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ORIGINAL ARTICLE

Frameshift Mutations of *HSPA4* and *MED13* in Gastric and Colorectal Cancers

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Abstract Frameshift mutation of genes containing mononucleotide repeats is a feature of gastric (GC) and colorectal cancers (CRC) with microsatellite instability (MSI). In the public genome database, we found that human *HSPA4* gene encoding a heats hock protein 70 protein (HSP70–4) and *MED13* gene had mononucleotide repeats in the coding sequences that could be targets for frameshift mutation in cancers with MSI. HSP70–4 is a member of HSP70 that is known to play a role in cell survival. MED13 is a member of MED genome-wide transcription regulators that function as a regulator for diverse biological processes. In this study, we analyzed the mutations in 79 GCs and 124 CRCs including high MSI (MSI-H) and microsatellite stable/low MSI (MSS/MSI-L) cases by single-strand conformation polymorphism analysis and DNA sequencing. We found frameshift mutations of *HSPA4* gene in two cancers (one GC and one CRC) and *MED13* gene in the other two cancers (one GC and one CRC). The frameshift mutations were deletions of one base (c.2396delA (p.Asn799MetfsX50)) in *HSPA4* and (c.2175delA (p.Lys725AsnfsX4)) in *MED13*. Each of *HSPA4* and *MED13* mutations were detected in GC with MSI-H (1/34: 2.9 %) and CRC with MSI-H (1/79: 1.3 %), but not in those with MSS. Our data show that unconventional *HSPA4* and *MED13* genes harbored frameshift mutations in GC and CRC with MSI. These mutations might possibly inactivate their functions and could be a feature of GC and CRC with MSI-H.

Keywords HSPA4 · HSP70 · MED13 · Mutation · Cancers · Microsatellite instability

Introduction

Heat shock proteins (HSPs) are a group of proteins induced by heat shock, which include five conserved classes, i.e., small HSPs, HSP40, HSP60, HSP70 and HSP90 [1]. HSPs function as intra-cellular chaperones by assisting correct folding of nascent and stress-accumulated misfolded proteins. HSPs allow the cells to survive stress conditions. HSP70 are a family of ubiquitously expressed heat shock proteins encoded by 13 genes (HSPA1A, HSPA1B, HSPA1L, HSPA2, HSPA4, HSPA4L, HSPA5–9, HSPA12A and HSPA14). Several lines of evidence indicate that HSP70 proteins promote cell survival and inhibit apoptosis [2]. HSP70s are overexpressed in several cancers [1, 3, 4]. The Mediator (MED), a genome-wide transcription regulator, is a multiprotein complex that promotes RNA polymerase II transcriptional activation through interactions with transcription factors bound with enhancers and promoter elements and with polymerase and the general initiation factors at the core promoter [1]. There are more than 20 MED complexes that exist in two compositionally distinct forms, cyclin-dependent kinase 8 (CDK8)-mediators and non-CDK8 core mediators. The CDK8 module consists of MED12, MED13, CDK8 and cyclin C (CCNC) proteins [5]. In addition, recent studies suggested that the mediator complex functions as a regulator for diverse biological processes, including differentiation, proliferation and tumorigenesis. For example, somatic mutations in *MED12* have been reported in

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uterine leiomyoma and breast fibroadenoma, suggesting that mutations in mediator genes may possibly play roles in tumor development [6, 7]. However, somatic mutation in *MED13* and *HSP70* genes has been rarely identified.

In a public genome database (http://genome.cse.ucsc.edu/), we found that both *HSPA4* (encoding HSP70–4) and *MED13* gene had mononucleotide repeats in the coding sequences that could be targets for frameshift mutation in cancers with microsatellite instability (MSI). Frameshift mutation of genes containing mononucleotide repeats is a feature of gastric (GC) and colorectal cancers (CRC) with MSI [8]. Approximately 10–20 % of GC and CRC are high MSI (MSI-H), and the cancers with MSI-H harbor mutations at nucleotide repeats in the coding sequences of cancer genes [8]. To date, however, it is not known whether *HSPA4* and *MED13* genes are mutationally altered in GC and CRC. In this study, we studied *HSPA4* and *MED13* mutations in GC and CRC.

