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LETTER TO THE EDITOR

Intratumoral Heterogeneity of *RPL22* Frameshift Mutation in Colorectal Cancers

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Dear Editor,

RPL22 gene encodes a cytoplasmic ribosomal protein that is a component of the large 60S subunit of ribosome. An earlier study identified that RPL22 protein functions as a haploinsufficient tumor suppressor [1]. *RPL22* inactivation promotes transformation by inducing expression of Lin28B [1]. A recent study discovered that *RPL22* was frequently mutated in colorectal cancer (CRC) and endometrial cancers by frameshift mutations in A8 repeat, especially those in microsatellite instability high (MSI-H) cancers [2]. Also, another study demonstrated a higher percentage of *RPL22* frameshift mutations in MSI-H gastric cancer (GC) [3]. These data suggest that *RPL22* is a tumor suppressor that is commonly inactivated in MSI-H cancers by mutations. Intratumoral heterogeneity (ITH) plays an important role in cancer development and progression and impedes proper diagnosis and treatment of cancers [4]. Currently, we are aware of the frequent mutations of *RPL22* in MSI-H cancers, but mutational ITH of *RPL22* remains elusive.

Genes are often observed to harbor frameshift mutations at monocleotide repeats in MSI-H cancers [2, 3]. The present study aimed to find whether *RPL22* gene harbored not only frameshift mutations within the A8 repeat but also ITH of the frameshift mutations. We analyzed the A8 repeat 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 [5].

We found *RPL22* somatic frameshift mutations in 16 CRCs (16/79, 20.3%) and 9 GCs (9/34, 26.5%) with MSI-H, but not in CRCs (0/45) and GCs (0/45) with MSS (Fisher's exact test, $p < 0.001$). These mutations were not detected in their normal tissues. The mutations consisted of 'A' deletion (c.44delA (p.Lys16Serfsx4)), 'A' duplication (c.44dupA (p.Lys16Glufsx9)) and 'AA' deletion (c.43_44delAA (p.Lys15Glufsx9)) in the coding region (Table 1). For ITH of the mutation, we studied 16 cases of CRCs with 4 to 7 regional fragments per CRC. Four of the 16 CRCs (25.0%) showed either the 'A' deletion (2 cases) or 'A' duplication (one case) or 'AA' deletion mutation in different tissue regions. One (case #34) of the 4 CRCs exhibited the 'A' duplication in 6 regions as well as the wild type (A8) in the other one region, indicating ITH of the *RPL22* mutation existed in CRC (Fig. 1). Clinical and histopathological parameters, however, could distinguish neither *RPL22* frameshift mutation (+) and (−) cancers, nor the ITH (+) and (−) cancers.

Our data here confirm the previous studies on the frequent involvement of *RPL22* frameshift mutations in GC and CRC. Furthermore, we report for the first time ITH of the *RPL22* frameshift mutation in CRC. The frameshift mutations of *RPL22* identified in this study would result in truncation of RPL22 protein, suggesting that RPL22 may be inactivated in MSI-H GCs and CRCs by the frameshift mutations. Based on the tumor suppressor functions of RPL22, the *RPL22* frameshift mutations appear to reduce the anti-tumor activities and contribute to tumor pathogenesis. However, ITH of the frameshift mutation in CRC might suggest a possibility that there could be a mixed or ameliorated effect of *RPL22* inactivation in MSI-H cancers. However, we were not able to find any distinguished clinicopathologic features of *RPL22*-mutated or ITH-positive cancers. It was probably due to small number of the mutated cases. Thus, further studies are needed to define the clinical implication of *RPL22* mutations and ITH in MSI-H cancers.

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Table 1 Summary of *RPL22* mutations in gastric and colorectal cancers

Location	Wild type	Mutation	MSI status of mutation cases (n)	Incidence in MSI-H cancers (%)	Nucleotide change (predicted amino acid change)
Exon 2	A8	A7	MSI-H (21)	Colorectal: 12/79 (15.2) Gastric: 9/34 (26.5)	c.44delA (p.Lys16Serfsx4)
	A8	A9	MSI-H (1)	Colorectal: 1/79 (1.3)	c.44dupA (p.Lys16Glufsx9)
	A8	A6	MSI-H (3)	Colorectal: 3/79 (3.8)	c.43_44delAA (p.Lys15Glufsx9)

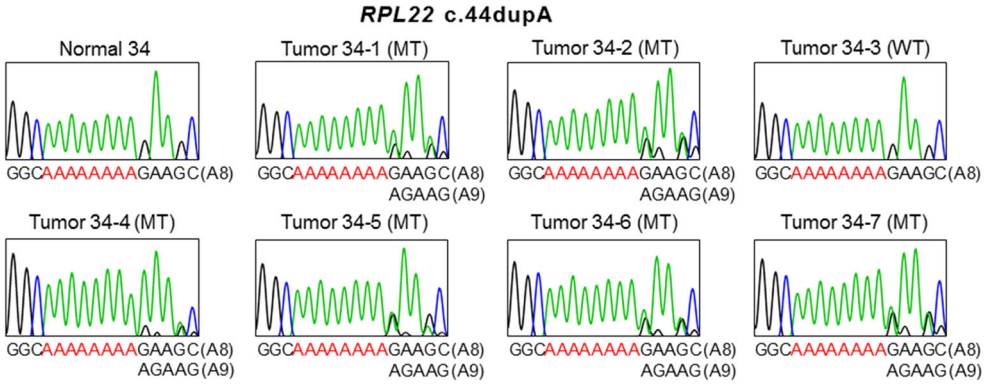

Fig. 1 Intratumoral heterogeneity of an *RPL22* frameshift mutation in a colon cancer. Sanger DNA sequencing analyses show *RPL22* c.44dupA mutation (MT) in 6 regional areas (34–1, −2, −4, −5, −6 and − 7) and wild-type (WT) in the other one area (34–3)

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Compliance with ethical standards

Conflicts of interest None to declare.

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