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LETTER TO THE EDITOR

Mutational Heterogeneity of *MED23* Gene in Colorectal Cancers

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To the Editor:

The Mediator (MED) complex is a multi-subunit complex that is required for regulating most RNA polymerase II transcripts through interactions with transcription factors bound at enhancers and promoter elements and with polymerase and the general initiation factors at the core promoter [1]. There are more than 20 MED complexes that exist in two compositionally distinct forms, i.e., CDK8-mediators and non-CDK8 core mediators. In addition to the roles in general transcription, MED complex might function as a regulator for diverse biological processes, including differentiation, proliferation and tumorigenesis, all of which are related to tumor development [1]. *MED12* somatic mutations are common in uterine leiomyoma and breast fibroadenoma, suggesting that mutations in MED genes may possibly play roles in tumor development [2, 3]. MED23 is a non-CDK8 core MED that is known for its alterations in diverse cancers [1]. MED23 is required in Ras-active lung cancer and is frequently upregulated in liver cancer [4, 5]. Also, the MED23 has tumor suppressive effects in esophageal cancer [6]. Together, these reports suggest that MED23 has either oncogenic or tumor suppressive function depending on tissue types. However, alterations of *MED23* in gastrointestinal cancers remain unknown. Cancer development initiates through a clonal expansion of a single cell, but the cells usually become heterogeneous after branching sub-clonal expansions, which leads to intra-tumor heterogeneity (ITH). This ITH contributes to tumor aggressiveness and may impede the accurate diagnosis/prognosis [7].

By searching the UCSC public genome database (http://genome.cse.ucsc.edu/), we identified that *MED23* gene harbors a mononucleotide repeat in the coding sequence that might serve as a target for mutation in gastric (GCs) and colorectal cancers (CRCs) exhibiting microsatellite instability (MSI) [8]. To see whether *MED23* gene harbored frameshift mutations in GC and CRC, we analyzed the A8 repeat in *MED23* exon 19 by polymerase chain reaction (PCR)-based single-strand conformation polymorphism (SSCP) assay. For this, we used methacarn-fixed tissues of 34 MSI-high (MSI-H) GCs, 56 MSI-low (MSI-L)/microsatellite-stable (MSS) GCs, 79 MSI-H CRCs and 62 MSI-L/MSS GCs. For 16 of the 79 MSI-H CRCs, we collected four to seven different tumor areas from the same patients and analyzed ITH of *MED23* mutation. In cancer tissues, malignant cells and normal cells were selectively procured from hematoxylin and eosin-stained slides by microdissection [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 [9].

On the SSCP, we observed aberrant bands of gene in six cases that included five CRCs and one GC. 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 *MED23* somatic mutations, which consisted of a frameshift mutation by deletion of a base (c.2276delA (p.Asn759MetfsX12)) (3 CRCs and a GC) or another frameshift mutation by duplication of a base (c.2276dupA (p.Asn759LysfsX7)) (2 CRCs) in the A8 repeat. They were detected in those with MSI-H (6/113: 5.3 %), but not in those

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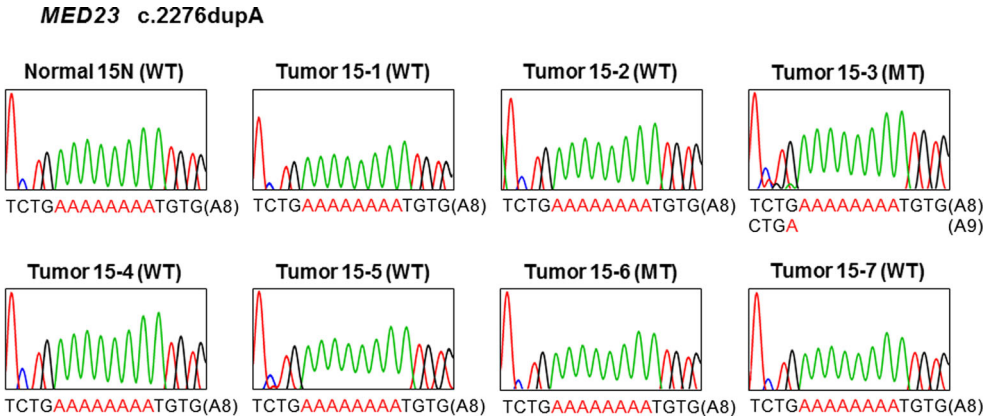

Fig. 1 Intratumoral heterogeneity of an *MED23* frameshift mutation in a colon cancer. Direct DNA sequencings show *MED23* c.2276dupA mutation (MT) in a regional biopsy (15-3) and wild-type (WT) *MED23* in the other six regional biopsies (15-1, 15-2, 15-4, 15-5, 15-6 and 15-7)

with MSI-L/MSS (0/118). The duplication mutation showed ITH in a CRC case, i.e., case #15 showed the c.2276dupA mutation in one out of seven regional biopsies (Fig. 1).

The frameshift mutations detected in the present study would result in premature stops of amino acid synthesis and hence resembles a typical loss-of-function mutation. Such inactivating mutation of *MED23* might be similar to MED23 downregulation in esophageal caners, which inhibited cellular apoptosis and promoted tumorigenecity [6]. Through our analyses, we noted ITH for *MED23* mutations in at least one of the CRC samples tested. Our data are in accordance with the earlier studies showing that genetic ITH for microsatellite markers, as well as repeat sequences within coding genes, may be encountered [10]. 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, we propose that the role of ITH in *MED23* should be clarified in conjunction with the elucidation of the role of *MED23* in CRC.

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Conflict of Interests The authors declare no competing interests.

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