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LETTER TO THE EDITOR

Frameshift Mutation of an Angiogenesis Factor *VEGFB* and its Mutational Heterogeneity in Colorectal Cancers

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To the Editor:

During tumor development and progression, angiogenic switch causes quiescent blood vessels to sprout new vessels that help sustain expanding neoplastic growths [1]. A well-known prototype of angiogenesis inducers is vascular endothelial growth factor (VEGF) protein family that is comprised of VEGFA, VEGFB, VEGFC and VEGFD, all of which may exhibit angiogenic functions. Angiogenic function of VEGFB has been identified in many studies, albeit less definite than that of VEGFA [2, 3]. With respect to the cancer, alterations of *VEGFB* gene are largely unknown. Promoter CpG methylation of *VEGFB* has been reported in ovarian cancers [4]. Cancer development initiates through a clonal expansion of a single cell. The resulting cell population usually becomes heterogeneous after branching sub-clonal expansions, which leads to intra-tumor heterogeneity (ITH). This ITH contributes to acquired tumor aggressiveness and may impede the accurate diagnosis/prognosis and the proper selection of cancer therapies [5].

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In a public genome database (http://genome.cse.ucsc.edu/), we found that human *VEGFB* had a mononucleotide repeat 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 cancer, endometrial cancer and colorectal cancers (CRCs) with MSI [6]. Somatic missense mutations of this gene have been identified in CRCs with MSI, whereas frameshift mutations have not [7]. To see whether *VEGFB* gene harbored frameshift mutations and mutational ITH in CRC, we analyzed the A8 repeat in *VEFGB* exon 5 by polymerase chain reaction (PCR)-based single-strand conformation polymorphism (SSCP) assay. For this, we used methacarn-fixed tissues of 79 MSI-high (MSI-H) CRCs and 45 MSI-low (MSI-L) or MSI-stable (MSS) CRCs. For 57 (16 MSI-H and 41 MSI-L/MSS) of the 124 CRCs, we collected four to seven different tumor areas from the same patients and analyzed ITH of *VEGFB* mutation. In cancer tissues, malignant cells and normal cells were selectively procured from hematoxylin and eosin-stained slides by microdissection [8, 9]. 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, 9].

On the SSCP, we observed aberrant bands of *VEGFB* gene in 11 CRCs. DNA from the patients' normal tissues showed no shifts in SSCP, indicating the aberrant bands had risen somatically. DNA sequencing analysis confirmed that the

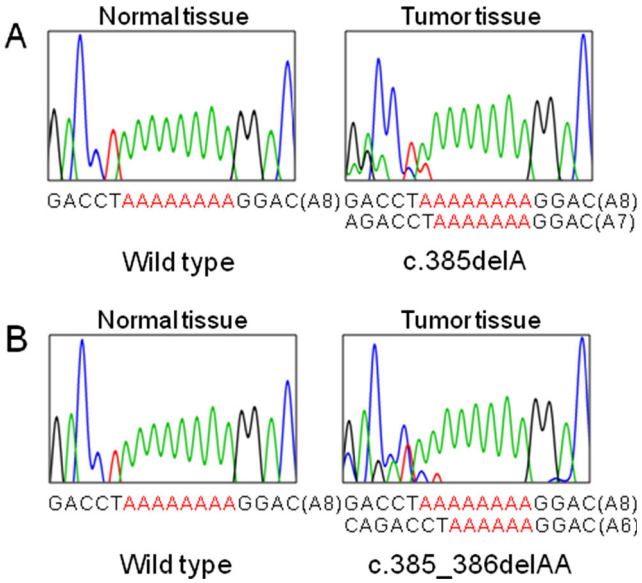

Fig. 1 *VEGFB* frameshift mutations in colon cancers. Direct DNA sequencing analyses show *VEGFB* c.385delA mutation (**a**) and c385_386delAA (**b**) in tumor tissues as compare to normal tissues

aberrant bands represented *VEGFB* somatic mutations, which consisted of a frameshift mutation by deletion of one base (c.386delA (p.Lys129ArgfsX5)) or another frameshift mutation by deletion of two bases (c.385_386delAA (p.Lys29GlyfsX24)) in the A8 repeat (Fig. 1). They were detected in CRCs with MSI-H (11/79: 13.9 %), but not in those with MSI-L/MSS (0/45) (Fisher's exact test, p=0.005). The frameshift mutation showed ITH in a CRC case, i.e., case #53 showed the c.386delA mutation in one out of seven regional biopsies (Fig. 2).

