High frequency of K-ras mutations in human colorectal hyperplastic polyps

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Abstract

Background—Hyperplastic polyps are common benign colorectal polyps, and are thought to have little association with malignant tumours in the colorectum. However, several reports suggest that some hyperplastic polyps may develop into colorectal neoplasms.

Aim—To clarify genetic alterations in colorectal hyperplastic polyps.

Methods—Twenty eight colorectal polyps having serrated components were resected from patients endoscopically. The K-ras gene mutations in codons 12 and 13 were analysed by PCR-RFLP. Intracellular p53 protein was immunostained by the avidin-biotin complex method.

Results—A mutation of the K-ras gene was detected in nine (47%) of 19 hyperplastic polyps, and five (56%) of nine adenomas. p53 protein nuclear accumulation was detected immunohistochemically in two (22%) of nine adenomas, but not in any of the 19 hyperplastic polyps.

Conclusion—Some hyperplastic polyps may be true neoplastic lesions, and could be precursors of malignant neoplasia.

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Keywords: hyperplastic polyps, K-ras, adenoma, colon, colorectal carcinogenesis.

Colorectal hyperplastic polyps are not generally regarded as neoplastic or premalignant lesions in the colon and rectum, and are not thought to have any malignant potential. However, coexisting adenomatous areas and hyperplasia in the same polyp have been reported.¹⁻¹⁰ In addition, hyperplastic polyps are more common in colons harbouring adenomas or carcinomas. This previous evidence suggests that hyperplastic polyps are related to colorectal neoplasms, and in certain cases, polyps may progress to cancer, following the so-called hyperplasia-adenoma-adenocarcinoma sequence.⁵⁻⁷ By contrast, the adenoma-adenocarcinoma sequence is the major pathway of colorectal carcinogenesis, and is thought to be associated with the accumulation of mutations or deletions both in oncogenes and in tumour suppressor genes.¹¹ Some mutations of various oncogenes and tumour suppressor genes may have already occurred, even in hyperplastic polyp-like early adenomas in the adenoma-adenocarcinoma sequence.¹¹ To date, however, no mutation of these genes has been reported in hyperplastic polyps. To clarify the genetic changes in hyper-

plastic polyps, we evaluated K-ras mutations and nuclear accumulation of p53 protein, which are common in colorectal adenomas and adenocarcinomas.

Methods

PATIENTS AND SPECIMENS

Twenty eight colorectal epithelial polyps were resected from patients undergoing colonoscopy during routine clinical practice at the National Cancer Center Hospital East from 1992 to 1995. All specimens were fixed with 10% buffered formalin. The specimens were stained with Carszsi's haematoxylin for light stereomicroscopy. The pit patterns were classified according to Kudo's classification,¹² and then embedded in paraffin wax. Each specimen was cut into 3 µm sections, and stained with haematoxylin and eosin. Histological diagnoses were made using the World Health Organisation classification.¹³

PREPARATION OF DNA SAMPLES

Based on the histopathological findings in each case, polyp tissue was cut from three serial 5 µm thick sections, and after deparaffinisation with xylene and ethanol, the genomic DNA was extracted from the polyp tissue by serial digestions with proteinase K and RNase A.¹⁴ To prevent contamination, we used a new needle to microdissect every time. A sample of 1-2 µl of the DNA solution was used as a template for the polymerase chain reaction (PCR).

