Some mixed hyperplastic adenomatous polyps (MHAPs) contain dysplastic lesions or even carcinomas. These polyps are considered to be different from ordinary hyperplastic polyps and may have a preneoplastic potential. We investigated APC and K-ras mutations in MHAPs of the colon and rectum, and also in colorectal adenomas and hyperplastic polyps to identify molecular differences between MHAPs, adenomas and hyperplastic polyps, using direct sequencing of mutation cluster regions (MCR) in APC and K-ras. No APC mutations were identified in 12 MHAPs and 8 hyperplastic polyps, whereas 10 of 27 (37.0%) adenomas showed somatic mutations. K-ras mutations were identified in one of 12 (8.3%) MHAPs, one of 8 (12.5%) hyperplastic polyps, and 10 of 27 (37.0%) adenomas. p53 mutation was found in a carcinoma arising in an MHAP. Mutations other than APC mutations may play a role in the development of MHAPs.

Key words: Mixed hyperplastic adenomatous polyp — Serrated adenoma — APC gene — K-ras gene — p53 gene

Colorectal carcinomas are considered to develop as a consequence of mutations in oncogenes and tumor suppressor genes, or DNA mismatch repair gene disruption. Such mutations are relevant to the adenoma-carcinoma sequence, de novo carcinogenesis or the RER pathway. In the case of the adenoma-carcinoma sequence, APC mutation is thought to play a key role in adenoma formation and inactivation of APC initiates colorectal carcinogenesis. In contrast, hyperplastic polyps have no malignant potential. Hyperplastic polyps have been examined for APC mutations by Jen et al. However, APC mutation was not identified in any hyperplastic polyps, while K-ras mutations were identified in both adenomas and hyperplastic polyps with almost the same frequency. Recently, the classification of MHAP was proposed by Longacre and Fenoglio-Preiser. MHAP has both hyperplastic and adenomatous appearance. It has been reported that 11% of MHAPs contain areas of intramucosal carcinoma.

Jeevatranam et al. reported a case of familial hyperplastic polyposis with multiple hyperplastic polyps, adenomas and MHAPs in the colon and rectum. Colorectal cancers developed in 6 relatives in this family. Therefore, MHAP is thought to be a preneoplastic lesion. In order to examine the molecular genetics of MHAPs, we analyzed APC mutations and K-ras mutations in MHAPs.

MATERIALS AND METHODS

Tumor samples. We obtained the following samples from Hamamatsu University School of Medicine, Matsuda Hospital, Seirei Sumiyoshi Hospital and Seirei Mikatabara Hospital: 12 paraffin-embedded MHAP samples (M1 to M12), 27 fresh colorectal adenoma samples (A1 to A27) including 18 tubular adenomas, 4 tubulo-villous adenomas and 5 villous adenomas, and 8 paraffin-embedded hyperplastic polyps (H1 to H8). Fresh specimens were stored at −70°C until they were used. All the specimens were reviewed by two pathologists.

In this study, the criteria for MHAPs, so-called serrated adenoma, were as follows: serrated glandular luminal appearance, increased mitotic activity in the upper zones of crypts, prominence of nuclei, goblet cell immaturity and nuclear pseudostratification. MHAP should be distinguished from a polyp with admixed hyperplastic glands.
and adenomatous glands as designated by Longacre and Fenoglio-Preiser. In other words, the latter polyp is a hyperplastic polyp with adenomatous aberrant crypt foci.

For the evaluation of the mitotic activity, Ki67 immunostaining was performed, because Ki67 is thought to be a marker of proliferating cells. Colorectal adenomas show a uniform distribution of Ki67-positive cells throughout the sections, including the cells of the adenoma surface, while Ki67 staining in the normal mucosa is confined to the middle third and lower third of the crypts. As MHAPs, we used samples which met the criteria of MHAP and were stained by anti-Ki67 antibody (Dako) not only on the bottom, but also sparsely in the upper zones of the crypts, using a modified peroxidase-antiperoxidase technique. Hyperplastic polyps and adenomas were also stained by anti-Ki67 antibody. All the hyperplastic polyps were stained only in the lower half of the crypts, and all the adenomas were stained profusely from the bottom to the upper zone of the entire glandular lesion.

