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LETTER TO THE EDITOR

Frameshift Mutation of *ASPM* Gene in Colorectal Cancers with Regional Heterogeneity

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To the Editor:

Recent advance in genome sequencing technologies such as whole-exome sequencing has provided huge data sets of somatic mutations in major cancer types [1]. The Pan-Cancer project integrated overall genome sequencing data, subsequently identified mutations across the cancers, found their organ specificity and identified molecular commonalities across tumor types [1, 2]. During the Pan-Cancer efforts, many of the mutations that occurred at a low frequency in a cancer type were shared by sets of cancer types and were predicted as significantly mutated genes [1–3]. One of such genes is *ASPM* that is the human ortholog of the *Drosophila melanogaster* abnormal spindle gene, which is essential for normal mitotic spindle function in the cells [4]. Germline mutations in *ASPM* are associated with microcephaly primary type 5 [4]. ASPM was initially identified as a protein that regulated neurogenesis but it was later known to be widely expressed in a variety of normal and malignant tissues [5]. ASPM is overexpressed in many cancers and considered a poor prognostic marker [6]. *ASPM* mutations have been identified in several cancers with a low prevalence, but it became a significantly mutated gene by the Pan-Cancer analysis [3]. Colorectal cancer (CRC) and gastric cancer (GC) harbored *ASPM* mutation in 4.35 % and 1.31 %, respectively [3], all of which were missense or splicing-site mutations (search at www.intogen.org).

Through a search in the UCSC public genome database (http://genome.cse.ucsc.edu/), we found that the *ASPM* gene harbors a mononucleotide repeat in the coding sequence that might serve as a target for mutation in GC and CRCs exhibiting microsatellite instability (MSI) [6]. To see whether *ASPM* gene harbored frameshift mutations of the repeat in GC and CRC, we analyzed the exon 18 A7 repeats in 34 GC with high MSI (MSI-H), 45 GC with stable MSI/low MSI (MSS/MSI-L), 76 CRC with MSI-H and 45 CRC with MSS/MSI-L by polymerase chain reaction (PCR) and single-strand conformation polymorphism (SSCP) assay as described previously [7]. In cancer tissues, malignant cells and normal cells were selectively procured from hematoxylin and eosin-stained slides by microdissection [7, 8]. 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, direct DNA sequencing reactions were performed in the cancers with mobility shifts in the SSCP as described previously [7, 8]. In addition, to see whether frameshift mutations in the *ASPM* gene repeat possess intra-tumor heterogeneity (ITH) that contributes to tumor aggressiveness [9], we analyzed 16 CRCs with four to seven regional biopsies per CRC.

On the SSCP, we observed aberrant SSCP bands of *ASPM* gene in three CRCs and two GCs. DNA from the patients' normal tissues showed no shifts in SSCP, indicating the aberrant bands had risen somatically. DNA sequencing analysis confirmed that the aberrant bands

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Table 1 Summary of the *ASPM* 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)
ASPM	Exon 18	A7	A6	MSI-H (4)	2/76 CRCs (2.6), 2/34 GCs (5.9)	c.5149delA (p. Ile1717fsx1)
		A7	A8	MSI-H (1)	1/76 CRCs (1.3)	c.5149dupA (p. Ile1717Asnfsx24)

represented *ASPM* somatic mutations, which consisted of a frameshift mutation by deletion of one base (c.5149delA (p. Ile1717fsX1)) or another frameshift mutation by duplication of one base (c.5149delA (p. Ile1717AsnfsX24)) in the A7 repeat (Table 1). They were detected in a GC (2/34) and CRCs (3/76) with MSI-H, but not in those with MSI-L/MSS (0/90) (Fisher's exact test, $p = 0.049$). The frameshift mutations showed ITH in two out of 16 CRC cases (12.5 %), i.e., two CRCs (tumors # 3 and 49) showed the c.5149delA mutation in one of the regional biopsies (#3–1 and 49–2), but there was no such mutation in the other five regional biopsies in each case (Fig. 1).

