STK11 Mutations in Peutz-Jeghers Syndrome and Sporadic Colon Cancer

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Abstract

A potential tumor suppressor gene, STK11, encoding a serine threonine kinase, has recently been identified on chromosome 19p13. Germ-line mutations of this gene have been found in patients with Peutz-Jeghers syndrome (PJS). To further investigate the relevance of STK11 mutations in PJS, we analyzed its coding sequence in nine patients and identified two deletions and three missense mutations. Because intestinal carcinomas have been observed to develop in association with PJS, we analyzed tumors from 71 patients for allelic deletions (loss of heterozygosity) and STK11 gene mutations, to elucidate the etiological role of STK11 gene in sporadic colorectal cancer. Loss of heterozygosity, evaluated using the microsatellite D19S886, was observed in 10 of 52 informative cases. No somatic mutations were detected except for a missense alteration in one tumor. Our data indicate the heterogeneity of PJS and the infrequent involvement of the STK11 gene in colorectal cancer.

Introduction

PJS is an autosomal dominant disease with variable expression and incomplete penetrance. The disorder is characterized by melanin spots in one or more sites, such as the lips, buccal mucosa, vulva, digits, and hamartomatous polyps of gastrointestinal tract, as well as susceptibility to a variety of tumors. Very recently, two groups isolated hamartomatous polyps of gastrointestinal tract, as well as susceptibility to a variety of tumors. Very recently, two groups (1,2) isolated the PJS gene, which was previously mapped to chromosome 19p13.3 (3). The gene, named STK11, encodes a serine threonine kinase and has been found in mutated form in 4 unrelated PJS patients and in several of the 13 PJS families studied thus far.

Several published reports have demonstrated that PJS predisposes carriers to cancers of the gastrointestinal tract and breast and other neoplasms (recently reviewed by Tomlinson and Houlston; Ref. 4). The observation of LOH in PJS polyps indicates that STK11 acts as a tumor suppressor gene. We cannot, therefore, exclude the possibility that mutations of the STK11 gene may play a role in the development of sporadic colorectal cancer.

Here, we aimed to further investigate the relevance of STK11 mutations in PJS and to elucidate the etiological role of STK11 gene mutations in sporadic colorectal cancer.

Materials and Methods

Patients. Among the group of polyposis carriers, nine patients were selected who had features consistent with PJS (intestinal hamartomatous polyps and typical mucocutaneous pigmentation). A family history was documented for patient F512, who had an affected sister, and patient F872, whose father presented freckling of the lips but no other clinical features of the disease. We also analyzed the only PJS family we have thus far collected; a previous investigation (data not shown) had revealed that their disease was unlinked to the markers located on chromosome 1p32-p34, the candidate site for the PJS gene proposed by Bali et al. (5), but was compatible with linkage to the marker D19S886 linked to the STK11 gene (3).

Freshly isolated lymphocytes or lymphoblastoid cell lines prepared from the patients were used as representative constitutional cells. In one patient, DNA was extracted from paraffin-embedded material.

Cytogenetic investigations performed in four patients (patients F222, F514, F557, and F872), including one member of the PJS family (patient F514), failed to reveal chromosome mutations.

DNA and RNA Preparation. High molecular weight genomic DNA was isolated according to the standard methods. Total RNA was purified using the High Pure RNA isolation kit (Boehringer). cDNA synthesis and PCR were performed in a one-step reaction with an optimized buffer and enzyme and without the need to add reagents between cDNA synthesis and PCR, thanks to the use of the Titan-one Tube reverse transcription-PCR system (Boehringer).

STK11 Mutation Analysis by SSCP and Sequencing. PCR amplification was performed using the series of oligonucleotide primers that amplify the coding region of the STK11 gene exon by exon, as reported by Jenne et al. (1). PCR was carried out as described previously (6) using denaturation cycles at 94°C for 5 min, followed by 35 annealing cycles for 30 s and extension at 72°C for 1 min. The annealing temperatures for each set of primers were: 60°C (exons 1 and 2), 63°C (exons 6, 7, and 8), 64°C (exons 3, 4, and 5), and 65°C (exon 9).

The PCR products were mixed with 95% formamide, 0.05% bromphenol blue, 0.05% xylene cyanole, and 0.02 M EDTA, denatured at 95°C for 5 min, and loaded onto precasted 122 mm x 110 mm x 0.5 mm GeneGel Excel (Pharmacia Biotech). Electrophoresis was conducted at 15 W and 25 mA at 12°C for 2 h. The gel bands were visualized by the silver staining method.

The PCR products were gel- and column-purified and directly sequenced. Sequence analysis was carried out using the Applied Biosystems Prism dye terminator cycle sequencing reaction ready kit (Perkin-Elmer Corp.). The products of cycle sequencing were electrophoresed on a 6% long range gel and analyzed on an Applied Biosystems model 377 automated DNA sequencer (Perkin-Elmer Corp.).

