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LETTER TO THE EDITOR

Frameshift Mutation of *MED25*, a Transcription Regulator, and its Mutational Heterogeneity in Colorectal Cancers

Yun Sol Jo[1] · **Min Sung Kim**[1] · **Nam Jin Yoo**[1] · **Sug Hyung Lee**[1]

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To the Editor:

MED12 somatic mutations are highly recurrent in uterine leiomyoma and breast fibroadenoma, suggesting that *MED* gene mutations might play roles in tumor development [1, 2]. The Mediator (MED) functions as a bridge to convey information from gene-specific regulatory proteins to the basal RNA polymerase II transcription machinery [3]. Mediator is recruited to promoters by direct interactions with regulatory proteins and serves as a scaffold for the assembly of a functional preinitiation complex with RNA polymerase II and the general transcription factors. There are more than 20 MED complexes that exist in two distinct forms, i.e., CDK8-mediators and non-CDK8 core mediators [3]. In addition to the roles in general transcription, the MEDs function as a regulator for diverse biological processes, including differentiation, proliferation and tumorigenesis that are related to tumor development [3]. MED25 (also known as ACID1, ARC92 and PTOV2), a non-CDK8 core MED, is functionally associated with the activation domains of multiple cellular and viral transcriptional activators, including the herpes simplex viral activator VP16, sterol regulatory element binding protein and NF-kappa B [4]. Transcriptional activity of RA receptor that plays a role in cancer therapy is enhanced by association of MED25 with CREB-binding protein [4]. However, alterations of *MED25* in cancers remain unknown. Cancer development initiates through a clonal expansion of a single cell, but the cells usually become heterogeneous after branching subclonal expansions, which leads to intra-tumor heterogeneity (ITH). This ITH contributes to tumor aggressiveness and may impede the accurate diagnosis/prognosis [5].

In a public genome database (http://genome.cse.ucsc.edu/), we found that human *MED25* had a mononucleotide repeat in the coding sequences that could be a target for frameshift mutation in cancers exhibiting microsatellite instability (MSI). Frameshift mutation of genes containing mononucleotide repeats is a feature of colorectal cancers (CRC) with MSI [6]. To date, however, it is not known whether *MED25* gene is mutationally altered in CRC with MSI. In this study, we analyzed a C7 repeat in the *MED25* exon 6 by polymerase chain reaction (PCR)-based single-strand conformation polymorphism (SSCP) assay. We used methacarn-fixed tissues of 89 high MSI (MSI-H) CRCs and 52 microsatellite-stable (MSS) CRCs. For 16 of the 89 MSI-H CRCs, we collected four to seven different tumor areas from the same patients and analyzed ITH of *MED25* 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 [7].

On the SSCP, we observed aberrant bands of *MED25* gene in seven 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 aberrant bands represented a recurrent *MED25* frameshift

✉ Sug Hyung Lee
suhulee@catholic.ac.kr

[1] Department of Pathology, College of Medicine, The Catholic University of Korea, 505 Banpo-dong, Socho-gu, Seoul 137-701, South Korea

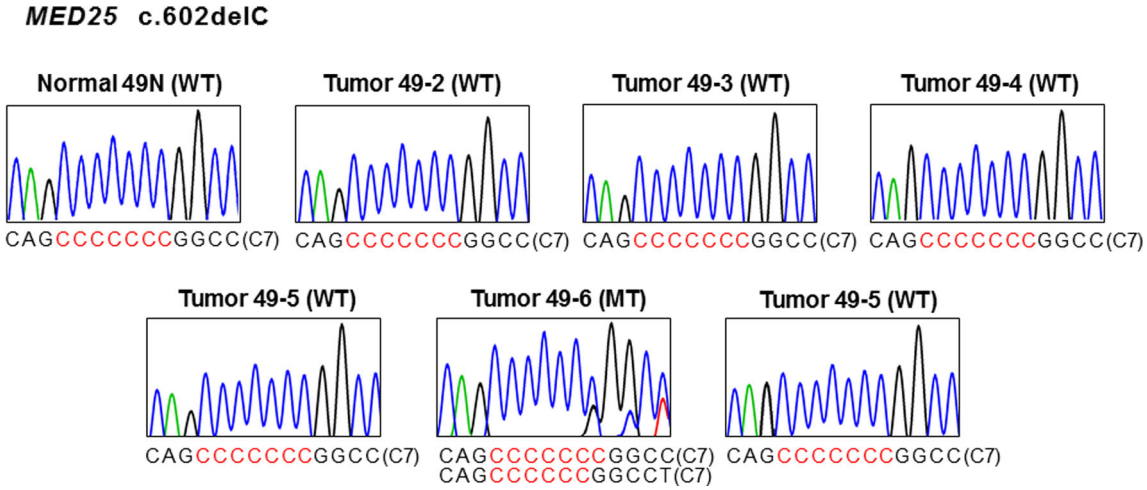

Fig. 1 Intratumoral heterogeneity of an *MED25* frameshift mutation in a colon cancer. Direct DNA sequencings show *MED25* c.620delC mutation (MT) in a regional biopsy (49–6) and wild-type (WT) *MED25* in the other five regional biopsies (49–2, 49–3, 49–4, 49–5 and 49–7)

