Genetic Changes and Histopathological Types in Colorectal Tumors from Patients with Familial Adenomatous Polyposis

Michiko Miyaki, Madoka Seki, Mieko Okamoto, Akiyoshi Yamanaka, Yoshiharu Maeda, Kiyoko Tanaka, Rei Kikuchi, Takeo Iwama, Tatsuro Ikeuchi, Akira Tonomura, Yusuke Nakamura, Ray White, Yoshio Miki, Joji Utsunomiya, and Morio Koike

Department of Biochemistry, The Tokyo Metropolitan Institute of Medical Science, 3-18-22 Honkomagome [M. M., M. S., M. O., K. T., R. K.], Department of Pathology, The Tokyo Metropolitan Komagome Hospital, 3-18-22 Honkomagome [A. Y., Y. M., M. B.], and Departments of Surgery [T. Iw.] and Cytogenetics, Medical Research Institute [T. Ik., A. T.], Tokyo Medical and Dental University, Yushima, Bunkyo-ku, Tokyo 113; Department of Biochemistry, Cancer Institute, Kamiikebukuro, Tashima-ku, Tokyo 170 [Y. N.], Japan; Howard Hughes Medical Institute and Department of Human Genetics, University of Utah Health Science Center, Salt Lake City, Utah 84132 [R. W.]; and Department of Surgery, Hyogo College of Medicine, Mukogawa-cho, Nishinomiya 663, Japan [Y. M., J. U.]

ABSTRACT

Loss of heterozygosity (LOH) and K-ras mutation were analyzed in 111 colorectal polyps and 26 invasive carcinomas from 40 patients with familial adenomatous polyposis of distinct histopathological types.

LOH, being <2% in moderate adenomas, was detected on chromosome 5q (20%) in severe adenomas, on 5q (26%) and 17p (38%) in intramuscosal carcinomas, and on 5q (52%), 17p (56%), 18 (46%), and 22q (33%) in invasive carcinomas. LOH on chromosome 5q occurred most frequently in the region close to the APC gene both in adenomas and carcinomas, and a loss of the normal allele of the APC gene was demonstrated in 3 cases. K-ras mutation markedly increased in the step of development from moderate (11%) to severe (36%) adenomas.

These results suggest the following mechanisms for the development of colon tumors in patients with familial adenomatous polyposis: (a) the heterozygous mutant/wild-type condition at the APC gene causes formation of mild or moderate adenoma; (b) the loss of the normal allele in the APC gene leads to a change from moderate to severe adenoma; (c) LOH on chromosome 17p contributes to the conversion of adenoma to intramuscosal carcinoma; (d) LOH on other chromosomes, such as 18 and 22q, are involved in the progression of intramuscosal carcinoma to invasive carcinoma; and (e) K-ras mutation may also affect the development of moderate to severe adenoma.

INTRODUCTION

Malignant change of normal cells proceeds via multiple steps involving changes in multiple genes, thereby affecting cell growth. In colon carcinogenesis two mechanisms are proposed: malignant conversion of adenomas to carcinomas or the proliferation of carcinoma cells that are not formed via adenomas. An autosomal dominant disorder, FAP, is characterized by the development of numerous adenomatous polyps and a high risk of colon carcinoma. Since the affected individuals have multiple adenomas at various intermediate stages between benign and malignant, analysis of genetic changes in these adenomas is useful to investigate the former mechanism, the adenoma-carcinoma sequence. These adenomas are produced by a heterozygous mutant APC gene, which has been mapped to chromosome 5q21-22 (1-4). A LOH has been found to occur on chromosome 5q in colon carcinomas from both FAP (5-7) and non-FAP patients (8-10), which suggests that LOH occurs not in adenoma formation but during development from adenoma to carcinoma. Vogelstein et al. (11) reported involvement of LOH at these chromosomes (5-7), suggesting that LOH occurs not in adenoma formation but during development from adenoma to carcinoma.

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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; SD, sodium dodecyl sulfate; DCC, deletion in colon cancer.

