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Advances in Brief

The *APC* Gene, Responsible for Familial Adenomatous Polyposis, Is Mutated in Human Gastric Cancer[1]

Akira Horii, Shuichi Nakatsuru, Yasuo Miyoshi, Shigetoshi Ichii, Hiroki Nagase, Yo Kato, Akio Yanagisawa, and Yusuke Nakamura[2]

Departments of Biochemistry [A. H., S. N., Y. M., S. I., H. N., Y. N.] and Pathology [Y. K., A. Y.], Cancer Institute, 1-37-1 Kami-ikebukuro, Toshima-ku, Tokyo 170, Japan

Abstract

Although gastric cancer is the most common cancer in the world, genetic changes during its carcinogenesis are not well understood. Since some gastric cancers are considered to originate from the intestinal metaplasia, it is likely that the adenomatous polyposis coli (*APC*) gene, the mutation of which causes adenomatous polyps in the colon, is associated with carcinogenesis of gastric cancer. Based on this idea, DNAs isolated from gastric cancers were examined by means of a RNase protection analysis coupled with polymerase chain reaction followed by sequencing of the polymerase chain reaction products. By screening nearly one-half of the coding region of the *APC* gene in 44 tumors, somatic mutations were detected in three tumors: a missense mutation, a nonsense mutation, and a 5-base pair deletion resulting in a frame shift which causes truncation of the gene product. These results suggest that the mutation of the *APC* gene also plays an important role during the carcinogenesis of at least some gastric cancers.

Introduction

Recently, the gene responsible for FAP,[3] termed *APC* (1–4), has been isolated and is found to be somatically mutated in sporadic colorectal cancers (2). Hense, the *APC* gene is considered to be one of the tumor suppressor genes associated with colorectal tumorigenesis. FAP patients often suffer from extracolorectal malignant tumors such as gastric and duodenal cancer (5). Although gastric cancer is the most common malignant tumor in the world (6), the risk for this disease in FAP patients is at least 10 times higher than that in the general population (5). Furthermore, frequent loss of heterozygosity on chromosome 5q, where the *APC* gene is located, have been detected in gastric carcinomas (particularly in well-differentiated type (7). Moreover, some differentiated types of gastric carcinoma are thought to originate from the intestinal metaplastic regions in gastric mucosa (8). Based on these observations, it is of great interest to examine whether or not the *APC* gene product plays a significant role in the proliferation of gastric mucosal cells.

Materials and Methods

Materials and DNA Preparation. A total of 44 pairs of primary gastric cancer tissue and corresponding normal tissues from patients at the Cancer Institute Hospital, Tokyo, were analyzed in this study. Thirty-four pairs of tissues (13 differentiated type and 21 undifferentiated type adenocarcinomas) were frozen in liquid nitrogen immediately after surgical operations and stored at −80°C until use. Ten pairs (signet ring cell carcinomas) were obtained from tissues attached to glass slide which were fixed in formaldehyde. DNA pairs from the frozen tissues were extracted as described (9), and from tissues attached to glass slides were extracted as described (10, 11).

RNase Protection Analyses. DNA sequence corresponding to exons and surrounding introns, if necessary, of the *APC* gene were amplified by PCR (12, 13). The PCR products were hybridized with ^{32}P-labeled RNA probes which represent normal genomic sequences corresponding to either the sense or antisense strand of PCR products. ^{32}P-Labeled RNA probes were produced by either T3 or T7 RNA polymerases (Stratagene, La Jolla, CA) under the conditions recommended by the supplier. The DNA-RNA hybrids were digested by an RNase A (Boehringer Mannheim GmbH., Mannheim, Germany) at final concentration of 25 μg/ml and electrophoresed in an 8% polyacrylamide-8M urea gel to detect extra bands. Details are described elsewhere (2, 14).

Sequencing. Template DNA for the sequencing reactions were prepared either by asymmetric PCR (15) or by purifying the DNA pools of at least 50 subclones which contained the PCR product at the *Eco*RV site of the pBluescriptSK(−) (Stratagene). Sequencing reactions using T7 DNA polymerase (Pharmacia LKB Biotechnology, Uppsala, Sweden) were performed by the chain termination method (16). Primers for sequencing reactions were the same as for PCR amplification.

