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ORIGINAL ARTICLE

Histone Demethylase Gene PHF2 Is Mutated in Gastric and Colorectal Cancers

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Abstract Alterations of genes involved in histone modification are common in cancers. A histone demethylase-encoding gene *PHF2* is considered a putative tumor suppressor gene (TSG). PHF2 is essential for p53-mediated TSG functions such as chemotherapy-mediated cancer cell killing. However, inactivating mutations of *PHF2* that could inactivate its functions are not reported in cancers. In a genome database, we observed that the *PHF2* gene possessed mononucleotide repeats, which could be mutated in cancers with high microsatellite instability (MSI-H). For this, we analyzed 124 colorectal cancers (CRCs) and 79 gastric (GCs) cancers for the mutations and their intratumoral heterogeneity (ITH). Twenty-two of 79 CRCs (27.8 %) and 7 of 34 GCs (20.6 %) harboring MSI-H exhibited frameshift mutations. However, we found no such mutations in microsatellite stable/low MSI (MSS/MSI-L) cancers. Also, we studied ITH for the detected frameshift mutations in 16 cases of CRCs and detected ITH in two (12.5 %) cases. Our data reveal that TSG gene *PHF2* harbors mutational ITH as well as the frameshift mutations in CRC and GC with MSI-H. Based on this, it is suggested that frameshift mutations of *PHF2* may play a role in tumorigenesis through its TSG inactivation in CRC and GC.

Keywords PHF2 · Tumor suppressor gene · Frameshift mutation · Cancer · Microsatellite instability · Intratumoral heterogeneity

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Introduction

Histones, the chief protein components of chromatin, are subject to post translational modifications by enzymes, including methylation, acetylation and ubiquitination [1]. Histone modification influences chromatin condensation, and poise genes for either transcriptional activation or repression. Histone methylation status is regulated by histone methyltransferase ("writer") and demethylases ("eraser") [2, 3]. Dysregulated histone methylation by mutational and expressional alterations of histone methyltransferases such as *KMT2A* and *KMT6* have been found in cancers [4, 5]. Also, histone demethylases are frequently altered in many cancers by mutational and expressional changes. For example, a histone demethylase *KDM6A* (also known as *UTX*) is frequently inactivated in kidney and bladder cancers by somatic mutations and considered a tumor suppressor gene (TSG) [6, 7]. There are two classes of histone demethylase genes, i.e. KDM1 family and Jumonji C family. PHF2, a plant homeodomain finger gene, is a member of the Jumonji C family. PHF2 demethylates H3K9-Me2 (lysyl dimethylation) and participates in epigenetic regulation of transcription [8]. *PHF2* gene is deleted and hypermethylated in breast cancer and its expression is lost in head and neck cancer [9, 10]. Functionally, PHF2 interacts with p53 and enhances p53-mediated gene expression and cell death [11]. In the absence of PHF2, anti-cancer drug-induced and p53-mediated cell killing is not efficient in cancer cells [11]. PHF2 expression is downregulated in colorectal (CRC) and gastric (GC) cancers [11]. Together, these data suggest that PHF2 may behave as a candidate TSG in CRC and GC.

About one third of GCs and CRCs are classified as high microsatellite instability (MSI-H) cancers [12]. Many TSGs harbor frameshift mutations at monocleotide repeats in MSI-H cancers [12]. In the human genome database, we observed that

the *PHF2* gene possess nucleotide repeats in coding sequences that might be mutated in MSI-H cancers. Frameshift mutations of *PHF2* gene, however, have not been reported in CRC and GC. Intratumoral heterogeneity (ITH) is a common phenomenon in cancers, which may result in cancer evolution and influence on clinical outcomes [13–15]. Thus, identification of genetic ITH is important in understanding clinicopathologic as well as biological features of the cancers. The current study aimed to find whether *PHF2* mutations are common and harbor ITH in MSI-H CRC and GC.