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Some 5q, LOH was observed in high frequency at chromosomes 17p, 18q, and 22q in colon carcinomas from FAP patients (5-7) and at 17p, 18q, and 8p and other chromosomes in sporadic carcinomas (10-13). These facts indicate that both the APC gene and multiple recessive oncogenes, or tumor suppressor genes, on other chromosomes contribute to the formation of malignant colon carcinomas; while, in contrast to carcinomas, adenomas in FAP patients showed very low frequencies of LOH at these chromosomes (5-7), suggesting that LOH occurs not in adenoma formation but during development from adenoma to carcinoma. Vogelstein et al. (14) reported involvement of LOH on chromosomes 5q, 17p, and 18q and ras mutation during development of colorectal tumors in sporadic cases, but they found no genetic changes in adenomas from FAP patients.

In other FAP adenomas a few examples of LOH have been reported (15), but the histopathological type of these adenomas has been unclear. In the present study we analyzed LOH in 111 FAP adenomatous polyps with distinct histopathological dysplasia and size, using the new markers close to the APC gene. These polyps were also analyzed for K-ras mutation and for LOH on chromosomes 17, 18, and 22, based on our previous observations of colorectal carcinomas from FAP patients (5-7). We detected a considerable frequency of LOH on chromosome 5q both in adenomas and intramuscosal carcinomas and a much higher frequency of LOH on 5q in invasive carcinomas than has been previously reported. We obtained evidence for loss of the normal allele of the APC gene in these FAP tumors, which supports the recessiveness of the APC gene. It was also found that the occurrence of genetic changes closely related to the grade of histopathological dysplasia.

MATERIALS AND METHODS

Tumor Specimens. In the present investigation 170 colon tumors from 59 patients were analyzed. One hundred eleven adenomatous polyps (3-30 mm in diameter) were obtained from 23 FAP patients, and 11 hamartomatous polyps (6-40 mm) were obtained from 2 patients with FAP and 2 patients with Peutz-Jeghers' syndrome. Twenty-six invasive adenocarcinomas were obtained from 22 FAP patients, and 22 sporadic adenocarcinomas were obtained from 19 non-FAP patients.

Seven FAP patients had both adenomas and invasive carcinomas. One to 18 tumor specimens were surgically resected from each patient. A portion (15-20%) of each tumor specimen was fixed with formaldehyde solution and submitted for histopathological analysis, and the remaining fraction was used for DNA analysis. These tumors were collected in Japan during the 9 years from 1981 to 1989.

Histopathological Diagnosis. All specimens were fixed with 10-17% neutral formalin and routinely processed by paraffin embedding. Serial sections were cut at 3 μm. The sections were stained by hematoxylin and eosin and Alcian blue-periodic acid-Schiff double stain (pH 2.5). Histopathological diagnoses were performed according to our usual criteria at The Tokyo Metropolitan Komagome Hospital. Adenomas, irrespective of histopathological type, consist of similar adenomatous
epithelium and were divided into three grades of dysplasia according to the cytological and architectural atypia, regardless of their size: mild, moderate, and severe. However, since the degree of dysplasia varies from area to area within the same lesion, we found it necessary to add another category of adenoma: moderate to severe dysplasia. Some adenomatous polyps had unquestionable focal carcinoma, called "focal carcinoma in adenoma." The term "intramucosal carcinoma" means cancer limited to the lamina propria. The term "invasive carcinoma" should be restricted to cancer that has invaded through the muscularis mucosae and into the submucosa, muscularis propria, or serosa, depending on the General Rules of the Japanese Research Society for Cancer of Colon and Rectum (16). Other than neoplastic polyps, a few cases of Peutz-Jeghers polyps were also examined. All specimens examined histopathologically only contained neoplastic epithelium, with no mixing of nonneoplastic epithelium.

DNA Preparation. High molecular weight DNA was extracted from each tumor specimen by the method of SDS-protease K and phenol-chloroform treatment. DNA from corresponding normal tissue was prepared from normal colon muscle, skin fibroblast cells, or lymphocytes.