Results

Initially, to determine whether or not the *APC* gene is transcribed in normal stomach, we examined expression of the *APC* gene in several rat normal tissues by means of reverse transcription-polymerase chain reaction assays (12, 17). The *APC* gene was expressed in a broad spectrum of organs including the stomach (data not shown).

Subsequently, parts of the DNA sequence of the *APC* gene were amplified by PCR (12) and the PCR products were analyzed by RNase protection analysis (14). Samples in which extra bands were detected were then sequenced to determine the specific DNA alteration (10). The region examined by RNase protection analysis in this study accounted for nearly one-half of the coding region corresponding to exon 8 to the 5′ one-half of exon 15 (codons 279 to 1666), a region in which over two-thirds of germ line mutations in FAP patients were observed (13). Among 44 cancers tested, sequence alterations were detected in three tumors, which proved to be somatic changes by comparison with corresponding normal tissues, using RNase protection analysis and/or DNA sequencing.

The sequence from tumor T5 (signet ring cell carcinoma) was GAA (glutamic acid) at codon 1120, although the DNA sequence of its normal tissue was GGA (glycine) (Fig. 1*A*). The absence of normal sequence in the tumor DNA indicates that two mutations at the *APC* locus occurred in this tumor tissue, a missense mutation of one allele and loss of the other allele. Tumor T9 (signet ring cell carcinoma) showed a 5-base pair deletion of either ATAAT or TAATA in ATAATA at codon

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[2] To whom requests for reprints should be addressed.

[3] The abbreviations used are: FAP, familial adenomatous polyposis; *APC* gene, adenomatous polyposis coli gene; PCR, polymerase chain reaction; LOH, loss of heterozygosity.

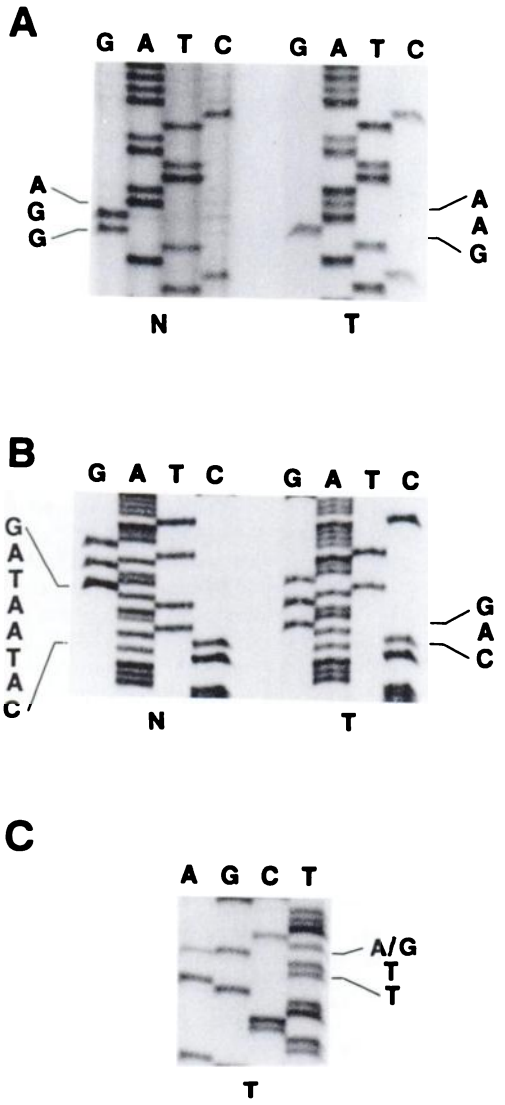

Fig. 1. Autoradiograhpies of sequencing results from part of the *APC* gene in gastric cancer patients. *N*, normal; *T*, tumor. (*A*) Tumor T5 showed a G to A transition causing a missense mutation of glycine (GGA) to glutamic acid (GAA) at codon 1120. (*B*) Tumor T9 showed a 5-base pair deletion of either ATAAT or TAATA in ATAATA (codon 1055–1056), resulting in a frame shift followed by a stop codon (TGA) 6–8 base pairs downstream. (*C*) Tumor T34 showed a C to T transition causing a nonsense mutation of glutamine (CAA) to a stop codon (TAA) at codon 1067. Sequence of the antisense strand is shown.

