ONCOLOGY REPORTS 1: 547-549, 1994

Activating mutations in the K-*ras* gene in ulcerative colitis and Crohn's disease

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Received February 10, 1994; Accepted February 28, 1994

Abstract. Patients with ulcerative colitis (UC) and Crohn's disease have an increased risk for developing cancer of the colon. Mutations in the K-*ras* gene are relatively frequent in specimens from patients with sporadic colon cancer, but less frequent in cases of cancer complicating ulcerative colitis. In order to study the problem further we used the polymerase chain reaction (PCR) technique followed by a restriction fragment length polymorphism (RFLP) assay, to detect mutations at codon 12 of K-*ras* in biopsy specimens from patients with UC or Crohn's disease. Six among 27 patients (22.2%) with UC and 2 of the 19 patients (10.5%) with Crohn's disease examined, carried a mutation at codon 12 of K-*ras*. Our results indicate that mutations in K-*ras* may be a genetic marker that would reveal the predisposition to colon cancer among this group of patients.

Introduction

Ulcerative colitis (UC) and Crohn's disease are inflammatory disorders which predispose patients for the development of colon cancer (1). The risk for the development of cancer is 20-30 fold elevated in patients with UC for 10-20 years, when compared to the general population. Crohn's disease exhibits a lower risk, estimated to be about one third of the rate found in UC (1,2).

Histologically, UC is associated with a dysplasia that may expand to affect large areas of the mucosa, in which multiple aneuploid cell clones coexist. A subpopulation of one of these clones has been proposed to give rise to an invasive carcinoma (1).

Genetic alterations such as *ras* and p53 mutations, allelic loss at loci on chromosomes 5q, 17q and 18q at relatively high frequences, have been reported in colon cancer (3). Some of these disorders are reported to be related etiologically to the promotion of the malignancy. It would be very helpful to determine whether some specific genetic alterations are related to the pathogenesis of invasive carcinoma in patients with UC or Crohn's disease and map the preneoplastic cells in the area of the dysplasia.

K-*ras*, H-*ras* and N-*ras* oncogenes are members of the *ras* family of genes. The three genes code for structurally and immunologically related proteins of 21 kD, known as *ras* p21. *ras* p21 is located on the inner side of the plasma membrane, possesses GTPse activity and is involved in a signal transduction pathway (4). Transforming mutations in *ras* genes are found in about 50% of the human epithelial neoplasms and they have a crucial role in the progression of the malignancy.

K-*ras* mutations are relatively frequent in colon cancer and in UC and Crohn's disease they have been reported to exist in various frequencies (5-7). The aim of the present study was to determine whether K-*ras* mutations play a role in the pathogenesis of invasive carcinoma in UC and Crohn's disease. We detected K-*ras* mutations at codon 12 in 22.2% of patients with UC and 10.5% in patients with Crohn's disease.

Materials and methods

Clinical specimens. Paraffin-embedded tissue sections from 27 biopsies from ulcerative colitis and 19 from Crohn's disease, were obtained from the Department of Gastroenterology of the University Hospital, Heraklion, Greece. Lesions with dysplasia or cellular atypia are shown in Table I (ulcerative colitis) and Table II (Crohn's disease).

DNA extraction. Three 10 μ thick sections from paraffin-embedded tissue were put in 1.5 ml Eppendorf tubes and 400 μl digestion buffer containing 100 mM NaCl, 10 mM Tris, 25 mM EDTA and 0.5% SDS was added. Samples were incubated with 100 mg/ml proteinase K in 60°C for 1 h and then extracted twice with equal volume of phenol:chloroform (1:1) and once with chloroform. Supernatants were transferred into fresh tubes and DNA was precipitated with 2.5 volumes of 100% ethanol and 1/20 volume of 5 M NaCl. DNA was recovered with centrifugation for 15 min at 4°C, washed twice with ice cold 70% ethanol dried and resuspended in 20 μl distilled water.

Oligonucleotide primers and PCR amplification. The oligonucleotide primers used for K-*ras* codon 12 and codon

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Key words: K-*ras* oncogene, ulcerative colitis, Crohn's disease

Table I. Cumulative data for ulcerative colitis.

Patient No.	K-*ras* mutation codon 12	K-*ras* mutation codon 13	High-grade dysplasia	Cellular atypia
1	-	-		
2	-	-		
3	-	-	+	
4	+			
5	-	-		
6	-	-		+
7	+			+
8	-	-		
9	-			
10	-	-	+	
11	-	-		
12	-			
13	-		+	
14	-	-		+
15	-			+
16	-	-		
17	-	-	+	
18	-		+	
19	-	-	+	
20	-			
21	-	-		
22	-	-		
23	-			
24	+	-		
25	+/+			
26	+			
27	+			

Table II. Cumulative data for Crohn's disease.

