Short communication

# Intratumoral heterogeneity for inactivating frameshift mutation of CUX1 and SIRT1 genes in gastric and colorectal cancers

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Both *CUX1* and *SIRT1* are considered tumor suppressor genes (TSGs), but it is not known whether *CUX1* and *SIRT1* alterations are different between high microsatellite instability (MSI-H) and microsatellite stable MSI (MSS) cancers. We identified frameshift mutations of *CUX1* in 4 cases of colorectal cancer (CRC) and of *SIRT1* in 1 case of gastric cancer (GC) and 3 cases of CRC. All of them were found in GC or CRC with MSI-H (3.5% of MSI-H for each gene), but neither in GC nor CRC with MSS. In addition, we analyzed intratumoral heterogeneity (ITH) of the *CUX1* frameshift mutation and found that two CRCs (12.5%) harbored regional ITH of the frameshift mutation. Our data indicate that there exist frameshift mutations of *CUX1* and *SIRT1* genes as well as ITH of *CUX1* frameshift mutation in MSI-H cancers, which together might play a role in tumorigenesis of GC and CRC with MSI-H.

**Key words:** *CUX1, SIRT1,* tumor suppressor, mutation, intratumoral heterogeneity

## Introduction

*CUX1* gene encodes a homeodomain protein that has both oncogene (cell cycle progression, cell migration and repair of DNA damages) and tumor suppressor gene (TSG) (repression of PI3K-AKT pathway and base excision repair) activities [1, 2, 3, 4, 5]. Loss of heterozygosity (LOH) at 7q22.1 where *CUX1* resides is common in many cancers [1]. Inactivating mutations of *CUX1* are present in many types of cancers [6]. Aside such evidence of TSG roles for *CUX1*, increased CUX1 expression is frequent in many cancers and is associated with poor survival [6]. These two opposing roles of CUX1 for cancer pathogenesis may indicate that several transcriptional targets and cellular functions of CUX1 as well as microenvironment affect tumorigenesis. SIRT1 is a member of sirtuin family of class III histone deacetylases (HDACs) that regulate many physiological processes, including cell proliferation, inflammation and metabolism [7]. Like CUX1, SIRT1 functions as both oncogene and TSG [7]. For the oncogenic function, SIRT1 can deacetylate p53 and thereby inhibits p53-dependent transcription or apoptosis [8]. For the TSG function, SIRT1 acts as an inhibitor of proliferation in colorectal cancers (CRC) [8]. About 10% of gastric cancer (GC) and CRC show microsatellite instability (MSI) phenotype that has defects in mismatch repair [9]. It is not known whether *CUX1* and *SIRT1* alterations are different between high-MSI (MSI-H) and microsatellite stable MSI (MSS) cancers.

Genes are often observed to harbor frameshift mutations at mononucleotide repeats in MSI-H cancers. There are mononucleotide repeats in *CUX1* (C7) and *SIRT1* (A7) of coding sequence that could be mutation targets in cancers with MSI-H. In addition, in-

tratumoral heterogeneity (ITH) plays an important role in cancer development and progression and impedes proper diagnosis and treatment of cancers [10]. The present study aimed to find whether *CUX1* and *SIRT1* genes harbored frameshift mutation within the repeat and ITH.

## Material and methods

We analyzed *C7* of CUX1 and *A7* of SIRT1 in 34 GCs with MSI-H, 45 GCs with MSS, 79 CRCs with MSI-H and 45 CRCs with MSS by polymerase chain reaction (PCR) and single-strand conformation polymorphism (SSCP) assay. After SSCP, Sanger DNA sequencing reactions were performed in the cancers with mobility shifts in the SSCP [11]. Pathologic features of the cancers are summarized in the supplement (Supplement 1). For 16 CRCs with MSI-H, we collected four to seven different tumor areas and one normal mucosal area from each frozen CRC specimen. They were analyzed for the detection of regional ITH of *CUX1* and *SIRT1* gene repeats. Approval of this study was obtained from the Catholic University of Korea, College of Medicine's institutional review board for this study.

## Results and discussion

SSCP and Sanger sequencing identified frameshift mutations of *CUX1* in 4 cases of CRC and those of *SIRT1* in 1 case of GC and 3 cases of CRC. All of them were found in GC or CRC with MSI-H (3.5% of MSI-H for each gene), but neither in GC nor CRC with MSS. These mutations were not detected in their normal tissues. All of the *SIRT1* mutations were the same deletion mutation (c.709delA (p.Arg-237GlufsTer11)), while the *CUX1* mutations included a deletion mutation (c.1289delC (p.Pro430LeufsTer27)) and a duplication mutation (c.1289dupC (p.Pro431SerfasTer16)). For ITH of the mutations, we studied 16 cases of CRCs with 4 to 7 regional fragments per CRC. Two of the 16 CRCs (12.5%) showed the deletion mutation of *SIRT1* (c.709delA) in different tissue regions. Also, another two (12.5%) showed the deletion mutation of *CUX1* (c.1289delC) in different tissue regions, indicating ITH of the *CUX1* and *SIRT1* mutation existed in CRC (Fig. 1). Clinical and histopathological parameters, however, could distinguish neither *CUX1* frameshift mutation (+) and (–) cancers nor *SIRT1* frameshift mutation (+) and (–) cancers. The parameters could distin-

