Pathol. Oncol. Res. (2015) 21:851–852
DOI 10.1007/s12253-014-9862-y

LETTER TO EDITOR

Mutation of HELLS, a Chromatin Remodeling Gene, Gastric and Colorectal Cancers

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Received: 22 April 2014 / Accepted: 22 October 2014 / Published online: 29 October 2014

To the Editor:

Chromatin remodeling is a dynamic modification process of chromatin architecture to allow access of genomic DNA to transcription machinery proteins, and control gene expression. ATP-dependent chromatin remodeling is a main mechanism for eukaryote chromatin remodeling [1]. *HELLS* (also known as *SMARC6* and *LSH*) gene encodes a protein related to the SNF2 family of chromatin-remodeling ATPase. This protein is essential for correct establishment of DNA methylation level and for efficient repair of DNA double-strand breaks [2]. Somatic mutations in chromatin remodeling genes have been detected in many cancers, including gastric, ovary and renal cancers [3]. For example, *ARID1A* in the SWI-SNF remodeling complex is mutated in many cancers and is considered a tumor suppressor gene [3]. Also, HELLS is known to be involved in maintenance of genome stability [2], loss of which is considered a hallmark of tumor cells. However, alterations of *HELLS i*n human cancers have not been reported, yet. In a public genome database (http://genome.cse.ucsc.edu/), we found that human *HELLS* 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 *HELLS* gene is mutated in GC and CRC, we analyzed an A8 repeat in exon 7 and another A8 repeat in exon 16 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 [5,6]. 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 [6].

On the SSCP, we observed aberrant bands of the *HELLS* gene in eight cancers (seven CRC and one GC). 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 *HELLS* somatic mutations. All of the mutations were frameshift mutations (deletion of one base) in each A8 repeat (c.461delA and c.1840delA) that would result in frameshifting changes with Asn154 as the first affected amino acid, changing to a Ile and cresting a new reading frame ending in a stop at position 29 from the Ile (p.Asn154IlefsX29) and that with Arg614 as the first affected amino acid, changing to a Glu and cresting a new reading frame ending in a stop at position 11 from the Glu (p.Arg614GlufsX11), respectively. They were detected in a GC with MSI-H (1/34; 3.0 %) and four of the CRC with MSI-H (7/99; 7.1 %), but none in those with MSS. There was a statistical difference in the *HELLS* frameshift mutation frequencies between the cancers with MSI-H (8/133) and MSS (0/90) (Fisher's exact test, $p = 0.015$).

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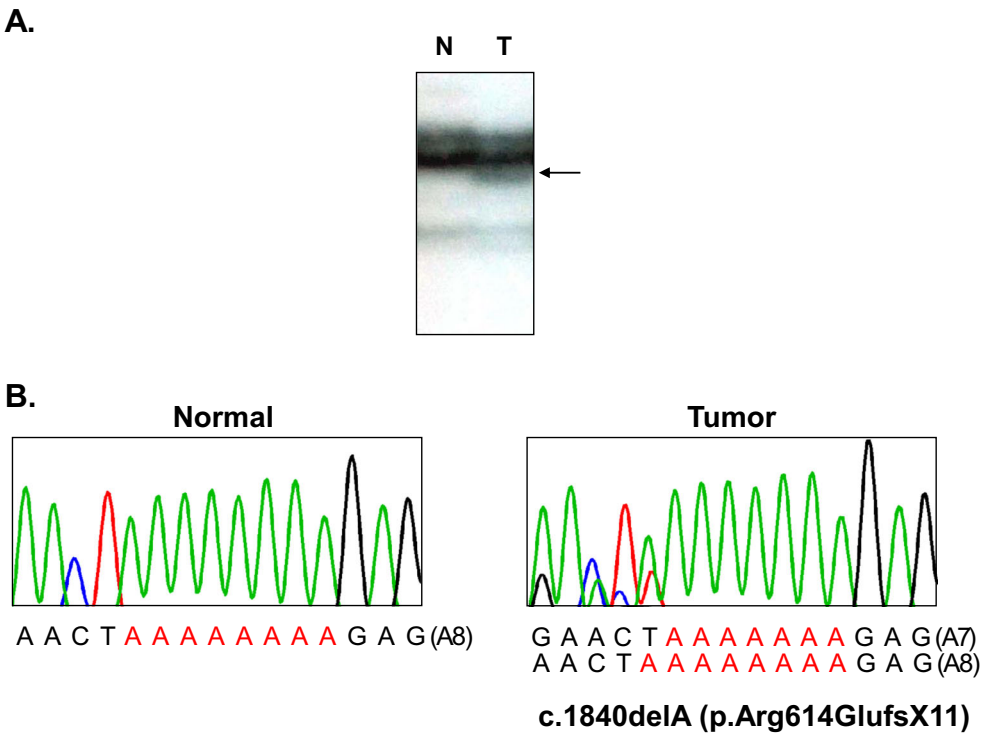

Fig. 1 Mutations of *HELLS* mononucleotide repeats in colon carcinomas with MSI-H. **a**. PCR product of *HELLS* exon 16 from a colon carcinoma show aberrant band (arrow in lane T) as compared to SSCP from normal tissue (N) of the same patient. **b**. Direct DNA sequencing analysis show a heterozygous A deletion within the A8 in tumor tissue as compare to normal tissue

The frameshift mutations in the present study would result in premature stops of amino acid translation in HELLS protein and hence resemble typical loss-of-function mutations, suggesting that *HELLS* would be inactivated in GC and CRC harboring the *HELLS* mutations. Expression of HELLS in normal tissues is restricted to thymus, testis and bone marrow, while it is upregulated in many cancers tissues [7]. Frequent deletions within the SNF2 domain of HELLS have been detected in human lymphoid malignancies [7]. However, the exact roles of HELLS in tumorigenesis remain unclear. Provided that *HELLS* is involved in maintenance of genome instability [2], we hypothesize that the *HELLS* inactivation by the frameshift mutations might alter the HELLS-mediated genome instability and contribute to tumorigenesis in the cancers harboring the mutations. In addition, previous loss of heterozygosity studies suggested that loss of one or more putative tumor suppressor genes at chromosome 10q23 where *HELLS* resides may be involved in the development of many cancers [8]. Although PTEN tumor suppressor gene has been identified in this locus, our data suggest a possibility that HELLS might be another candidate tumor suppressor gene in GC and CRC with MSI-H. However, to further extend the roles of HELLS in tumorigenesis, especially for the cancers with MSI-H, further studies on HELLS mutation and expression are required based on our observation.

Acknowledgments This work was supported by a grant from National Research Foundation of Korea (2012R1A5A2047939).

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