The characteristics of the samples are summarized in Table I. Carcinomas were observed in two MHAP cases: one was a carcinoma in an MHAP and the other was an advanced carcinoma synchronized with multiple adenomas, MHAPs and hyperplastic polyps. The microscopic appearance of MHAP with focal cancer is shown in Fig. 1.

DNA extraction DNA was extracted from fresh frozen tissues, as described elsewhere.

Six 10 µm sections were cut from each paraffin-embedded tissue block, and one section was stained with HE. Using the HE-stained section as a guide, adenoma, hyperplastic polyp, MHAP and normal tissues were microdissected, and DNA was extracted as described in a previous article. Analysis of tumors for APC mutations MCR (codons 1286 to 1513) of APC was analyzed by direct sequencing. In brief, two overlapping fragments of APC covering codons 1260 to 1410 and 1410 to 1547 were amplified by PCR using 0.3 µl of 5 u/µl Taq DNA polymerase (Boehringer Mannheim, Mannheim, Germany) in 25 µl of 10 mM Tris pH 8.8, 2.5 mM MgCl₂, 1.5 µM of each of the appropriate primers (upstream fragment, 5'-AGACTTATGTGTAAGATAC-3' and 5'-ATGGTTTCACCTTGAACCGGA-3'; downstream fragment, 5'-TCTCTCTTCTCTGTGTTAC-3' and 5'-CATTGATCTTGAG-3'). Amplifications were performed for 37 cycles of denaturation (30 s at 95°C), annealing (2 min at 50°C) and extension (5 min at 70°C).

All of the PCR products were purified using C3TTK filtration tubes (Millipore, Tokyo). Purified PCR products were sequenced using internal primers as follows: forward sequencing primer for the upstream fragment 5'-CATTATCATCCTTGTGTAAGACGG-3' and reverse sequencing primer 5'-TTCACCTTGAAACGGAGCTTG-3'; forward sequencing primer for the downstream fragment 5'-ACCTGTATTTTCAGAGTTGC-3' and reverse sequencing primer 5'-GGAGGCATTATCTTCAAATGCTC-3'; each primer was labeled at the 5' end with rhodamine with a Thermo Sequenase cycle sequencing kit (Amersham, Tokyo) according to the manufacturer’s protocol. Detection was carried out by using an FMBIO 100 (Takara, Kyoto).

Analysis of LOH of APC In three cases (M1, M7 and M12), of which two were associated with cancer. LOH of the APC locus was analyzed. A CA repeat marker is located 30–70 kb downstream from APC, and can be amplified by PCR. Another CA repeat marker is located upstream from APC, and can also be amplified by PCR. In the three cases, DNAs of normal mucosa tissue, MHAP tissue and cancer tissue were amplified by PCR at the 2 CA repeat markers, and the PCR products were separated by denaturing polyacrylamide gel electrophoresis.

K-ras mutation analysis Exon 1 of the K-ras was amplified for all the samples using forward primer 5'-ACCTTATGTGTAAGATACGTTCT-3' and reverse primer 5'-AGAGAAACCTTATCTGATTC-3'. Direct sequencing of the PCR products was carried out to identify K-ras mutations, using an FMBIO 100 (Takara).

p53 mutation analysis Direct sequencing of exon 5 to exon 8 of the p53 gene was amplified for two cases: a focal carcinoma in MHAP (M1) and a synchronous advanced carcinoma associated with multiple MHAPs (M7). Each exon of the p53 gene was amplified by PCR as described above, with the following primers: (exon 5, 5'-ATGGTTTCATCTTTGCTGCGGCG-3' and 5'-CCATTAAATACCTCACAACGCAAT-3'; exon 6, 5'-CTAGATGAGCCAGTGAG-3' and 5'-GGAGAAAGCCCGCTCCCTATT-3'; exon 7, 5'-CTCCCCAAAGGCGCACTG-3' and 5'-TGAGGAGCAGTAAGGAGATTC-3'; exon 8, 5'-AACCTGTGACTTCTCTCACTC-3' and 5'-ACCTGTGACTTCTCTCACTC-3'). After purification of the PCR products, direct sequencing of the PCR products was performed, as described for APC direct sequencing, with 5' biotinylated inner primers: (exon 5, forward primer 5'-CAACCTGCTCTCCTCTCTCCT-3' and reverse primer 5'-CAAGGACCCCATGCTGAAAACAGA-3'; exon 6, forward primer 5'-CTTCTTCTTGAGCTGATCTC-3' and reverse primer 5'-TTACTGCTTCTGCTTCTC-3').
Table I. Characteristics of MHAPs, Adenomas and Hyperplastic Polyps