Analysis of LOH. DNA from tumor and normal tissue of each patient was digested with an appropriate restriction endonuclease, electrophoresed in 1% agarose gel, and transferred to a nitrocellulose membrane. The membrane was prehybridized for 2 h at 42°C in 50% formamide, 0.75 M NaCl, 2 mM EDTA, 0.1 M 1,4-piperazinediethanesulfonic acid (pH 6.8), 1× Denhardt’s solution, and 100 μg/ml denatured salmon sperm DNA and hybridized with 32P-labeled probe in the same solution as the prehybridization. After hybridization the membrane was washed three times at room temperature in 50% formamide/0.3 M sodium citrate (pH 6.0) (2× SSC), then in 0.2% SDS/2× SSC, and exposed to X-ray film at −70°C. LOH was judged by loss or decrease in density of one of a pair of bands on X-ray film. To estimate the sensitivity of LOH in Southern blot hybridization of DNA samples from whole adenoma specimens, radioactivity in a pair of bands on membrane was measured directly with an AMBIS radioanalytic imaging system in several cases. Comparison of radioactivity with the density of the corresponding bands on X-ray film indicated that LOH was visible on X-ray film when the tumor specimens included >25% tumor cells with LOH. The following probes and enzymes were used: for chromosomes 5q, L1.4 (DSS4) (EcoRI) (17), M4 (DSS6) (BamHI) (18), α227 (DSS37) (PstI) (4), 111p11 (DSS71) (TaqI) (1), YN5.48-4 (DSS81) (MspI) (3), L5.34 (TaqI),* 4-fms (CSF1R) (EcoRI) (20), and λMS8 (DSS43) (HindII) (21); for chromosome 17p, YNZ22 (D17S30) (PstI, MspI, BamHI, TaqI) (22), HF12-2 (D17S1) (MspI) (23), hp53B (TP53) (BglII) (24), MCT5.1 (D17S31) (MspI) (25), and THH59 (D17S4) (TaqI) (22); for chromosome 18, L2.7 (D18S6) (PstI) (26), HF12-62 (D18S1) (TaqI) (23), 26, HH64 (PALB) (MspI) (27), and OS4 (D18S5) (PstI) (28); for chromosome 22, HuCRI (IgL) (EcoRI) (29, 30), V3.3 (IGLV) (BamHI) (31), 22/34 (D22S9) (TaqI) (30), and 22C1-18 (D22S10) (PstI) (32).

Analysis of Kras Mutation. The 89 base pairs in K-ras-2 (33) sequences in DNA samples were amplified by Taq polymerase using primers 5'-ATGACCTGAATATAAACTTGT-3' and 3'-TAAGTCTCGGCGAGACG-5' from corresponding normal tissue with the same primer sets. DNA was amplified in a total DNA sample without amplification. DNA was dot blotted on a nitrocellulose membrane, prehybridized in a solution containing 50% formamide/0.3 M sodium phosphate (pH 7.0), 0.9 M NaCl, 2 mM EDTA, 0.3% SDS, and 100 μg/ml salmon sperm DNA at 56°C for 2 h, and hybridized with 32P-labeled 19-mer deoxynucleotide probes at 56°C for 20 h in the same solution of hybridization. After hybridization the membrane was washed three times at 56°C in the same solution, except for the absence of salmon sperm DNA, and exposed to X-ray film. Only the dense spot was judged as positive for mutation. The following antisense 19-mer deoxynucleotide probes at 56°C for 20 h in the same solution of hybridization were used: at codon 12, ACA (Cys), TCA (Ser), GCA (Arg), CAA (Val), CTA (Asp), and CGA (Ala) and at codon 13, CTG (Asp).

RESULTS

Loss of Heterozygosity and Kras Mutation in Colorectal Tumors with Distinct Histopathological Type. Each sample of 111 adenomatous polyps (3–30 mm in diameter) from FAP patients was precisely diagnosed histopathologically, and polyps were classified into 5 groups: moderate adenoma (54 tumors), moderate to severe adenoma (11 tumors), severe adenoma (22 tumors), carcinoma in adenoma (7 tumors), and intramucosal carcinoma (17 tumors). Colorectal polyps (6–40 mm) from patients with Peutz-Jeghers' syndrome were diagnosed as hamartomas (11 tumors). These polyps and invasive carcinomas (26 tumors) from FAP patients and invasive carcinomas (22 tumors) from sporadic cases were analyzed for LOH on chromosomes 5q, 17p, 18, and 22q, the chromosome regions at which LOH was most frequently observed in colorectal carcinomas from FAP patients in our previous investigations (5–7). The frequency of point mutation at codon 12 or 13 of the K-ras gene was also measured since the mutation at this region was frequently observed in sporadic colon carcinomas (34, 35). Histopathological diagnosis and DNA analysis were done independently, and after completion the results from the two analyses were combined.

