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LETTER TO THE EDITOR

Frameshift Mutation of *FXR1* Encoding a RNA-Binding Protein in Gastric and Colorectal Cancers with Microsatellite Instability

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To the Editor:

Fragile X mental retardation syndrome-related protein 1 (FXR1) gene encodes an RNA binding protein that is a critical regulator of post-transcriptional gene expression in differentiation, development and immunity [1]. Because FXR1 coordinates networks of RNA–protein and protein–protein interactions that link RNA metabolism to signal transduction pathways, altered function of FXR1 is expected to contribute to development of human diseases including cancer [1]. For example, association of FXR1 with RNA-protein complex (a target-specific miRNA and AGO2) is important in regulation of the post-transcriptional gene expression [2]. FXR1 resides on chromosome 3q26–29 which is frequently amplified in many cancers [1]. Elevated FXR1 expression is observed in several cancers, including colon and lung cancers and is associated with poor outcome of the patients [3]. Functionally, overexpression of FXR1 is critical for cell growth, migration and invasion, indicating that FXR1 possesses oncogenic activities (Table 1).

Microsatellite instability (MSI) is the condition of impaired DNA mismatch repair (MMR) that results in deletion or insertion of bases in mono- or dinucleotide repeats. MSI phenotype is most common in colorectal (CRC) and gastric (GC) cancers [4]. There is a mononucleotide repeat (A8) in the *FXR1* coding sequence that could be a target for frameshift mutation in cancers with MSI. To find whether *FXR1* gene harbored frameshift mutation within the repeat, we analyzed the A8 repeat of in 34 GCs with high MSI (MSI-H), 45 GCs with microsatellite stable/low MSI (MSS/MSI-L), 79 CRCs with MSI-H and 45 CRCs with MSS/MSI-L by polymerase chain reaction (PCR) and single-strand conformation polymorphism (SSCP) assay. Radioisotope ([^{32}P]dCTP) was incorporated into the PCR products for detection by autoradiogram. The PCR products were subsequently displayed in SSCP gels. After SSCP, Sanger DNA sequencing reactions were performed in the cancers with mobility shifts in the SSCP [5].

In the SSCP, we found aberrantly migrating bands in 14 CRCs and four GCs, but not in their normal tissues. DNA sequencing analysis confirmed that the aberrant bands represented *FXR1* somatic mutation that was a deletion (c.116delA (p. Asn391IlefsX14)) within the repeat. The mutation was detected in CRCs (14/79, 17.7%) and GCs (4/34, 11.8%) with MSI-H, but not in GCs (0/45) and CRCs (0/45) with MSS/MSI-L (Fisher's exact test, $p = 0.001$). Clinical and histopathological parameters, however, could not distinguish the *FXR1* mutation (+) and (−) cancers. In addition, to find whether the *FXR1* mutation harbor intra-tumor heterogeneity (ITH) that is known to play a role in evolution as well as treatment resistance in cancers, we studied 16 cases of CRCs with four to seven regional biopsies per CRC. Three of the 16 CRCs (18.8%) showed the deletion mutation (c.116delA) in different tissue regions, indicating ITH of the *FXR1* mutation existed in CRC.

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Table 1 Intratumoral heterogeneity of *FXR1* mutation 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.	WT	WT	WT	WT	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	WT	n.d.	WT	WT	n.d.	WT	WT	Wild type	Non-ITH
CRC43	WT	c.116delA	WT	n.d.	n.d.	WT	n.d.	Mutation	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	c.116delA	WT	Mutation	ITH
CRC49	n.d.	WT	WT	WT	WT	WT	WT	Wild type	Non-ITH
CRC51	WT	WT	WT	WT	n.d.	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	c.116delA	WT	Mutation	ITH

n.d.: not done, ITH: Intratumoral heterogeneity, CRC: colorectal cancer

The frameshift mutation identified in this study is predicted to result in a truncating FXR1 protein and thus resemble a typical loss-of-function mutation, suggesting that FXR1 may be inactivated in MSI-H GCs and CRCs by this frameshift mutation. Provided that FXR1 possesses oncogenic activities, the *FXR1* frameshift mutation appears to reduce the tumorigenesis. It is hypothetically possible that the *FXR1* frameshift mutation partially explains the better prognosis of CRC and GC with MSI-H than those with MSS [4]. Also, this study identified ITH of the frameshift mutation in CRCs, suggesting possibilities that the mutation occurred during tumor progression rather than during tumor development and that such ITH could influence on the cancer progression [6]. Although ITH is known to be important in clinical outcome of cancer patients, it was not possible to define clinical feature of the ITH case in this study due to small number of the mutated cases. Based on our preliminary data, further studies are needed to define the clinical implication of *FXR1* mutation.

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Compliance with Ethical Standards

Conflict of Interests The authors declare no competing interests.

References

1. Raheja R, Gandhi R (2016) FXR1: linking cellular quiescence, immune genes and cancer. Cell Cycle 15:2695–2696
2. Vasudevan S, Steitz JA (2007) AU-rich-element-mediated upregulation of translation by FXR1 and Argonaute 2. Cell 128:1105–1118
3. Qian J, Hassanein M, Hoeksema MD, Harris BK, Zou Y, Chen H, Lu P, Eisenberg R, Wang J, Espinosa A, Ji X, Harris FT, Rahman SM, Massion PP (2015) The RNA binding protein FXR1 is a new driver in the 3q26-29 amplicon and predicts poor prognosis in human cancers. Proc Natl Acad Sci U S A 112:3469–3474
4. Imai K, Yamamoto H (2008) Carcinogenesis and microsatellite instability: the interrelationship between genetics and epigenetics. Carcinogenesis 29:673–680
5. Oh HR, An CH, Yoo NJ, Lee SH (2015) Frameshift mutations of MUC15 gene in gastric and its regional heterogeneity in gastric and colorectal cancers. Pathol Oncol Res 21:713–718
6. Marusyk A, Almendro V, Polyak K (2012) Intra-tumour heterogeneity: a looking glass for cancer? Nat Rev Cancer 12:323–334