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Frameshift Mutation of the *STK11* Gene in a Sporadic Gastrointestinal Cancer with Microsatellite Instability

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Germline mutations of the *STK11* gene lead to emergence of hamartomas in the gastrointestinal tract of patients with Peutz-Jeghers syndrome, who bear an increased risk of malignancies of the gastrointestinal tract, genital tract, and other organs. We analyzed 80 sporadic colorectal cancers, six small-intestinal cancers, and 40 gastric cancers for somatic mutations of *STK11* by SSCP methods. Among them only one colorectal cancer, which showed a phenotype of microsatellite instability, was found to possess a deleterious mutation in this gene, a frameshift involving deletion of one base at codons 279–281. This region of the gene contains a mononucleotide-repeat sequence, CCCCCC. The other allele of *STK11* had been lost in this tumor. If the *STK11* gene is one of the mutational targets of microsatellite instability, its inactivation may be associated with tumor development in a small proportion of colorectal cancers.

Key words: STK11 — Microsatellite instability — Gastrointestinal cancer

PJS, an autosomal dominant condition, is characterized by hamartomatous polyposis throughout the gastrointestinal tract and by melanin spots on the lips, buccal mucosa, fingers, and toes.[1, 2)] Patients affected with PJS are at increased risk for malignancies of the gastrointestinal tract, genital tract, and other organs.[3–5)] The PJS locus was mapped to the telomeric region of chromosome *19p* by linkage analysis.[6)] Subsequently, germline mutations in a gene encoding a serine/threonine kinase, *STK11*, were found to be responsible for PJS in some patients.[7, 8)] Hamartomatous polyps from PJS patients often display LOH of the telomeric region of the short arm of chromosome 19, supporting the idea that *STK11* is a tumor suppressor gene.[6)] Adenomas/carcinomas are thought to develop from hamartomatous lesions present in the stomach, duodenum, small intestine, and/or colon of PJS patients.[9, 10)] These lines of evidence have suggested that mutations in the *STK11* gene may be associated with development of sporadic gastrointestinal tumors. To investigate this possibility, we analyzed *STK11* for somatic mutations in a large panel of gastrointestinal cancers.

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The abbreviations used are: PJS, Peutz-Jeghers syndrome; PCR, polymerase chain reaction; SSCP, single-strand conformation poymorphism; LOH, loss of heterozygosity.

MATERIALS AND METHODS

Subjects We extracted genomic DNAs from frozen samples of 80 colorectal cancers, six cancers of the small intestine, and 40 gastric cancers by a standard method.[11)] Genomic DNAs of matched normal tissue were extracted from peripheral blood or peritumoral intestinal tissues.

PCR-SSCP analysis Eight primer pairs corresponding to intronic sequences were designed to amplify the entire coding region of *STK11* from genomic DNA, and PCR-SSCP protocols were performed in the manner reported previously.[12)] Each 20 μl PCR mixture contained 10–50 ng of genomic DNA, 2 μl of 10× PCR buffer, 250 μM of each dNTP, 0.5 μM of each primer, 5% DMSO, and 1 unit of *Taq* polymerase (TaKaRa, Otsu). PCR amplifications were performed in a thermocycler (Perkin Elmer-Cetus 9600, Norwalk, CT) with denaturation at 94°C for 4 min followed by 35 cycles of 94°C for 30 s, 58–62°C for 30 s, 72°C for 30 s, and final extension at 72°C for 3 min. The PCR products of exons 1, 2, and 4+5 (as intron 4 is small, exons 4 and 5 were amplified together) were digested with appropriate restriction enzymes to achieve higher sensitivity for the SSCP analysis. The 450 bp PCR product for exon 1 was digested with *Rsa*I into fragments of 223 bp and 227 bp, the 413 bp product for exon 2 was digested with *Pvu*II (280-bp and 133-bp fragments), and the 503-bp product for exons 4+5 was cleaved by *Apa*I (257 bp and 246 bp). A 3 μl aliquot of each PCR product was added to 6 μl of loading buffer (95% formamide, 10 mM EDTA,

0.25% bromophenol blue, 0.25% xylene cyanol) and denatured for 10 min at 90°C. The samples were electrophoresed at 4°C in non-denaturing polyacrylamide gels (5% polyacrylamide, 0.5× TBE and 5% glycerol). After electrophoresis, gels were stained with SYBER Green II (FMC Bioproducts, Rockland, ME) and visualized with an FMBIO II Multi-View fluorescence image analyzer (TaKaRa).

