NCI criteria (18). The MSI-H cancers were randomly selected among MSI-H cancers resected between 1999 and 2004. Similarly, the MSI-L cancers were randomly selected among MSI-L cancers resected between 1999 and 2008. Malignant cells and normal cells were selectively procured from hematoxylin and eosin-stained slides using a 30G1/2 in hypodermic needle by microdissection as described previously (19,20). All of the patients with the cancers were Koreans. Approval for the study was obtained from the Catholic University of Korea, College of Medicine's institutional review board.

In exon 22 of the *TTK* gene, there is one A9 and one A7. We designed a primer pair that could amplify a region encompassing these two repeats (forward and reverse, respectively): (5'-TAT AGTGGTGGTGAAAGTCA-3' and 5'-TG TAGATTTCCAC AGGGAT-3') (product size: 150 bps). In exon 5, there are 2 A7s. We used a primer pair for these two repeats (forward and reverse, respectively): (5'-ATTATCTTTTGCTTTGTTAG-3' and 5'-GAT AAATTCTTCTTTTCCTC-3') (product size: 162 bps). Genomic

**Table 1.** Summary of *TTK* mutations in the gastric and colorectal cancers with MSI

Gene	Location	Repeats (wild type)	Repeats (Mutation)	Incidence in MSI-H cancers (%)	Nucleotide change (predicted amino acid change)
<i>TTK</i>	Exon 22	A9	A8	Gastric: 8/30 (26.7) Colorectal: 8/33 (24.2)	c.2560delA (p.Arg854GlyfsX39)
<i>TTK</i>	Exon 22	A9	A10	Gastric: 0/30 (0) Colorectal: 1/33 (3.0)	c.2560dupA (p.Arg854LysfsX11)
<i>TTK</i>	Exon 22	A7	A6	Gastric: 1/30 (3.3) Colorectal: 1/33 (3.0)	c.2571delA (p.Lys857AsnfsX36)
<i>TTK</i>	Exon 22	A9+A7	A8+A6	Gastric: 2/30 (6.7) Colorectal: 2/33 (6.1)	c.[2560delA (+) 2571delA] (p.p.Arg854GlyfsX10)
<i>TTK</i>	Exon 5	A7, A7	No mutation	Gastric: 0/30 (0) Colorectal: 0/33 (0)	—
			Total	Gastric: 11/30 (33.3) Colorectal: 12/33 (34.3)	

DNA from cancer and from normal cells were isolated separately, and analyzed for potential mutations in the repeat sequences by polymerase chain reaction (PCR) with the specific primer pairs. Each PCR reaction was performed under standard conditions in an 8  $\mu$ L reaction mixture. Radioisotope ( $[^{32}\text{P}]\text{dCTP}$ ) was incorporated into PCR products for detection by SSCP autoradiogram. Other procedures of PCR and SSCP analysis were performed as described previously (19,20). Direct DNA sequencing reactions were performed in the cases with the mobility shifts in the SSCP (tumor vs. normal DNA). Sequencing of the PCR products was carried out using a capillary automatic sequencer (ABI Prism Genetic Analyzer, Applied Biosystem, Foster City, CA). We repeated the experiments twice, including PCR, SSCP and DNA sequencing analysis to ensure the specificity of the results.

## Results

The GC with MSI-H consisted of 13 diffuse-type, 12 intestinal-type and 5 mixed-type carcinomas by Lauren's classification, while the GC with MSI-L consisted of 7 diffuse-type, 7 intestinal-type and 1 mixed-type carcinomas. The GC with MSI-H consisted of 3 early (EGC) and 27 advanced (AGC) GC, while the GC with MSI-L consisted of 1 EGC and 14 AGC. The TNM stages of the GC with MSI-H were 17 stage I, 6 stage II, 6 stage III and 1 stage IV, while those of the GC with MSI-L were 5 stage I, 4 stage II, 4 stage III and 2 stage IV. The CRC originated from cecum [MSH-H (N=5), MSI-L (N=0)], ascending colon [MSH-H (N=17), MSI-L (N=2)], transverse colon [MSH-H (N=9), MSI-L (N=2)], sigmoid colon [MSH-H (N=2), MSI-L (N=4)] and rectum [MSH-H (N=0), MSI-L (N=7)]. The TNM stages of the CRC with MSI-H were 5 stage I, 11 stage II, 13 stage III and 4 stage IV, while those of the CRC with MSI-L were 2 stage I, 4 stage II, 7 stage III and 2 stage IV.

Genomic DNA isolated from normal and tumor tissues of the 45 GC and 48 CRC with MSI were analyzed for detection of frameshift mutations within 4 mononucleotide repeats of *TTK* gene in the coding DNA sequences by PCR-SSCP analysis. Overall, we detected aberrant bands in 11 of the 45 GC and 12 of the 48 CRC. None of the corresponding normal samples showed evidence of mutations by SSCP, indicating the aberrant bands had arisen somatically (Fig. 1A). Direct DNA sequencing analysis of the cancers with the aberrant bands in the SSCP led to identification of 23 *TTK* frameshift mutations (Fig. 1B). All of the mutations were detected in the cancers with MSI-H (35.4%; 23/65) (Table 1), but not in those with MSI-L. In the MSI-H cancers, the mutations were seen in the GC with MSI-H (11/30; 36.7%) and the CRC with MSI-H (12/35; 34.3%). There was a strong statistical difference of *TTK* mutation frequency between the cancers with MSI-H (23/65) and the cancers with MSI-L (0/30) (Fisher's exact test,  $p < 0.001$ ).