Materials and Methods

Tissue Samples and Microdissection

In this study, we used 124 CRCs and 79 GCs. The CRCs included 79 CRCs with MSI-H, 45 CRCs with microsatellite stable/low MSI (MSS/MSI-L), 34 GCs with MSI-H and 45 GCs with MSS/MSI-L (Table 1). Approval for this study was obtained from institutional review board of the Catholic University of Korea. . These cases over-represent MSI-H cancers, because we collected separately MSI-H and MSS/MSI-L cancers in different times. We adopted an MSI evaluation by using five mononucleotide repeats (BAT25, BAT26, NR-21, NR-24 and MONO-27) [16]. For 16 CRCs with MSI-H, we used 4–7 regional cancer areas per CRC for ITH evaluation. These multi-regions were examined under light microscope and used to detect mutational ITH of *ZMPSTE24*. Cancer and matched normal cells were micro-dissected as described in our earlier reports [17, 18].

Single Strand Conformation Polymorphism (SSCP) and DNA Sequencing Analyses

Targets of this mutation study were two A8 repeats (an A8 in exon 12 and another A8 in exon 21) in *PHF2*. Genomic DNA from matched normal and cancer cells was separately amplified by polymerase chain reaction (PCR) with radioisotope ([^{32}P]dCTP). We determined aberrant motility on SSCP (FMC Mutation Detection Enhancement system; Intermountain Scientific, Kaysville, UT, USA) by visual inspection. The aberrant migrated products were further analyzed by Sanger DNA sequencing. Other procedures for PCR-SSCP were defined in our earlier reports [19, 20].

Results

Mutational Analysis

Genomic DNAs of 124 CRCs and 79 GCs were studied to detect frameshift mutations within the nucleotide repeats of *PHF2* (an A8 in exon 12 and another A8 in exon 21) by the PCR-SSCP analyses. In SSCP, we observed aberrant bands in 29 cases (26 cases for the A8 in exon 12 and 3 cases for the A8 in exon 21) (Table 2 and Fig. 1). Normal tissues of matched patients revealed no aberrant migration in SSCP, which indicated that the aberrant bands had somatically risen (Fig. 1). Sanger DNA sequencing confirmed somatic mutation of *PHF2* gene (Fig. 1), which was either a deletion or a duplication (insertion) mutation in the A8 repeats that would lead to termination of PHF2 translation (Table 2). Twenty-two of 79 CRCs (27.8 %) and 7 of 34 GCs (20.6 %) carrying MSI-H showed frameshift mutations in *PHF2*. Any of the cancers did not harbor two or more frameshift mutations of the repeats simultaneously. These mutations were found in MSI-H cases (29/113), but there was not any in MSS/MSI-L cases (0/90) (Table 2) (Fisher's exact test, $p < 0.001$) (Fig. 2).

Table 1 Summary of pathologic features of gastric and colorectal 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

Table 2 Summary of *PHF2* frameshift 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)
PHF2	Exon 12	A8	A7	MSI-H (25)	Colorectal: 20/79 (6.3) Gastric: 5/34 (5.9)	c.1475delA (p. Lys492Argfsx6)
			A6	MSI-H (1)	Gastric: 1/34 (2.9)	c.1474_5delAA (p. Lys492Aspfsx21)
	Exon 21	A8	A7	MSI-H (1)	Colorectal: 1/79 (1.3)	c.2858delA (p. Lys953Argfsx7)
			A8	MSI-H (2)	Colorectal: 1/79 (1.3) Gastric: 1/34 (2.9)	c.2858dupA (p. Ser954Glufsx9)
			Total	MSI-H (29)	Colorectal: 22/79 (27.8) Gastric: 7/34 (20.6)	

MSI-H high microsatellite instability

Intratumoral Heterogeneity of PHF2 Frameshift Mutations

From 92 of 16 CRCs (4–7 fragments per CRC) with MSI-H were analyzed for the ITH status of the *PHF2* frameshift mutations. In two (CRC41 and CRC49), one of the frameshift mutations of *PHF2* (c.1475delA) exhibited ITH. One CRC (CRC41) showed the mutation in two of five regions, but no such mutation in the other three. Likewise, the other (CRC49) exhibited the mutation in three of six regions (Table 3). Collectively, the ITH of *PHF2* frameshift mutation was identified in 2 (12.5 %) of 16 CRC cases.