The data from the present analyses are summarized in Fig. 1. The frequency of LOH in moderate adenomas was <2%, and no LOH was detected in hamartomatous polyps. In more advanced severe adenomas, including moderate to severe adenomas, LOH on chromosome 5q was detected at a frequency of 20%, but there was little LOH on other chromosomes. Intramucosal carcinomas, including carcinoma-in-adenomas, exhibited LOH on chromosomes 5q at 26% and on 17p at 38%, LOH on chromosome 18 or 22 being <7%. Invasive carcinomas from FAP patients showed high frequencies of LOH on chromosomes 5q (52%), 17p (56%), 18 (46%), and 22q (33%). Sporadic carcinomas also exhibited the high frequencies on chromosomes 5q (60%), 17p (74%), 18 (63%), and 22q (50%). The pattern of occurrence of LOH closely correlated with the histopathological grade in this study, as indicated in Fig. 1, but
it did not correlate well with polyp size. There were many large polyps (20–30 mm) diagnosed as intramucosal carcinoma, but several intramucosal carcinomas, showing LOH in chromosomes 5q and 17p, were smaller than 10 mm in diameter. Large hamartomatous polyps (~40 mm) had no LOH on chromosomes 5, 17, 18, or 22.

K-ras mutation was detected in 11% of moderate adenomas but not in hamartomas. A high frequency of mutation was observed in severe adenomas (36%), but the frequency did not increase during development to intramucosal carcinomas (26%). Invasive carcinomas showed a high frequency of K-ras mutation (44%).

The cumulative number of genetic changes, including LOH on chromosomes 5, 17, 18, and 22, and K-ras mutations increased with an increase in the histopathological grade of FAP tumors (Fig. 2). Although only 4% of moderate adenomas had multiple changes, 13% of severe adenomas, 21% of intramucosal carcinomas, and 66% of invasive carcinomas had two or more genetic changes. The majority of sporadic carcinomas (86%) had multiple genetic changes. Two cases had both primary carcinoma in the colon and metastasis in the liver. The metastatic carcinoma from FAP patient PLK27 had LOH on chromosome 18, while the primary carcinoma in the colon had no LOH on this chromosome. In the sporadic case COK86, the metastasis in the liver had LOH on chromosomes 5q, 17p, 18, and 22q, while the primary carcinoma in the colon exhibited LOH on 5q and 17p but not on 18 or 22q. These cases also indicate that the more advanced carcinomas have more LOH and that LOH on chromosomes 18 and 22 were involved in a late stage of the progression of colon carcinomas.

Nature of LOH on Chromosome 5 in FAP Patients. LOH on chromosome 5 was analyzed in 170 colorectal tumors using 7 restriction fragment length polymorphism probes mapped to chromosome 5, including closely flanking markers for the APC gene in 5q21–22, YN5.48, LS5.34, C11p11, and z227. Loss in both invasive carcinomas and polyps occurred most frequently in the region near the APC gene (Tables 1 and 2). With respect to all tumors analyzed, the highest LOH was observed at YN5.48, which detected the loss in invasive carcinomas at 61% (19 of 31) and that in polyps at 13% (8 of 64). One moderate adenoma, 5 severe adenomas, 6 intramucosal carcinomas, and 12 invasive carcinomas from FAP patients and 12 sporadic carcinomas showed LOH for one or more probes on chromosome 5. Three other FAP adenomas also showed the loss on 5q, the histopathological types of which were not analyzed. Examples of lost regions are shown in Table 1. More than 87% (21 of 24) of FAP tumors and 100% (9 of 9) of sporadic carcinomas with LOH on chromosome 5 exhibited the loss at 5q21–22. The frequencies of LOH were lower at more proximal regions including M4 (5q13) and at a more distal regions including fms (5q33) and MnS8 (5q34-pter). Only a few tumors had loss on the short arm (L1.4), and loss on the entire chromosome 5 was rare. A difference in the range of loss was not observed between adenomas and carcinomas or between FAP carcinomas and sporadic carcinomas. Although it was difficult to determine exactly the mechanism of LOH by densitometry of the remaining allele, semiquantitative analysis around the APC locus suggested that 3 tumors lost heterozygosity through the loss and duplication or mitotic recombination giving two copies of remaining allele (homozygosity), and 9 tumors showed LOH through deletion of one allele (hemizygosity).

