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LETTER TO THE EDITOR

TRIO Gene Encoding Trio Rho Guanine Nucleotide Exchange Factor Harbors Frameshift Mutations of in Gastric and Colorectal Cancers

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Dear Editor,

Rho GTPases are involved in major cellular functions including cell motility, growth and differentiation. TRIO, one of the Rho guanine nucleotide exchange factors (GEFs), contains three functional domains: a serine/threonine kinase domain and two GEF domains [1], suggesting that TRIO may play a crucial role in signaling pathways that control cell proliferation. In several cancers, TRIO expression is significantly increased and is correlated with poor prognosis [2]. In addition, many cancers are reported to harbor somatic mutations of *TRIO* gene that include truncating mutations as well as missense mutations [3, 4]. However, the functional consequences of the *TRIO* mutations are largely unknown.

About 10% of gastric cancer (GC) and colorectal cancer (CRC) show microsatellite instability (MSI) phenotype that has defects in mismatch repair [5]. *TRIO* gene has mononucleotide repeats (A7, T8 and C7) in their coding sequences that could be targets for frameshift mutation in cancers with MSI. Intratumoral heterogeneity (ITH) plays an important role in cancer development and progression and impedes proper diagnosis and treatment of cancers [6]. In this study, we analyzed somatic frameshift mutation of *TRIO* gene and its mutational ITH in GC and CRC. We analyzed the G7 repeat in 34 GCs with MSI-H, 45 GCs with MSS, 79 CRCs with MSI-H and 45 CRCs with MSS by polymerase chain reaction (PCR) and single-strand conformation polymorphism (SSCP) assay. After SSCP, Sanger DNA sequencing reactions were performed in the cancers with mobility shifts in the SSCP [7].

We found *TRIO* somatic frameshift mutations in GCs (5/34, 14.7%) and CRCs (11/79, 13.9%) with MSI-H, but not in CRCs (0/45) and GCs (0/45) with MSS ($p < 0.001$). All of the mutations were deletion or duplication mutations in the repeats that would cause premature stops, which would lead to termination of amino acid translation. For ITH of the mutation, we studied 16 cases of CRCs with 4 to 7 regional fragments per CRC. One CRC showed a frameshift mutation in the T8 (T8 to T7), which exhibited ITH of the mutation (mutation in four of seven regional biopsies and wild type in the other three) (Tables 1 and 2). Another CRC showed a frameshift mutation in the C7 (C7 to C6), but the mutation was identified in all regional biopsies, indicating no ITH of the mutation in this CRC.

The frameshift mutations identified in this study would result in truncation of TRIO protein, suggesting that TRIO may be inactivated in MSI-H GCs and CRCs by this frameshift mutation. Recent studies suggested evidence that TRIO might have oncogenic activity [1, 2]. Also, our data on inactivating *TRIO* mutations in MSI-H cancers may suggest that TRIO could have tumor suppressor activities. However, because to our knowledge there is no definite experimental data on TRIO functions in MSI-H tumor development, it is not possible to guess consequences of the frameshift mutations of *TRIO*. On the other hand, provided that TRIO activation promotes oncogenic activities, the

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Table 1 Summary of *TRIO* 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)
TRIO	Exon 22	A7	A8	MSI-H (1)	Gastric: 1/34 (2.9)	c.3657dupA (p.Cys1220MetfsX7)
		A7	A6	MSI-H (5)	Gastric: 2/34 (5.9)	c.3657delA (p.Lys1219AsnfsX43)
					Colorectal: 3/79 (3.8)	
	Exon 41	T8	T7	MSI-H (3)	Gastric: 1/34 (2.9)	c.6092delT (p.Leu2031fsX1)
					Colorectal: 2/79 (2.5)	
	Exon 48	C7	C8	MSI-H (2)	Gastric: 1/34 (2.9)	c.7050dupC (p.Val2351ArgfsX90)
					Colorectal: 1/79 (1.3)	
		C7	C6	MSI-H (5)	Colorectal: 5/79 (6.3)	c.7050delC (p.Val2351CysfsX62)

frameshift mutations in *TRIO* may reduce the oncogenic activities of cancer cells, thereby putatively providing a rationale for the better prognosis of MSI-H CRCs and GCs compared to its corresponding MSS cases [5]. In the present study we found ITH of *TRIO* frameshift mutation despite the low incidence (1 of 16 CRCs (6.3%)). Due to the small number of cases with the ITH, it is not possible to define clinical feature of the ITH of *TRIO* mutation and thus it is imperative to analyze a larger cohort to define the clinical features of the ITH.

Table 2 Intratumoral heterogeneity of *TRIO* frameshift mutation in gastric and colorectal cancers

Case	Regional biopsy sites						
	#1	#2	#3	#4	#5	#6	#7
CRC3	Wild type	Wild type	Wild type	Wild type	Wild type	Wild type	-
CRC15	TRIO mutation	TRIO mutation	TRIO mutation	TRIO mutation	TRIO mutation	TRIO mutation	TRIO mutation
CRC26	Wild type	Wild type	-	Wild type	Wild type	Wild type	-
CRC27	Wild type	Wild type	Wild type	Wild type	Wild type	Wild type	Wild type
CRC34	Wild type	Wild type	Wild type	Wild type	Wild type	-	Wild type
CRC35	Wild type	Wild type	-	-	-	Wild type	Wild type
CRC39	Wild type	Wild type	Wild type	Wild type	-	Wild type	Wild type
CRC41	Wild type	-	Wild type	Wild type	-	Wild type	Wild type
CRC43	Wild type	Wild type	Wild type	-	-	Wild type	-
CRC45	Wild type	Wild type	Wild type	Wild type	Wild type	Wild type	Wild type
CRC47	Wild type	Wild type	Wild type	Wild type	Wild type	Wild type	Wild type
CRC48	Wild type	-	-	Wild type	Wild type	Wild type	Wild type
CRC49	TRIO mutation	Wild type	TRIO mutation	TRIO mutation	Wild type	TRIO mutation	Wild type
CRC51	Wild type	Wild type	Wild type	Wild type	Wild type	Wild type	Wild type
CRC53	Wild type	Wild type	Wild type	Wild type	Wild type	Wild type	Wild type
CRC55	Wild type	Wild type	-	-	Wild type	Wild type	Wild type

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Compliance with Ethical Standards

Conflicts of Interest and Financial Sponsorship and Support None.

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