Frequent Somatic Mutations of the \textit{APC} Gene in Human Pancreatic Cancer

Akira Horii, Shuichi Nakatsuru, Yasuo Miyoshi, Shigetoshi Ichii, Hiroki Nagase, Hiroshi Ando, Akio Yanagisawa, Eiju Tsuchiya, Yo Kato, and Yusuke Nakamura


Abstract

The \textit{APC} (adenomatous polyposis coli) gene is responsible for familial adenomatous polyposis and is also associated with the development of sporadic tumors of the colon and stomach. To investigate whether or not mutations of \textit{APC} play any role in tumors arising in other organs, we examined somatic mutations of this gene in sporadic (nonfamilial) renal cell carcinomas, hepatocellular carcinomas, and cancers of the lung and pancreas. DNAs isolated from tumors were examined by means of a RNase protection analysis, coupled with the polymerase chain reaction followed by DNA sequencing of the polymerase chain reaction products. By screening a part of the \textit{APC} coding region, we detected somatic mutations in four of ten pancreatic cancers; each of these mutations would yield a truncated \textit{APC} product due to a 1- or 5-base pair deletion. These results imply that mutations in \textit{APC} contribute to carcinogenesis in the pancreas.

Introduction

The gene responsible for familial adenomatous polyposis, termed \textit{APC} \textsuperscript{3} (1–4), was isolated in 1991. \textit{APC} is considered to be a tumor suppressor gene for the following reasons: (a) LOH at the \textit{APC} locus has been observed frequently in colorectal and gastric tumors (5, 6); and (b) somatic mutations of \textit{APC} have been identified in sporadic forms of these tumors (2, 7, 8). Although the somatic mutations reported thus far were detected only in tumors arising in the gastrointestinal tract, whether \textit{APC} plays a role in the proliferation of cells in other organs is still an open question. Frequent LOH at loci on chromosome 5q, where \textit{APC} is located, have been reported in renal cell carcinomas (9, 10), hepatocellular carcinomas (11, 12), and cancers of the lung (13–15); however, that \textit{APC} is the gene responsible for development of these types of tumor has not been proved. To address these questions, we have examined the mutation cluster region (MCR) (7) of \textit{APC} for somatic mutations in sporadic tumors from kidney, liver, lung, and pancreas which represent a common cancer with poor prognosis. The results we report here contribute significantly to a growing understanding of the role of the \textit{APC} gene in human disease.

Materials and Methods

DNA Preparation. A total of 84 primary tumor tissues, consisting of 14 renal cell carcinomas, 5 hepatocellular carcinomas, 55 cancers of the lung (1 large cell carcinoma, 5 adenocarcinoma, 36 adenocarcinomas, and 13 tumors of unknown histopathological diagnosis), and 10 pancreatic cancers (7 well differentiated adenocarcinomas, 2 moderately differentiated adenocarcinomas, and 1 poorly differentiated adenocarcinoma) were analyzed in this study. In each case, corresponding noncancerous tissues, either from surrounding normal tissues or from peripheral WBC, were collected. All of the pancreatic DNA samples, from both tumor and normal tissues, were obtained from tissues attached to glass slides in slices 10 μm thick which had been fixed in formalin and embedded in paraffin; under a microscope, cells were excised from ten slides of each tissue specimen and pooled (16). Other tissues were obtained during surgery and were frozen immediately in liquid nitrogen and stored at −80°C until use. Genomic DNAs from tissues attached to glass slides were extracted as described elsewhere (17) and from other tissues as described by Sato et al. (18).

PCR Amplification. DNA sequence corresponding to certain parts of the coding region of \textit{APC} were amplified by PCR (19). Primer sets for PCR amplification were described previously (20).

RNase Protection Analyses. To detect mutations efficiently, RNase protection analyses were performed according to the method of Winter et al. (21) with some modification (22). In brief, PCR products were hybridized with \textsuperscript{32}P-labeled RNA transcripts which represent normal \textit{APC} sequence and digested by an RNase A (Boehringer Mannheim GmbH, Mannheim, Germany) at a final concentration of 25 μg/ml. This enzyme cleaves RNA at mismatches within DNA-RNA hybrids. Digested products were electrophoresed in an 8% polyacrylamide-8 μm urea gel and autoradiographed to detect any extra bands. RNA transcripts corresponding to each strand of genomic DNA were examined to increase the efficiency and accuracy of this method.

DNA Sequencing. PCR products which showed different patterns by RNase protection analyses were subsequently sequenced. Template DNAs for the sequencing reactions were prepared either by asymmetrical PCR (23) or by purification of pooled DNA from at least 50 subclones which contained the PCR product at the EcoRV site of the pBluescript II SK (−) (Stratagene, La Jolla, CA). Sequencing reactions on both strands were performed (24, 25) to confirm mutations.

Results

We first examined expression of \textit{APC} in several normal tissues by means of reverse transcription-PCR assays. Expression of actin was also examined as a control of RNA preparation. We detected expression of \textit{APC} in a broad spectrum of organs including kidney, liver, lung, and pancreas (data not shown). Then, parts of \textit{APC} were amplified by PCR, and the products were analyzed by a RNase protection assay. Results of abnormalities detected by the RNase protection analysis are shown in Fig. 1. Samples in which extra bands were detected were then sequenced to determine the specific DNA alterations. Because the amount of DNA was limited for some samples, it was impossible to examine the entire coding region of this very large gene; therefore, we first screened the samples for somatic mutations within the "mutation cluster region" of \textit{APC} (between codons 1286 and 1513). This nearly 700-base pair region is the site where more than two-thirds of the somatic mutations in colorectal tumors were observed to date (7). Then, we extended the region for screening in samples where the amount of available DNAs permitted. Table 1 summarizes this information.

