Frequent Somatic Mutations in Serine/Threonine Kinase 11/Peutz-Jeghers Syndrome Gene in Left-sided Colon Cancer

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Abstract

We analyzed somatic mutation and loss of heterozygosity (LOH) in the serine/threonine kinase 11 (STK11)/Peutz-Jeghers syndrome gene in 49 colorectal tumors in three different stages of a dysplasia-carcinoma sequence. We detected LOH in 10 of 19 (52.6%) informative colorectal cancers at loci D19S886 and/or D19S883, but no LOH was observed in 25 informative adenomas. We detected a total of 9 somatic mutations [7 of 13 (53.8%) left-sided colon cancers and 2 of 7 (28.6%) left-sided adenomas with high-grade dysplasia], but no mutations were detected in right-sided colon tumors. Of the nine mutations, one was a frameshift mutation (the same mutation detected in Peutz-Jeghers syndrome family previously), and the other eight were missense mutations. This results indicate that STK11 is a tumor suppressor gene and that genetic changes of STK11 play an important role in left-sided colon cancer carcinogenesis.

Introduction

PJS is a rare autosomal dominant disorder with varying degrees of penetrance, characterized by gastrointestinal hamartomatous polyps and mucocutaneous melanin pigmentation; these polyps are thought to be nonmalignant disturbances of superfluous tissue (1, 2). Such patients are, however, at an increased risk of developing both gastrointestinal and nongastrointestinal cancers. Giardiello et al. (3) estimated that these patients have an 18-fold higher risk of malignancy than the general population. Recently, the PJS gene, STK11, encoding a novel serine/threonine kinase and residing on chromosome 19p13.3 at a distance of 190 kb proximal to D19S886, was identified. Numerous germ-line mutations were detected in individuals affected by PJS (4-7). These findings suggest that genetic changes of STK11 might also be associated with the development of a sporadic form of colorectal cancer.

Colorectal tumors provide an excellent opportunity to study tumor progression because most carcinomas appear to arise from adenomas, and tumors at various stages in the development of the adenoma-carcinoma sequence can be easily obtained for analysis (8). Therefore, we collected colorectal tumors in three different stages: adenomas with low-grade dysplasia (defined as mild and moderate dysplasia), adenomas with high-grade dysplasia (defined as severe dysplasia or carcinoma in situ), and invasive cancers, characterized according to the guidelines of the National Polyp Study Group (9).

Here, we performed a PCR-based LOH and mutation analysis of the STK11 gene in a series of 49 colorectal tumors in three different stages of the dysplasia-carcinoma sequence to determine whether STK11 genetic alterations could be involved in colorectal tumor development and, if so, determine to which stage it is linked.

Materials and Methods

Materials. Paraffin-embedded histological sections of 26 colorectal adenomas (14 low-grade dysplasias, 6 left-sided and 8 right-sided; 12 high-grade dysplasias, 7 left-sided and 5 right-sided) and 23 invasive colorectal cancers (13 left-sided and 10 right-sided) were obtained from the Catholic University Medical College-affiliated hospital (Seoul, Korea). The term "invasive cancer" means, strictly, a cancer that has invaded beyond the muscularis mucosa. The line of demarcation between the right and left colon has been defined by the embryonic division line at the junction of the proximal two-thirds and the distal one-third of the transverse colon. None of the patients had a family history of PJS, familial adenomatous polyposis, or HNPCC.

Microdissection. Tumor cells were selectively procured from H&E-stained slides using a 30G12 hypodermic needle (Becton Dickinson, Franklin Lakes, NJ) affixed to a micromanipulator, as described previously (10, 11). We also obtained inflammatory cells or normal mucosal epithelium for corresponding normal DNA from the same slides in all cases.

DNA Extraction. DNA extraction was performed by a modified single-step DNA extraction method, as described previously (10-12).