The *TTK* mutations were observed in both A9 and A7 of exon 22, but in neither of the two A7s of exon 5 (Table 1). The mutations were either deletion or duplication of one base in the repeats that would result in premature stops of amino acid synthesis (Table 1). Of note, 2 GC and 2 CRC displayed 2 sequence variations (mutations) in 1 individual cancer c.[2560delA(+)]2571delA].

For the mutations, we analyzed the clinicopathologic data of the patients (demographic data, subtype, location, grade, stage, and metastasis). As for the stage, the 11 GC with the *TTK* mutations consisted of 7 TNM I, 1 stage II and 3 stage III cancers, while the 12 CRC with the mutations consisted of 1 stage I, 4 stage II, 5 stage IIIA and 2 stage IV cancers. However, there was no significant association of the mutations with the clinicopathologic data, including the TNM stage (Fisher's exact test,  $p > 0.05$ ). One of the 11 *TTK* mutations in the GC was detected in EGC.

## Discussion

The known frequency of frameshift mutations of cell cycle-related genes in cancers (7-10) led us to analyze *TTK* for the detection of somatic mutations in GC and CRC tissues. Our study disclosed that the GC with MSI-H (11/30; 36.7%) and the CRC with MSI-H (12/35; 34.3%) harbored *TTK* frameshift mutations in the repeat sequences. The frameshift mutations identified in this study would lead to premature stops of *TTK* protein synthesis and hence resemble a typical loss-of-function mutation. *TTK* plays crucial roles in the mitotic checkpoint by regulating kinetochore (13,14). Also, *TTK* phosphorylates the p53 and mediates the p53-dependent postmitotic checkpoint (15). The frameshift mutation of *TTK* might alter the protein structure of *TTK*, deregulate the cell cycle control of *TTK*, and contribute to cancer development.

In earlier studies from Western countries, *TTK* mutations in the A9 repeat have been detected in GC with MSI-H (2/6 (33.3%) and 5/18 (27.8%)) (16,17) and CRC with MSI-H (2/17 (11.8%)) (16). Compared to these data, *TTK* mutations in the A9 detected in the present study is not statistically different (Fisher's exact test,  $p > 0.05$ ), confirming that the A9 mutation may be common in GC and CRC from both Western and Asian countries. Although most frameshift mutations in cancers with MSI have been screened in genes with 8 to 10-bp-long mononucleotide repeats (4-10), 7-bp-long mononucleotide repeats have also been reported to harbor mutations in cancers with MSI (21,22). In this study, we detected 6 mutations in the A7 of exon 22 (2 c.2571delA and 4 c.[2560delA(+)-2571delA]), indicating that

*TTK* is mutated not only in the A9, but also in the A7.

We found that the patients with the *TTK* mutations did not show any difference from others with regards to known clinicopathologic characteristics, suggesting that there may be a similar mechanism equivalent to *TTK* mutation in the cancer cells. We found *TTK* mutations at EGC and at low TNM stages of GC and CRC, suggesting that the mutations might affect the pathogenesis at a relatively early stage of the cancers.

Genomic instability is simply classified into chromosomal instability (CIN) and MSI (1-3). It is intriguing that *TTK*, a CIN-related gene, is mutated in MSI. There is evidence that MSI and CIN may not be mutually exclusive, and that both 'CIN-negative and MSI-negative' and 'CIN-positive and MSI-positive' cancers may exist (23, 24). Frequent somatic mutations of other CIN-related genes in MSI-H cancers and the *TTK* mutations identified in the present study might possibly explain the overlapping of MSI and CIN in the cancers. Alternatively, it can be hypothesized that the CIN-related genes with the mutations might play roles in the mismatch repair pathway.

## Conclusion

Our data showed that frameshift mutations of *TTK* are common in both GC and CRC with MSI-H. The frameshift mutations of *TTK* may alter the structure of *TTK* and deregulate its cell cycle control in cancers with MSI-H.