Patient No.	K-*ras* mutations at codon 12	High-grade dysplasia	Cellular atypia
1	-		+
2	-		
3	-		
4	-		
5	-		
6	-	+	
7	-		
8	-	+	
9	-		
10	-		
11	-		
12	-	+	
13	-		
14	+		
15	-	+	
16	-		+
17	+	+	
18	-		
19	-		

13 were as follows: 5' end primer-5' ACTGAATAT AAACTTGTGGTAGTTGGACCT3' and 3' end primer-5' TCAAAGAATGGTCCTGGACC3' (8). 2 µl of the extracted DNA of each sample was amplified in a volume of 50 µl reaction mixture as follows: The reaction solution contained 35 mM $MgCl_2$, 100 mM Tris HCl pH 8.3, 500 mM KCl, 0.1% gelatin, 200 µM of each dNTP, 500 ng of each primer and 1.25 U Taq polymerase (Gibco BRL). Amplification parameters were: Denaturation at 94˚C for 40 sec, primer annealing at 60˚C for 45 sec and extention at 72˚C for 50 sec, increasing the extention time by 1 sec per cycle, up to 40 cycles.

RFLP analysis. 10-20 µl aliquots of the amplification products were digested for 3 h with 20 U *Bst*N1 in 60˚C for codon 12 and another 10-20 µl were digested overnight with *Hph*1 in 37˚C for codon 13. The enzymes were purchased from New England Biolabs. Digestion products were electrophoresed through a 2% agarose gel, stained with ethidium bromide and photographed on a UV light transilluminator.

Results

We analyzed 27 specimens from patients with chronic ulcerative colitis (UC) and 19 specimens from patients with Crohn's disease for the presence of K-*ras* mutations employing a PCR-RFLP analysis. Genomic DNA from the colon carcinoma cell line SW480, which is homozygous for a codon 12 K-*ras* mutation, was used as a positive control. Our results are summarised in Table I (ulcerative colitis) and Table II (Crohn's disease).

We found 5 heterozygous (patient numbers 4, 7, 24, 26 and 27) (18.5%) and 1 homozygous (patient number 25) or hemizygous (3.7%) mutation in codon 12 of K-*ras* among the 27 specimens from UC and 2 heterozygous (patients 14 and 17) (10.5%) mutations among the 19 specimens from Crohn's disease.

No correlation was found between K-*ras* mutations and high-grade dysplasia or cellular atypia in either ulcerative colitis or Crohn's disease samples, however the number of the specimens is low.

Representative results of the PCR-RFLP analysis are shown in Fig. 1 (ulcerative colitis) and Fig. 2 (Crohn's disease).

It is unlikely that the finding of K-*ras* mutations in our study represent PCR artifacts because DNA was extracted from various samples independently and the RFLP analysis which was positive for the mutations was performed twice for each sample. The results were identical on each occasion.

We also examined 15 specimens from UC for mutations in codon 13 of K-*ras* (8). All the samples tested were negative for mutations (see Table I).

Discussion

The fact that K-*ras* mutations in colon cancer are a relatively frequent genetic event, suggests that these mutations may play an important role in the tumorigenic progression of dysplasia from UC. The total number of K-*ras* mutations in

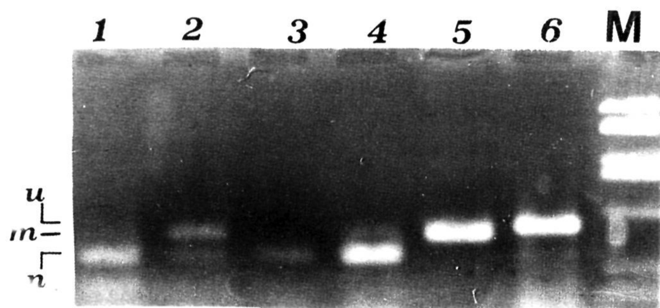

Figure 1. K-*ras* amplification products from ulcerative colitis digested with *Bst*N1 and electrophoresed through a 2% agarose gel. M: pUC18/*Hae*III, lane 6: undigested PCR product, lane 5: positive control (SW480), lanes 2 and 4: positive samples, lanes 1 and 3: negative samples. u: uncut, m: mutant, n: normal.