Materials and Methods

Tissue Samples and Microdissection

For mutation analysis, 79 sporadic GCs and 124 sporadic CRCs were used in this study. Of them, 54 CRCs were frozen tissues and the other 149 tissues were methacarn-fixed tissues. The GCs consisted of 34 GCs with MSI-H, 45 GCs with microsatellite stable/low MSI (MSS/MSI-L), 79 CRCs with MSI-H and 45 CRCs with MSS/MSI-L. Our samples over-represent MSI-H cancers compared to MSS cancers, which is not in agreement with the incidences of GC and CRC in ordinary cohorts. This is because we collected these two groups separately for different duration. The MSI evaluation system used five mononucleotide repeats (BAT25, BAT26, NR-21, NR-24 and MONO-27), tumoral MSI status of which was characterized as: MSI-H, if two or more of these markers show instability, MSI-L, if one of the markers shows instability and MSS, if none of the markers shows instability [9]. Our samples over-represented MSI cancers compared to MSS cancers since we recruited additional MSI-H cancers. The tumor cell purities of the tissues were at least 70 %. Pathologic features of the cancers are summarized in Table 1. The histologic features of CRC with MSI-H, including mucinous histology, tumor infiltrating lymphocytes, medullary pattern, and Crohn's like inflammation, were evaluated in all blocks of all cases by a pathologist. 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 [10, 11]. DNA extraction was performed by a modified single-step DNA extraction method by proteinase K treatment [12]. DNA extraction was done by a modified single step DNA extraction method [13].

Table 1 Summary of pathologic features of the cancers

Feature	MSI-H	MSS/MSI-L
Gastric carcinomas		
Total cases	34	45
TNM stage		
I	13	15
II	13	18
III	7	11
IV	1	1
Lauren's subtype		
Diffuse	4	28
Intestinal	20	18
Mixed	3	6
Indeterminate	7	3
EGC vs. AGC		
EGC	3	4
AGC	31	41
Colorectal carcinomas		
Total cases	79	45
TNM stage		
I	15	6
II	29	20
III	32	16
IV	3	3
Location		
Cecum	16	0
Ascending colon	46	3
Transverse colon	12	2
Descending & sigmoid colon	4	17
Rectum	1	23

EGC early gastric cancer, *AGC* advanced gastric cancer, *TNM* tumor, lymph node, metastasis, *MSI-H* high microsatellite instability, *MSI-L* low microsatellite instability, *MSS* stable microsatellite instability

Briefly, the cells obtained in 20 μl of DNA extraction buffer were incubated at 52 °C for 1 or 2 days. The mixture was boiled for 10 min to inactivate the proteinase K, and 1 μl of this solution was used as DNA template for polymerase chain reaction (PCR) amplification. Approval of this study was obtained from the Catholic University of Korea, College of Medicine's institutional review board for this study.

Single Strand Conformation Polymorphism (SSCP) Analysis

We analyzed the mononucleotide repeats of *HSPA4* (one A7 repeat in exon 19) and *MED13* (one A7 repeat in exon 10) in their coding sequences. Genomic DNA from the microdissected cells was isolated, and was amplified by PCR with specific primer pairs. Radioisotope ([^{32}P]dCTP) was incorporated into the PCR products for detection by autoradiogram. After

SSCP, mobility shifts on the SSCP gels (FMC Mutation Detection Enhancement system; Intermountain Scientific, Kaysville, UT, USA) were determined by visual inspection. Direct DNA sequencing reactions in both forward and reverse sequences were performed in the cancers with the mobility shifts in the SSCP using a capillary automatic sequencer (3730 DNA Analyzer, Applied Biosystem, Carlsbad, CA, USA). When mutations in the genes were suspected by SSCP, analysis of an independently isolated DNA from another tissue section of the same patients was performed to exclude potential artifacts originated from PCR. Other procedures for PCR-SSCP were described in our previous reports [10, 11].

Results and Discussion

Genomic DNAs isolated from normal and tumor tissues of the 79 GC and 124 CRC were analyzed for detection of mutation in the nucleotide repeats of *HSPA4* (one A7 repeat in exon 19) and *MED13* (one A7 repeat in exon 10) by PCR-SSCP analysis. On the SSCP, we observed aberrant bands of *HSPA4* gene in two cancers (one GC and one CRC) and *MED13* gene in the other two cancers (one GC and one CRC). DNA from the patients' normal tissues showed no shifts in SSCP, indicating the aberrant bands had risen somatically. DNA sequencing analyses confirmed that the aberrant bands represented an *HSPA4* somatic mutation and an *MED13* somatic mutation, which were frameshift mutations by deletion of one base (c.2396delA (p.Asn799MetfsX50)) in *HSPA4* and (c.2175delA (p.Lys725AsnfsX4)) in *MED13* (Fig. 1). In terms of tissue origins, there was no statistical difference in mutation frequencies between GCs and CRCs (Fisher's exact test, $p = 0.349$). There was no significant association of the mutations with the clinicopathologic data of the patients (age, sex, histologic grade and stage). In the cancers with MSI-H, there was no correlation between histological features of the tumors (histologic grade, subtypes, mucinous histology, medullary pattern and tumor-infiltrating lymphocytes) and presence of the mutations.