## References

- Grady WM. Genomic instability and colon cancer. *Cancer Metastasis Rev.* 2004;23:11-27.
- Gorringe KL, Chin SF, Pharoah P, Staines JM, Oliveira C, Edwards PA, et al. Evidence that both genetic instability and selection contribute to the accumulation of chromosome alterations in cancer. *Carcinogenesis.* 2005;26:923-30.
- Imai K, Yamamoto H. Carcinogenesis and microsatellite instability: the interrelationship between genetics and epigenetics. *Carcinogenesis.* 2008;29:673-80.
- Markowitz S, Wang J, Myeroff L, Parsons R, Sun L, Lutterbaugh J, et al. Inactivation of the type II TGF-beta receptor in colon cancer cells with microsatellite instability. *Science.* 1995;268:1336-8.
- Rampino N, Yamamoto H, Ionov Y, Li Y, Sawai H, Reed JC, et al. Somatic frameshift mutations in the BAX gene in colon cancers of the microsatellite mutator phenotype. *Science.* 1997;275:967-9.
- Schwartz S Jr, Yamamoto H, Navarro M, Maestro M, Reventos J, Perucho M. Frameshift mutations at mononucleotide repeats in caspase-5 and other target genes in endometrial and gastrointestinal cancer of the microsatellite mutator phenotype. *Cancer Res.* 1999;59:2995-3002.
- Miquel C, Jacob S, Grandjouan S, Aime A, Viguier J, Sabourin JC, et al. Frequent alteration of DNA damage signalling and repair pathways in human colorectal cancers with microsatellite instability. *Oncogene.* 2007;26:5919-26.
- Vassileva V, Millar A, Briollais L, Chapman W, Bapat B. Genes involved in DNA repair are mutational targets in endometrial cancers with microsatellite instability. *Cancer Res.* 2002;62:4095-9.
- Lewis KA, Bakkum-Gamez J, Loewen R, French AJ, Thibodeau SN, Cliby WA. Mutations in the ataxia telangiectasia and rad3-related-checkpoint kinase 1 DNA damage response axis in colon cancers. *Genes Chromosomes Cancer.* 2007;46:1061-8.
- Wang Z, Cummins JM, Shen D, Cahill DP, Jallepalli PV, Wang TL, et al. Three classes of genes mutated in colorectal cancers with chromosomal instability. *Cancer Res.* 2004;64:2998-3001.
- Mills GB, Schmandt R, McGill M, Amendola A, Hill M, Jacobs K, et al. Expression of *TTK*, a novel human protein kinase, is associated with cell proliferation. *J Biol Chem.* 1992;267:16000-6.
- Fisk HA, Mattison CP, Winey M. Human Mps1 protein kinase is required for centrosome duplication and normal mitotic progression. *Proc Natl Acad Sci U S A.* 2003;100:14875-80.
- Huang H, Hittle J, Zappacosta F, Annan RS, Hershko A, Yen TJ. Phosphorylation sites in BubR1 that regulate kinetochore attachment, tension, and mitotic exit. *J Cell Biol.* 2008;183:667-80.
- Xu Q, Zhu S, Wang W, Zhang X, Old W, Ahn N, et al. Regulation of kinetochore recruitment of two essential mitotic spindle checkpoint proteins by Mps1 phosphorylation. *Mol Biol Cell.* 2009;20:10-20.
- Huang YF, Chang MD, Shieh SY. *TTK*/hMps1 mediates the p53-dependent postmitotic checkpoint by phosphorylating p53 at Thr18. *Mol Cell Biol.* 2009;29:2935-44.
- Greenman C, Stephens P, Smith R, Dalgliesh GL, Hunter C, Bignell G, et al. Patterns of somatic mutation in human cancer genomes. *Nature.* 2007;446:153-8.
- Mori Y, Sato F, Selaru FM, Oлару A, Perry K, Kimos MC, et al. Instability typing reveals unique mutational spectra in microsatellite-unstable gastric cancers. *Cancer Res.* 2002;62:3641-5.
- Boland CR, Thibodeau SN, Hamilton SR, Sidransky D, Eshleman JR, Burt RW, et al. A National Cancer Institute Workshop on Microsatellite Instability for cancer detection and familial predisposition: development of international criteria for the determination of microsatellite instability in colorectal cancer. *Cancer Res.* 1998;58:5248-57.
- Lee SH, Shin MS, Park WS, Kim SY, Kim HS, Han JY, et al. Alterations of Fas (Apo-

- 1/CD95) gene in non-small cell lung cancer. *Oncogene*. 1999;18:3754-60.
20. Lee JW, Soung YH, Kim SY, Lee HW, Park WS, Nam SW, et al. PIK3CA gene is frequently mutated in breast carcinomas and hepatocellular carcinomas. *Oncogene*. 2005;24:1477-80.
21. Kang MR, Kim MS, Oh JE, Kim YR, Song SY, Kim SS, et al. Frameshift mutations of autophagy-related genes ATG2B, ATG5, ATG9B and ATG12 in gastric and colorectal cancers with microsatellite instability. *J Pathol*. 2009;217:702-6.
22. Kim MS, Kim SS, Ahn CH, Yoo NJ, Lee SH. Frameshift mutations of Wnt pathway genes AXIN2 and TCF7L2 in gastric carcinomas with high microsatellite instability. *Hum Pathol*. 2009;40:58-64.
23. Vauhkonen H, Vauhkonen M, Sajantila A, Sipponen P, Knuutila S. DNA copy number aberrations in intestinal-type gastric cancer revealed by array-based comparative genomic hybridization. *Cancer Genet Cytogenet*. 2006;167:150-4.
24. Jones AM, Douglas EJ, Halford SE, Fiegler H, Gorman PA, Roylance RR, et al. Array-CGH analysis of microsatellite-stable, near-diploid bowel cancers and comparison with other types of colorectal carcinoma. *Oncogene*. 2005;24:118-29.