| Patients | Age | Sex | Location | Diameter (cm) | Shape | Other condition |
|----------|-----|-----|----------|--------------|-------|-----------------|
| M1       | 77  | F   | T        | 3.0×2.5      | FL    | cancer in MHAP  |
| M2       | 47  | M   | S        | 1.5          | P     |                 |
| M3       | 49  | F   | R        | 1.5×1.0      | P     |                 |
| M4       | 54  | M   | T        | 0.6          | SP    |                 |
| M5       | 65  | M   | S        | 1.2          | P     |                 |
| M6       | 54  | M   | S        | 1.5          | P     |                 |
| M7       | 65  | M   | R        | 2.0×1.5      | FL    | diffuse hyperplastic polyposis with synchronous advanced cancer |
| M8       | 53  | M   | T        | 1.3          | P     |                 |
| M9       | 50  | F   | D        | 0.4          | SP    |                 |
| M10      | 61  | M   | R        | 0.6          | SP    |                 |
| M11      | 50  | M   | D        | 1.0          | SP    |                 |
| M12      | 58  | M   | R        | 0.9          | SP    | multiple MHAPs  |
| A1       | 64  | M   | S        | 0.7          | P     | T               |
| A2       | 55  | M   | R        | 6.0×5.0      | FL    | V               |
| A3       | 57  | F   | S        | 2.5          | P     | TV              |
| A4       | 66  | F   | S        | 4.0×2.2      | FL    | TV              |
| A5       | 71  | F   | S        | 6.0×3.5      | FL    | V               |
| A6       | 63  | M   | S        | 1.5          | P     | T               |
| A7       | 73  | F   | A        | 3.0          | FL    | TV              |
| A8       | 72  | F   | C        | 2.0×2.0      | FL    | T               |
| A9       | 63  | M   | R        | 2.0×3.0      | FL    | T               |
| A10      | 71  | F   | T        | 3.0×3.0      | FL    | V               |
| A11      | 29  | F   | S        | 0.8          | P     | T               |
| A12      | 56  | M   | A        | 0.7          | P     | T               |
| A13      | 29  | F   | A        | 0.7          | P     | T               |
| A14      | 40  | M   | S        | 1.0          | P     | T               |
| A15      | 75  | M   | A        | 1.2×0.8      | P     | T               |
| A16      | 70  | F   | R        | 1.5          | P     | T               |
| A17      | 66  | F   | S        | 1.0          | SP    | T               |
| A18      | 69  | M   | R        | 1.5          | P     | T               |
| A19      | 67  | F   | S        | 0.5          | P     | T               |
| A20      | 57  | F   | S        | 2.0×1.3      | P     | T               |
| A21      | 48  | M   | R        | 1.2          | P     | T               |
| A22      | 55  | M   | R        | 2.1×1.5      | SP    | T               |
| A23      | 59  | M   | S        | 1.0×1.5      | P     | T               |
| A24      | 54  | M   | S        | 1.2          | P     | T               |
| A25      | 89  | M   | S        | 5.0×6.0      | FL    | V               |
| A26      | 65  | M   | R        | 3.0×1.5      | SP    | TV              |
| A27      | 47  | F   | R        | 2.5          | FL    | TV              |
| H1       | 52  | M   | S        | 0.6          | SP    |                 |
| H2       | 52  | F   | S        | 3.0          | SP    |                 |
| H3       | 64  | F   | A        | 2.0          | SP    |                 |
| H4       | 69  | M   | R        | 0.5          | SP    |                 |
| H5       | 24  | M   | R        | 0.5          | SP    |                 |
| H6       | 58  | M   | D        | 0.4          | S     |                 |
| H7       | 50  | M   | A        | 0.4          | S     |                 |
| H8       | 45  | M   | S        | 0.6          | S     |                 |

