

Mutations of the APC Gene Occur during Early Stages of Gastric Adenoma Development

Gen Tamura,¹ Chihaya Maesawa, Yasushi Suzuki, Hiroshi Tamada, Mamoru Satoh, Satoshi Ogasawara, Masahiro Kashiwaba, and Ryoichi Satodate

Department of Pathology, Iwate Medical University School of Medicine, 19-1 Uchimarui, Morioka 020, Japan

Abstract

Mutations of the adenomatous polyposis coli (APC) gene have recently been shown to play an important role in colorectal tumorigenesis. We investigated mutations of the APC gene in 30 gastric adenomas obtained endoscopically. Mutations of the APC gene were examined by polymerase chain reaction-single-strand conformation polymorphism analysis followed by sequencing of the polymerase chain reaction products. Mutations were detected in 20% (6 of 30) of gastric adenomas. In addition, deletion of the remaining allele that subsequently led to complete inactivation of the APC gene was confirmed in one-half (3 of 6) of the tumors with APC gene mutations. Sequencing analysis confirmed that the mutations resulted in truncation of the gene products or in an amino acid change. The incidences of mutations of the APC gene remained constant regardless of the size or degree of histological atypia. Our observations suggest that mutations of the APC gene, similarly to those in colorectal tumorigenesis, occur during the early stages of gastric adenoma development.

Introduction

The APC² gene has recently been isolated and mapped to 5q21 (1, 2). Germline mutations of the APC gene have been found in patients with familial adenomatous polyposis (3, 4), and somatic mutations of this gene have been detected not only in patients with colorectal carcinoma (3, 5) but also in patients with pancreatic carcinoma (6) and signet-ring cell carcinoma of the stomach (7). In addition, allelic loss of 5q and loss of heterozygosity at the APC gene locus have frequently been detected in patients with carcinomas of the colorectum (5), esophagus (8), stomach (9, 10), lung (11, 12), kidney (13), and liver (14). These observations suggest that this gene is involved in the genesis of common human malignancies, as has been described previously for the p53 tumor suppressor gene (15). More recently, Nakatsuru *et al.* (16) have reported that mutations of the APC gene occur frequently in differentiated carcinoma of the stomach which has been postulated to sequentially arise from gastric adenoma (17). Powell *et al.* (18) have reported that APC gene mutations occur early during colorectal tumorigenesis because this gene was frequently (63%) mutated in colorectal adenomas. To clarify the role of APC gene mutations in the development of gastric adenoma, which is thought to be the precursor lesion of differentiated gastric carcinoma (19), we have investigated mutations of the APC gene in 30 gastric adenomas.

Materials and Methods

Thirty gastric adenomas were obtained endoscopically from 30 patients. Tissues were fixed in 10% buffered formalin, embedded in paraffin, cut into

3- μ m-thick sections, stained with hematoxylin and eosin, and then graded histologically (20). Gastric adenoma DNA was extracted according to methods described previously (21) from ten serial sections of adenomas; each case was confirmed by hematoxylin and eosin-stained sections to avoid contamination of normal DNA. The 5' half of exon 15 (codons 653–1673) was divided into 8 segments, which included the mutation cluster region of colorectal carcinoma (5); these segments were amplified by PCR using primers designed by Miyoshi *et al.* (4) and listed in Table 1. PCR was performed with 40 cycles of 0.5 min at 95°C, 2 min at 51°C, and 2 min at 70°C. The PCR products were subjected to SSCP analysis. The shifted bands detected by SSCP were separated from the other PCR products, eluted from the polyacrylamide gel, and amplified by PCR using the same primers (22). The PCR products were then purified and sequenced using the double stranded DNA cycle sequencing system (GIBCO BRL, Life Technologies, Inc., Gaithersburg, MD), with the same primers as used in PCR.