1055-6 (Fig. 1*B*), resulting in generation of a new stop codon (TGA) immediately downstream caused by the frame shift. The results from DNA sequencing also suggest two separate mutations occurred in this tumor at the *APC* locus. Tumor T34 (poorly differentiated adenocarcinoma) showed a nonsense mutation from CAA (glutamine) to TAA (stop codon) at codon 1067 (Fig. 1*C*), resulting in a truncated gene product. Although still retaining a normal sequence, it is not clear whether it was mutated only once at the *APC* locus (nonsense mutation) or if the normal allele was also lost: DNA from this material was extracted from the frozen tissue *en masse* and histopathological examination of this tumor showed a large proportion of normal stroma and inflammatory cells infiltrating into the cancer tissue. The above results are summarized in Table 1.

Discussion

According to certain histopathological criteria (18, 19), gastric carcinomas are divided into two categories; differentiated type (intestinal type) and undifferentiated type (diffuse type). We anticipated some mutations of the *APC* gene in differentiated type carcinomas, as (*a*) some differentiated adenocarcinomas are considered to originate from intestinal metaplasia in stomach (8); and (*b*) LOHs on the long arm of chromosome 5 in differentiated adenocarcinomas were reported (7, 20). On the other hand, there is no report of frequent LOHs or gene amplifications for undifferentiated types of primary gastric cancer; associated genetic changes have been reported only for cell lines: amplification of the K-*sam* gene in the gastric cancer cell line KATO-III which originated from a signet ring cell carcinoma (6, 21), and a point mutation of c-Ha-*ras* gene in cell line BCG-823 (22). However, unexpectedly, *APC* gene mutations were detected only in undifferentiated types of gastric carcinomas, among which signet ring cell carcinomas without surrounding intestinal metaplastic regions seemed to be relatively common, as shown in Table 1. Since cancer cells are surrounded with a large amount of stroma cells in undifferentiated types of gastric carcinomas, especially in signet ring cell type, it is very difficult to detect LOH when DNA is extracted from the tumor as a mass, but is possible when cancer cells are carefully collected, as we did in this study.

The mutations detected here were located in relatively small part of exon 15. Since exon 15 is extremely large, covering codons 654 through 2843 (77% of the whole coding region), it is probable that this exon encodes one of the important domains of the gene product. Alternatively, this region may be a hot spot of mutation for targeting by carcinogens. The fact that more than one-half of the germ line mutations were detected in this region (13) also supports these arguments. In agreement with Knudson's two-hit hypothesis (23), a missense mutation in tumor T5 and a 5-base pair deletion in tumor T9 were each detected in one allele, together with loss of other allele. Since this would result in absence of normal *APC* gene product in these tumor cells, these mutations have probably played a significant role in the development of these tumors.

At the outset of these experiments we expected somatic mutations of the *APC* gene in differentiated types of gastric cancers. However, no mutations have yet been detected in the 13 differentiated-type gastric cancers we examined. Other tu-

Table 1 *Somatic mutations of the APC gene in gastric cancers*

Tumor	Histological diagnosis	Intestinal metaplasia	Nucleotide change	Codon	Type of mutation
T5[a]	Sig[b]	No	GGA to GAA	1120	Missense mutation (Gly to Glu) and an allele loss
T9[a]	Sig	No	CATAATAG to CAG	1055–1056	5-base pair deletion causing a frame shift and an allele loss
T34[c]	Por	—[d]	CAA to TAA	1067	Nonsense mutation (Gln to Ter)

[a] DNA was extracted from the slide glass.
[b] Sig, signet ring cell carcinoma; Por, poorly differentiated adenocarcinoma.
[c] DNA was extracted from the tumor as a mass.
[d] Unable to determine due to heterogeneities in this case.

mor suppressor genes which are associated with well-differentiated adenocarcinoma of the stomach may be located on chromosome 5q. Alternatively, since we did not examine the entire coding region, there could be another important domain(s) in the *APC* gene closely associated with well-differentiated adenocarcinoma. Further screening to identify all possible mutations of the *APC* gene will be required to address this question.

