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LETTER TO EDITOR

HMCN1, a cell polarity-related gene, is somatically mutated in gastric and colorectal cancers

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To the Editor:

HEMICENTIN1 (*HMCN1*) gene, also known as FBLN6 and ARMD1, encodes an extracellular protein of the immunoglobulin superfamily. The HMCN1 protein consists of a von Willebrand A domain, tandem immunoglobulin modules, tandem EGF modules, and a fibulin carboxy-terminal module [1]. The HMCN in *C. elegans* has wide functions in cell contacts that are required for cell migration and invasion, and in contacts at hemidesmosome-mediated cell junctions [1, 2]. Although HMCN1 in vertebrate is assumed to have a similar function to HMCN of *C. elegans*, its role in human remains unknown. Of note, a linkage analysis identified a mutation in human HMCN1 that segregated with the phenotype of age-related macular degeneration [3]. Based on its role in *C. elegans*, the *HMCN1* mutation is likely to interfere with the hemicentin assembly process.

The HMCN in *C. elegans* is involved in maintenance of cell polarity [1], loss of which is considered a hallmark of tumor cells. However, alterations of HMCN1 in human cancers have not been reported, yet. In a public genome database (http://genome.cse.ucsc.edu/), we found that human *HMCN1* gene had mononucleotide repeats in its 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 [4].

To see whether the mononucleotide repeat in human *HMCN1* gene is mutated in GC and CRC, we analyzed an A7 repeat in the exon 7 and an A7 repeat in exon 42 by polymerase chain reaction (PCR)-based single strand conformation polymorphism (SSCP) assay. For this, we used methacarn-fixed tissues of 34 GC with high MSI (MSI-H), 45 GC with stable MSI (MSS), 99 CRC with MSI-H and 45 CRC with MSS. In cancer tissues, malignant cells and normal cells were selectively procured from hematoxylin and eosin-stained slides by microdissection [5]. Radioisotope ([^{32}P]dCTP) was incorporated into the PCR products for detection by autoradiogram. The PCR products were subsequently displayed in SSCP gels. After SSCP, direct DNA sequencing reactions were performed in the cancers with mobility shifts in the SSCP as described previously [5].

Next, to see whether On the SSCP, we observed aberrant bands of the *HMCN1* gene in five cancers. DNA from normal tissue showed no shifts in SSCP, indicating the aberrant bands had risen somatically (Fig. 1). DNA sequencing analysis confirmed that aberrant bands represented *HMCN1* somatic mutations. All of the mutations were frameshift mutation (deletion or duplication of one base) in the A7 repeats (c.1000delA and c.6557dupA) that would result in a frameshifting change with Thr334 as the first affected amino acid, changing to a Gln and cresting a new reading frame ending in a stop at position 26 from the Gln (p. Thr334GlnfsX26) and that with Asn2186 as the first affected amino acid, changing to a Lys and cresting a new reading frame ending in a stop at position 16 from the Lys (p. Asn2186LysfsX16). They were detected in a GC with MSI-H (1/34; 3.0 %) and four of the CRC with MSI-H (4/99; 4.0 %), but none in those with MSS. HMC1 protein is altered in the cancers, we performed immunohistochemistry using tissue microarray (TMA) blocks that contained paraffin-embedded GC (N=100) and CRC (N=100) tissues. Each case has cores representing cancers as well as those representing corresponding normal epithelial tissues. The TMA included CRC with MSI-H (N=20) and GC with MSI-H (N=20). In the