These mutations were detected in cancers with MSI-H, but not in those with MSS/MSI-L. The *HSPA4* mutations were detected in a GC with MSI-H (1/34: 2.9 %) and a CRC with MSI-H (1/79: 1.3 %), but not in those with MSS. Also, the *MED13* mutations were detected in a GC with MSI-H (1/34: 2.9 %) and a CRC with MSI-H (1/79: 1.3 %), but not in those with MSS.

The frameshift mutations detected in the present study would result in a premature stop of amino acid synthesis in the HSP70–4 and MED13 proteins and hence resembles a typical loss-of-function mutation. These data suggest that HSP70–4 and MED13 are inactivated in GC and CRC harboring the frameshift mutations. At this stage, however, consequence of these mutations in cancers remains unknown. Because HSP70–4 is one of the HSP70 proteins that facilitates cancer cell survival in cancer cells by inhibiting apoptosis and promoting proliferation [2], *HSPA4* might possess an oncogenic activity. Provided that *HSPA4* behaves as an oncogene, the *HSPA4* frameshift mutation appears to reduce the survival activity of cancer cells. It is theoretically possible that the *HSPA4* frameshift mutation at least partially explains better prognosis of CRC and GC with MSI-H than those with MSS [8]. However, due to the low incidence in tumors,

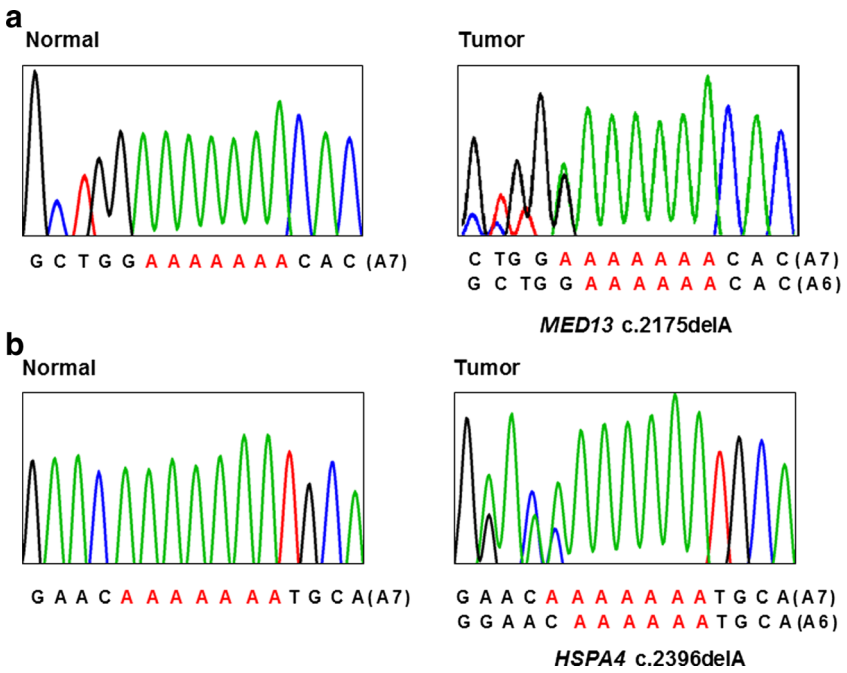

Fig. 1 Mutations of *MED13* A7 repeat and *HSPA4* A7 repeat in colon carcinomas with MSI-H. A. Direct DNA sequence analysis of *MED13* shows a heterozygous A deletion within the A7 in tumor tissue as compared to normal tissue. B. Direct DNA sequence analysis of *HSPA4* shows a heterozygous A deletion within the A7 in tumor tissue as compared to normal tissue

clinical significance of the mutations may be limited. As for *MED13*, its roles in cancer development are not known. MED13 interacts with both CCNC and CDK8, which are known to possess dual roles (oncogene and tumor suppressor gene) [13]. The uterine leiomyoma-linked mutation in *MED12* disrupts mediator-associated CDK activity and promotes tumorigenesis [14]. Whether *MED13* frameshift mutation similarly disrupts the CDK activity should further be studied.

In this study, we identified *HSPA4* and *MED13* frameshift mutations in GC and CRC with MSI-H. The mutation rates are low (2.9 % in GC and 1.3 % in CRC). However, because these mutations are specific for MSH-H phenotype, the oncogenic role of the frameshift mutations in MSI-H should be further studied.

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Compliance with Ethical Standards

Conflict of Interests The authors declare no competing interests.

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