The high frequency of loss in the region near the APC gene suggests that the loss of heterozygosity in the APC gene is involved in colon carcinogenesis in both FAP and non-FAP patients; however, it is not yet clear whether both alleles of the APC gene are lost or inactivated in colon carcinomas, as predicted by Knudson (36). In the present study several cases were found in which a specific allele was lost in multiple tumors from a single FAP patient. As shown in Fig. 3, both adenoma A5 and carcinoma Ca from FAP patient PLK58 lost allele 2 of M4. Adenomas A1 and A3 from PLK62 lost the same allele 3 of YN5.48. All three adenomas A1, A2, and A3 from PLK70 lost allele 1 of YN5.48. Both adenoma A2 and carcinoma Ca from PLK72 lost allele 2 of YN5.48. In contrast to these FAP patients, sporadic case COK26 showed a loss in different alleles of YN5.48 in two independent carcinomas, Ca1 and Ca2; the former was produced in the ileocecal region and the latter was formed in the sigmoid colon.

In three FAP families, a normal or mutant allele of the APC gene could be assigned by inheritance of particular restriction patterns in family members, using the closely linked marker which has been reported to show no recombination with APC (3). Fig. 4 demonstrates examples in which the loss of a normal allele of the APC gene was unequivocally shown to occur. Adenoma A in FAP patient PLK46 lost allele 1 of YN5.48 and allele 2 of LS5.34. The lost alleles were inherited from the unaffected mother M46; therefore, the lost alleles of YN5.48 and LS5.34 appeared to congregate with the normal allele of the APC gene. Both carcinoma Ca and adenoma A2 in FAP patient PLK72 lost allele 2 of YN5.48, which was inherited from the unaffected mother M72. In the case of FAP patient PLK70, allele 2 of YN5.48 and allele 1 of LS5.34 were retained after LOH in adenomas A1, A2, and A3. Since these alleles were inherited by her affected niece PLK69, the mutant allele of the APC gene was assumed to be retained with these alleles in three adenomas. There was one more FAP family with Gardner syndrome in which the loss of a normal allele of the
APC gene could be demonstrated in one desmoid and two colorectal tumors.5

Nature of LOH on Chromosomes 17, 18, and 22 in FAP Patients. The frequencies of LOH at loci on chromosomes 17, 18, and 22 in the polyps and invasive carcinomas are summarized in Table 2. Polyps included moderate and severe adenomas, carcinoma-in-adenomas, and intramucosal carcinomas from FAP patients, and carcinomas included invasive carcinomas from FAP and sporadic cases.

LOH on chromosome 17 occurred most frequently at the distal region of the short arm. The frequency at YNZ22 (17p13.3) was 69% in invasive carcinomas and 8% in polyps, the high values being obtained from analysis using four restriction endonucleases. A high frequency of LOH was also observed at the hp53B locus (17p13.1) in carcinomas (57%), and this region was lost in almost all FAP tumors having the loss on chromosome 17p. These data are consistent with the proposal that the p53 gene is a target for LOH on chromosome 17p (37-39). The more proximal region, including D17S31 and D17S1, showed lower frequencies in carcinomas, such as 44 and 43%, respectively, and LOH at D17S4 in the long arm was low (20%).

The frequency of LOH on chromosome 18 was highest (63%) at the OS4 locus, which was mapped to 18q21.3 ~ qter. LOH at the short arm, including D18S6 and D18S1, was lower, 47 and 25%, respectively. Fearon et al. (40) recently isolated the DCC gene as a candidate for the target gene in LOH on chromosome 18q21.3. The increased frequency of LOH at OS4 in FAP carcinomas suggests the involvement of the DCC gene in LOH on 18q, but the specific region of the loss on chromosome 18 in each FAP tumor could not be determined, since no FAP tumors had partial loss on chromosome 18q.

A high frequency of LOH on chromosome 22 was observed at the 22q11 region including IGLC, IGLV, D22S9, and D22S10, as indicated in Table 2.

Nature of Kras Mutations in FAP Tumors. The point mutation in the K-ras gene increased during development from moderate (11%) to severe (36%) adenoma as shown in Fig. 1, and it also increased with an increase in the size of the adenomas including moderate and severe dysplasia. The frequencies were 13% in adenomas smaller than 10 mm, 26% in those between 10 and 20 mm, and 29% in those larger than 20 mm. K-ras mutation was also high in advanced carcinomas (44%), but the direction of mutation was not the same as that in adenomas. As indicated in Table 3, moderate and severe adenomas contained mutations GGT (Gly) to GAT (Asp), GGT to TGT (Cys), GGT to GTT (Val), GGT to GCT (Ala), and GGT to AGT (Ser) at codon

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Table 2. Frequencies of loss of heterozygosity at loci on chromosomes 5, 7, 18, and 22 in colorectal polyps and carcinomas.

