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LETTER TO THE EDITOR

Frameshift Mutations of *CAB39L*, an Activator of *LKB1* Tumor Suppressor, in Gastric and Colorectal Cancers

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To the Editor:

Calcium binding protein 39-like (CAB39L) is a core component of LKB1 tumor suppressor complex and activates LKB1 [1]. Activation of LKB1 plays crucial roles maintaining cell polarity and loss of LKB1 leads to disorganization of cell polarity and facilitates tumor growth. Germline mutations in *LKB1* are associated with Peutz-Jeghers syndrome characterized by polyps in the gastrointestinal tract and other neoplasms [2]. Somatic mutations of *LKB1* gene were also found in many tumors [3]. Also, CAB39L binds and activates STK24, a STE20 family protein kinase [4]. STK24 not only inhibits cell cycle progression by phosphorylating NDR kinase, but also promotes cell death [5]. These data indicate that that CAB39L is possibly involved in cancer-related pathways and suggest that inactivation of CAB39L might be related to tumorigenesis. However, its implications in cancer development are not unknown.

In a public genome database (http://genome.cse.ucsc.edu/), we found that human *CAB39L* had mononucleotide repeats in the coding sequences that could be targets for frameshift mutation in cancers with microsatellite instability (MSI). Frameshift mutation of genes containing mononucleotide repeats is a feature of gastric (GC) and colorectal cancers (CRC) with MSI [6]. To date, however, it is not known whether *CAB39L* gene is mutationally altered in GC and CRC. In this study, we analyzed an A7 repeat in the *CAB39L* exon 2 by polymerase chain reaction (PCR)-based single strand conformation polymorphism (SSCP) assay. For this, we used methacarn-fixed tissues of 34 GC with high MSI (MSI-H), 45 GC with stable MSI (MSS), 89 CRC with MSI-H and 45 CRC with MSS. For 16 of the 89 CRC with MSI, we collected four to seven different tumor areas from the same patients and analyzed intratumoral heterogeneity (ITH) of *CAB39L* mutation. In cancer tissues, malignant cells and normal cells were selectively procured from hematoxylin and eosin-stained slides by microdissection [7]. 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 [8].

On the SSCP, we observed aberrant bands of *CAB39L* gene in two cancers (a GC and a CRC). 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 represented *CAB39L* somatic mutations, which were a frameshift mutation by duplication of one base (c.10dupA (p.Met4AsnfsX5)) and another frameshift mutation by deletion of one base (c.10delA (p.Met4CysfsX15)) in the A7 repeat. They were detected in a GC with MSI-H (1/34: 2.9 %) and a CRC with MSI-H (1/89: 1.1 %), but not in those with MSS. The mutational ITH analysis in the 16 CRCs (4–7 fragments per case) with MSI-H identified that the same CRC described above harbored ITH

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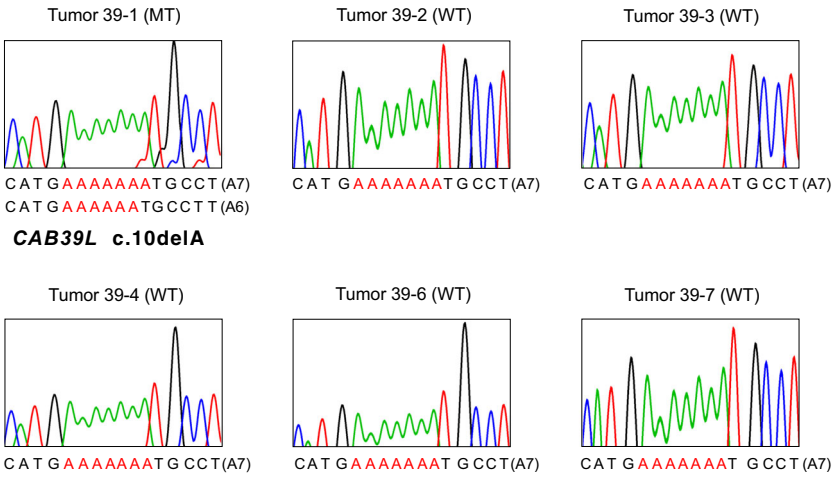

Fig. 1 Intratumoral heterogeneity of *CAB39L* frameshift mutation in colon cancers. Direct DNA sequencings show *CAB39L* c.10delA mutation (MT) in a regional biopsy (39–1) and wild-type (WT) *CAB39L* in the other five regional biopsies (39–2, 39–3, 39–4, 39–6 and 39–7)

of *CAB39L* frameshift mutation. This CRC case (#39) showed the *CAB39L* mutation in one of six regional biopsies (Fig. 1).

In the COSMIC database (http://cancer.sanger.ac.uk/cancergenome/projects/cosmic/), one c.10dupA and another c.10delA are listed in CRC, but there is no mutation data on *CAB39L* in GC. Our study first demonstrated a *CAB39L* frameshift mutation in GC. MSI-H tumors are notoriously prone to mutations in microsatellites and these mutations often represent passenger events with no phenotypic or functional consequences. It is difficult to identify which somatic mutations are drivers and which are passengers. Frequency of the mutation and/or predicted effects of mutation on the encoded protein could be used to define a driver mutation [9]. The frameshift mutations detected in the present study would result in premature stops of amino acid synthesis in CAB39L proteins and hence resembles a typical loss-of-function mutation. Because earlier data suggested possibilities that CAB39L (inhibition of cell cycle progression and promotion of apoptosis) might possess tumor suppressor activities, it can be inferred that the frameshift mutations detected in this study might contribute to cancer development by inhibiting the tumor suppressor activities. Our study did not disclose functional (e.g. changes of specific gene expression) or clinical consequences (e.g. clinical outcomes, MSI-status correlation). Therefore, whether they are 'functional' or 'passenger' mutations remains to be further studied. We also found ITH of *CAB39L* mutation in a CRC. Presence of genetic ITH may have implications for predictive and prognostic biomarker strategies. Genetic ITH contributes to acquire aggressiveness in cancers and may impede accurate diagnosis and proper selection of cancer therapies [10]. Roles of ITH of *CAB39L* mutation remain to be clarified in conjunction with the identification of biological functions of CAB39L in cancers. Also, our study suggests that mutational ITH should be considered in clinical setting for the evaluation of GC and CRC with MSI-H.

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