Results

Mutations of the APC gene were detected in 20% (6 of 30) of gastric adenomas by PCR-SSCP, 4 in segment 5 and 2 in segment 6 (Fig. 1). In 3 of 6 tumors with mutations, the 2 bands corresponding to the normal allele were lost, suggesting complete inactivation of the APC gene. Direct DNA sequencing confirmed that 4 (GA 3, GA 12, GA 19, GA 27) of 6 mutations resulted in truncation of gene products and the remaining 2 (GA 8, GA 24) resulted in an amino acid change (Fig. 2). Although it is possible that the amino acid substitution at the codon 1301 detected in two tumors (GA 8, GA 24) is an inherited polymorphism, no such sequence polymorphism has been reported previously (3–7). The results of sequencing analysis are summarized in Table 2. The incidence of APC gene mutations remained constant in regardless of the size of adenoma or degree of histological atypia (Table 3). Mutation with loss of the remaining allele occurred even in a tumor with a diameter of 4 mm and with mild histological atypia (GA 19).

Discussion

The frequent loss of heterozygosity of 5q in gastric carcinoma (9, 10) suggests the existence of one or more tumor suppressor genes at this locus. Horii *et al.* (7) have detected mutations of the APC gene, mapped to 5q21, in 3 of 44 (6.8%) gastric carcinomas using RNase protection analysis and sequencing analysis of PCR products. All three tumors with APC mutations were of the histologically undifferentiated type (signet-ring cell carcinoma) and were located in segment 4, outside the mutation cluster region of colorectal carcinoma. More recently, Nakatsuru *et al.* (16) have reported that the APC gene was mutated in 20% (9 of 36) of differentiated gastric carcinomas, including very well-differentiated adenocarcinoma in which 41% (7 of 17) carried mutations.

In our present observations, the APC gene was mutated in 20% of gastric adenomas, and loss of the remaining allele subsequently led to complete inactivation of the APC gene in one-half of the tumors with APC gene mutations. The incidence of APC gene mutations remained

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¹ To whom requests for reprints should be addressed.

² The abbreviations used are: APC, adenomatous polyposis coli; PCR, polymerase chain reaction; SSCP, single strand conformation polymorphism.

Table 1 Oligonucleotide primers (5'-3')^a

Segment	Codons	Upstream	Downstream
1	653-751	CAATCATATTATGCCTTTTGTGTC	GATGGCAAGCTTGAGCCAG
2	735-884	CGAAGTACAAGGATGCCAAT	CAGTGGTGGAGATCTGCAA
3	862-1022	AACTACCATCCAGCAACAGA	TCTAGTTCTCCATCATTATCAT
4	998-1141	TCAATACCCAGCCGACCT	GGCTTATCATCTTCATAGTCA
5	1125-1284	GTAAGCCAGTCTTTGTGTC	CAGCTGATGACAAAGATGAT
6	1260-1410	AGACTTATTGTGTAGAAGATAC	ATGGTTCACCTCTGAACGGA
7	1389-1547	TCTGTCAAGTTCACCTTGATAG	CATTTGATTCTTTAGGCTGC
8	1516-1673	ACAGAAAGATGTGGAATTAAG	TTCTCCAGCAGCTAACTCAT

^a These primers were designed by Miyoshi *et al.* (see Ref. 4).