Discussion

Earlier studies have identified that the chromosomal region 9q22.2–31.2, where *PHF2* resides, is frequently deleted in

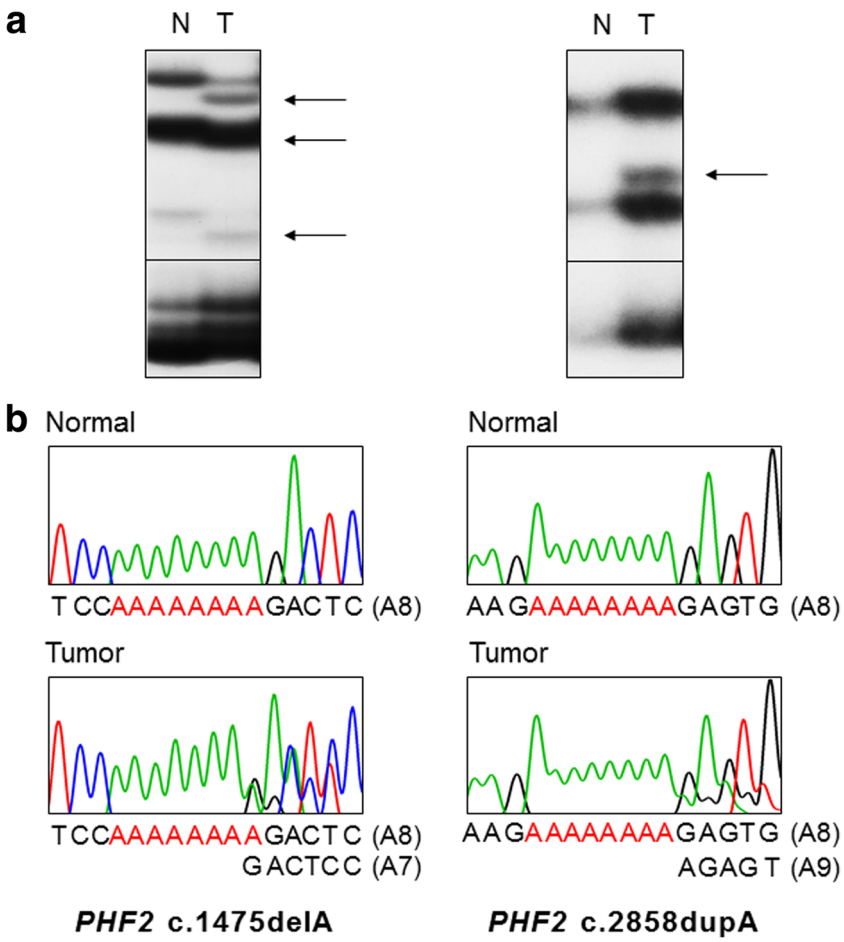

Fig. 1 Representative SSCP and DNA sequencing of *PHF2* frameshift mutations. SSCP (A) and DNA sequencing analyses (B) of the A8 repeats of *PHF2* gene from tumor (Lane T) and normal tissues (Lane N). **a** In the SSCP, the arrows (Lane T) indicate aberrant bands compared to the SSCP from normal tissues (N). Deletion in the A8 of exon 12 (*left*) and duplication in the A8 of exon 21 (*right*) mutations. **b** Direct DNA sequencing analyses of the PCR products of the deletion (*left*) and duplication (*right*) mutations show heterozygous mutations in tumor tissues as compare to normal tissues

