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Somatic mutations of candidate tumor suppressor genes folliculin-interacting proteins *FNIP1* and *FNIP2* in gastric and colon cancers

To the Editor

Both FNIP1 (folliculin-interacting preotein1) and FNIP2 form a complex with folliculin (FLCN), which interacts with AMP-activated protein kinase (AMPK) and regulates energy metabolism in cells [1]. Germline mutations of the *FLCN* result in Birt–Hogg–Dubé syndrome, an inherited cancer syndrome characterized by increased risks for multiple tumors, indicating that FLCN may be a tumor suppressor gene (TSG) [2]. Mice deficient of *Fnip1* and/or *Fnip2* display tumors in several organs, suggesting their roles as TSGs [3].

Mutations in DNA mismatch repair genes such as MLH1, MSH2 or MSH6 result in defects in repairing errors in DNA repetitive sequences, producing microsatellite instability (**MSI**) phenotype mainly in colorectal (CRC), gastric (GC) and endometrial cancers [4]. Cancers with MSI can exhibit frameshift mutations at repeat sequences in the coding region, which may inactivate TSGs and contribute to tumorigenesis [4]. In the genome database, we observed that both *FNIP1* and *FNIP2* genes harbor coding sequence repeats that might inactivate these genes in MSI cancers.

In the present study, we analyzed one T7 (exon 10) and one A8 (exon 14) repeats in *FNIP1,* and one A7 (exon 11) in *FNIP2* coding sequences by polymerase chain reaction (PCR)-based single strand conformation polymorphism (SSCP) analysis. We studied 34 GCs with high MSI (MSI-H), 45 microsatellite stable (MSS) GCs, 100 CRCs with MSI-H and 45 MSS CRCs. In the cancer tissues, malignant cells and normal cells were selectively procured by microdissection [5]. Radioisotope ([^{32}P]dCTP) was incorporated into the PCR products, which were subsequently displayed in SSCP gels and analyzed with direct DNA sequencing [5].

We found *FNIP1* frameshift mutations in 6 (6%) CRCs and 2 (5.9%) GCs and *FNIP2* frameshift mutations in one GC (2.9%) (Table 1). DNA from their matched normal tissues did not show any evidence for mutation in Sanger sequencing, indicating the mutations had risen somatically. These mutations were either deletion or duplication of one base in nucleotide repeats that would result in frameshifts of amino acid translation. The mutations were detected in 9 cancers (9/134) with MSI-H, but not in those with MSS (0/90) ($P < 0.01$). Of the 100 CRCs with MSI-H analyzed, we further analyzed multi-regional areas in 16 CRCs (96 areas, 4–7 areas per case). Two of the 16 CRCs (12.5%) revealed different mutation status of the *FNIP1* c.2196delA deletion in the regional areas (1 wild and 5 mutant areas in one CRC, 1 wild and 4 mutant areas in the other CRC), indicating there was intratumoral heterogeneity (ITH) of the frameshift mutation. There was no *FNIP2* mutation ITH in the CRCs. We could not find any significant histological difference among the ITH regions.

The frameshift mutations identified in our study would translate premature stops in FNIP1 and FNIP2. Subsequently, TSG functions of FNIP1 and FNIP2 might be inactivated by the mutations, which could play a role in cancer development [3]. In our data, *FNIP1* frameshift mutation is increased compared to *FNIP2* frameshift mutation, which is in agreement with the previous observation that *Fnip1*-deficient mice but not *Fnip2*-deficient mice displayed significant increase of tumor development [3]. We identified mutational ITH of *FNIP1* in CRCs, suggesting a possibility that loss of the FNIP1 TSG may have regional heterogeneity that could be further selected and influence the clinical outcomes. However, it should be further confirmed in a larger cohort.

Table 1
Summary of *FNIP1* and *FNIP2* 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)
FNIP1	A8	A7	MSI-H (6)	Colorectal: 6/100 (6.0)	c.2196delA (p.Lys732AsnfsX23)
		A9	MSI-H (2)	Gastric: 2/34 (5.9)	c.2196dupA (p.Pro733ThrfsX4)
FNIP2	A7	A6	MSI-H (1)	Gastric: 1/34 (2.9)	c.1301delA (p.Asn434ThrfsX14)

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Declaration of Competing Interest

None to declare.

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