Association of APC Gene Mutations and Histological Characteristics of Colorectal Adenomas

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ABSTRACT

Fifty-nine colonic adenomas and 6 hyperplastic colonic polyps were analyzed by single-strand conformation polymorphism analysis for mutations in the adenomatous polyposis coli gene (APC). Frameshifts and premature stop codons in at least one copy of APC were detected in 25 of these adenomas. Five adenomas carried 2 APC mutations. No mutations in APC were found in any of the 6 hyperplastic polyps. The detection of APC mutations increased with size and degree of dysplasia and in rectal as compared to colonic adenomas, although the association was not statistically significant. The frequency of detectable APC mutations was higher in tubulovillous and villous adenomas (10 of 13) than in tubular adenomas (15 of 45) (odds ratio, 6.67; 95% confidence limits, 1.39–41.83; P = 0.005). The significance of the association between the detection of APC mutations and a villous architecture was confirmed in multivariate analysis (relative risk, 6.67; 95% confidence limits, 1.54–28.8; P = 0.005). In conclusion, APC mutation plays a role in adenoma progression; its frequency is significantly higher in lesions with a more villous morphology.

INTRODUCTION

The majority of colorectal carcinomas arise through the malignant conversion of adenomatous polyps (1). It has been shown that large adenomas and those with a villous morphology are more likely to contain cancer and, by inference, have a greater malignant potential (2, 3). The frequency with which a villous growth pattern is observed usually increases with the size of the tumor (1). These observations suggest that, usually, the emergence of the villous phenotype is not an early event in the sequence of normal mucosa to adenoma. However, the grade of dysplasia is important in placing a lesion in the adenoma-to-carcinoma progression (1, 4). A genetic model of colorectal tumorigenesis has proposed that K-ras and chromosome 5q gene mutations occur early in adenoma formation, while chromosome 17p and 18q alterations are late events (5). At present, the biological relationships among these mutational events are unclear, although accumulation rather than order seems to be important.

The cloning of the APC3 gene (APC) on chromosome 5q (6–9), mutations in which are responsible for familial APC (10), has allowed the evaluation of this gene in the development of sporadic colorectal tumors. There have been 2 reports (11, 12) of a high frequency of APC mutation in sporadic adenomas (5 of 8 and 10 of 16, respectively) and adenocarcinomas (33 of 43 and 15 of 25, respectively) of the colon, confirming that mutations of APC play a role in the early stages of colorectal tumorigenesis. The vast majority of these somatic APC mutations introduce a premature termination signal into the open reading frame of the gene. More than half of these mutations cluster within a small region of APC that represents <10% of the coding sequence. This region is designated “MCR” for “mutation cluster region” (11).

Many tumors in which APC mutations were found carried more than one APC mutation, implying that alterations of both alleles had occurred. This was shown either by the presence of a second unique APC sequence alteration or as LOH. Ichii et al. (13) found that 5 of 7 small adenomas from the same APC patient (in whom the germ line APC mutation had been identified) had lost the normal allele. They concluded that inactivation of both APC alleles is essential for the development of an early-stage adenoma. More recently, they reported somatic APC mutations in 22 of 65 adenomas from 6 APC patients; the frequency of these somatic APC mutations was the same regardless of size or histopathological classification (14). In contrast, Miyaki et al. (15) reported that LOH of 5q markers is low (<2%) in “moderate adenomas” and increases in “severe adenomas” (20%), intramucosal carcinomas (26%), and invasive carcinomas (52%). These authors suggested that heterozygosity for mutation of APC is associated with mild or moderate adenomas and that the loss of the normal allele is associated with a change from a moderate to a severe adenoma.

The function(s) of the APC protein is (are) unknown, although the cellular localization of the APC protein is cytoplasmic (16). It has been shown in vitro that some domains of the APC protein can form homodimers (16). Also, some truncated APC proteins can be associated with wild-type protein (17), suggesting a dominant negative ability for mutant APC protein. A model similar to that for p53 mutations in tumors can be proposed, in which the initial reduction of normal APC protein due to, the formation of wild-type/mutant complexes, confers growth advantage and then is followed by loss of all normal protein through LOH (18). Western blot analysis has revealed that 24 of 32 colon carcinoma cell lines contain truncated APC proteins, while 27 of 32 contain no full-length protein (16).

In this study, the association of APC mutation with known predictors of carcinoma in an adenoma (size, type, and degree of dysplasia) was investigated. Fifty-nine adenomas and 6 hyperplastic polyps were evaluated for mutation by SSCP analysis. In 8 cases, areas with different degrees of dysplasia and/or early cancer were analyzed independently. We observed a significant association between the detection of APC mutation and a villous architecture in this sample group.


