Concurrent Mutations of K-ras Oncogene at Codons 12 and 22 in Colon Cancer

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K-ras mutation is the most common oncogenic alteration in various human cancers including colorectal carcinomas. Point mutations have the potential to activate the K-ras gene if they occur in the critical coding sequences. Almost all of these mutations have been localized in codons 12, 13 and 61. We report a case of colon cancer presenting point mutations at both codons 12 and 22 of the K-ras gene. PCR-SSCP and subsequent sequencing revealed that GGT (glycine, wild-type) to AGT (serine) substitution at codon 12 and CAG (glutamine, wild-type) to CGG (arginine) substitution at codon 22 occurred in the same allele.

Key words: K-ras gene – colon cancer – double point mutation – PCR-SSCP

GENETIC SUMMARY

Disorder: Colon cancer
Ethnicity of patient: Japanese
Gene: K-ras
GeneBank accession number: L00045
Type of DNA variant: Somatic point mutations
Mutation: GGT (Gly, wild-type) to AGT (Ser) substitution at codon 12 and CAG (Gln, wild-type) to CGG (Arg) substitution at codon 22 of the K-ras gene
Allelic frequency: Not tested
Methods of mutation detection: PCR-SSCP and direct sequencing

CASE REPORT AND GENETIC ANALYSIS

The ras mutation is the most common oncogenic alteration in human cancers. K-ras mutation is present in up to 40% of colorectal adenomas and carcinomas (1,2). K-ras gene has transforming activity after single point mutation within its coding sequence. Mostly, these mutations have been localized in codons 12, 13 and 61. Rare variants of point mutations at codons 11, 18 and 59 have been reported in a few human tumors (3–5). Recently, Tsukuda et al. (6) reported a point mutation at codon 22 in a primary colon cancer and suggested that this mutation might be advantageous for the growth of tumor cells. Here we report a case of colon cancer presenting point mutations of the K-ras oncogene at codons 12 and 22 concurrently in the same allele.

An 82-year-old female was diagnosed with ascending colon cancer and underwent surgical resection. Histological diagnosis was well differentiated adenocarcinoma. We previously reported microsatellite instability (MSI) status (MSI-negative) and methylation profile (partial methylation) of the hMLH1 promoter region in tumor tissues of this case (7). PCR-SSCP analysis for the K-ras gene revealed two significant shifted bands in the tumor sample (Fig. 1). Direct sequencing of this tumor-derived PCR product identified two point mutations which caused a substitution of serine for glycine (GGT to AGT) at codon 12 and arginine (Arg) for glutamine (CAG to CGG) at codon 22 (Fig. 2). To confirm whether these mutations occurred in the same allele or not, each of the two shifted bands were cut from the gel, reamplified and subjected to SSCP analysis and direct sequencing (Figs 1 and 2). It was shown that DNA fragments amplified from these two bands had the same mobility shift in SSCP, indicating that they are complementary single-strands. Furthermore, sequencing analysis confirmed that these two mutations were detected in...
the same electropherogram without contamination of the wild-type sequence. These findings indicate that mutations at codons 12 and 22 were localized in the same allele. Previously, Tsukuda et al. reported that the novel point mutation at codon 22 was C to A transversion substituting lysine (Lys) (AAG) for glutamine (CAG) (6). The tumorigenecity of K-ras Lys22 transformants in nude mice was significantly less potent than that of K-ras Val12 transformants. In this study, it was not identified whether this K-ras Arg22 mutation gives rise to tumorigenesis. Further studies are needed to elucidate the biological activity of this combination of point mutations at codons 12 and 22. To our knowledge, this is the first report of K-ras point mutations occurring concurrently in the same allele.

METHODS FOR MUTATION DETECTION

PCR-SSCP and PCR/direct sequencing was performed with the following conditions and parameters:

PCR primer, forward: 5′ ACT GAA TAT AAA CTT GTG GTA GTT GGA CCT 3′.
PCR primer, reverse: 5′ TA A TAT GTC GAC TAA AAC AAG ATT TAC CTC 3′.

Size of PCR product: 135 bp.

**Thermal cycle profile:**
40 cycles of 96°C, 1 min/55°C, 1 min/73°C 30 s.
Final extension: 72°C, 7 min.

The 5′ end of the forward primer was labeled with indodi-carbocyanine (Cy5) fluorescent dye.

PCR-SSCP: non-RI SSCP analysis as described previously with minor modifications (8,9).

![Figure 1](image1.png)

**Figure 1.** Detection of point mutations of the K-ras gene by PCR-SSCP analysis. N, DNA derived from normal colonic mucosa; T, DNA derived from tumor sample, presenting mutated alleles (arrows a and b); Ta and Tb, PCR product reamplified from shifted bands (arrows a and b) in tumor sample (T), respectively, showing enrichment of the mutated allele.

![Figure 2](image2.png)

**Figure 2.** Direct sequencing of the PCR product using reverse PCR primer. Each sample is the same as indicated in Fig. 1. PCR product derived from the mutated allele showed substitutions of GGT (Gly, wild-type) to AGT (Ser) at codon 12 and CAG (Gln, wild-type) to CGG (Arg) at codon 22 in the same allele.
Electrophoresis: 15% polyacrylamide gel, 18°C.
Sequencing primer: same as the reverse PCR primer.

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