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GENETIC CHANGES IN MULTI-STEP DEVELOPMENT OF COLORECTAL CANCER

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INDEXING WORDS

colorectal cancer ; ras gene ; chromosome aberration

SYNOPSIS

We studied activated mutations of K-ras gene in three forms of colorectal tumors, i.e., 45 specimens of colorectal adenoma (CA), 10 of 'cancer in adenoma' (CIA), and 24 of colorectal cancer (CC), and in 15 of gastric cancer (GC) as controls. Chromosome aberrations were also examined in 7 specimens of CA, 3 of CIA, 8 of CC, and 7 of GC.

Mutation of K-ras Codon 12 was observed in 12 (26.7%) of the 45 specimens of CA, 6 (60.0%) of the 10 specimens of CIA, 6 (25.0%) of the 24 specimens of CC, and 1 (6.7%) of the 15 specimens of GC. In CA, its frequency increased with the degree of histological atypism. In CA and CIA, its frequency increased with the increase in short diameter.

The most frequent chromosome aberration was the numerical excess of chromosome 7. Numerical deficiencies of chromosomes 17 and 18 or structural abnormalities of 17p+ and 18q+ were noted in 1 specimen each of CA and CIA, and 2 of CC. Thus, aberrations of these two chromosomes were concurrent. 5q− was observed in 1 specimen each of CA and CC.

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These findings were not contradictory to the multi - step carcinogenesis model of the colorectum based on the hypothesis that carcinogenesis requires activation of an oncogene by mutation accompanied by defects of several genes that might normally inhibit tumorigenesis.

INTRODUCTION

Cancer is considered to occur as a result of a series of genetic changes that gradually disrupt the normal control of cell proliferation[12]. Since most colorectal cancers are considered to arise from adenomas[19], their genetic changes can be studied on tumors at various stages of malignant transformation. Several types of genetic changes have been reported to occur in the most advanced form of the colorectal neoplasms, i.e., cancer.

One important genetic change is mutation of the ras gene. This gene codes for a protein, with a molecular weight of 21,000, that possesses intrinsic GTPase activity and signal transmission ability, and binds to the cell membrane. DNA extracted from human solid cancer cell lines has been reported to cause malignant trasformation of NIH3T3 cells in vitro with a mutation of a single base at a particular site of the gene[1),7)]. Various investigations have clarified that such point mutations are observed in 40 - 70% of malignant colorectal neoplasms[2),4),5),22)].

Another form of genetic change in colorectal neoplasms is deletion of alleles on the chromosomes. Deletion of alleles in the 5q chromosome has been reported in individuals with colorectal adenoma in the general population. Recent studies on the cell and molecular levels have shown that alleles in 17p and 18q are often deleted in colorectal cancer patients. Since these sites include the p53 gene and deletion in the colorectal carcinoma gene (DCC gene) respectively, simultaneous inactivation of these suppressor genes is considered to be involved in the development of colorectal cancer[10]. We tried to understand whether mutation of the ras gene also occurs in benign colorectal adenomas, at what stage of the development of colorectal neoplasms these genetic defects occur, and whether tumors that show defects in chromosomes 17 and 18 also show defects in chromosome 5 or mutation of the ras gene.

MATERIALS AND METHODS

1. Patients and tumors

We examined 45 specimens of colorectal adenoma (CA), 10 of 'cancer in

adenoma' (CIA), 24 of colorectal cancer (CC) for the presence of K-ras Codon 12 mutations. For comparison, 15 specimens of gastric cancer (GC) were examined. N-ras Codons 12 and 13 were also studied in 11 specimens of CA, 2 of CIA, and 4 of CC. 7 specimens of CA, 3 of CIA, 8 of CC, and 7 of GC were examined for chromosome aberrations. These specimens were selected from the lesions endoscopically or surgically resected at Hyogo Medical Center for Adults from the general group of patients excluding those with familial adenomatous polyposis. Of these specimens, 4 of CA, 2 of CIA, 7 of CC, and 5 of GC were examined for both mutations and chromosome aberrations. The criterion for selection of the specimen was the presence of over 90% neoplasm (less than 10% normal colorectal or gastric mucosa). The adenomatous portion was subclassified into 3a, 3b, and 3c according to the degree of histological atypism (Fig. 1).

