Coexistence of Somatic and Germ-Line Mutations of APC Gene in Desmoid Tumors from Patients with Familial Adenomatous Polyposis

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Abstract

Desmoid tumors, which are locally invasive with recurrence but without metastasis, are frequently observed in patients with familial adenomatous polyposis after abdominal surgery or during pregnancy. This study analyzed mutation of the adenomatous polyposis coli gene in 8 desmoid tumors from 7 familial adenomatous polyposis patients using polymerase chain reaction-single-strand conformation polymorphism and the direct sequencing method. Seven somatic mutations, 1 somatic allele loss, and 6 germ-line mutations were detected. The majority of adenomatous polyposis coli gene mutations were deletions of 1 to 19 base pairs in exon 15, and all mutations led to the formation of stop codons. A somatic mutation with repair replication. No mutation was detected in exons 1 to 2 of H-ras, K-ras, and N-ras genes and in exons 5 to 8 of p53 gene, in these tumors. The simultaneous existence of somatic and germ-line alterations of adenomatous polyposis coli gene observed in all 8 tumors strongly suggests that inactivation of both alleles of adenomatous polyposis coli gene is involved in the development of desmoid tumors.

Introduction

Although FAP is characterized by numerous colorectal adenomas, it is also recognized as a growth disorder affecting the whole body (1). Extracolonic manifestations include gastric and duodenal polyps, desmoid tumors, osteoma, thyroid tumors, adenocortical tumors, brain tumors, retinal pigmentation, and tumors in other organs. Some of these benign tumors become malignant if left untreated. A high incidence of desmoid tumors has been observed in FAP patients, as high as 8-12% (2), most of which arise during the 2 years following abdominal surgery. These desmoids grow to a very large size and often recur many times but without metastasis. Desmoid tumors are indicated in Table 1.

Materials and Methods

Desmoid Tumors. Eight desmoid tumors were obtained from 7 FAP patients and histopathologically diagnosed as desmoid. Clinical data of individual tumors are indicated in Table 1.

PCR-SSCP Analysis. DNA was extracted from each desmoid tumor and the corresponding normal tissue or peripheral blood using pronase K, sodium dodecyl sulfate, and phenol-chloroform. DNA samples were amplified for SSCP analysis of APC gene using PCR (5 min at 97°C, 1 time; 1 min at 95°C, 1 min at 58°C or 60°C, and 1 min at 72°C for 35 or 50 cycles; and 10 min at 72°C, 1 time). The reaction mixture (5 μl) contained 200 ng of genomic DNA, the proper pair of each 0.2 μM primer, 25 μM each deoxynucleoside triphosphate, 1× PCR buffer, Taq polymerase (Perkin-Elmer Cetus, Norwalk, CT), and [α-32P]dTTP. Oligodeoxynucleotide primers used to amplify the 15 coding exons of APC gene, including 76 primers for 38 regions, were the same as those reported (8). Primers to amplify the exons 5 to 8 of p53 gene and conditions for PCR were those previously reported (12). Primers used to amplify exons 1 and 2 of H-ras, K-ras, and N-ras genes were from Takara Biochemicals (Kyoto). PCR products were diluted 10-fold with formamide-dye solution (95% formamide, 20 mM EDTA, 0.05% bromophenol blue, and 0.05% xylene cyanol), and a 2-μl sample of the diluted reaction mixture was heated for 5 min at 80°C followed by electrophoresis in 5% polyacrylamide gel containing 5% glycerol, as described (13). After electrophoresis at 20–25°C, gel was exposed to x-ray film at −70°C. PCR was performed at least twice for each sample and only the reproducible cases were taken.