mutation (deletion of one base) in the C7 repeat (c.602delC) that would result in a frameshift mutation (p. Pro201ArgfsX13). They were detected in the CRCs with MSI-H (7/89: 7.9 %), but not in those with MSS (0/52) (Fisher's exact test, $p = 0.036$). The frameshift mutation showed ITH in three CRC cases. Case #49 showed the mutation in one out of six regional biopsies (Fig. 1). Likewise, cases #34 and #39 showed one of seven and one of six regional biopsies, respectively.

The aim of this study was to find *MED25* mutation that might alter the MED module. The frameshift mutation detected in the current study would result in a premature stop of amino acid synthesis and hence resembles a typical loss-of-function mutation. Based on this observation, it can be thought that truncated MED25 by the mutation might disrupt the proper formation of the MED module. Similarly, uterine leiomyoma-linked mutation in *MED12* disrupts mediator-associated CDK activity [8]. Through our analyses, we noted ITH for *MED25* mutations in three CRC samples tested. The data are in agreement with the earlier observation showing that genetic ITH for microsatellite markers, as well as repeat sequences within coding genes, may be encountered [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. Therefore, we propose that the role of ITH in *MED25* should be clarified in conjunction with the identification of the role of MED genes in CRC.

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References

1. Mäkinen N, Mehine M, Tolvanen J, Kaasinen E, Li Y, Lehtonen HJ, Gentile M, Yan J, Enge M, Taipale M,Aavikko M, Katainen R, Virolainen E, Böhling T, Koski TA, Launonen V, Sjöberg J, Taipale J, Vahteristo P, Aaltonen LA (2011) MED12, the mediator complex subunit 12 gene, Is mutated at high frequency in uterine leiomyomas. Science 334: 252–255.
2. Lim WK, Ong CK, Tan J, Thike AA, Ng CC, Rajasegaran V, Myint SS, Nagarajan S, Nasir ND, McPherson JR, Cutcutache I, Poore G, Tay ST, Ooi WS, Tan VK, Hartman M, Ong KW, Tan BK, Rozen SG, Tan PH, Tan P, Teh BT (2014) Exome sequencing identifies highly recurrent MED12 somatic mutations in breast fibroadenoma. Nat Genet 46:877–880
3. Poss ZC, Ebmeier CC, Taatjes DJ (2013) The Mediator complex and transcription regulation. Crit Rev Biochem Mol Biol 48:575–608
4. Lee HK, Park UH, Kim EJ, Um SJ (2007) MED25 is distinct from TRAP220/MED1 in cooperating with CBP for retinoid receptor activation. EMBO J 26:3545–3557
5. Marusyk A, Almendro V, Polyak K (2012) Intra-tumour heterogeneity: a looking glass for cancer? Nat Rev Cancer 12:323–334
6. Imai K, Yamamoto H (2008) Carcinogenesis and microsatellite instability: the interrelationship between genetics and epigenetics. Carcinogenesis 29:673–680
7. Yoo NJ, Kim HR, Kim YR, An CH, Lee SH (2012) Somatic mutations of the KEAP1 gene in common solid cancers. Histopathology 60:943–952
8. Turunen M, Spaeth JM, Keskitalo S, Park MJ, Kivioja T, Clark AD, Mäkinen N, Gao F, Palin K, Nurkkala H, Vähärautio A, Aavikko M, Kämpjärvi K, Vahteristo P, Kim CA, Aaltonen LA, Varjosalo M, Taipale J, Boyer TG (2014) Uterine leiomyoma-linked MED12 mutations disrupt mediator-associated CDK activity. Cell Rep 7:654–660
9. Calin GA, Gafà R, Tibiletti MG, Herlea V, Becheanu G, Cavazzini L, Barbanti-Brodano G, Nenci I, Negrini M, Lanza G (2000) Genetic progression in microsatellite instability high (MSI-H) colon cancers correlates with clinico-pathological parameters: A study of the TGRbetaRII, BAX, hMSH3, hMSH6, IGFIIR and BLM genes. Int J Cancer 89:230–235