Sex: M, male; F, female. Location: C, cecum; A, ascending colon; T, transverse colon; D, descending colon; S, sigmoid colon; R, rectum. Shape: P, pedunculated type; SP, semi-pedunculated type; FL, flat type. Pathological type: T, tubular adenoma; TV, tubulo-villous adenoma; V, villous adenoma.
Fig. 1. A mixed hyperplastic adenomatous polyp (M1). A. Focal cancer in MHAP. HE ×25. B. Marked staining on the bottom of crypts, sparse staining in the middle and upper zone of crypts for Ki67 in MHAP, and diffuse and strong staining for Ki67 in the focal cancer.
Table II. Mutation Analysis of \(K\)-\textit{ras}, \(APC\), \(p53\) for MHAPs, Adenomas and Hyperplastic Polyps

| Case no. | Examined lesion | \(K\)-\textit{ras} codon | mutation | \(APC\) codon | mutation | \(p53\) codon | mutation |
|----------|----------------|--------------------------|----------|----------------|----------|----------------|----------|
| M1       | MHAP           | ND                       | ND       | ND             | ND       | ND             | ND       |
|          | focal ca       | ND                       | ND       | ND             | ND       | 174            | CGC→CAC |
| M2       | MHAP           | ND                       | ND       | ND             | ND       | ND             | ND       |
| M3       | MHAP           | ND                       | ND       | ND             | ND       | ND             | ND       |
| M4       | MHAP           | ND                       | ND       | ND             | ND       | ND             | ND       |
| M5       | MHAP           | ND                       | ND       | ND             | ND       | ND             | ND       |
| M6       | MHAP           | ND                       | ND       | ND             | ND       | ND             | ND       |
| M7       | MHAP           | ND                       | ND       | ND             | ND       | ND             | ND       |
|          | syn ca         | ND                       | ND       | ND             | ND       | ND             | ND       |
| M8       | MHAP           | 12                       | GGT→TGT | ND             | ND       | ND             | ND       |
| M9       | MHAP           | ND                       | ND       | ND             | ND       | ND             | ND       |
| M10      | MHAP           | ND                       | ND       | ND             | ND       | ND             | ND       |
| M11      | MHAP           | ND                       | ND       | ND             | ND       | ND             | ND       |
| M12      | MHAP           | ND                       | ND       | ND             | ND       | ND             | ND       |
| A1       | ad             | ND                       | 1393     | CTT→CTT       | ND       | ND             | ND       |
| A2       | ad             | 12                       | GGT→GAT | ND             | ND       | 1370           | AAA→TAA |
| A3       | ad             | ND                       | ND       | ND             | ND       | ND             | ND       |
| A4       | ad             | 12                       | GGT→TGT | ND             | ND       | ND             | ND       |
| A5       | ad             | 12                       | GGT→GT  | ND             | ND       | ND             | ND       |
| A6       | ad             | ND                       | 1411     | AGT→AGG       | ND       | ND             | ND       |
| A7       | ad             | ND                       | ND       | ND             | ND       | ND             | ND       |
| A8       | ad             | 12                       | GGT→GAT | ND             | ND       | ND             | ND       |
| A9       | ad             | ND                       | 1309     | GAAAGAT→GAT   | ND       | ND             | ND       |
| A10      | ad             | 12                       | GGT→GAT | 1370           | AAA→TAA |
| A11      | ad             | ND                       | ND       | ND             | ND       | ND             | ND       |
| A12      | ad             | ND                       | ND       | ND             | ND       | ND             | ND       |
| A13      | ad             | ND                       | 1309     | GAA→TAA       | ND       | ND             | ND       |
| A14      | ad             | 12                       | GGT→GT  | ND             | ND       | ND             | ND       |
| A15      | ad             | ND                       | 1438     | AAA→ACA       | ND       | ND             | ND       |
| A16      | ad             | ND                       | ND       | ND             | ND       | ND             | ND       |
| A17      | ad             | ND                       | ND       | ND             | ND       | ND             | ND       |
| A18      | ad             | ND                       | ND       | ND             | ND       | ND             | ND       |
| A19      | ad             | 12                       | GGT→GAT | ND             | ND       | ND             | ND       |
| A20      | ad             | ND                       | 1438     | AAC→AACC      | ND       | ND             | ND       |
| A21      | ad             | 12                       | GGT→GAT | ND             | ND       | ND             | ND       |
| A22      | ad             | ND                       | 1376     | TATGT→GAT     | ND       | ND             | ND       |
| A23      | ad             | ND                       | 1363     | AAA→TAA       | ND       | ND             | ND       |
| A24      | ad             | 12                       | GGT→GT  | 1450           | CAG→GA  |
| A25      | ad             | 12                       | GGT→GAT | ND             | ND       | ND             | ND       |
| A26      | ad             | ND                       | ND       | ND             | ND       | ND             | ND       |
| A27      | ad             | ND                       | ND       | ND             | ND       | ND             | ND       |
| H1       | HP             | ND                       | ND       | ND             | ND       | ND             | ND       |
| H2       | HP             | ND                       | ND       | ND             | ND       | ND             | ND       |
| H3       | HP             | ND                       | ND       | ND             | ND       | ND             | ND       |
| H4       | HP             | ND                       | ND       | ND             | ND       | ND             | ND       |
| H5       | HP             | ND                       | ND       | ND             | ND       | ND             | ND       |
| H6       | HP             | ND                       | ND       | ND             | ND       | ND             | ND       |
| H7       | HP             | ND                       | ND       | ND             | ND       | ND             | ND       |
| H8       | HP             | 12                       | GGT→GCT | ND             | ND       | ND             | ND       |