<table>
<thead>
<tr>
<th>Tumors</th>
<th>Ch5</th>
<th>D5S4</th>
<th>D5S6</th>
<th>D5S37</th>
<th>D5S71</th>
<th>D5S81</th>
<th>LS5.34</th>
<th>CSF1R</th>
<th>D5S43</th>
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<tbody>
<tr>
<td>Polyps</td>
<td>12/95(13)</td>
<td>1/59(2)</td>
<td>4/67(6)</td>
<td>0/14(0)</td>
<td>0/11(0)</td>
<td>8/64(13)</td>
<td>5/19(26)</td>
<td>4/49(8)</td>
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</tr>
<tr>
<td>Carcinomas</td>
<td>24/43(56)</td>
<td>1/29(3)</td>
<td>9/32(28)</td>
<td>6/11(55)</td>
<td>3/6(50)</td>
<td>19/31(61)</td>
<td>3/5(60)</td>
<td>4/11(36)</td>
<td>1/17(6)</td>
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LOH informative tumors (%)

<table>
<thead>
<tr>
<th>Tumors</th>
<th>Ch17</th>
<th>D17S30</th>
<th>YNZ22</th>
<th>hp53B</th>
<th>D17S31</th>
<th>MCT35.1</th>
<th>D17S51</th>
<th>HF12-2</th>
<th>D17S4</th>
<th>THH59</th>
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<tr>
<td>Polyps</td>
<td>11/108(10)</td>
<td>8/97(8)</td>
<td>3/22(14)</td>
<td>3/46(6)</td>
<td>1/39(3)</td>
<td>1/46(2)</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carcinomas</td>
<td>28/44(64)</td>
<td>27/39(69)</td>
<td>8/14(57)</td>
<td>7/16(44)</td>
<td>6/14(43)</td>
<td>5/25(20)</td>
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LOH informative tumors (%)

<table>
<thead>
<tr>
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<th>D18S1</th>
<th>L.27</th>
<th>D18S5</th>
<th>PALB</th>
<th>LS5.34</th>
<th>CSF1R</th>
<th>D18S5</th>
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<tbody>
<tr>
<td>Polyps</td>
<td>4/92 (4)</td>
<td>1/30 (3)</td>
<td>2/65 (3)</td>
<td>1/55 (2)</td>
<td>3/51 (6)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carcinomas</td>
<td>22/42 (52)</td>
<td>4/16 (25)</td>
<td>9/19 (47)</td>
<td>9/18 (50)</td>
<td>12/19 (63)</td>
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LOH informative tumors (%)

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<tr>
<th>Tumors</th>
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<th>IGLV</th>
<th>IGLC</th>
<th>D22S10</th>
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<td>Polyps</td>
<td>2/86 (2)</td>
<td>0/64 (0)</td>
<td>0/69 (0)</td>
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<td>1/93 (1)</td>
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<tr>
<td>Carcinomas</td>
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<td>10/20 (50)</td>
<td>9/27 (33)</td>
<td>15/30 (50)</td>
<td>11/29 (38)</td>
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Fig. 3. Loss of the same allele on chromosome 5q in multiple colon tumors from a single FAP patient. DNA samples were digested by BamH1 or Msp1 and hybridized with 32P-labeled M4 or YN5.48 as described in "Materials and Methods." N, normal tissue; A, adenoma; Ca, carcinoma.

12, while advanced carcinomas contained significantly higher GGT to GAT (Asp) mutations compared to other types.