ANALYSIS OF K-ras MUTATION

Mutations in K-ras (codons 12 and 13) were screened by mismatched primer mediated PCR amplification for 40 cycles, followed by restriction fragment length polymorphism (RFLP) analysis using specific restriction endonucleases (PCR-RFLP) detected as described previously.¹⁴ For codon 12, PCR products encoding the wild type and mutant sequences were distinguished as 114 base pair (bp) and 143 bp fragments, respectively, by digestion with the restriction enzyme MvaI (Takara, Kyoto, Japan). For codon 13, the wild type was cleaved into 125 bp and 32 bp fragments by the restriction enzyme BglI (Takara, Kyoto, Japan), whereas the PCR product having the mutant sequence could not be digested by this enzyme. Human placental DNA (Sigma, St Louis, MO, USA) was used as a wild type control. And mutant DNA in codon 12 derived from the human colon adenocarcinoma cell line

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SW480 (American Type Culture Collection, Rockville, MD, USA) and DNA samples extracted from colorectal carcinomas, which were known to harbour a heterozygous point mutation at K-ras codon 13 (GGC to GAC; data not shown) were used as mutant controls. We always used aerosol resistant tips for PCR to prevent contamination. The mixtures with several ratios of mutant (SW480 DNA) to wild type (human placental) DNA were amplified by PCR to determine the sensitivity for detecting mutated K-ras gene. Mutation in K-ras is detectable by this method when mutated DNA comprised more than 10% of genomic DNA.

DETECTION OF p53 PROTEIN

All polyps were assessed for nuclear accumulation of p53 protein. Sections were deparaffinised, heated in a hot water bath at 90°C for 20 minutes in phosphate buffered saline (PBS), and then stained using a 1:1000 dilution of rabbit polyclonal antibody to human p53 protein, RSP53 (Nichirei, Tokyo, Japan), by the avidin-biotin-peroxidase complex method. Human lung carcinoma tissue, known to contain p53 protein, was used as a positive control (data not shown). When all epithelial cells of one crypt or more than 20% of 500 epithelial cells in a section were stained with the antibody, the polyp was scored as p53 positive.

STATISTICAL ANALYSES

Statistical analysis was performed using computer assisted Student's *t* test with Stat View software for the Macintosh computer, version 4.0 (Abacus Concepts, Berkeley, CA, USA). A value of *p*<0.05 was considered significant.

Clinicopathological data

Case	Age	Sex	Site of polyp	Size (mm)	Type of pit	K-ras mutation (codon)
Hyperplastic polyp:						
1	64	M	A	6	a	12
2	61	F	A	8	a	12
3	57	M	R	5	c	-
4	71	F	A	6	a	12
5	51	F	A	5	a	-
6	43	M	R	4	a	12
7	67	M	R	2	a	-
8	81	F	A	4	a	12
9	67	M	S	4	a	12
10	43	M	T	8	a	13
11	41	M	R	5	a	-
12	71	M	A	4	a	-
13	60	M	R	6	a	-
14	74	M	C	4	a	-
15	59	M	R	6	a	-
16	61	M	R	7	a	12
17	77	F	R	4	a	12
18	70	M	R	3	a	-
19	46	M	R	4	c	-
Adenoma:						
1	68	M	R	24	g	12
2	52	M	C	10	a	12
3	79	M	R	4	g	-
4	67	M	R	7	a+g	12
5	45	F	R	8	o	12
6	76	M	R	4	g	-
7	56	F	R	10	a	12
8	66	M	S	5	o+g	-
9	49	M	R	2	a	-

C=caecum; A=ascending colon; T=transverse colon; S=sigmoid colon; R=rectum; a=small and large asteroid pits; o=oval pit; g=gyrus-like pit; c=cerebriform surface.