Somatic mutations were detected in pancreatic cancers, whereas no mutation has thus far been detected in other type of tumors. In four of ten pancreatic cancers where mutations were...
APC mutations in pancreatic cancer

Fig. 1. Results of RNase protection analysis. Abnormal patterns were detected in each lane of tumor, which indicated the presence of somatic mutations. PN, and PT, DNA samples obtained from normal and tumor tissue, respectively.

Table 1 Summary of screening experiments

<table>
<thead>
<tr>
<th>Origin of tumor</th>
<th>Examined area (by codons)</th>
<th>Tumors examined</th>
<th>Somatic mutations detected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney</td>
<td>279-1666</td>
<td>14</td>
<td>0</td>
</tr>
<tr>
<td>Liver</td>
<td>279-1666</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Lung</td>
<td>582-1666</td>
<td>55</td>
<td>0</td>
</tr>
<tr>
<td>Pancreas</td>
<td>742-1666</td>
<td>10</td>
<td>4</td>
</tr>
</tbody>
</table>

detected, we proved that they were somatic events by comparing the mutant DNAs with samples isolated from corresponding normal tissues, by means of RNase protection analyses and/or DNA sequencing. In tumor PT9 (well differentiated adenocarcinoma), we detected a 5-base pair deletion of either GAATT or AATTG from the GGAATTGG sequence at codons 857-859 (Fig. 2A); this change generated a new stop codon (TGA) immediately downstream, due to a frame shift. In tumor PT4 (well differentiated adenocarcinoma), a deletion of C from GCCCA was detected at codon 1006; and in tumors PT6 (moderately differentiated adenocarcinoma) and PT10 (well differentiated adenocarcinoma), a deletion of A from CAAAC was detected at codon 1444. Again, in each case a new stop codon was created immediately downstream. From DNA sequencing results of tumor PT10, bands corresponding to the normal DNA sequence were very faint on the sequencing gel, indicating loss of the normal APC allele in this tumor (Fig. 2B). These results are summarized in Table 2.

Discussion

In previous studies, we found somatic mutations of APC in nonfamilial colorectal tumors (2, 7) and gastric cancers (8). In the present study, we searched for somatic mutations of APC in sporadic tumors arising in organs outside the gastrointestinal tract. We chose to examine tumors of kidney, liver, lung, and pancreas for the following reasons: (a) frequent LOH at sites on chromosome 5q has been reported in tumors of kidney (9, 10), liver (11, 12) and lung (13-15); (b) tissues of these four organs express the APC gene; and (c) cancers of these four organs are relatively frequent malignancies with poor prognosis.

Among the tumors tested, we could detect somatic mutations only in pancreatic cancers. Until now, reported genetic alterations in pancreatic tumors have been limited to point mutations of K-ras (16, 26-31) and p53 (32, 33) and loss of expression of DCC (34). In the course of colorectal tumorigenesis, an accumulation of genetic alterations within genes on chromosomes 5q (APC, MCC), 12p (K-ras), 17p (p53), and 18q (DCC)

Table 2 Somatic mutations of APC in pancreatic cancers

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Codon</th>
<th>Nucleotide change</th>
<th>Type of mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>PT4</td>
<td>1006</td>
<td>GCCCA—GCCA</td>
<td>1-base pair deletion</td>
</tr>
<tr>
<td>PT6</td>
<td>1444</td>
<td>CAAAAC—CAAAC</td>
<td>1-base pair deletion</td>
</tr>
<tr>
<td>PT9</td>
<td>857</td>
<td>GGAATTGG—GGAATTGG</td>
<td>5-base pair deletion</td>
</tr>
<tr>
<td>PT10</td>
<td>1444</td>
<td>CAAAAC—CAAAC</td>
<td>1-base pair deletion with allelic loss</td>
</tr>
</tbody>
</table>

a W, well differentiated tubular adenocarcinoma; M, moderately differentiated tubular adenocarcinoma.
is considered to be crucial (35). In the present study, we found somatic mutations of APC in four of ten pancreatic cancers. Taking our results and the evidence from previous reports into consideration, it appears that combinations of genetic alterations in APC, K-ras, p53, and DCC could be important for tumorigenesis in the pancreas, as they are in the colon. In these two organs, the same or similar mechanism(s) may exist in the course of carcinogenesis.

No somatic APC mutation has thus far been detected in the other tumors tested here, although all of 14 renal cell carcinomas and 5 hepatocellular carcinomas and 7 of 55 lung cancers had lost one allele at this locus on chromosome 5q (8-10, 15). Since we did not examine the entire coding region of APC, other domain(s) of the gene could be closely associated with tumors in these types of tissue. However, we think it is more likely that a tumor suppressor gene(s) other than APC, but also located on chromosome 5q, may be responsible for these tumors. Further study will be required to address that question.

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References


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