DISCUSSION

By a combination of DNA analysis and precise histopathological diagnosis of colorectal polyps, our present study revealed the close correlation between LOH and histopathological type, as summarized in Fig. 1. Numerous mild and moderate adenomas were present in FAP patients, but these adenomas had quite a low frequency of LOH, indicating that the heterozygous mutant APC gene causes adenoma formation. LOH on chro-
mosome 5q was observed at the stage of severe adenomas (20%) in FAP patients, the loss occurring most frequently at the region close to the APC gene. Similar LOH on 5q has been previously observed by Vogelstein et al. (14) as an early event in class II adenomas during development of sporadic colorectal carcinomas, but these authors have detected no LOH in adenomas from FAP patients. The FAP adenomas in their study seem to be like the mild or moderate dysplasia, which showed little LOH, in our study. The loss on chromosome 5q was detected at a considerable extent in intramucosal carcinomas (26%) and at a high frequency in invasive carcinomas (52%) in this study (Fig. 1). The LOH on 5q, observed throughout stages from adenoma to invasive carcinoma in both FAP patients and sporadic cases, suggests that LOH in the APC gene is specific to colorectal tumors. However, it has been unclear whether LOH leads to a complete loss of activity of the APC gene or to half loss. In the present study we obtained evidence for the loss of a normal allele of the APC gene in several cases of severe adenomas, intramucosal carcinomas, and advanced carcinomas from FAP patients (Fig. 4). The loss of the same allele at the specific locus on 5q in multiple tumors from a single FAP patient (Fig. 3) also indicates the possible loss of the normal allele. Loss of a normal allele thus seems to unmask the recessive abnormal allele of the APC gene as has been shown for the RB (retinoblastoma) gene in hereditary retinoblastoma (41).

In addition to LOH on chromosome 5q, LOH on chromosome 17p, including the p53 gene locus, was markedly increased during development of severe adenomas (3%) to intramucosal carcinomas (38%) (Fig. 1). This suggests that loss of the second allele of the APC gene is essential, but not sufficient, for carcinoma formation in FAP patients and that additional LOH at the p5q gene contributes to the change of adenomas into intramucosal carcinomas. The high frequencies of LOH at the region close to the APC gene (61%) and at the p5 locus (57%) in invasive carcinomas (Table 2) indicate cooperation among these LOH in colon carcinogenesis. A gradual increase in the frequency of loss on chromosome 17p has also been reported during development of sporadic colorectal tumors (14). LOH on 17p has been shown to associate with many other adult tumors, including lung, breast, and brain tumors, and it has been predicted that the loss results in the recessive expression of a tumor suppressor gene, the p53 gene, since the remaining allele of this gene has been mutated (38–40). Whether the p53 gene in FAP carcinomas is inactivated by deletion and mutation is to be investigated further.

LOH on chromosomes 18 (46%), in addition to those on 5q and 17p, also occurred in invasive carcinomas (Fig. 1). This loss appears to be involved in the step of progression from intramucosal carcinoma to invasive carcinoma, since the frequencies of LOH on 18 were very low (<7%) in intramucosal carcinomas. Involvement of LOH on chromosome 18 in the progression was also supported by the fact that metastatic carcinoma in the liver exhibited additional LOH on chromosome 18 compared to the primary carcinoma in the colon of the same patient. Vogelstein et al. observed LOH on chromosome 18q at an earlier stage in the development of sporadic carcinomas than in the present cases of FAP tumors. Such a difference in the time for the appearance of LOH on 18q may result from the difference in the classification of tumors between the two studies or from the difference in genetic background between FAP and sporadic cases. Recently, Fearon et al. (40) isolated the DCC gene from the 18q region, frequently lost in sporadic colorectal carcinomas, and they showed evidence that this gene may be a tumor suppressor gene. If a certain non-FAP case has a germinal mutation in the DCC gene, as predicted in the case of hereditary non-polyposis colon cancer syndrome [Lynch syndrome (42)], the LOH on 18q may occur at an earlier stage in carcinogenesis.

LOH on chromosome 22q11 was observed at a considerable frequency in invasive carcinomas (33%) but not in intramucosal carcinomas (Fig. 1). Accordingly, 22q loss appeared to be involved in the stage of progression of colorectal carcinomas. LOH in the same region has been detected frequently in other tumors, including acoustic neuromas (43) and benign and malignant meningiomas (44). The region (22q11.1–13.1) lost in these tumors has been suggested to contain the gene for hereditary neurofibromatosis 2, a possible tumor suppressor gene (45). Loss at this tumor suppressor gene may confer a growth advantage on tumors of neural crest origin and also on colorectal carcinomas.