Results

The Table shows the clinicopathological characteristics of 28 colorectal polyps. The mean age of patients (21 men and seven women) was 61 years (ranging from 43 to 81 years). The incidence of polyps was higher in the rectum than in the colon. Histologically, 19 of 28 colorectal polyps were diagnosed as hyperplastic polyps, and nine of 28 as adenomas. Some hyperplastic polyps showed slightly elongated and stratified nuclei (Figure), but they could not be diagnosed as serrated adenomas. Seventeen hyperplastic polyps showed small or large asteroid pits. However, two hyperplastic polyps did not fit into Kudo's classification, and were tentatively called "cerebriform". Adenomas showed various pit patterns: asteroid, oval, gyrus-like pit, and a composite type (Table). All but the adenoma in case 1 were no more than 10 mm in diameter. The size of the adenomas (8.2 (SD) 6.5 mm) was significantly larger than that of hyperplastic polyps (5.0 (1.6 mm); *p*<0.05). On PCR-RFLP analysis, K-ras mutations in codon 12 were detected in eight (42%) of 19 hyperplastic polyps and five (56%) of nine adenomas; a K-ras mutation in codon 13 was detected in one (5%) of 19 hyperplastic polyps; and no mutation in codon 13 was detected in adenomas. The total frequency of K-ras mutations was nine (47%) of 19 hyperplastic polyps and five (56%) of nine adenomas. Accumulation of p53 protein in the nuclei was found in two (22%) of nine adenomas (cases 1 and 4); this was not found in any of the 19 hyperplastic polyps. APC gene mutations were searched for in the other seven colorectal hyperplastic polyps, but none was found.

Discussion

It is generally accepted that hyperplastic polyps are benign lesions without malignant potential and do not develop into colorectal neoplasms. Our study is the first to identify genetic alterations (K-ras mutations) in hyperplastic polyps of the colon and rectum. Similarly, K-ras mutation occurs in the hyperplastic foci of the pancreatic duct and hyperplastic aberrant crypt foci (ACF) of the colorectal epithelium, which are thought to be early precursor lesions of pancreatic and colorectal cancer.¹⁵⁻¹⁹ K-ras gene mutations may be responsible for the hyperplastic change, and may not contribute to immediate carcinogenesis of the pancreas and colorectum. However, K-ras mutations were identified in colorectal adenomas and adenocarcinomas, and are thought to play an important part in colorectal carcinogenesis.¹¹ In this study, the frequency of K-ras mutation in hyperplastic polyps was similar to that of adenomas. Furthermore, hyperplastic polyps show positive staining for carcinoembryonic antigen (CEA) with greater intensity than the normal epithelium, increased bcl-2 expression on immunohistochemistry, and reduced secretion of O-acetylated sialomucin, which are important features of colorectal neoplasia.²⁰⁻²² Therefore, some hyperplastic polyps may be neoplastic lesions, and develop into colorectal tumours.

Hyperplastic polyps: microscopic views of vertical axis, stained with haematoxylin and eosin (original magnification A, B $\times$ 100, C $\times$ 200). Case 9 (A) harbours a K-ras mutation, case 19 (B, C) has no K-ras mutation.

This transition from hyperplastic polyps to colorectal tumours is supported by previous reports based on histological findings.¹⁻¹⁰ The coexistence of hyperplastic, adenomatous, or carcinomatous components in the same polyps was shown in these reports. None of the hyperplastic polyp nuclei contained accumulated p53 protein. The p53 gene mutation may be responsible for carcinogenesis of the colon and rectum at a late stage. Moreover, there was

no APC mutation present in seven colorectal hyperplastic polyps. In addition to K-ras mutation, some unknown genetic alterations are probably needed for hyperplastic polyps to develop into colorectal neoplasms. In this study, there was no difference in size between hyperplastic polyps with K-ras mutation and those without. We investigated the relation between hyperplastic polyps harbouring K-ras mutation and their pit patterns. There was no

correlation between K-ras mutation and the pit pattern. Almost all of the hyperplastic polyps contained small or large asteroid pits. Therefore, by endoscopy hyperplastic polyps harbouring K-ras mutation were indistinguishable from those without K-ras mutation. Histologically, slight differences in the nuclear morphology of cryptic cells were found in hyperplastic polyps (Figure). A precise analysis of the histological features in hyperplastic polyps is needed. Hyperplastic polyps have not been regarded as precancerous lesions, because there was no evidence that the lesions were directly linked to colorectal neoplasia. However, it is now clear that about half of all hyperplastic polyps harbour K-ras mutation, and it is necessary to examine thoroughly the proposed hyperplasia-adenoma-adenocarcinoma sequence.

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