Sequencing of the Mutated Strand. Abnormal single-stranded DNA fragments were extracted with distilled water from the corresponding bands on PCR-SSCP gels as described (14). The DNA fragments were amplified through the asymmetrical PCR (15) in 100 μl of mixture under the same conditions as those for PCR-SSCP analysis, with the exception that the ratio of primers was 100/1 or 1/100 for sense and antisense primers. The amplified DNA samples were purified using QIAGEN spin 20 column (Qiagen Inc., Chatsworth, CA) and sequenced with the dyeoxy chain-termination reaction using Sequenase Version 2.0 (United States Biochemical Co., Cleveland, OH). Primers used for sequencing were the same as those in PCR-SSCP. Sequencing was performed more than twice for each DNA fragment.

Results

Clinical data regarding the 8 desmoid tumors of the present study are shown in Table 1. Five desmoids were in abdominal wall and 3 were in the mesentery, 5 of which developed 6 to 36 months after abdominal surgery. One desmoid was newly formed 10 months after surgical removal of the first desmoid. The size of 5 tumors was 2–10 cm in maximum diameter, and 3 tumors had grown to weigh as much as ~1700 g. Recurrence was observed in 4 cases, although none of these desmoids was tumorigenic in nude mice.

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2 To whom requests for reprints should be addressed.

3 The abbreviations used are: FAP, familial adenomatous polyposis; APC, adenomatous polyposis coli; LOH, loss of heterozygosity; PCR, polymerase chain reaction; SSCP, single-strand conformation polymorphism.
DNA fragments in the abnormal bands were reamplified by asymmetrical PCR and then sequenced, and an example of the sequencing is shown in Fig. 1C.

Six germ-line mutations and 7 somatic mutations were determined in the regions that included exons 15-C, 15-E, 15-G, 15-H, and 15-I of APC gene, as listed in Table 2. Three germ-line mutations existed close together at codons 1061, 1105, and 1110 in 15-E in PLK59, PLK56, and PLK111, where somatic mutation was not detected. Both germ-line mutations at 1061 and 1105 led to the formation of a stop codon (TAA) at 1125–1126. The 5-base deletion (ACAAA) at codon 1062–1063 has been frequently observed as a germ-line mutation in FAP families (16, 17), but the germ-line mutation of PLK59 was a 4-base deletion (AACAA). Germ-line mutation in PLK126 was A to T transversion at 848, and that in PLK111 was C to G transversion at 1110. A germ-line mutation of a 5-base deletion at codon 1309–1311 of 15-G in PLK124 led to a stop codon at 1313–1314 (TAG).

Around the codons from 1452 to 1470 of 15-H, six mutations were detected: one germ-line mutation in PLK42; and five somatic mutations in desmoid tumors from patients PLK56, PLK124, PLK126, and PLK150. Each case had a different pattern of deletion that included 1–19-base deletions, but 5 of the 6 different mutations produced the same stop codon (TAA) at downstream codon 1472–1473. PLK150-Desmoid and PLK150-Desmoid-R from the same patient had different somatic mutations at codons 1458 and 1470, respectively. The mutant bands observed in PLK150-Desmoid were not detected in PLK150-Desmoid-R in the PCR-SSCP analysis, which indicated that these two desmoids had originated from different cells. The somatic mutation in PLK59-Desmoid, occurring at codon 1581–1584 with an 8-base deletion (TGCCATGC), produced a stop codon (TAA) at 1589–1590.

The somatic mutation in the desmoid tumor from PLK111 was found to include the repetition of 82 base pairs from codon 1399 to 1426 in exon 15-H. Such an insertion of a long sequence exhibited abnormal bands that moved much slower than normal bands in PCR-SSCP (Fig. 1B). The insertion of the 82 base pairs between the first A and the second G of codon 1426 caused the formation of a stop codon (TGA) at 1407–1408 in the inserted sequence, as indicated in the lower part of Table 2. Such a type of somatic mutation has not previously been reported in the APC gene. The slowly electrophoresed abnormal bands were also detected in this tumor when another PCR primer set was used (data not shown).

The 8 desmoids analyzed had both somatic and germ-line alterations of the APC gene, although germ-line mutations in a FAP patient PLK150 has not yet been determined. None of the 8 desmoids had more than 2 mutations within exons 1 to 15 of the APC gene.