\(ad\), adenoma; MHAP, mixed hyperplastic adenomatous polyp; HP, hyperplastic polyp; focal ca, focal cancer in MHAP; syn ca, synchronous cancer; ND, not detected; blank, not examined.
primers. The PCR products were separated by denaturing polyacrylamide gel electrophoresis.

RESULTS

Analysis of APC mutations and LOH The results of APC analysis in colorectal tumors are shown in Tables II and III. Ten of 27 (37.0%) adenomas showed somatic mutations but no mutation was identified in 12 MHAPs and 8 hyperplastic polyps.

No LOH of the APC locus was identified in any of three cases (M1, M7 and M12), using the two CA repeat microsatellite markers near the APC (Table III).

Analysis of K-ras mutations K-ras mutations were observed in only one of 12 (8.3%) MHAPs, while they were observed in 10 of 27 (37.0%) adenomas and in 1 of 8 (12.5%) hyperplastic polyps (Tables II and III).

Analysis of p53 mutations and LOH We performed an immunohistochemical study of M1 (carcinoma in MHAP) using anti p53 antibody (data not shown). The carcinoma lesion was strongly stained, but the surrounding MHAP lesion was hardly stained. Further, as shown in Fig. 2 and Tables II and III, p53 mutation was identified only in the focal carcinoma, not in the surrounding MHAP portion. LOH of p53 was identified in the cancer, but not in the surrounding MHAP of M1. In M7, neither p53 mutation nor LOH was identified in the benign portion of the MHAP or in the synchronized cancer (Tables II and III), and in M12, no LOH of p53 was identified in the MHAP.

DISCUSSION

In order to determine the role of the APC gene in MHAPs, we examined MHAPs, adenomas and hyperplastic polyps. It has been reported that 32 to 75% of somatic mutations of adenomas occur in the MCR. Therefore, we examined the MCR in this study. However, no APC mutation was identified in 12 MHAPs. It is likely that adenomatous appearance is not always related to APC gene mutation, and MHAPs may involve mutations other than APC gene mutation.

In our study, the frequency of K-ras mutation in MHAPs was less than that of adenomas. Jen et al. found K-ras mutations in 22% of hyperplastic polyps and in 25% of adenomas. Thus, K-ras mutation may be implicated in MHAP, but K-ras is not a key gene for MHAP formation.

We also examined p53 mutations in two cases where MHAP was associated with cancer (M1 and M7). p53 mutation was identified only in the cancerous portion in M1, in which the cancer appeared to have developed within the MHAP, not in the benign MHAP portions. Thus, p53 may play an essential role in the transformation of MHAP to carcinoma.

Recently, Allen proposed that colorectal carcinogenesis occurs through the LOH pathway and the RER pathway. The LOH pathway is followed by APC inactivation, and a typical disease is familial adenomatous polyposis. The RER pathway is initiated by an inherited or somatic mutation within one of the DNA mismatch repair genes such as hMSH2, hMLH1, hPMS1, hPMS2 and hMSH6.
References

1) Hamilton, S. R. The molecular genetics of colorectal neoplasia. *Gastroenterology*, **105**, 3–7 (1993).

2) Jacoby, R. F., Marshall, D. J., Kailas, S., Schlack, S., Harms, B. and Love, R. Genetic instability associated with adenoma to carcinoma progression in hereditary nonpolyposis colon cancer. *Gastroenterology*, **109**, 73–82 (1995).