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References

1. Kinzler, K. W., Nilbert, M. C., Su, L-K., Vogelstein, B., Bryan, T. M., Levy, D. B., Smith, K. J., Preisinger, A. C., Hedge, P., McKechnie, D., Finniear, R., Markham, A., Groffen, J., Boguski, M. S., Altschul, S. F., Horii, A., Ando, H., Miyoshi, Y., Miki, Y., Nishisho, I., and Nakamura, Y. Identification of FAP locus genes from chromosome 5q21. Science (Washington DC), *253:* 661–665, 1991.
2. Nishisho, I., Nakamura, Y., Miyoshi, Y., Miki, Y., Ando, H., Horii, A., Koyama, K., Utsunomiya, J., Baba, S., Hedge, P., Markham, A., Krush, A. J., Peterson, G., Hamilton, S. R., Nilbert, M. C., Levy, D. B., Bryan, T. M., Preisinger, A. C., Smith, K. J., Su, L-K., Kinzler, K. W., and Vogelstein, B. Mutations of chromosome 5q21 genes in FAP and colorectal cancer patients. Science (Washington DC), *253:* 665–669, 1991.
3. Groden, J., Thliveris, A., Samowitz, W., Carlson, M., Gelbert, L., Albertsen, H., Joslyn, G., Stevens, J., Spirio, L., Robertson, M., Sargeant, L., Krapcho, K., Wolff, E., Burt, R., Hughes, J. P., Warrington, J., McPherson, J., Wasmuth, J., Paslier, D. L., Abderrahim, H., Cohen, D., Leppert, M., and White, R. Identification and characterization of the familial adenomatous polyposis coli gene. Cell, *66:* 589–600, 1991.
4. Joslyn, G., Carlson, M., Thliveris, A., Albertsen, H., Gelbert, L., Samowitz, W., Groden, J., Stevens, J., Spirio, L., Robertson, M., Sargeant, L., Krapcho, K., Wolff, E., Burt, R., Hughes, J. P., Warrington, J., McPherson, J., Wasmuth, J., Paslier, D. L., Abderrahim, H., Cohen, D., Leppert, M., and White, R. Identification of deletion mutations and three new genes at the familial polyposis locus. Cell, *66:* 601–613, 1991.
5. Utsunomiya, J. The concept of hereditary colorectal cancer and the implications of its study. *In*: J. Utsunomiya and H. T. Lynch (eds.), Hereditary Colorectal Cancer, pp. 3–16. Tokyo, Japan: Springer-Verlag, 1990.
6. Hattori, Y., Odagiri, H., Nakatani, H., Miyagawa, K., Naito, K., Sakamoto, H., Katoh, O., Yoshida, T., Sugimura, T., and Terada, M. K-*sam*, an amplified gene in stomach cancer, is a member of the heparin-binding growth factor receptor genes. Proc. Natl. Acad. Sci. USA, *87:* 5983–5987, 1990.
7. Sano, T., Tsujino, T., Yoshida, K., Nakayama, H., Haruma, K., Ito, H., Nakamura, Y., Kajiyama, G., and Tahara, E. Frequent loss of heterozygosity on chromosomes 1q, 5q, and 17p in human gastric carcinomas. Cancer Res., *51:* 2926–2931, 1991.
8. Jaervi, O., and Lauren, P. On the role of heterotopias of the intestinal epithelium in the pathogenesis of gastric cancer. Acta Pathol. Microbiol. Scand., *29:* 26–44, 1951.
9. Sato, T., Tanigami, A., Yamakawa, K., Akiyama, F., Kasumi, F., Sakamoto, G., and Nakamura, Y. Allelotype of breast cancer: cumulative allele losses promote tumor progression in primary breast cancer. Cancer Res., *50:* 7184–7189, 1990.