Since K-ras gene and p53 gene mutations have frequently been detected in colorectal tumors from FAP patients (7, 12), examination of mutations of exons 1 and 2 of H-ras, K-ras, and N-ras genes and of exons 5 to 8 of p53 gene, in the desmoid tumors, was done using PCR-SSCP method. However, no mutation was detected for these genes in any of the 8 desmoid tumors.

Discussion

All of the 8 desmoid tumors analyzed in this study had somatic alterations in APC gene: 7 had mutations and 1 had loss of normal allele, in addition to the germ-line mutations. Also all mutations led to alterations in APC gene: 7 had mutations and 1 had loss of normal allele, in addition to the germ-line mutations. The simultaneous existence of two genetic alterations in individual desmoids suggests that both alleles of APC gene were inactivated in these tumors, although it is not yet fully confirmed that the mutations...
SOMATIC AND GERM-LINE APC MUTATIONS IN DESMOIDS

Fig. 1. PCR-SSCP and direct sequencing of APC gene in DNAs of desmoid tumors and normal tissues from FAP patients. Specific sequences of APC gene were amplified from genomic DNAs from desmoid tumors and normal tissues using PCR, and denatured PCR products were separated electrophoretically followed by the direct sequencing as described in “Materials and Methods.” A, PCR-SSCP patterns in exon 15-E. B, PCR-SSCP patterns in exon 15-H. D, desmoid; N, normal tissue; number is individual FAP patient; Arrows, normal bands. C, sequencing of DNA fragments eluted from the normal and abnormal PCR-SSCP bands in exon 15-H. In A, germ-line mutations are seen in both desmoid and normal tissues from PLK56, PLK59, and PLK111. In B, germ-line mutation is seen in both desmoid and normal tissue of PLK42, and somatic mutations are seen in desmoids from PLK56, PLK111, PLK124, and in PLK150-desmoid.

exist on both alleles. In PLK42-Desmoid, it was clear that somatic allele loss and germ-line mutation existed in the two different APC alleles.

The cause of the production of somatic mutations is still unclear, but it can be assumed from the nature of mutations. The current study observed that almost all somatic and germ-line mutations in desmoid tumors are of the same nature, in that the deletions occur between or within areas of repeated sequences of exon 15, except for two germ-line mutations with one base change. In the region from 1458 to 1465, four deletions were detected as follows: 2-base deletion at 1461–1462 CCTACTGCTGAA(AA)AGAGAGAG; 4-base deletion at 1462–1465 CCTACTGCTGAAAGAG(AGAG)AG; 10-base deletion at 1458–1461 CC(TACTGCTGAA)AGAGAGAGAG; and 19-base deletion at 1458–1464 CC(TACTGCTGAAAGAGAGAGAG)AG.

Somatic mutation of a 1-base deletion at 1470 GC(T)GC, somatic mutation of an 8-base deletion at 1581–1584 ATTATTTTC(GGCCATGC)CAACAA, and germ-line mutations of a 1-base deletion at 1105–1106 GG(G)G; a 4-base deletion at 1061–1063 AA(AACA)AA, and a 5-base deletion at 1309–1311 AAAAG( AAAAG)TTGGAA, were also between repeated sequences. The 82-base repeat from codon 1399 to 1426 of the APC sequence in PLK111-Desmoid strongly suggests that this novel genetic alteration occurred due to a DNA replication error. These somatic mutations occurring in desmoid tumors appear to be caused by misalignment of the DNA strands during replication in the proliferating cells after abdominal surgery or by repair replication after incision of DNA in musculoaponeurotic soft tissue. It should be noted that the second desmoid tumor PLK150-Desmoid-R, formed 10 months after surgical removal of the first tumor 150-Desmoid, had a somatic mutation which was different from the mutation in the first desmoid. This fact suggests that the somatic mutation of the APC gene in the second desmoid newly occurred after removal of the first desmoid.