The frameshift mutations detected in the present study would result in premature stops of amino acid synthesis in VEGFB protein and hence resembles a typical loss-of-function mutation. Because earlier data suggested that VEGFB possessed angiogenic activities, it can be inferred that the frameshift mutations identified in this study might inhibit the angiogenic activities. Provided that the angiogenic activities are essential for cancer cell survival, the frameshift mutations might reduce aggressiveness of cancer cells, suggesting a rationale for explaining better prognosis of CRC with MSI-H than those with MSS [6]. Through our analyses, we noted ITH for *VEGFB* mutations in at least one of the CRC samples tested. These data are in accordance with previous studies showing that genetic ITH for (non-coding) microsatellite markers, as well as repeat sequences within coding genes, may be encountered [10]. However, no definite differences were observed between the CRCs with or without *VEGFB* gene mutation-related ITH and the different clinicopathologic parameters tested. Therefore, we propose that its role should be clarified in conjunction with the elucidation of the role of *VEGFB* in cancer. Presence of genetic ITH may have implications for predictive and prognostic biomarker strategies. 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. Therefore, when performing mutation analyses in cancers with MSI-H, multi-regional biopsies should be considered for a better evaluation of their mutation status.

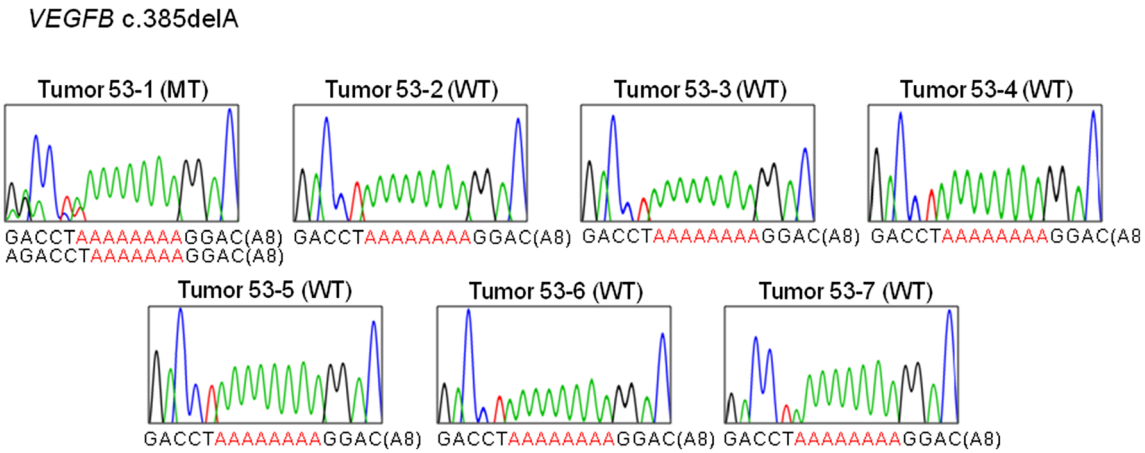

Fig. 2 Intratumoral heterogeneity of a *VEGFB* frameshift mutation in a colon cancer. Direct DNA sequencings show *VEGFB* c.385delA mutation (MT) in a regional biopsy (53–1) and wild-type (WT) *VEGFB* in the other six regional biopsies (53–2, 53–3, 53–4, 53–5, 53–6 and 53–7)

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