Direct sequencing Aberrant bands revealed by SSCP analysis were excised from the gel and amplified under the PCR conditions described above. The products were purified with a Suprec-02 (TaKaRa) and sequenced directly in both strands using an ABI 377 DNA automated sequencer and a Dye Terminator Cycle Sequencing FS Ready Reaction Kit (Perkin Elmer-Cetus, Norwalk, CT).

Analysis of microsatellite instability Genomic DNA samples from tumors were analyzed for microsatellite instability at the *BAT-26* mononucleotide-repeat locus by PCR amplification. This locus can identify the status of microsatellite instability very effectively and it was reported to be 96% sensitive to microsatellite instability, with 100% specific responsibility for microsatellite instability.[13, 14] Using forward primer 5′-TGACTACTTTTGACTTCAGCC-3′ and rhodamine-labeled reverse primer 5′-AACCATTCAACATTTTTAACCC-3′ (10 pmol each), PCR-amplification was performed with 50 nmol of each deoxynucleotide, 2 μl of 10× PCR buffer, 1 unit of *Taq* polymerase (TaKaRa), and 10–50 ng of DNA in a total volume of 20 μl. PCR conditions were 94°C for 4 min followed by 35 cycles (94°C for 30 s, 56°C for 30 s, and 72°C for 30 s) and final extension at 72°C for 3 min. PCR products were separated on a 5.6 *M* urea/32% formamide/8% polyacrylamide gel, and visualized with an FMBIO II Multi-View fluorescence image analyzer (TaKaRa).

LOH analysis of *19p13.3* DNAs from tumors and normal tissues were amplified at three microsatellite loci (*D19S886*, *D19S883*, *D19S878*) in the *19p13.3* region, using primers derived from the Genethon linkage map. One primer of each pair was labeled with ^{32}P-ATP, and PCRs were performed for 35 cycles in 20-μl volumes containing 10–50 ng of DNA, 5 pmol of radiolabeled primer, 5 pmol of unlabeled primer, 2 μl of 10× PCR buffer, 50 nmol of each deoxynucleotide, and 1 unit of *Taq* polymerase (TaKaRa). PCR amplifications were performed with denaturation at 94°C for 4 min and 35 cycles of 94°C for 30 s, 56°C for 30 s, 72°C for 30 s, and final extension at 72°C for 3 min. The products were separated on 6% denaturing polyaclylamide gels and visualized by autoradiography.

RESULTS

We analyzed the entire coding region of the *STK11* gene in a panel of 126 sporadic gastrointestinal cancers consisting of 80 colorectal cancers, six cancers of the small intestine, and 40 gastric cancers, by the SSCP method. Only one colorectal (T33) and one small-intestinal cancer (SI5) showed aberrant bands (Fig. 1). Analysis of the sequences in question detected two somatic mutations: a 1-bp deletion within codons 279–281 (exon 6) in T33 (Fig. 2) and a C-to-G substitution at codon 32 (exon 1) in SI5. These nucleotide changes were present only in the tumor tissues. The C-to-G substitution in SI5 caused no amino-acid change, but the deletion in T33 would probably fatally truncate the STK11 gene product.

Codons 279–281 contain the six-cytosine repetitive sequence that is frequently mutated in germline DNAs of PJS patients.[12] Mononucleotide-repeat sequences often undergo somatic frameshift mutations in colorectal cancers with microsatellite instability. We examined the gastrointestinal cancers of our panel for mutations in the *BAT-26* sequence, a reliable DNA marker for microsatellite instability. Thirteen of the 80 colorectal cancers, one of the six small-intestinal cancers, and four of the 40 gastric cancers showed microsatellite instability by mutation in the *BAT-26* repeat sequence. T33, a colorectal cancer with a somatic frameshift mutation of STK11, was among the tumors mutated at *BAT-26* (Fig. 3).