In contrast to such a predominance of microdeletions in the APC gene in desmoids, point mutations, which appeared to be mainly caused by mutagens, were dominant in the p53 gene in colorectal tumors from FAP patients, as has been previously described (12). This difference in the characteristic of mutation suggests a difference in the contribution of environmental mutagens or carcinogens between APC mutation and p53 mutation. It can be presumed that the major cause of APC mutation is replication error and that of p53 mutation is DNA lesion by mutagens.

Although there are many repeated sequences in the APC gene, the region where mutations are frequently detected appears to be not only in the most easily mutable sequence, but also in the important regions with regard to the development of tumors or loss of function of APC protein. The region from codon 1452 to 1470, which frequently experiences somatic mutation in desmoid tumors, falls within the region where somatic mutations have frequently been detected in colorectal tumors (19, 20). Mutation at codon 1309–1311 has been the most frequent germ-line mutation in FAP patients previously described (16, 17, 18), and we also detected the same mutation in PLK124 and another 15 independent FAP families.4 The mutation at codon 1062 has been assumed to be the second most frequent germ-line mutation (16, 17), and around this codon three germ-line mutations were also observed in the present study.

The absence of mutations in H-ras, K-ras, and N-ras genes and in p53 gene in these tumors suggests that inactivation of the APC gene is significant for induction of the aggressive growth of desmoid tumors of frequent recurrence. This fact appears to be an important clue to the molecular mechanisms of desmoid formation. Desmoid tumors are defined as benign fibrous tumors which do not metastasize, and it is reasonable to assume that p53 mutation is absent, since p53 mutation is usually involved in the change from benign to malignant nature, in colorectal tumors (12). Furthermore, it has been assumed that K-ras mutation contributes to rapid growth of both benign and malignant colorectal tumors because of the greater frequency of K-ras mutation in large polyps in FAP patients (7). However, despite the lack of any ras mutation, some desmoid tumors grow very rapidly. It is necessary to determine if inactivation of the APC gene is the only

4 Unpublished data.
cause of such aggressive growth. Moreover, desmoid tumors contain a significant amount of fibrous components, and the mechanism of the increased production of fiber or collagen should also be examined.

The present finding of two-hit inactivation of the APC gene in desmoid tumors is significant for understanding the mechanism of extracolonic manifestations and also for understanding the function of the APC gene as a tumor suppressor.

References


Table 2 Germ-line and somatic mutations of the APC gene in desmoid tumors from FAP patients

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Codon</th>
<th>Mutation</th>
<th>Somatic mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLK42-Desmoid</td>
<td>1462–1465</td>
<td>(AGAG) del</td>
<td>Loss of normal allele H</td>
</tr>
<tr>
<td>PLK56-Desmoid</td>
<td>1105–1106</td>
<td>(G) del</td>
<td>1461–1462</td>
</tr>
<tr>
<td>PLK59-Desmoid</td>
<td>1061–1063</td>
<td>(AACA) del</td>
<td>1581–1584</td>
</tr>
<tr>
<td>PLK111-Desmoid</td>
<td>1110</td>
<td>T(CA) → (TGA) del</td>
<td>1399–1426</td>
</tr>
<tr>
<td>PLK124-Desmoid</td>
<td>1309–1311</td>
<td>(AAAGA) del</td>
<td>1452</td>
</tr>
<tr>
<td>PLK126-Desmoid</td>
<td>848</td>
<td>(AAATA) → (TAA) del</td>
<td>1458–1561</td>
</tr>
<tr>
<td>PLK150-Desmoid-R</td>
<td>1458–1464</td>
<td>(TACTCTGAAAAGAGAGAG) del</td>
<td>1470</td>
</tr>
</tbody>
</table>

* All mutations led to stop codons.
* The loss was detected both in primary and recurring desmoid.
* Sequence of somatic mutation, which includes the 82-base pair repeat from codon 1399 to 1426, in PLK111-Desmoid is as follows:

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(CTG TCG ATT GCC AGC TTC GTT CAG AGT GAA TG A ATG GTA AGT GGC ATT ATA AGC CCC AGT GAT CTT CCA GAT A)GC
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