LOH Analysis. Tumor and corresponding normal DNA from each slide were amplified by thermal cycler (MJ Research Institute, Watertown, MA) with three microsatellite markers (Research Genetics, Huntsville, AL), D19S886, D19S883, and D19S555, in the 19p13.3 region. Each PCR was generally performed under standard conditions in a 10 µl of reaction mixture containing 1 µl of template DNA, 0.4 µM each primer, 125 µM each dNTP, 1.5 mM MgCl₂, 0.4 units of Taq polymerase, 0.5 mM [³²P]dCTP (Amersham, Buckinghamshire, United Kingdom), and 1 µl of 10X buffer. The reaction mixture was denatured for 5 min at 95°C and incubated for 35 cycles (denaturing at 95°C for 30 s, annealing at 57°C for 90 s, and extending at 72°C for 90 s), with some variations in the annealing temperature. Final extension was continued for 10 min. Reaction products (2 µl) were then denatured and electrophoresed in 6% polyacrylamide gel containing 7 M urea. After electrophoresis, the gels were transferred to 3-MM Whatman paper, dried, and subjected to autoradiography using Kodak-OMAT film (Eastman Kodak, Rochester, NY).

SSCP Analysis and DNA Sequencing. Twelve sets of Primers (Table 1) covering nine exons of STK11 gene were designed by using the OLG0 software program (Version 5.0; National Bioscience Inc., Plymouth, MN) according to the genomic sequence of STK11, which was obtained from GenBank accession nos. AF023984, AF032985, and AF032986. PCR amplifications were performed under exactly the same conditions as described above, with the exception of the annealing temperature (Table 1). The amplified DNA was mixed with an equal volume of formamide loading dye (95% formamide, 20 mM EDTA, 0.05% bromophenol blue, and 0.05% xylene cya...
Table 1 Primers for amplification and sequencing of STK11

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
<th>Fragment size (bp)</th>
<th>Annealing temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>STK1A-F</td>
<td>5’-CTACGGGCTGGGCGGCAGGACT-3’</td>
<td>163</td>
<td>64</td>
</tr>
<tr>
<td>STK1A-R</td>
<td>5’-CTCGGCCGCCGCTGCTGGAAG-3’</td>
<td>150</td>
<td>60</td>
</tr>
<tr>
<td>STK1B-F</td>
<td>5’-ACCTCTCCCAGACGCTGAGAC-3’</td>
<td>140</td>
<td>60</td>
</tr>
<tr>
<td>STK1B-R</td>
<td>5’-CTACGGGCTGGGCGGCAGGACT-3’</td>
<td>151</td>
<td>58</td>
</tr>
<tr>
<td>STK1C-F</td>
<td>5’-GCCCTCGGGACCTGCACTGAT-3’</td>
<td>173</td>
<td>66</td>
</tr>
<tr>
<td>STK1C-R</td>
<td>5’-GCCCTCGGGACCTGCACTGAT-3’</td>
<td>218</td>
<td>64</td>
</tr>
<tr>
<td>STK1K-F</td>
<td>5’-CTACGGGCTGGGCGGCAGGACT-3’</td>
<td>231</td>
<td>64</td>
</tr>
<tr>
<td>STK1K-R</td>
<td>5’-CTACGGGCTGGGCGGCAGGACT-3’</td>
<td>240</td>
<td>64</td>
</tr>
<tr>
<td>STK19A-F</td>
<td>5’-CTACGGGCTGGGCGGCAGGACT-3’</td>
<td>204</td>
<td>64</td>
</tr>
<tr>
<td>STK19A-R</td>
<td>5’-CTACGGGCTGGGCGGCAGGACT-3’</td>
<td>195</td>
<td>64</td>
</tr>
</tbody>
</table>

Results

LOH of 19p13.3. The fixed marker order and composite map information for three marker loci were obtained from the genetic location database (http://cedar.genetics.soton.ac.uk/public_html) at the University of Southampton (Southampton, United Kingdom; Ref. 13).