10. Yanagisawa, A., Kato, Y., Ohtake, K., Kitagawa, T., Ohashi, K., Hori, M., Takagi, K., and Sugano, H. c-Ki-*ras* point mutations in ductectatic-type mucinous cystic neoplasms of the pancreas. Jpn. J. Cancer Res., *82:* 1057–1060, 1991.
11. Goelz, S. E., Hamilton, S. R., and Vogelstein, B. Purification of DNA from formaldehyde fixed and paraffin embedded human tissue. Biochem. Biophys. Res. Commun., *130:* 118–126, 1985.
12. Baker, S. J., Preisinger, A. C., Jessup, J. M., Paraskeva, C., Markowitz, S., Wilson, J. K. V., Hamilton, S., and Vogelstein, B. *p53* gene mutations occur in combination with 17p allelic deletions as late events in colorectal tumorigenesis. Cancer Res., *50:* 7717–7722, 1990.
13. Miyoshi, Y., Ando, H., Nagase, H., Nishisho, I., Horii, A., Miki, Y., Mori, T., Utsunomiya, J., Baba, S., Peterson, G., Hamilton, S. R., Kinzler, K. W., Vogelstein, B., and Nakamura, Y. Germ-line mutations of the *APC* gene in 53 familial adenomatous polyposis patients. Proc. Natl. Acad. Sci. USA, in press, 1992.
14. Kinzler, K. W., Nilbert, M. C., Vogelstein, B., Bryan, T. M., Levy, D.B., Smith, K. J., Preisinger, A. C., Hamilton, S. R., Hedge, P., Markham, A., Carlson, M., Joslyn, G., Groden, J., White, R., Miki, Y., Miyoshi, Y., Nishisho, I., and Nakamura, Y. Identification of a gene located at chromosome 5q21 that is mutated in colorectal cancers. Science (Washington DC), *251:* 1366–1370, 1991.
15. Gyllensten, U. B., and Erlich, H. A. Generation of single-stranded DNA by the polymerase chain reaction and its application to direct sequencing of the HLA-DQA locus. Proc. Natl. Acad. Sci. USA, *85:* 7652–7656, 1988.
16. Sanger, F., Nicklen, S., and Coulson, A. R. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA, *74:* 5463–5467, 1977.
17. Noonan, K. E., and Roninson, I. B. RNA phenotyping by enzymatic amplification of randomly primed cDNA. Nucleic Acids Res., *16:* 10366, 1988.
18. Japanese Research Society for Gastric Cancer. The general rules for the gastric cancer study in surgery and pathology. Jpn. J. Surg., *11:* 127–145, 1985.
19. Lauren, P. The two histological main types of gastric carcinoma, diffuse and so called intestinal type carcinoma. An attempt at a histo-clinical classification. Acta Pathol. Microbiol. Scand., *64:* 31–49, 1965.
20. Wada, M., Yokota, J., Mizoguchi, H., Sugimura, T., and Terada, M. Infrequent loss of chromosomal heterozygosity in human stomach cancer. Cancer Res., *48:* 2988–2992, 1988.
21. Nakatani, H., Sakamoto, H., Yoshida, T., Yokota, J., Tahara, E., Sugimura, T., and Terada, M. Isolation of an amplified DNA sequence in stomach cancer. Jpn. J. Cancer Res., *81:* 707–710, 1990.
22. Deng, G., Lu, Y., Chen, S., Miao, J., Lu, G., Li, H., Cai, H., Xu, X., E, Z. and Liu, P. Activated c-Ha-*ras* oncogene with a guanine to thymine transversion at the twelfth codon in a human stomach cancer cell line. Cancer Res., *47:* 3195–3198, 1987.
23. Knudson, A. G. Mutation and cancer: statistical study of retinoblastoma. Proc. Natl. Acad. Sci. USA, *68:* 820–823, 1971.

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