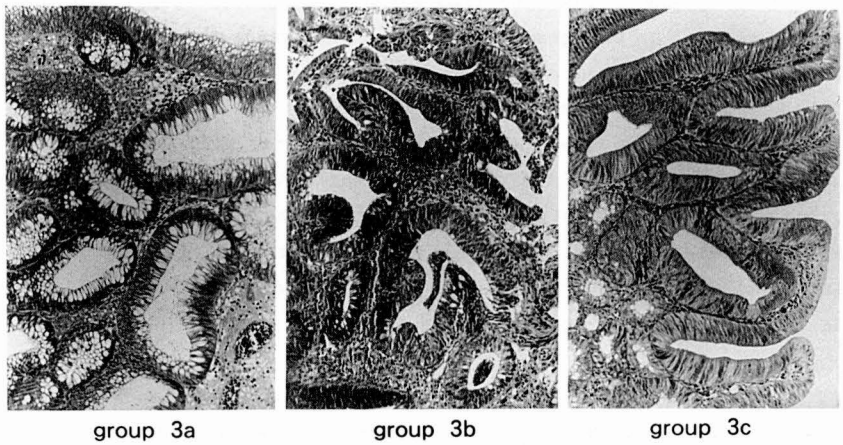

Fig. 1 Histological subclassification of the adenomatous portion

In group 3a, the epithelial cells had a high cylindrical shape, and a relatively oval nucleus arranged close to the basal side. In group 3b, the epithelial cells were elevated with nuclei of slightly various sizes arranged in the basal 1/2 of the epithelium, but with slightly irregular axis. In group 3c, the epithelial cells were cylindrical or cubic, with irregular round nuclei occupying over 1/2 the height of the epithelium, and with a markedly irregular axis.

2. Detection of mutations

1) DNA extraction from the tissue

DNA was purified by digestion with proteinase K, extracted with phenol-chloroform, precipitated with ethanol, and dissolved in TE buffer[11]. Samples were stored at 4℃.

2) Synthetic oligonucleotide

For detection of the K-ras gene, the 118 base containing Condon 12 was amplified using two primers; 5-dGGCCTGCTGAAAATGACTGA-3' as primer Ⅰ and 5'-dTTGTTGGATCATATTCGTCC-3' as primer Ⅱ. For detection of the N-ras gene, the 101 base containing Codons 12 and 13 was amplified using 5'-dCTGGTGTGAAATGACTGAGT-3 as primer Ⅰ and 5'-dCATCTACAAAGTGGTTCTGG-3' as primer Ⅱ[11]. These oligonucleotides were synthesized by a synthesizer model 380A, Applied Biosystems (Foster City, CA). The oligonucleotide probe for Codon 12 of the K-ras gene was obtained from Katayama Biomedicals (Osaka, Japan), that for Codon 12 of the N-ras gene from Kurabo (Neyagawa, Japan), and that for Codon 13 of the N-ras gene from Special Reference Laboratory (Hachioji, Japan).

3) Polymerase chain reaction(PCR)

The DNA extracted from the tissue was incubated with 0.5 μg/μl of the primer in the presence of Taq polymerase. For PCR, we used the modification reported by Saiki et al., 1988[15]. Briefly, the procedure consisted of denaturation at 94 ℃ for 1 minute, annealing at 60 ℃ for 2 minutes, and extension at 72 ℃ for 3 minutes on an automated heat-block (DNA thermal cycler; Cetus), repeated for a total of 40 times.

4) Hybridization

To 3μl of the PCR product was added 3μl of the denaturing solution (0.5M NaOH + 1.5M NaCl + 2.5mM EDTA-2Na). After a 10-minute incubation at room temperature, the solution mixture was dropped onto a nylon membrane with a dot-blot manifold (BRL, Gaithersburg, MD) and hybridized to a panel of 19-mer synthetic oligonucleotide probes which are representative of the normal Codons 12 and 13 as well as of all possible activating mutations affecting these codons.