We analyzed T33 for allelic deletions of three markers at chromosome *19p13.3* (*D19S886*, *D19S883*, and *D19S878*). As the tumor revealed LOH at *D19S886* and *D19S878*, both alleles of the *STK11* gene appeared to have been inactivated.

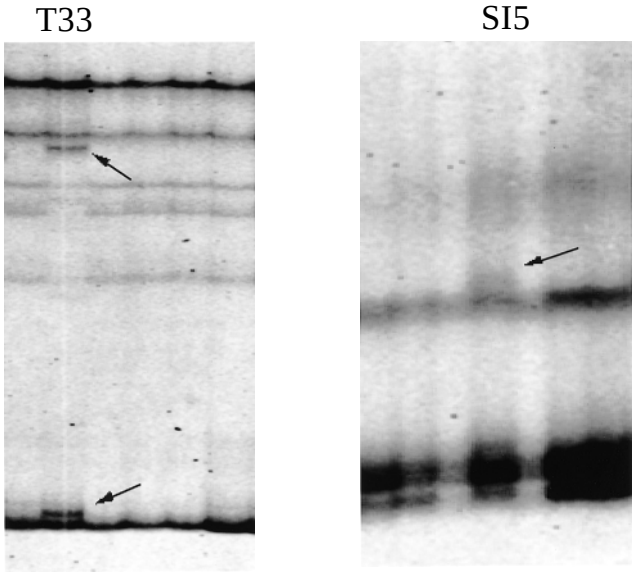

Fig. 1. SSCP analysis of the *STK11* gene. T33 and SI5 showed aberrant bands in exons 6 and 1, respectively.

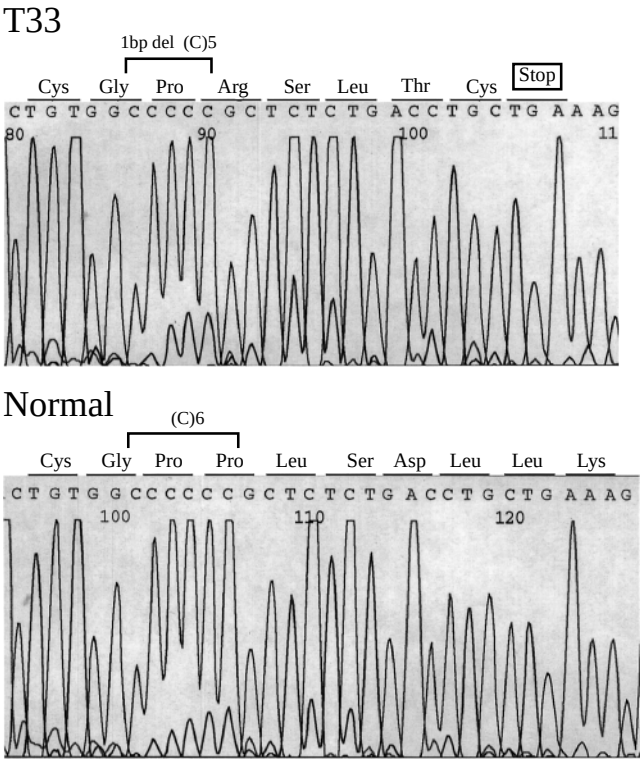

Fig. 2. Sequence analysis of the aberrant band detected in T33, revealing a 1-bp (C) deletion at codon 279–281. This region of *STK11* contains a six-cytosine repeat sequence in normal DNA but in T33, deletion of one of those cytosines leads to a stop codon downstream.

Tumor T33 originated in the ascending colon of a 58-year-old female with no family history or personal prior history of malignancies. Histological study of the tumor, classified as Dukes' B, revealed a moderately differentiated adenocarcinoma with no adenomatous or hamartomatous elements, and without any unique features.