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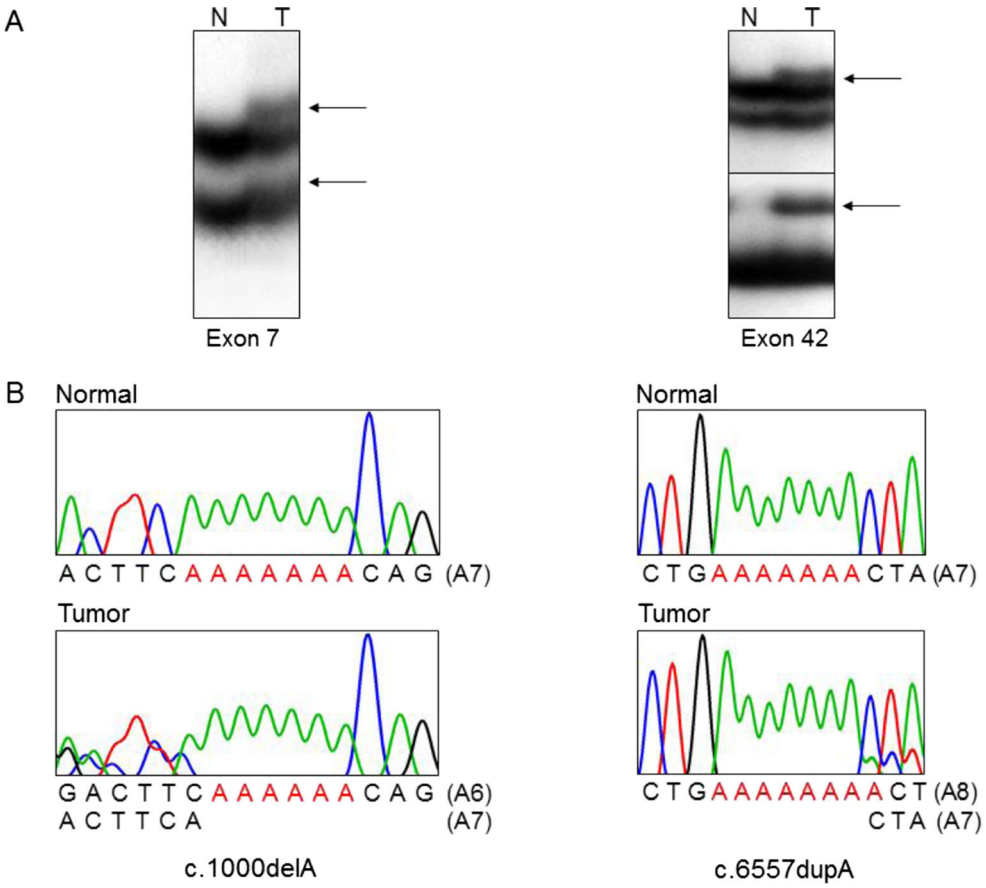

Fig. 1 Mutations of *HMCN1* mononucleotide repeats in colon carcinomas with MSI-H. A. PCR products of *HMCN1* exon 7 from a colon carcinoma and exon 42 from another colon carcinoma show aberrant bands (arrows in lane T) as compared to SSCP from normal tissue (N) of the same patients. B. Direct DNA sequence analyses show a heterozygous A deletion within the A7 (left) and a heterozygous A duplication within the A7 (right) in tumor tissue as compare to normal tissue

immunohistochemistry, we used ImmPRESS System (Vector Laboratories, Burlinggame, CA, USA) with rabbit polyclonal antibody against human HMCN1 (Sigma, St. Louis, USA; dilution 1/50). Other procedures for mutation and immunohistochemistry were described in our previous report [6]. In the immunohistochemistry, positive HMCN1 immunostaining was observed in normal gastric and colonic mucosa, while it was observed in 62 % and 58 % of the GC and CRC, respectively. Statistically, there was no difference of the immunopositivity between the cancers with MSI-H (23/40) and MSS (87/160) (Fisher's exact test, $p>0.05$).

The frameshift mutations detected in the present study would result in premature stops of amino acid synthesis in *HMCN1* protein and hence resembles a typical loss-of-function mutation, suggesting that *HMCN1* is inactivated in GC and CRC with the mutations. Also, we found that HMCN1 expression was lost about 60 % of GC and CRC irrespective of the MSI status. At this stage, consequence of the *HMCN1* inactivation by the frameshift mutation and loss of expression in tumorigenesis remains unknown. Provided that *HMCN1* is involved in maintenance of cell polarity, the *HMCN1* inactivation might alter the HMCN1-mediated cell polarity and possibly contribute to tumorigenesis. To extend the knowledge of HMCN1 in tumorigenesis, further studies on HMCN1 mutation and expression are required based on our observation.

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