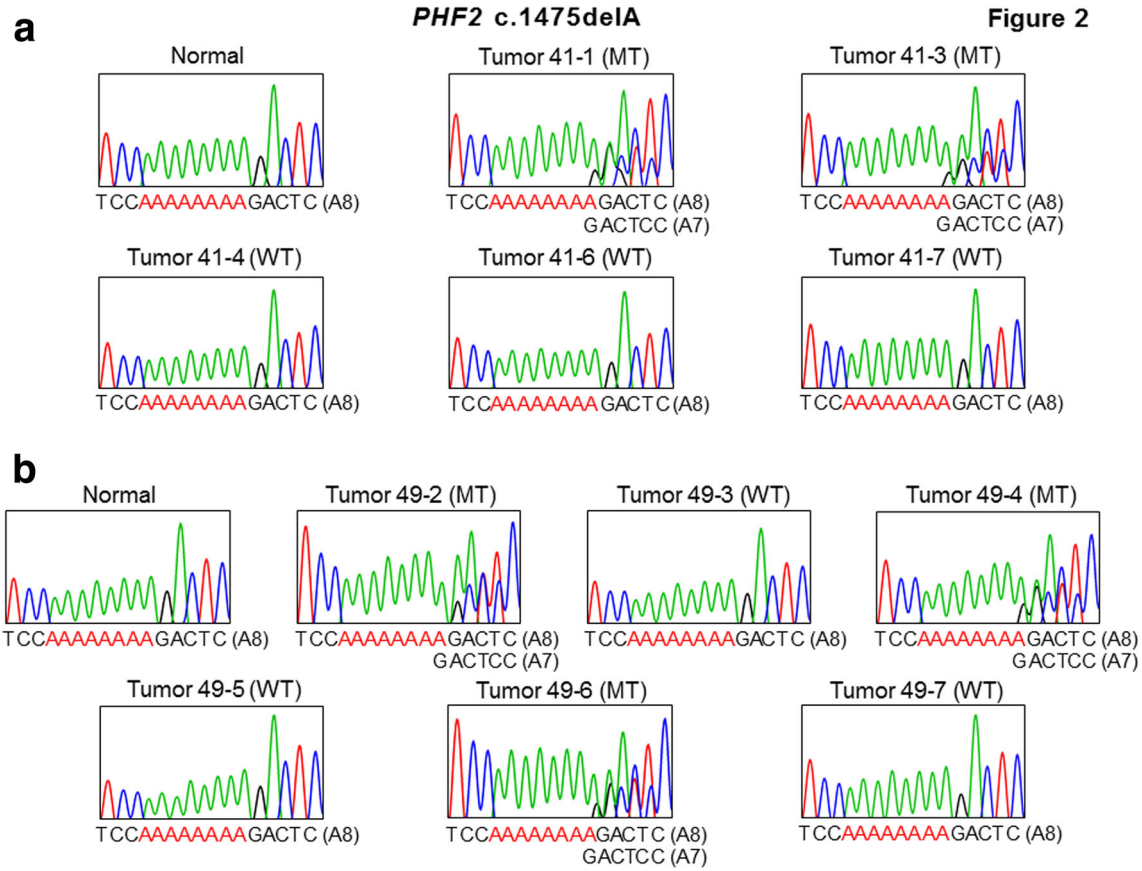

Fig. 2 Intratumoral heterogeneity of *PHF2* frameshift mutation in scolon cancer. **a** Direct DNA sequencings show *PHF2* c.1475delA mutation (MT) in two regional biopsies (41–1 and 41–3) and wild-type (WT) in the other three regional biopsies (41–4, 41–6 and 41–7). **b** Direct DNA sequencings show *PHF2* c.1475delA mutation (MT) in three regional biopsies (49–2, 49–4 and 49–6) and wild-type (WT) in the other three regional biopsies (49–3, 49–5 and 49–7)

Table 3 Intratumoral heterogeneity of *PHF2* frameshift mutations in colorectal cancers