MATERIALS AND METHODS

Tissue Samples. Biopsies from 60 patients (39 males, 21 females; median age, 63 years; range, 33–88 years) with 59 sporadic colorectal adenomas and 6 hyperplastic polyps were collected during endoscopy. In 33 cases, tissue from normal rectal mucosa and/or peripheral blood were collected. In 4 cases, 2 synchronous adenomas from the same patient were analyzed. In one case, one hyperplastic polyp and one adenoma from the same patient were analyzed.

The entire lesion was used for DNA extraction in all hyperplastic polyps and in adenomas up to 6 mm in size (n = 17); 3 random forceps biopsy samples were taken from the head of polyps up to 10 mm (n = 6), and 5 samples were taken from those larger than 10 mm (n = 28). No attempt to isolate different components (tubular and villous) by microdissection was made. In addition, in 8 of 12 adenomas containing invasive carcinoma, histologically guided samples were taken from the selected sectors corresponding to adenoma tissue with low-grade dysplasia, high-grade dysplasia, and/or early cancer, regardless of histological type.

Histological type was defined according to World Health Organization criteria (19) as tubular (branching neoplastic tubules occupying at least 80% of the tumor), villous (at least 80% of the tumor composed of leaf- or finger-like processes of lamina propria covered by epithelium), and tubulovillous (each architectural configuration contributing to >20% of tumor mass). Dysplasia (low grade or high grade) and the presence of infiltrating adenocarcinoma were defined according to World Health Organization criteria (19).

DNA was extracted from fresh tumor specimens and corresponding fresh normal rectal tissue using standard methods (20).

SSCP Analysis. Oligonucleotide primer pairs used for polymerase chain reaction-SSCP analysis of the APC-coding region have been published (6, 21). DNA samples were amplified with polymerase chain reaction and subjected to SSCP analysis according to the method of Varesco et al. (22).

The entire APC-coding region was characterized in 32 adenomas; in the remaining cases, 35% of the coding region (nucleotide position 1960–4923), including the mutation cluster region, was analyzed (11).

Sequencing of SSCP Conformers. SSCP conformers were sequenced with the dideoxy termination method as previously described (20).

Statistical Analysis. The association between the presence of APC mutation and each of the clinical and pathological characteristics of the adenomas was evaluated using the OR. The 95% exact confidence limits were calculated (23). Tests of significance were based on the χ² test for heterogeneity (or the Fisher test, when appropriate) and on the χ² for trend (24). In order to identify those covariates that were independently associated with the presence of APC mutations in an adenoma, a multivariate logistic regression model was fitted to the data. This allowed the simultaneous adjustment for the confounding effects of the clinical and pathological characteristics. All covariates were categorical, and the lowest level of each was used as the reference stratum (25).

For the definition of site, large intestine was classified as rectum, distal colon (up to the splenic flexure), and proximal colon (proximal to the splenic flexure).

RESULTS

SSCP analysis of the APC gene in 26 of 59 colorectal adenomas showed bands with altered mobilities due to APC mutations, as confirmed by sequence analysis. Five contained 2 different mutations (it is unknown whether these are on the same or different APC alleles). No mutations were found in 6 of 6 hyperplastic polyps. In general, sequence alterations were similar to those reported previously: nonsense mutations, n = 10; insertions, n = 4; deletions, n = 15; and duplications, n = 1 (Table 1). In addition, a 3-base pair, in-frame deletion at nucleotide position 5427 was found in case 595.

The somatic origin of mutations was confirmed in all but one sample in the 33 cases for which DNA from normal rectal mucosa or peripheral blood was available. In case 595, both normal mucosa and peripheral blood DNAs carried the same 3-base pair, in-frame deletion that was found in the adenoma. Because the functional significance of this germ line sequence alteration is uncertain, this case was excluded from all subsequent statistical analyses, leaving 25 of 58 cases with APC mutations.

<table>
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<tr>
<th>Sample</th>
<th>Type/ dysplasia</th>
<th>Size (mm)</th>
<th>Mutation</th>
<th>Nucleotide position</th>
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</table>

* T, tubular; TV, tubulovillous; V, villous; L, low grade; H, high grade; AC, adenocarcinoma.

† Previously described (28).

‡ Degree of dysplasia corresponding to the most severe degree in the entire adenoma.

§ BP, base pair; DUPL, duplication.

Chain-terminating mutations were found in 14 of 32 adenomas in which the entire coding region was examined.

Areas with different degrees of dysplasia and/or adenocarcinoma were examined separately in 8 malignant adenomas. APC mutations, when present (3 cases), were detected in all areas examined (Table 2).