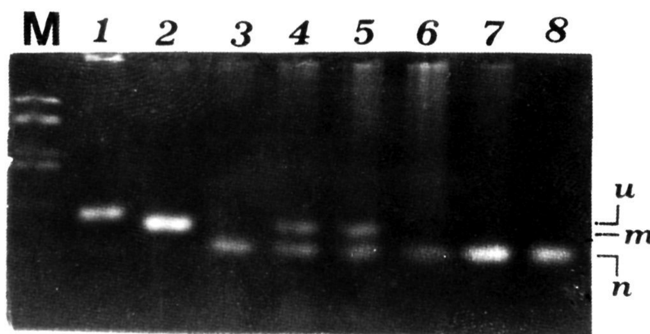

Figure 2. K-*ras* amplification products from Crohn's disease digested with *Bst*N1 and electrophoresed through a 2% agarose gel. M: pUC18/*Hae*III, lane 1: undigested PCR product, lane 2: positive control (SW 480), lanes 3, 6, 7 and 8: negative samples, lanes 4 and 5: positive samples. u: uncut, m: mutant, n: normal.

specimens from UC in our study was 6 among 27 specimens (22.2%).

The results obtained from specimen number 25 in which only the mutant band for the codon 12 of K-*ras* appeared (Fig. 1, lane 2), agrees with previous results that allelic losses is a fairly common genetical event in the progression of colon malignancy (9). Thus, it seems possible that this sample is not homozygous for the mutation but an allelic loss occured in its heterozygous previous state which left the mutant K-*ras* allele alone, in the cellular DNA. The lack of mutations in codon 13 of K-*ras* in 15 specimens of UC examined (Table I) shows a preference for codon 12 mutations of the K-*ras* gene in UC.

Specimens from patients with Crohn's disease carried K-*ras* codon 12 mutations at a lower frequence than UC (2 out of 19 specimens) (10.5%). This finding agrees with the observation that Crohn's disease exhibits a lower risk for the development of colon cancer compared to UC (2).

We suggest that codon 12 K-*ras* mutations may be implicated in the promotion of colon cancer from UC and Crohn's disease. Screening patients for K-*ras* mutations combined with another genetic marker, such as the p53 onco-suppressor gene (10-12), may cluster patients with UC and Crohn's disease into subpopulations of increased risk for the development of colon cancer.

Furthermore, our results suggest that tumor progression between sporadic colon carcinoma and carcinomas arising in chronic ulcerative colitis or Crohn's disease may follow a different genetic pathway.

References

1. Dobbins WO: Dysplasia and malignancy in inflammatory bowel disease. Ann Rev Med 35: 33-48, 1984.
2. Greenstein AJ, Sachar DB, Smith H, Janovitz HD, Aufses AH: A comparison of cancer risk in Chron's disease and ulcerative colitis. Cancer 48: 2742-2745, 1981.
3. Fearon ER and Vogelstein B: A genetic model for colorectal tumorigenesis. Cell 61: 759-767, 1990.
4. Spandidos DA: (Ed) *Ras* Oncogenes. Plenum Press, New York, London, pp1-323, 1989.
5. Bell SM, Kelly SA, Hoyle JA, Lewis FA, Taylor GR, Thompson H, Dixon MF and Quirke P: c-K-*ras* gene mutations in dysplasia and carcinomas complicating ulcerative colitis. Br J Cancer 64: 174-178, 1991.
6. Chen J, Compton C, Cheng E, Fromowitz F and Viola MV: c-K-*ras* mutations in dysplastic fields and cancers in ulcerative volitis. Gastroenterology 102: 1983-1987, 1992.
7. Burmer GC, Levine DS, Kulander BG, Haggitt RC, Rubin CE and Rabinovitch PS: c-K-*ras* mutations in chronic ulcerative colitis and sporadic colon carcinoma. Gastroenterology 99: 416-420, 1990.
8. Spandidos DA, Liloglou T, Arvanitis D and Gourtsoyiannis NC: *ras* gene activation in human small intestinal tumors. Int J Oncol 2: 513-518, 1993.
9. Vogelstein B, Fearon ER, Hamilton SR, Kern SE, Preisinger AC, Leppert M, Nakamura Y, White R, Smits AMM and Bos JL: Genetic alterations during colorectal-tumor development. New Engl J Med 319: 525-532, 1988.
10. Greenwald BD, Harpaz N, Yin J, Huang Y, Tong Y, Brown VL, McDaniel T, Newkirk C, Pesau JH and Meltzer SJ: Loss of heterozygosity affecting the p53, Rb and *mcc/acp* tumor suppressor gene loci in dyspastic and cancerous ulcelative colitis. Cancer Res 52: 741-745, 1992.
11. Yin J, Harpaz N, Tong Y, Huang Y, Laurin J, Greenwald BD, Hontanosaw M, Newkirk C and Meltzer SJ: p53 point mutations in dysplastic and cancerous ulcerative colitis lesions. Gastroenterology 104: 1633-1639, 1993.
12. Taylor HW, Boyle M, Smith SC, Bustin S and Williams NS: Expression of p53 in colorectal cancer and dysplasia complicating ulcerative colitis. Br J Surg 80: 442-444, 1993.