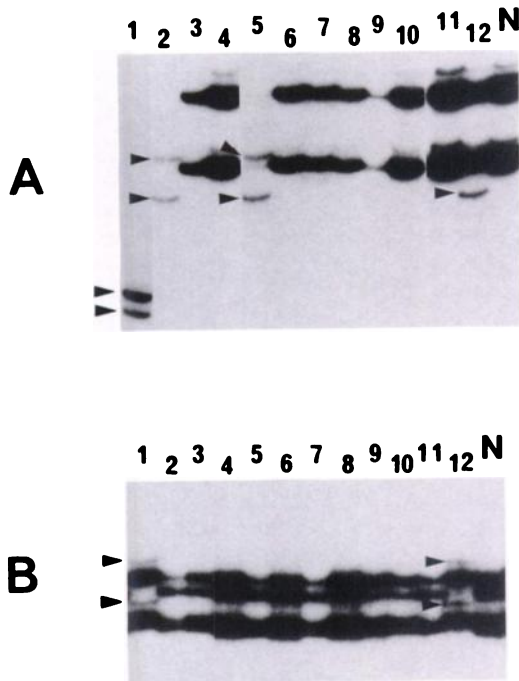


Fig. 1. PCR-SSCP analysis of segments 5 (A) and 6 (B) in several gastric adenomas. Arrowheads, mobility shifts. (A) Lanes 1-12, gastric adenomas (Lane 1, GA 3; Lane 2, GA 14; Lane 5, GA 19; Lane 12, GA 27); N, normal DNA. Two bands corresponding to the normal allele were lost in Lanes 1, 2, and 5. (B) Lanes 1-12, gastric adenomas (Lane 1, GA 8; Lane 12, GA 24); N, normal DNA.

Table 2 Mutations of the APC gene in gastric adenoma

Case	Codon	Nucleotide change
GA 3	1156-1157	4-base pair deletion (AAGA)
GA 8	1301	ACC (Thr) to AGC (Ser)
GA 12	1191	A G deletion
GA 19	1191	A G deletion
GA 24	1301	ACC (Thr) to AGC (Ser)
GA 27	1196	A G insertion

Table 3 Incidence of APC gene mutations according to the size and histological atypia in 30 gastric adenomas

	Incidence of APC gene mutations (%)
Size in diameter	
≤5 mm	25 (3/12)
6-10 mm	10 (1/10)
≥11 mm	25 (2/8)
Histological atypia	
Mild	19 (3/16)
Moderate	22 (2/9)
Severe	20 (1/5)

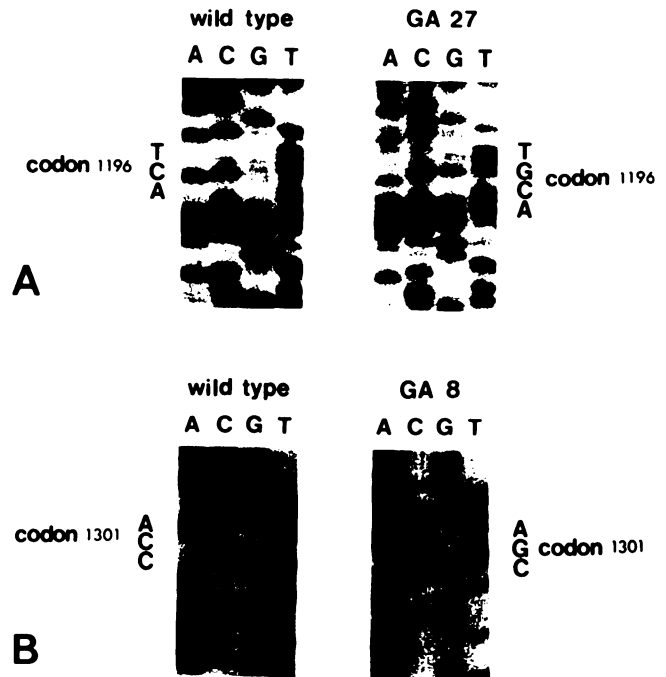


Fig. 2. Sequencing autoradiographs of segments 5 (A) and 6 (B). Arrowheads, mutations. A G insertion at codon 1196 is seen in GA 27 (A) and a point mutation (C to G) at codon 1301 is seen in GA 8 (B).

constant in regardless of the size or grade of atypia. In addition, the incidence of APC mutations in gastric adenoma demonstrated here is the same as the incidence reported by Nakatsuru *et al.* (16) in differentiated gastric carcinoma.

During colorectal tumorigenesis, mutations of the APC gene are thought to occur as early events because mutations have been detected in adenomas as small as 5 mm in diameter and because the frequency of mutations has remained constant as tumors progressed from benign to malignant stages (18). Thus, the condition of the APC gene may be critical during gastric and colorectal tumorigenesis, although the incidence of mutations is relatively low in gastric tumors. In conclusion, mutations of the APC gene occur during early stages of gastric adenoma development and may play a crucial role in the genesis of this tumor.

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