3. Analysis of chromosome aberrations

The tissue was cut into pieces and collagenase solution was added. Cells (1×10^6) were transplanted into a culture bottle containing growth proliferation medium and incubated at 37 ℃ at 5% CO_2. When the cells showed adherent growth, a colcemide solution was applied and treatment was continued overnight. After adjusting the cell concentration with Carnoy's solution, one drop was applied onto a slide glass. The cells were treated with Giemsa.

RESULTS

1. Mutations of the ras gene

We examined the mutations of the ras gene in the DNA of the 94 specimens (Tables 1, 2, 3), which consisted of 45 of CA, 10 of CIA, 24 of CC, and 15 of GC. Mutations of the K-ras Codon 12 were detected in 26.7% of CA (13.6% in group 3a, 38.1% in group 3b, 50.0% in group 3c), 60.0% of CIA, 25.0% CC, and 6.7% of GC. Mutation of the N-ras gene was detected in only 1 CC which was a mutation of Codon 12 (Table 4). The detection rate of mutations increased with the advance in the stage of tumor, from group 3a, 3b, 3c, to CIA, but it decreased in CC which is histologically more advanced in stage.

No tendency was detected for the type of mutation for either CA, CIA, or CC (Table 4). Mutations were not detected in either of 2 CA or 1 CIA with villous components (Table 5). The frequency of mutations in CA and CIA increased with the increase in short diameter of the tumor. That is, mutations were detected in 9 of the 33 specimens (27.3%) with a short diameter of less than 10mm, 6 of the 17 specimens (35.3%) with that of 10mm to 20mm, and 3 of the 5 specimens (60.0%) with that of over 20mm. Fig. 2 shows the results of the analysis of the ras gene mutations.

2. Chromosome aberrations

The most frequent chromosome aberration was a numerical excess of chromosome 7 detected in 3 of CA, 1 of CIA, and 3 of CC (Tables 6, 7, 8). The genetic changes referred to here are a numerical excess of chromosome 7, a deficient allele in chromosomes 5, 17, and 18, and a mutation of the ras gene.

In CA, tumor No.2 had only -17 and -18, tumor No. 3 had only 5q$-$, and tumors 4, 5, and 6 had only +7. That is, they had only one of the above factors of genetic changes (aberrations of chromosomes 17 and 18 occurred simultaneously in our observation, and they are regarded to be one factor). In CIA, tumor No.8 had a mutation of the K-ras Codon 12 along with -17 and -18, and tumor No.9 had only +7. In CC, tumor No.11 had +7 and a mutation of the K-ras Codon 12, tumor No.16 had +7 and 17p+, -18, 18q+, and tumor No.17 had +7 and -17, -18 (standard is tetraploid). That is, with the advance in the stage of tumor, from CA, CIA, to CC, the genetic changes occurring in combination seemed to increase. However, mutations of the K-ras Codon 12 were detected less frequently in CC lesions. Interestingly, +7 was seen in both of the CA specimens, tumors No.5 and 6, extracted from the same patient.