Patients who were heterozygous for a given marker were considered informative. Twenty-five of 26 cases of adenomas and all 23 cases of invasive carcinomas were informative for at least one of the markers studied, and the results are summarized in Table 2. We observed no LOH at all in 25 informative adenomas with low- or high-grade dysplasia. In invasive carcinomas, 9 of 17 (52.9%), 6 of 15 (40%), and 6 of 22 (27.3%) informative cases showed allelic loss at the markers D19S886, D19S883, and D19S565, respectively. The autoradiograms of two selected cases showing LOH are displayed in Fig. 1. Six cases, including cases 10 and 16, both of which had one noninformative maker, showed allelic loss at all three markers (Fig. 1b). Three cases (cases 8, 14, and 19) revealed allelic loss at only one marker, D19S886 (Fig. 1a); this single region showed allelic loss in all left-sided colon cancers with STK11 mutations, except in cases 20 and 23. Case 20 revealed loss of heterozygosity at D19S886 but showed allelic loss at D19S883 and D19S565. The location of the STK11 gene relative to these markers is between D19S886 and D19S565. The composite map distance between the two markers D19S886 and D19S565 is only 0.54 Mb, and STK11 resides at a distance of 0.19 Mb proximal to D19S886, spanning over 23 kb. Therefore, we concluded that case 20 might also contain one allele deletion of the STK11 gene. There was no significant difference in allelic loss frequencies between the left-sided and right-sided colon cancers (Fisher’s exact test, P = 1.000).

Mutation Analysis of the STK11 Gene. We detected 9 somatic mutations of the STK11 gene in 26 adenomas and 23 invasive carcinomas, as summarized in Table 2, and two representative cases with aberrant bands and mutations are shown in Fig. 2. No adenomas with low-grade dysplasia had mutations of the STK11 gene (0 of 14), whereas 2 of 12 (16.7%) adenomas with high-grade dysplasia and 7 of 23 (30.4%) invasive carcinomas had mutations. There was a signific-

Table 2 STK11 mutations and LOH in histopathological subtypes of colorectal tumor

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Site</th>
<th>Histological type</th>
<th>LOH</th>
<th>STK11 mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cecum</td>
<td>Mucinous</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Cecum</td>
<td>WD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Cecum</td>
<td>PD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Cecum</td>
<td>Mucinous</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Cecum</td>
<td>WD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Ascending</td>
<td>MD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Ascending</td>
<td>MD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Ascending</td>
<td>MD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Ascending</td>
<td>MD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>STK10</td>
<td>Transverse (proximal two-thirds)</td>
<td>MD</td>
<td></td>
</tr>
</tbody>
</table>

* MD, moderately differentiated; WD, well differentiated; PD, poorly differentiated; DEL, deletion; FS, frameshift; □, retention of heterozygosity; ■, LOH; □, noninformative; *, CpG island.
compared to normal control tissue. Transition at nucleotide 595 in tumor tissue as position 286. In case 19 (a), there was a G —¿* A reading frameshift and a premature stop codon at nucleotide 842 (C) in exon 6, which led to a

performed using DNA eluted from each of ab bands, c and d. Cyclic sequencing analyses were

analysis of DNA from tumors (Lanes T) and normal samples (Lanes N) from cases 15 (a) and 19 (b). Arrowheads, abnormal electrophoresis bands. c and d, cyclic sequencing analyses were performed using DNA eluted from each of abnormal bands. Case 15 (c) revealed a deletion of nucleotide 842 (C) in exon 6, which led to a reading frameshift and a premature stop codon (TGA) at position 286. The other eight mutations were missense mutations (Fig. 2, b and d). Of these missense mutations, seven mutations were of the C:G —¿» T:A transitional type, and of these, six were located at the dipyrimidine sequences and five were located at the CpG site.