Tumor Tissue Samples and Allelic Deletion Analysis. Seventy-one frozen sporadic adenocarcinomas (41 classified as Dukes’ B stage, 19 classified as Dukes’ C stage, and 11 classified as Dukes’ D stage) were analyzed. Tumor tissue samples were obtained at surgery and freshly frozen. Gross cryostat dissection was limited to areas consisting mainly of neoplastic cells. Paried normal DNA was obtained from frozen normal colon or peripheral blood. All of the samples were screened for LOH using the polymorphic marker D19S886 mapped to chromosome 19p13.3 linked to the PJS locus (3). SSCP mutational analysis of all of the samples and DNA sequencing of the variant conformers were performed as in the PJS patients.

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3 The abbreviations used are: PJS, Peutz-Jeghers syndrome; LOH: loss of heterozygosity; SSCP, single strand conformation polymorphism.
Results and Discussion

In nine PJS patients, the entire coding region of the STK11 gene was amplified from germ-line DNA samples using nucleotide primer pairs flanking each exon. The PCR products were analyzed on SSCP gels, and those with altered mobility were sequenced. To optimize the detection of mutations, we also sequenced the entire cDNA prepared from lymphblastoid cell lines of four PJS patients. Mutations were found in four patients (Table 1). Sequence analysis of the variant band (Fig. 1A) observed in patient PJF263 showed a 4-bp deletion in exon 4, leading to a reading frameshift and a stop at codon 286 that dramatically disrupted the catalytic domain of the truncated protein.

One patient (patient PJF512) presented a 6-bp deletion in exon 4 (Fig. 1B) that caused a nontruncating change; the same mutation was found in her PJS affected brother. In two other PJS patients (patients PJ1 and PJG42), two missense mutations were identified that are predicted to cause amino acid substitution within the conserved catalytic domain at codons 251 and 304, respectively. Although these were the only changes detected in the entire coding sequence of the STK11 gene in the four patients, it remains to be demonstrated whether they are responsible for the PJS phenotype or are merely a simple polymorphism. These DNA variants were not observed in 91 controls (182 chromosomes) from the general population, but at present, the possibility that we are dealing with rare polymorphisms cannot be ruled out. However, a nontruncating deletion of 9 bp at codons 303-306 (exon 7) has also been reported by Hemminki et al. (2) in a PJS patient. In five PJS patients the search for mutations was unsuccessful, although in three of them, sequencing of each of the nine exons and their flanking region, as well as of the entire cDNA, was performed. In addition, in polypl from patient PJF222 and patient PJ2 no allele loss at D19S886 was observed. The paucity of germ-line mutations in this series of patients may depend on the fact that analysis of the coding sequence of the gene was not exhaustive or even on the occurrence of mutations in regulatory regions or on the transcriptional silencing (via methylation) of one allele. The existence of large genomic deletions involving the STK11 locus can be excluded, as the five patients were found to be heterozygous for intronic polymorphisms. Misdiagnosis of the syndrome seems likewise unlikely, due to the accurate selection of all of the patients performed by specialist clinicians. A plausible explanation for the failure to observe mutations in PJS patients may be the genetic heterogeneity of the PJS, as suggested by two linkage analysis studies (7, 8) performed in a large number of PJS families.

To elucidate the etiological role of the STK11 gene in sporadic colorectal cancer, we screened a series of 71 colorectal adenocarcinomas for LOH and mutational analysis.

Ten (19.2%) of the 52 informative sporadic colorectal neoplasms, characterized with the D19S886 polymorphic marker, showed deletions in the area of the STK11 gene. No second-hit mutations were found in the coding region and splicing junctions of the gene in any of the carriers of deletions. Sequencing of all variant bands identified by SSCP analysis performed on the 71 tumors and normal DNA paired samples, enabled identification of a somatic mutation, a C→A transversion predicting a substitution of a proline for a histidine, in only one tumor (T32).

Our data confirm the lack of somatic mutations of the STK11 gene recently reported in sporadic colorectal cancer, as well as in breast and testicular tumors (9, 10).

The lack of somatic mutations of the STK11 gene in sporadic colorectal cancers is not surprising because increasing evidence shows that some hereditary forms of colorectal cancer may develop through genetic changes different from those occurring in the sporadic forms (11).

On the other hand, the germ-line STK11 mutations, responsible for PJS, are more often associated with benign rather malignant colorectal neoplasms, and in general, the cardinal lesions in PJS are benign, with only subsets of patients developing malignancies (4).

Finally, it is possible that the growth advantage conferred on the colorectal cells by mutations occurring in the STK11 gene is negligible; thereafter, the development of the tumors is predominantly determined by mutations of other genes that have not yet been identified.

The sequencing of many SSCP band variants revealed the presence of several benign polymorphisms of the STK11 gene, i.e., an amino acid substitution, K48N in exon 1, and six silent nucleotide substitutions in introns 2, 5, 7, and 8 (Table 2).

These results indicate the heterogeneity of PJS and the lack of any apparent contribution of the STK11 gene to the development of sporadic colorectal cancer.

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References

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