Major clinical and pathological parameters in this series of cases were evaluated. Larger adenomas were more likely to be tubulovillous or villous (P = 0.0002) and were located more frequently in the rectum (P = 0.005). High-grade dysplasia and the presence of adenocarcinoma was observed more frequently in these adenomas (P = 0.02 and P = 0.0002, respectively) as well as in all of those removed from the rectum (P = 0.005; data not shown).

The frequency of detectable APC mutations is shown in Table 3 and is correlated with other characteristics of these adenomas. An increase in the frequency of detectable APC mutations was observed as size and degree of dysplasia increased. However, this trend was not statistically significant. In addition, an increase in detectable mutations was observed in rectal adenomas relative to colonic adenomas. APC mutations were detected in 15 of 45 tubular adenomas and in 10 of 13 tubulovillous or villous adenomas (OR, 6.67; 95% CI, 1.39–41.83; P = 0.005). Seven of 12 adenomas containing carcinoma showed APC mutations. When all characteristics were fitted to a multiple logistic regression model, the association between histology and detectable APC mutations was confirmed (relative risk, 6.67; 95% CI, 1.54–28.8; P = 0.005).
A similar result was found in univariate and multivariate analyses when only the 32 cases, in which the entire APC-coding region was examined for mutations, were analyzed (OR, 6.00; exact 95% CL, 0.91—39.6; P = 0.09 and relative risk, 6.00; 95% CL, 0.91—39.6; P = 0.09). This could be explained by a different distribution of the histopathological characteristics in our series of adenomas. Alternatively, it could be due to a lower sensitivity of SSCP analysis as compared with either RNase protection analysis (11) or sequence analysis (12). However, this analysis confirms that a relatively high number (5 of 16) of diminutive adenomas (up to 5 mm in size) contain APC mutations. These results indicate that APC mutation is an early event in some sporadic colorectal tumors.

Our data show that adenomas with a villous component have an increased probability of carrying detectable APC mutations as compared to tubular adenomas (Table 3). A trend toward this increased frequency of detectable APC mutations is seen also as adenoma size and dysplasia increase. In adenomas of APC patients, Ichii et al. (14) found the same frequency of somatic APC mutations regardless of histopathological characteristics. This discrepancy could be due to the fact that the progression of APC adenomas follows an uniform pathway as compared to sporadic ones. In the latter, different pathways may be responsible for tumorigenesis, as has been suggested by Thibodeau et al. (26) and Ionov et al. (27). Alternatively, a villous component of an adenoma could carry 2 or more APC mutations giving a greater likelihood of detecting, by SSCP, at least one of them.

In 5 adenomas of our series, 2 different APC somatic sequence alterations were detected (Table 1). The possibility that 2 somatic changes occurred at the same allele was not ruled out. These 5 adenomas were more advanced lesions: 2 adenomas were 14- and 15-mm tubular lesions, one was a 25-mm villous adenoma, and 2 were 7- and 10-mm malignant adenomas. These data indicate that inactivation of the second APC allele by sequence alteration is uncommon in early-stage sporadic lesions. However, a small subset of early adenomas should be present that carry 2 APC mutations if the work of Ichii et al. (13, 14) is correct. Again, this discrepancy could be explained by the existence of a larger spectrum of carcinogenetic pathways in sporadic as compared to APC adenomas. Alternatively, it could be due to an underestimation of APC mutations using SSCP.

Finally, in 8 adenomas in which regions of adenocarcinoma as well as low- or high-grade dysplasia were separately analyzed (Table 2), APC mutations, when present, were detected in all areas examined. According to previous published data, the role of APC mutation in the non-APC adenoma-carcinoma sequence can be explained by 2 alternate hypotheses: (a) APC mutations, in colorectal tumorigenesis, are early events that correlate with adenoma formation (12, 13, 14). The concordance for the presence of APC mutations among regions with different degree of dysplasia in the same adenoma reported in this study (Table 2) is in agreement with this hypothesis; (b) APC mutations occur early or late in tumorigenesis; accumulation rather than order is important during progression to late adenoma stages (5). The frequency of APC mutations in our series increased with size, villous component, and degree of dysplasia but was almost the same in villous and malignant adenomas. This finding, although inconclusive because of the small number of cases, favors the second hypothesis.

In conclusion, our data suggest that APC mutations confer a growth advantage to small sporadic adenomas through villous component expansion and that second mutational events at the APC locus are not common in early sporadic adenomatous lesions. This suggests the hypothesis that a high proportion of sporadic adenomas follows a different tumorigenic pathway as compared to APC adenomas.

REFERENCE


APC MUTATIONS IN SPORADIC COLORECTAL ADENOMAS


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