3) Allen, J. I. Molecular biology of colorectal cancer: a clinician’s view. *Prospect Colon Rectal Surg.*, **8**, 181–202 (1995).

4) Miyoshi, Y., Nagase, H., Ando, H., Horii, A., Ichii, A., Nakatsuru, S., Aoki, T., Miki, Y., Mori, T. and Nakamura, Y. Somatic mutations of the APC gene in colorectal tumors: mutation cluster region in the APC gene. *Hum. Mol. Genet.*, **4**, 229–233 (1992).

5) Jen, J., Powell, S. M., Papadopoulos, N., Smith, K. J., Hamilton, S. R., Vogelstein, B. and Kinzler, K. W. Molecular determinants of dysplasia in colorectal lesions. *Cancer Res.*, **54**, 5523–5526 (1994).

6) Longacre, T. A. and Fenoglio-Preiser, C. M. Mixed hyperplastic adenomatous polyps/serrated adenomas. *Am. J. Surg. Pathol.*, **14**, 524–537 (1990).

7) Jeevaratnam, P., Cottier, D. S., Browett, P. J., van De Water, N. S., Pokos, V. and Jass, J. R. Familial giant hyperplastic polyps in terms of APC and K-ras mutational analysis. The mean ages of the MHAP group and the hyperplastic polyt group were slightly different at 56.9±8.4 and 51.8±12.8 (Student’s t test; P=0.05), respectively. The mean size of MHAPs was 1.3±0.68 cm and that of hyperplastic polyps was 0.9±0.9 cm (Student’s t test; P>0.05). These data are consistent with the idea that hyperplastic polyps may develop into MHAP. A clonal change may originate in a hyperplastic polyp and progress into MHAP, or an admixed hyperplastic polyp may be a transient lesion in the formation of an MHAP. By examining the gene alterations of MHAPs, hyperplastic polyps and admixed hyperplastic polyps, the question may be solved.

Although MHAP is rare, analysis of gene abnormalities associated with this tumor may reveal a novel pathway from normal mucosa to neoplastic lesions. In view of the possibility that large MHAPs, hyperplastic polyps and hyperplastic polyposis may become malignant, it should be considered whether treatment is appropriate.

Acknowledgments

We thank Dr. Shinichi Nakamura for his advice and critical review, Prof. Toshio Ohki for his critical review, and Mr. Norio Suzuki for his technical assistance.

(Received October 24, 1997/Revised December 15, 1997/ Accepted December 16, 1997)
13) 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).

14) Spirio, L., Joslyn, G., Nelson, L., Leppert, M. and White, R. A CA repeat 30–70 KB downstream from the adenomatous polyposis coli (APC) gene. *Nucleic Acids Res.*, 19, 6348 (1991).

15) Wijnen, J., Tops, C., Breukel, C., Leeuwen, C. V., Goverde, A., van Der Kliff, H., Fodde, R. and Khan, P. M. CA repeat polymorphism from YAC JW25 at the D5S318 locus, distal to adenomatous polyposis coli (APC). *Nucleic Acids Res.*, 19, 6965 (1991).

16) Jones, M. H. and Nakamura, Y. Detection of loss of heterozygosity at the human TP53 locus using a dinucleotide repeat polymorphism. *Genes Chromosom. Cancer*, 5, 89–90 (1992).

17) Miyaki, M., Konishi, M., Kikuchi Yanoshita, R., Enomoto, M., Igaru, T., Tanaka, K., Muraoka, M., Takahashi, H., Amada, Y., Fukayama, M., Maeda, Y., Iwama, T., MISHIMA, Y., MOni, T. and Koike, M. Characteristics of somatic mutation of adenomatous polyposis coli gene in colorectal tumors. *Cancer Res.*, 54, 3011–3020 (1994).

18) Vogelstein, B., Fearon, E. R., Hamilton, S. R., Kern, S. E., Preisinger, A. C., Leppert, M., Nakamura, Y., White, R., Smits, A. M. M. and Bos, J. L. Genetic alterations during colorectal-tumor development. *N. Engl. J. Med.*, 319, 525–532 (1988).

19) Urbanski, S. J., Marcon, N., Kossakowska, A. E. and Bruce, W. R. Mixed hyperplastic adenomatous polyps—an underdiagnosed entity. *Am. J. Surg. Pathol.*, 8, 551–556 (1984).