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LETTER TO THE EDITOR

Somatic Mutations and Intratumoral Heterogeneity of Cancer-Related Genes *NLK, YY1* and *PA2G4* in Gastric and Colorectal Cancers

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Abstract
Many genes act as both tumor suppressor gene (TSG) and proto-oncogene depending on cellular context and cancer type. *Nemo-like kinase* (*NLK)* encoding a serine/threonine kinase, *Yin Yang 1* (*YY1*) encoding a zinc-finger transcription factor and *PA2G4* encoding an ErbB3 binding protein have both of these two opposing functions. In the present study, we analyzed *NLK, YY1* and *PA2G4* frameshift mutations in sporadic GC and CRC with high microsatellite instability (MSI-H). Also, regional intratumoral heterogeneity (ITH) of frameshift mutations of these genes was analyzed in CRCs. We found frameshift mutations of *NLK, YY1* and *PA2G4* in CRC and GC with MSI-H (17/132: 12.9%), but not in those with MSS (0/90). Two (12.5%), one (6.3%) and one (6.3%) CRC (s) of the 16 CRCs exhibited ITH of *NLK, YY1* and *PA2G4* mutations among the 4–7 regions, suggesting that ITH of the frameshift mutations might be frequent in the CRCs. These results suggest that frameshift mutations of *NLK, YY1* and *PA2G4* along with the ITH might contribute to MSI-H cancer pathogenesis.

Keywords *NLK* · *YY1* · *PG2G4* · Mutation · Cancer · Microsatellite instability

Dear Editor,

Two main types of genes involved in cancer pathogenesis are proto-oncogenes and tumor suppressor genes (TSGs). Proto-oncogenes normally help cancer cell development and metastasis while TSGs inhibit these processes by slowing down cell division, repairing DNA mistakes and inducing cell death [1]. However, many genes display both proto-oncogenic and TSG functions during cancer pathogenesis. For example, many genes involved in autophagy pathways possess both functions depending on cellular context [2]. *Nemo-like kinase* (*NLK)* encodes a serine/threonine kinase that is involved in the regulation of multiple transcription factors for Wnt and Notch signaling pathways [3]. NLK knock-down suppresses cell growth and tumorigenesis in some cancers, but it results in opposite effects in other cancers [3]. Yin Yang 1 (YY1) is a zinc-finger transcription factor that regulates expression of many genes involved in cancer pathogenesis (cell growth, survival and epithelial to mesenchymal transition) [4]. YY1 is over-expressed in many cancers, but sometimes under-expressed in other cancers [4]. PA2G4, also known as ErbB3 binding protein 1, is known to inhibit tumor growth in prostate cancer, but it can promote tumor growth in colon cancer [5], indicating its dual roles in cancer pathogenesis.

Approximately 10–20% of gastric (GC) and colorectal (CRC) are high microsatellite instability (MSI-H) cancers that exhibit genetic hypermutability caused by impaired DNA mismatch repair [6]. Many TSGs harbor frameshift mutations at monocleotide repeats in MSI-H cancers, which results in suppression of TSG and promotes cancer development [6]. However, proto-oncogenes may have monocleotide repeats that could be targets for the frameshift mutations as well. In the human genome database, we observed that *NLK, YY1* and *PG2G4* genes have nucleotide repeats in the coding areas that might be altered in MSI-H cancers. In the present

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Table 1 Summary of pathologic features of gastric and colorectal cancers

Feature	MSI-H	MSS
Gastric carcinomas		
Total cases	32	45
TNM stage		
I	11	15
IIA, B, C	10, 2, 1	15, 3, 0
IIIA, B, C	1, 5, 1	2, 8, 1
IVA	1	1
WHO subtype		
Signet-ring cell	1	2
poorly cohesive carcinoma	3	17
Tubular	16	14
Papillary	2	1
Mucinous	8	2
Mixed	10	9
Colorectal carcinomas		
Total cases	100	45
TNM stage		
I	22	6
IIA, B, C	29, 6, 1	16, 3, 1
IIIA, B, C	5, 32, 2	3, 11, 2
IVA	3	3
Location		
Cecum	20	0
Ascending colon	63	3
Transverse colon	12	2
Descending & sigmoid colon	4	17
Rectum	1	23

TNM: tumor, lymph node, metastasis, MSI-H: high microsatellite instability, MSS: stable microsatellite instability

study, we analyzed a T7 repeat in *NLK,* an A8 in YY1 and an A8 in *PA2G4* by polymerase chain reaction (PCR)-based single strand conformation polymorphism (SSCP) analysis. We used 32 GCs with MSI-H, 45 GCs with microsatellite stable (MSS), 100 CRC with MSI-H and 45 CRCs with MSS (Table 1). In cancer tissues, malignant cells and normal cells were selectively procured by microdissection [7]. Radioisotope ([^{32}P]dCTP) was incorporated into the PCR products, which were subsequently displayed in SSCP gels [7]. After SSCP, mobility shifts on the SSCP gels (Intermountain Scientific, Kaysville, UT, USA) were determined by visual inspection. Direct DNA sequencing reactions in both forward and reverse sequences were performed in the cancers with the mobility shifts in the SSCP using a capillary automatic sequencer (Applied Biosystem, Carlsbad, CA, USA).

We found frameshift mutations of *NLK, YY1* and *PA2G4* in 7, 7 and 3 cases in CRC and/or GC, respectively (Table 2). 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 repeats that would result in frameshift of amino acids. The incidences of the mutations were 1–6% in GC and CRC (Table 2). The mutations were detected in 17 cancers (17/132: 12.9%) with MSI-H, but not in those with MSS (0/90). Additionally, we analyzed 16 cases of CRCs with 4 to 7 regional fragments per CRC to detect intratumoral heterogeneity (ITH) of these mutations. Two (12.5%), one (6.3%) and one (6.3%) CRC (s) of the 16 CRCs exhibited ITH (different wild-type and mutated sequences) of *NLK, YY1* and *PA2G4* among the 4–7 regions, suggesting that ITH of the frameshift mutations might be frequent in the CRCs.

The frameshift mutations (premature amino acid stops) in the present study resemble a typical inactivating mutation. Based on the earlier data that showed TSG functions of *NLK, YY1* and *PA2G4* [3–5], these mutations could play a role in cancer development by inhibiting the TSG activities. However, the other aspect of gene functions (proto-oncogenes) as well as ITH of the frameshift mutations suggest that there could be a mixed effect of the inactivating mutations in the pathogenesis of MSI cancers.

Table 2 Summary of *NLK, YY1* and *PA2G4* 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)
NLK	T7	T6	MSI-H (7)	Colorectal: 6/100 (6.0) Gastric: 1/32 (3.1)	c.576delT (p.Phe192LeufsX30)
YY1	A8	A9	MSI-H (3)	Colorectal: 1/100 (1.0) Gastric: 2/32 (6.2)	c.690dupA (p.Asp231ArgfsX3)
		A7	MSI-H (4)	Colorectal: 3/100 (3.0) Gastric: 1/32 (3.1)	c.690delA (p.Asp231IlefsX25)
PA2G4	A8	A7	MSI-H (3)	Colorectal: 3/100 (3.0)	c.1115delA (p.Lys372ArgfsX16)

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Compliance with Ethical Standards

Conflict of Interest None to declare.

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