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LETTER TO THE EDITOR

Intratumoral heterogeneity of *FLCN* somatic mutations in gastric and colorectal cancers

Hyun Ji Son[1] · Eun Ji Choi[1] · Nam Jin Yoo[1] · Sug Hyung Lee[1]

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Dear Editor,

FLCN gene encoding folliculin protein is a cytoplasmic guanine exchange factor associated with Birt-Hogg-Dubé (BHD) syndrome, the clinical manifestations of which include multiple tumors and pulmonary cysts [1]. Folliculin interacts with AMP-activated protein kinase (AMPK) and regulates energy metabolism in cells [1]. Activation of AMPK-related mTOR pathway may be responsible for tissue lesions [1]. In BHD syndrome, skin and kidney tumors are common, but colon tumors have also been reported in some individuals with BHD [2]. Frameshift mutations in the C8 mononucleotide repeat in exon 8 are the most common inactivating mutation in BHD individuals [1, 2]. The same frameshift mutations of *FNCL* are detected in sporadic colorectal cancer (CRC) with high microsatellite instability (MSI-H) (16% (5/32) of one study, 0% (0/5) of the other study) [3, 4]. Together, *FLCN* is considered a tumor suppressor gene (TSG).

Approximately 10–20% of gastric (GC) and CRC are MSI-H cancers that exhibit genetic hypermutability caused by impaired DNA mismatch repair [5]. Many TSGs harbor frameshift mutations at monocleotide repeats in MSI-H cancers, which results in suppression of TSG and promotes cancer development [6]. In the present study, we analyzed the C8 repeat in *FLCN* in sporadic GC and CRC by polymerase chain reaction (PCR)-based single strand conformation polymorphism (SSCP) analysis. We used 32 sporadic GCs with MSI-H, 45 GCs with microsatellite stable (MSS), 100 CRC with MSI-H and 45 CRCs with MSS. Also, we analyzed 16 cases of MSI-H CRCs with 4 to 7 regional fragments per CRC to detect intratumoral heterogeneity (ITH) of these mutations. In cancer tissues, malignant cells and normal cells were selectively procured by microdissection [6]. Radioisotope ([^{32}P]dCTP) was incorporated into the PCR products, which were subsequently displayed in SSCP gels and analyzed with direct DNA sequencing [6].

In the analyses, we found frameshift mutations of the C8 of *FLCN* in 2 GCs and 24 CRCs (Table 1). The mutations were detected in cancers with MSI-H (GC: 2/32 (6.3%), CRC 24/100 (24%), GC + CRC: 26/132 (19.7%)), but not in those with MSS (0/90) (P<0.001). DNA from the patients' normal tissues showed no evidence of mutation in Sanger sequencing, indicating the mutations had risen somatically. These mutations were deletion or duplication of one base in the C8 repeat that would result in frameshift of amino acids. For the ITH of the mutations, 4 (25%) of the 16 CRCs exhibited ITH (case 1: c.1285delC in 2 regions and wild type in 4 regions, case 2: c.1285delC in 4 regions and wild type in 1 region, case 3: c.1285dupC in 3 regions and wild type in 4 regions, Case 4: c.1285dupC in 4 regions and wild type in 3 regions), suggesting that ITH of the *FNCN* frameshift mutations may be common in CRCs. However, probably due to the small number (n = 4) with the ITH, we were not able to find any clinical difference between ITH and non-ITH cases.

In the present study, we identified *FLCN* frameshift mutations of the C8 in sporadic GC and CRC as well as ITH of these mutations in CRC. Our data for mutation incidence in MSI-H is not statistically different with previous data [3, 4], indicating that *FLCN* frameshift mutation is common in sporadic MSI-H CRCs. Also, there was a significant difference of the mutations between the cancers with MSI-H and MSS, confirming that associations of the mutations with MSI-H were specific. We newly found that ITH of *FLCN* mutation is common (25%) in CRCs, suggesting that the *FLCN* mutation occurred during tumor progression rather than as an early event. Cancer ITH could result in poor clinical outcomes in patients since rare clones can accomplish dominance during tumor progression [7]. Based on the TSG functions of *FLCN* in cancer, our data suggest that loss of *FLCN* TSG as well as its ITH could be further selected and influence the clinical outcomes in affected cases. For this, further study is needed in a larger cohort with ITH of *FLCN* mutation.

✉ Sug Hyung Lee
suhulee@catholic.ac.kr

[1] Department of Pathology, College of Medicine, The Catholic University of Korea, 505 Banpo-dong, Socho-gu, 137-701 Seoul, Korea

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

Gene	Wild type	Mutation	MSI status of the mutation cases (n)	Incidence in MSI-H cancers (%)	Nucleotide change (predicted amino acid change)
FLCN	C8	C9	MSI-H (9)	Colorectal: 8/100 (8.0) Gastric: 1/32 (3.1)	c.1285dupC (p.His429ProfsX27)
		C7	MSI-H (17)	Colorectal: 16/100 (16.0) Gastric: 1/32 (3.1)	c.1285delC (p.His429ThrfsX39)

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Compliance with Ethical Standards

Conflicts of interest None to declare.

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