DISCUSSION

In PJS, a hereditary polyposis syndrome, patients are at increased risk of gastrointestinal cancers and malignancies of multiple other organs.[3–5] Recently mutations of *STK11*, the gene encoding serine/threonine kinase 11, were shown to be responsible for PJS,[7, 8] but the function of this serine/threonine kinase remains unclear as regards tumor development. The hamartomatous polyps in gastrointestinal tracts of PJS patients are thought to have the potential for becoming adenomas or carcinomas,[9, 10] and many of them display LOH in the telomeric region of *19p13.3* where *STK11* is located.[6] Such observations imply that *STK11* is a tumor suppressor gene.

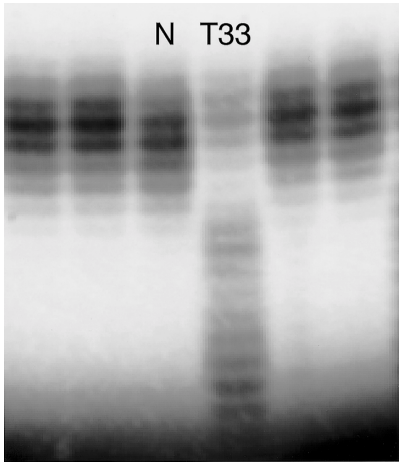

Fig. 3. Microsatellite instability indicated by mutation at the *BAT-26* locus in T33 colorectal cancer. This locus is 96% sensitive to replication errors, with 100% specific responsibility for microsatellite instability.[13, 14] N, normal tissue DNA matched to T33.

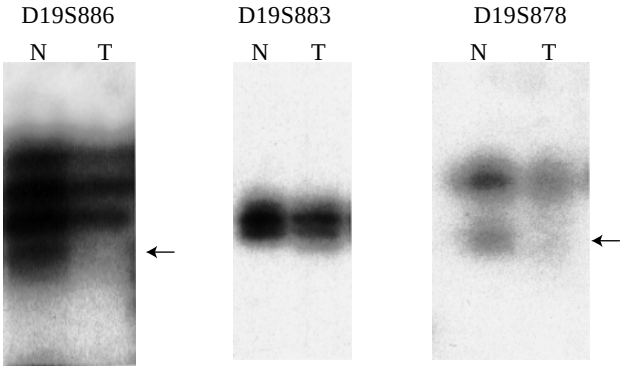

Fig. 4. Loss of heterozygosity for *STK11* locus, *19p13.3* telomeric markers *D19S886* and *D19S878* in tumor T33. *D19S883* was not informative. N, normal tissue DNA; T, tumor DNA.

However, several studies have indicated that somatic mutations of STK11 are rare in sporadic colorectal cancers,[15–17] gastric cancers,[18] breast cancers[19] and testicular tumors.[15] Only Dong *et al.* have reported frequent somatic mutations of *STK11* in left-sided colorectal tumors, including frameshift mutations involving codons 279–281.[20] These codons contain the six-cytosine mononucleotide-repeat sequence that appears to be a mutational hotspot in PJS patients.[12] Mononucleotide-repeat sequences are frequently targets of somatic frameshift mutations in colorectal cancers with microsatellite instability. However, we detected a frameshift mutation within codons 279–281 in only one among the 13 colorectal cancers in our panel that

showed instability by mutation at the *BAT-26* locus. This frameshift would cause truncation of the STK11 gene product, and probably abolish its function, because codons 279–281 lie within the catalytic-core domain of this serine/threonine kinase.[8] Furthermore, this colorectal tumor (T33) showed LOH at markers flanking *STK11* (*D19S886* and *D19S878*), indicating that both alleles of *STK11* had been inactivated.

In tumors with microsatellite instability, frameshift mutations preferentially accumulate in various oncogenes during tumor progression. The (A)10 of transforming growth factor-β receptor II (TGFβRII),[21] (G)8 of insulin-like growth factor II receptor (IGFIIR)[22] and (G)8 of the *BAX* gene[23] are frequent mutational targets of microsatellite instability. Since tumor T33 showed no frameshift mutations at any of these three locations (data not shown), we consider the *STK11* gene to be a novel target of microsatellite instability. As such, it may play an important role in development and progression of colorectal tumors having a microsatellite-mutator phenotype, although the frequency of this particular event appears to be very low.

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