The *ASPM* frameshift mutations detected in this study would result in premature stops of amino acid synthesis (Table 1) and hence resembles a typical loss-of-function mutation. *ASPM* appears to possess dual roles (oncogene and tumor suppressor gene) probably depending on cell types and contexts [4, 5, 10]. In pancreatic cancer and glioblastoma, ASPM is overexpressed and contributes to cell proliferation [4, 5]. In small cell lung cancer, however, *ASPM* gene was frequently altered by not only missense but also nonsense mutations [10], indicating that inactivation of *ASPM* gene possibly contributed to tumorigenesis as well. Because it is not known whether the *ASPM* frameshift mutations stimulate or suppress GC and CRC tumorigenesis, further functional studies should be required. Also, we noted ITH for *ASPM* mutations in at least two of the 16 CRC tested. Presence of genetic ITH may have implications for predictive and prognostic biomarker strategies [9]. In the context of clinical practice, our ITH data suggest that there could be an under- or over-estimation of the occurrence of frameshift mutations in MSI-H cancers. Because of the small numbers of the ITH, we were not able to define the differences in clinicopathologic parameters between the CRCs with or without *ASPM* gene mutation-related ITH. Therefore, analysis of mutational ITH of *ASPM* gene in a larger cohort is needed to identify its clinical meanings.

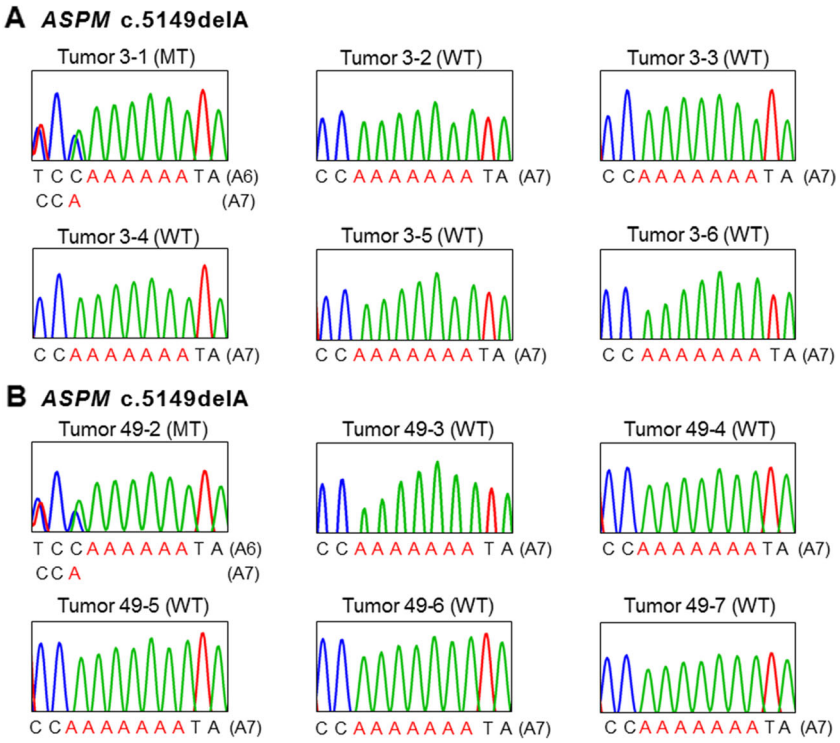

Fig. 1 Intratumoral heterogeneity of *ASPM* frameshift mutation in colon cancers. **a** Direct DNA sequencing data show *ASPM* c.5149delA mutation (MT) in a regional biopsy (3–1) and wild-type (WT) in the other biopsies. **b** Direct DNA sequencing data show *ASPM* c.5149delA mutation (MT) in a regional biopsy (49–2) and wild-type (WT) in the other biopsies

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Compliance with Ethical Standards

Conflict of Interest The authors declare no competing interests.

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