Case	Regional biopsy sites							Mutation status	ITH status
	#1	#2	#3	#4	#5	#6	#7		
CRC3	WT	WT	WT	WT	WT	WT	n.d.	Wild type	Non-ITH
CRC15	WT	WT	WT	WT	WT	WT	WT	Wild type	Non-ITH
CRC26	WT	WT	n.d.	n.d.	WT	n.d.	n.d.	Wild type	Non-ITH
CRC27	WT	WT	WT	WT	WT	WT	WT	Wild type	Non-ITH
CRC34	WT	WT	WT	WT	WT	WT	WT	Wild type	Non-ITH
CRC35	WT	WT	n.d.	n.d.	n.d.	WT	WT	Wild type	Non-ITH
CRC39	WT	WT	WT	WT	n.d.	WT	WT	Wild type	Non-ITH
CRC41	c.1475delA	n.d.	c.1475delA	WT	n.d.	WT	WT	Mutation	ITH
CRC43	WT	WT	WT	n.d.	n.d.	WT	n.d.	Wild type	Non-ITH
CRC45	WT	WT	WT	WT	WT	WT	WT	Wild type	Non-ITH
CRC47	WT	WT	WT	WT	WT	WT	WT	Wild type	Non-ITH
CRC48	WT	n.d.	n.d.	WT	WT	n.d.	WT	Wild type	Non-ITH
CRC49	n.d.	c.1475delA	WT	c.1475delA	WT	c.1475delA	WT	Mutation	ITH
CRC51	WT	WT	WT	WT	WT	WT	WT	Wild type	Non-ITH
CRC53	WT	WT	WT	WT	WT	WT	WT	Wild type	Non-ITH
CRC55	WT	WT	n.d.	n.d.	WT	WT	WT	Wild type	Non-ITH

n.d. not done

human cancers including CRC [21], suggesting that this region might harbor a TSG. A TSG is a gene that protects a cell from cancer development, and when mutated, the mutated TSG would lead the cell to cancer development. Inactivating frameshift mutation in the repeat sequence of a TSG is frequent in MSI-H CRCs and GCs [17]. These findings led us to further dissect the genetic alterations of *PHF2* gene in CRC and GC. This study by us identified that both MSI-H GC and CRC exhibited *PHF2* frameshift mutations (25.7 %). Our data suggest that the TSG activities in *PHF2* may be lost by various mechanisms including inactivating mutations identified in this study as well as the deletion and hypomethylation identified in earlier studies [9–11].

The PHF2 protein consisted of an N-terminal PHF domain (7–53 amino acid residues), a Jumonji C domain (201–336 residues) and a C-terminal p53-interacting domain (820–1096 residues) [11]. The *PHF2* mutations identified in the present study would lead to loss of amino acids after the residue 492 or 954 (Table 2), which would delete the C-terminal domain. An earlier study reported that the C-terminal domain-deleted PHF2 profoundly inhibited TSG activities, including chemotherapy-induced anti-cancer effects [11]. Therefore, it is reasonable to assume that the *PHF2* mutations in this study might probably inhibit TSG activities and contribute to cancer development. A previous study reported that PHF2 expression is downregulated in GC and CRC [11], too. These reports, together, may indicate that PHF2 is inactivated by multiple mechanisms, including somatic mutation and protein loss in CRC and GC.

Genomic instability in a cancer may result in an elevated level of somatic mutations and contribute to ITH development by providing a pool of mutations upon which selection can act in a given microenvironment [14]. While the majority of the mutations in a cancer appear to be passenger mutations, only a small fraction represents driver mutations, conferring a selective advantage to the cancer [22]. In this study, we identified mutational ITH of *PHF2* frameshift mutation in 2 (12.5 %) of 16 CRCs, which is consistent with previous studies that had reported frequent mutational ITH in CRCs with MSI-H [23]. Functionally, *PHF2* is known to possess TSG activities [11]. The modest incidence of mutational ITH of *PHF2* (12.5 %) and functional relevance on TSG inactivation may indicate that the ITH in *PHF2* frameshift mutation might possibly contribute to the pathogenesis and influence the clinical outcomes. However, it should be further studied how the ITH change during the progression and the ITH play a causal role in tumor pathogenesis. We were not able to find any significant difference between cancers with the ITH and those without it, probably because the number of cases with the ITH was small. Analysis of a larger cohort with the mutations is required in future studies.

PHF2 is known to ensure cell death in response to chemotherapy [11]. Thus, evaluation of PHF2 expression and mutation status might provide a clue to delineate chemotherapy response in GC and CRC. In this point, In this point, our data may reveal information on biological mechanisms as well as therapeutic strategies in cancer cells.

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Compliance with Ethical Standards

Conflicts of Interest and Financial Sponsorship and Support none.

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