K-ras mutations were detected at the early stage, as LOH on chromosome 5q was detected. The frequency of K-ras mutation at codon 12 or 13 remarkably increased during development from moderate adenoma (11%) to severe adenoma (36%) and increased with an increase in adenoma size. However, K-ras mutation had no relation to the conversion of adenomas to carcinomas, since the frequency was not increased during change from severe adenomas into intramucosal carcinomas. The high frequency in invasive carcinomas (44%) indicated that K-ras mutation also contributes to the malignancy of FAP carcinomas. These data are consistent with previous observations in sporadic colorectal adenomas and carcinomas (14, 34, 35). However, the type of mutation in carcinomas was not completely the same as that in adenomas in the present investigation (Table 3). Adenomas contained various types of mutation, while advanced carcinomas contained significantly high GGT (Gly) to GAT (Asp) compared to other types. This suggests that mutation to aspartic acid is more advantageous for malignant growth of colorectal carcinomas.

The cumulative number of genetic changes, including LOH on chromosomes 5q, 17p, 18, and 22q, and K-ras mutation, increased with an increase in the histopathological grade of colorectal tumors (Fig. 2). The great majority of invasive car-

### Table 3 Kras mutations and histopathological types in colorectal tumors from FAP patients

<table>
<thead>
<tr>
<th>Histopathological types (no. of tumors)</th>
<th>Total No. of tumors with mutation (%)</th>
<th>No. of tumors with mutation:</th>
<th>At codon 12 Gly(GGT) to:</th>
<th>At codon 13 Gly(GGC) to Asp(GAC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenomas with moderate dysplasia (54)</td>
<td>6 (11)</td>
<td>2 2 1 0 1 0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adenomas with moderate to severe and severe dysplasia (33) Carcinoma-in-adenomas and intramucosal carcinomas (23)</td>
<td>12 (36)</td>
<td>4 1 4 3 0 0</td>
<td>2 2 1 0 0 1</td>
<td></td>
</tr>
<tr>
<td>Invasive carcinomas (25)</td>
<td>11 (44)</td>
<td>8 1 1 0 0 1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**GENETIC CHANGES AND HISTOPATHOLOGICAL TYPES IN FAP TUMORS**

The cumulative number of genetic changes, including LOH on chromosomes 5q, 17p, 18, and 22q, and K-ras mutation, increased with an increase in the histopathological grade of colorectal tumors (Fig. 2). The great majority of invasive car-

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cinenomas from FAP and sporadic cases had multiple genetic changes, and the more advanced invasive carcinomas showed more LOH. These results suggest a significant role of inactivation of multiple tumor suppressor genes and activation of ras gene during the development and progression of colorectal tumors in both FAP and sporadic cases.

In conclusion, the following steps are proposed in colorectal carcinogenesis in FAP patients: (a) The heterozygous mutant/wild-type condition at the APC gene causes formation of mild or moderate adenoma; (b) the loss of a normal allele in the APC gene leads to a change from moderate to severe adenoma; (c) LOH on chromosome 17p contributes to the conversion of adenoma to intramucosal carcinoma; (d) LOH on other chromosomes, such as 18 and 22q, are involved in the progression of intramucosal carcinoma to invasive carcinoma; and (e) K-ras mutation may also affect the development of moderate to severe adenoma. These data indicate that the LOH analysis may be helpful in the diagnosis of colorectal tumors, which has previously been done only by histopathological methods.

It appears that the high incidence of colorectal carcinomas in FAP patients originates from a high incidence of adenoma formation by the heterozygous mutant/wild-type condition at the APC gene and from a tendency toward alteration of both alleles of APC gene in FAP adenomas in which one of a pair of APC genes is intrinsically abnormal. LOH on other chromosomes and ras mutation seems to be not specific for FAP patients, but the presence of a large number of adenomas in FAP mucosa may result in an increased frequency of conversion from adenoma to carcinoma by these genetic lesions.

The mechanism of adenoma formation by heterozygous mutation in the APC gene is still unclear. The normal APC gene is supposed to be a growth regulatory gene, and inactivation of one copy of two alleles may give rise to hyperproliferation in the colorectal mucosa through a threshold effect produced by fluctuating levels of gene product, as predicted by Solomon et al. (8). This effect may also be manifested in extracolonic tissues of FAP patients, since these patients have a high incidence of various tumors, such as gastric and duodenal polyps, osteomata, and desmoid tumors, and thyroid and brain tumors (46). In a desmoid tumor with recurrence we have detected the loss of APC genes is intrinsically abnormal. LOH on other chromosomes and ras mutation seems to be not specific for FAP patients, but the presence of a large number of adenomas in FAP mucosa may result in an increased frequency of conversion from adenoma to carcinoma by these genetic lesions.

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Michiko Miyaki, Madoka Seki, Mieko Okamoto, et al.


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