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.Arg237GluSTOP11)), while the CUX1 mutations included a deletion mutation (c.1289delC (p.Pro430LeufsTer27)) and a duplication mutation (c.1289dupC (p.Pro431SerfsTer16)). 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. Conserved 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.

The authors declare no conflict of interest.

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References