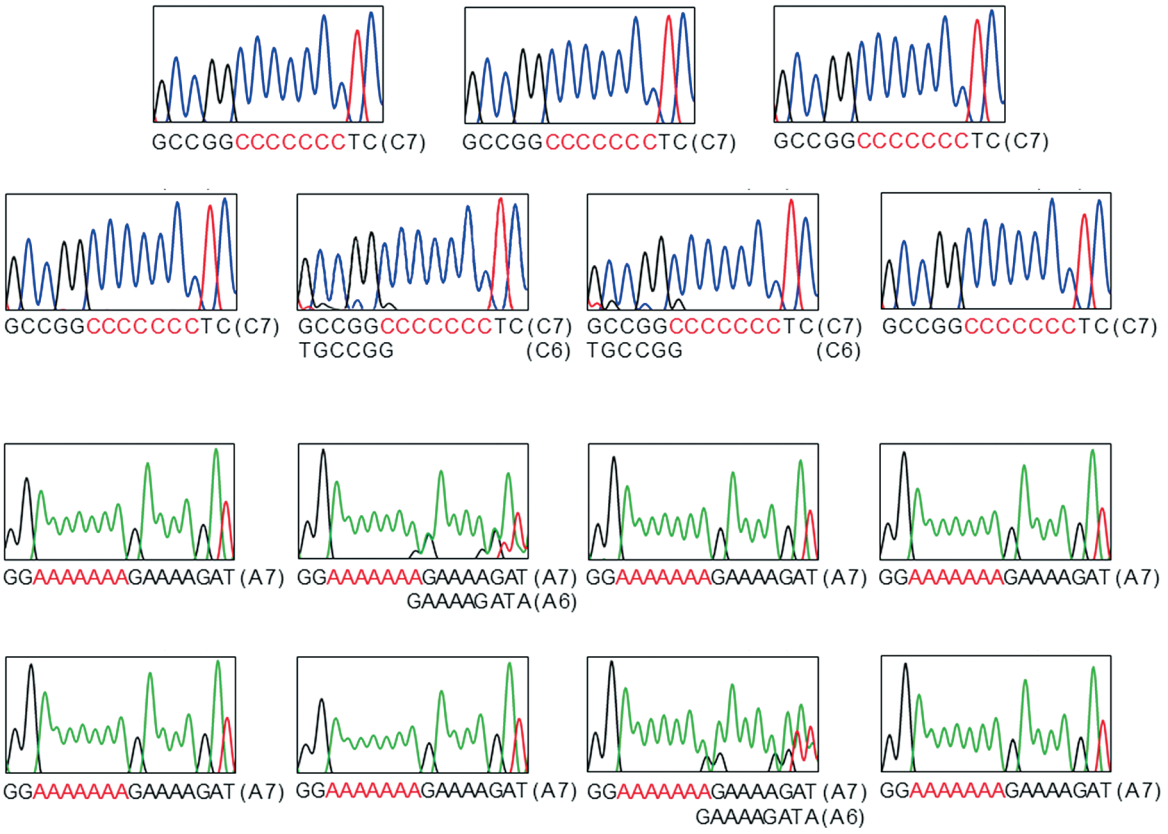


**Fig. 1.** Intratumoral heterogeneity of CUX1 and SIRT1 frameshift mutations in colon cancers. A) Direct DNA sequencing shows CUX1 c.1289delC mutation (MT) in 2 regional areas (49-5 and -6) and wild-type (WT) in the other 4 areas (49-2, -3, -4 and -7). B) Direct DNA sequencing shows SIRT1 c.709delA mutation (MT) in 2 regional areas (53-1 and -6) and wild-type (WT) in the other 5 areas (53-2, -3, -4, -5 and -7)

guish neither *CUX1* ITH (+) and (–) cancers nor *SIRT1* ITH (+) and (–) cancers.

The frameshift mutations identified in this study would result in truncation of CUX1 and SIRT1 proteins, suggesting that they may be inactivated in MSI-H GCs and CRCs by the frameshift mutations. However, incidence of the mutations was low and identified only in MSI-H cancers. Conservable proportions of the frameshift mutations (12.5% for each gene) exhibited ITH in CRCs. ITH of the frameshift mutation in the CRCs might suggest a possibility that there could be a mixed or ameliorated effect of *CUX1* and *SIRT1* mutation effect in MSI-H cancers. However, we were not able to find any distinguished clinicopathologic features of *CUX1/SIRT1*-mutated or ITH-positive cancers. It was probably due to small number of the mutated cases. Based on our preliminary data, further studies are needed to define the clinical implication of *CUX1* and *SIRT1* mutations and their ITH in MSI-H cancers.

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