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LETTER TO THE EDITOR

WRN, the Werner Syndrome Gene, Exhibits Frameshift Mutations in Gastric and Colorectal Cancers

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To the Editor

Werner syndrome, an autosomal recessive disorder causing premature aging, is caused by truncating mutations in *Werner syndrome gene* (*WRN*) that encodes a DNA helicase with exonuclease activity [1]. Patients with Werner syndrome have an increased cancer incidence as well, suggesting that the lack normal *WRN* function affects tumorigenesis [2]. Both helicase and exonuclease activities of WRN protein contribute to DNA repair in cells [3]. Also, cells with defective WRN show genomic instability [4]. These features are frequently observed in cancers, suggesting a possibility of *WRN* gene alterations in cancers. However, it remains unknown whether inactivating mutation of *WRN* is common in gastric cancer (GC) and colorectal cancer (CRC).

About one third of GC and CRC are classified as high microsatellite instability (MSI-H) cancers [5]. Many tumor suppressor genes such as *BAX* and *TGFBR2* harbor frameshift mutations at monocleotide repeats in MSI-H cancers [5]. In the human genome database, we observed that *WRN* gene possesses nucleotide repeats in coding sequences that might be mutated in MSI-H cancers. In this study, we analyzed an A8 repeat in exon 2 and an A7 repeat in exon 28 of *WRN* by polymerase chain reaction (PCR)-based single strand conformation polymorphism (SSCP) assay. In this study, we used 79 GCs and 124 CRCs. The GCs were 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. In cancer tissues, malignant cells and normal cells were selectively procured by microdissection. Radioisotope ([^{32}P]dCTP) was incorporated into the PCR products, which were subsequently displayed in SSCP gels and analyzed with direct DNA sequencing [6]. Additionally, to see whether the *WRN* mutations possess intra-tumor heterogeneity (ITH) that contributes to tumor aggressiveness [7], we studied 16 CRCs with four to seven regional biopsies per CRC.

In the SSCP, we found aberrantly migrating bands in three GCs and three CRCs, but not in their matched normal samples. DNA sequencing analysis confirmed that the aberrant bands represented *WRN* somatic mutations, which consisted of frameshift mutations by a deletion (c.15delA (p. Lys5AsnfsX15)) in exon 2 and another deletion (c.3382delA (p. Ser1128ValfsX34)) in exon 28 within the repeat (Table 1). The mutations were found in GCs (3/34, 8.8%) and CRCs (3/79, 3.8%) with MSI-H (3/113, 5.3%), but not in GCs (0/45) and CRCs (0/45) with MSS/MSI-L (Fisher's exact test, $p = 0.028$). The frameshift mutation in exon 2 showed ITH in one of 16 CRCs (6.3%). A CRC (#41) showed the c.15delA mutation in three regional biopsies (#41–1, 41–3 and 41–4), but there was no such mutation in the two regional biopsies (#41–6 and 41–7) (Fig. 1). We could not find any significant histological difference among the ITH regions in this case.

Cancer-related functions (DNA repair and maintenance of genomic stability) and increased cancer incidence in Werner syndrome [1] led to us to analyze inactivating mutations of *WRN* gene in GC and CRC. In the present study, we found that six cases (5.3%) of GCs and CRCs with MSI-H harbored *WRN* frameshift mutations, indicating that *WRN* is mutated

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Table 1 Summary of *WRN* mutations in gastric and colorectal cancers

Gene	Location	Wild type	Mutation	MSI status of the mutation cases (n)	Incidence in MSI-H cancers (%)	Nucleotide change (predicted amino acid change)
WRN	Exon 2	A8	A7	MSI-H (3)	Colorectal: 1/79 (1.3)	c.15delA (p. Lys5AsnfsX15)
	Exon 28	A7	A6	MSI-H (3)	Gastric: 3/34 (8.8) Colorectal: 2/79 (2.5)	c.3382delA (p. Ser1128ValfsX34)

MSI-H high microsatellite instability

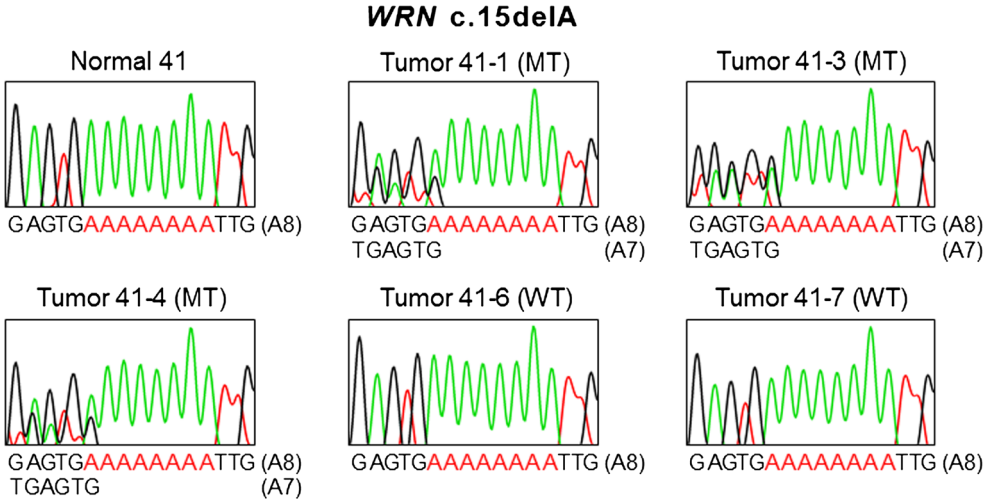

Fig. 1 Intratumoral heterogeneity of *WRN* frameshift mutation in a colon cancer. A: Sanger DNA sequencing analyses show *WRN* c.15delA mutation (MT) in 3 regional areas (41–1, −3 and −4) and wild-type (WT) in the other two areas (41–6 and −7)

in some GCs and CRCs with MSI-H. The *WRN* mutations would result in truncation of WRN protein and hence resembled a typical loss-of-function mutation. The truncated WRN mutants might inactivate the tumor suppressor functions of WRN (DNA repair and maintenance of genomic stability) and might contribute to tumorigenesis of MSI-H cancers. How the WRN inactivation affects the MSI-H phenotype should be further clarified in future studies. We also found ITH of *WRN* frameshift mutation in a CRC (Fig. 1), suggesting a possibility that the *WRN* mutation occurred during tumor progression rather than during tumor development in this case. Although ITH is known to be important in clinical outcome of cancer patients [7], it was not possible to define clinical feature of the ITH case in this study due to the small number. Further studies are needed to define the clinical implication of ITH in *WRN* mutation.

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Compliance with Ethical Standards

Conflicts of Interest None to declare.

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