Discussion

Colorectal cancer occurs both as a hereditary disorder and as a sporadic case. Familial disorders that cause susceptibility to colorectal cancer include familial adenomatous polyposis and HNPCC (14). PJS is another autosomal dominant disorder and is also associated with an increased risk of gastrointestinal carcinomas (3). Recently, the gene responsible for PJS was identified as STK11, which encodes a novel serine/threonine kinase of 433 amino acids (6, 7). Because most colorectal carcinomas appear to arise from adenomas, studies of different stages of colorectal neoplasia may shed light on the genetic alterations involved in tumor progression (9, 15). These facts led us to examine the genetic alterations of STK11 gene in a sporadic form of colorectal tumors undergoing three different stages of a dysplasia-carcinoma sequence: adenoma with low-grade dysplasia, adenoma with high-grade dysplasia, and invasive carcinoma.

We have analyzed allelic deletion in 23 colorectal cancers, most frequently at loci D19S886 (52.9%) and D19S883 (40%) and less commonly at D19S565 (27.3%). But we observed no LOH at all in 25 informative adenomas. STK11 resided at a distance 0.19 Mb proximal to D19S886. The composite map distance between D19S886 and proximal marker D19S883 is only 0.54 Mb (11, 13). Therefore, we suspect that one allele of the STK11 gene must also have been deleted in case 20, despite retention of heterozygosity at D19S886. Because these two markers lie in close proximity to the STK11 gene, these two markers were beneficial for analyzing allelic loss of this gene. When we compared the frequencies of LOH by tumor site in invasive cancers, there was no significant difference between left- and right-sided colon cancer (Fisher’s exact test, P = 1.000).

In observing the dysplasia-carcinoma sequence in its tumor development stages, mutations in STK11 were first detected in adenomas with high-grade dysplasia at low frequency (16.7%), but they were detected in invasive carcinomas with increasing frequency (30.4%). However, no mutation was detected in 14 adenomas with low-grade dysplasia. Because the mutations were first detected in adenoma with high-grade dysplasia, STK11 might be involved in tumor promotion and/or progression rather than initiation in the process of tumorigenesis. Interestingly, all nine mutations were detected exclusively in left-sided colon tumors, including two adenoma cases. Therefore, calculating the mutation rate values of STK11 obtained from left-sided colon tumors results in a cancer mutation rate of 53.8% (7 of 13) and an adenoma with high-grade dysplasia rate of 28.6% (2 of 7). With the exception of case 23, all seven left-sided colon cancers with mutation have allelic loss at D19S886 and/or D19S883; unfortunately, the marker at D19S886 was noninformative in case 23. These results strongly support the notion of earlier work showing that the STK11 is a tumor suppressor gene (6, 7) and also suggest that the genetic changes of both alleles of STK11 play an important role in the
conversion of high-grade dysplasia into invasive carcinoma, especially in the carcinogenesis of left-sided colon cancer. The large majority of mutations in STK11 detected in PJS families are frameshift mutations that result in the truncation of the protein (6, 7); this also strongly supports the suggestion that STK11 is a tumor suppressor gene. Here, we found one frameshift mutation. Case 15 carried a also strongly supports the suggestion that STK11 is a tumor suppressor gene. Here, we found one frameshift mutation. Case 15 carried a

Same nucleotide 842 (C) was substituted by a T in case gene. Here, we found one frameshift mutation. Case 15 carried a also strongly supports the suggestion that STK11 is a tumor suppressor gene. Here, we found one frameshift mutation. Case 15 carried a

Even with a small number of cases, because we frequently observed genetic changes in both alleles of STK11 gene in left-sided colon cancer, we concluded that the STK11 gene is a tumor suppressor gene and that genetic alterations of STK11 play an important role in tumor promotion and progression in left-sided colon cancer carcinogenesis. However, further studies on a large patient population will be important to verify these initial observations, and identification of the biological function of STK11 will certainly broaden our understanding of the pathogenesis of not only hereditary PJS but also of relevant sporadic forms of cancer.

References


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