Table 1. Ras-gene mutations in colorectal adenoma

tumor No.	age (yr)	sex*	size (mm)	location‡	histology‡*	histological grade of atypia	ras mutations	nucleotide and amino acid sequence
1	59	M	13×10	R	T.A.	group 3a	K12	GTT (Val)
2	44	M	19×12	R	T.A.	group 3a	(–)	
3	62	M	12×10	R	T.A.	group 3a	K12 K12	AGT (Ser) GAT (Asp)
4	55	M	10×6	T	T.A.	group 3a	(–)	
†5	55	M	12×7	A	T.A.	group 3a	(–)	
6	69	M	7×6	T	T.A.	group 3a	K12	GAT (Asp)
†7	62	M	12×4	T	T.A.	group 3a	(–)	
†8	43	F	8×7	R	T.A.	group 3a	(–)	
†9	59	M	9×9	S	T.A.	group 3a	(–)	
†10	58	F	15×7	D	T.A.	group 3a	(–)	
11	64	F	9×6	T	T.A.	group 3a	(–)	
12	60	F	10×6	T	T.A.	group 3a	(–)	
13	70	M	10×7	T	T.A.	group 3a	(–)	
14			5×3	R	T.A.	group 3a	(–)	
15	56	M	17×11	D	T.A.	group 3a	(–)	
16	43	M	7×5	D	T.A.	group 3a	(–)	
17	49	M	7×6	A	T.A.	group 3a	(–)	
18	61	M	10×10	T	T.A.	group 3a	(–)	
19	70	M	10×8	A	T.A.	group 3a	(–)	
20			5×5	S	T.A.	group 3a	(–)	
21	66	M	7×5	D	T.A.	group 3a	(–)	
22	55	M	9×6	S	T.A.	group 3a	(–)	
23			22×15	D	T.A.	group 3b	(–)	
24	19	M	23×20	R	T.A.	group 3b	(–)	
25	48	F	12×7	S	T.A.	group 3b	(–)	
26	40	M	8×7	A	T.A.	group 3b	K12 K12	GCT (Ala) GTT (Val)
27	68	M	17×12	A	T.A.	group 3b	K12	GTT (Val)
28	48	F	16×7	A	T.A.	group 3b	K12	GAT (Asp)
29	59	M	8×7	S	T.A.	group 3b	K12	GAT (Asp)
†30	60	M	15×10	S	T.A.	group 3b	K12	GTT (Val)
†31	53	M	18×13	S	T.A.	group 3b	(–)	
†32	57	M	7×5	S	T.A.	group 3b	K12	AGT (Ser)
33	64	M	10×9	S	T.A.	group 3b	K12 K12	AGT (Ser) TGT (Cys)
34			7×6	R	T.A.	group 3b	(–)	
†35	36	M	11×9	C	T.A.	group 3b	K12	GTT (Val)
36	63	M	9×5	D	T.A.	group 3b	(–)	
37	65	M	20×17	R	T.A.	group 3b	(–)	
38	71	F	10×6	R	T.A.	group 3b	(–)	
39	47	M	20×15	R	T.A.	group 3b	(–)	
40			15×12	S	T.A.	group 3b	(–)	
41	55	M	12×8	S	T.A.	group 3b	(–)	
†42	57	F	18×8	R	V.A.	group 3b	(–)	
†43	61	M	15×14	S	V.A.	group 3b	(–)	
44	65	M	7×6	S	T.A.	group 3c	(–)	
45	42	M	28×24	T	T.A.	group 3c	K12	GAT (Asp)

* M : male F : female
‡ C : cecum A : ascending T : transverse D : descending S : sigmoid R : rectum
‡* T.A. : tubular adenoma V.A. : villous adenoma
† specimen which was examined for N-ras Codons 12 and 13

Table 2. Ras-gene mutations in 'cancer in adenoma'

tumor No.	age (yr)	sex*	size of their adenomas (mm)	location of tumor‡	histology of their adenomas‡*	histological grade of atypia of their adenomas	ras mutations	nucleotide and amino acid sequence
1	56	M	20 × 18	S	T.A.	group 3a	(–)	
2	54	F	25 × 20	C	T.A.	group 3b	K12	GTT (Val)
3	67	M	12 × 8	R	T.A.	group 3b	(–)	
4	57	M	15 × 15	D	T.A.	group 3b	K12	GTT (Val)
†5	66	M	19 × 14	A	V.A.	group 3b	(–)	
†6	57	M	9 × 6	R	T.A.	group 3b	K12 K12	AGT (Ser) GTT (Val)
7	74	M	17 × 14	D	T.A.	group 3b	K12	GTT (Val)
8	69	F	24 × 20	S	T.A.	group 3c	K12	GTT (Val)
9	41	M	25 × 23	R	T.A.	group 3c	(–)	
10	53	M	11 × 9	R	T.A.	group 3c	K12	GAT (Asp)

* M : male F : female
‡ C : cecum A : ascending T : transverse D : descending S : sigmoid R : rectum
‡* T.A. : tubular adenoma V.A. : villous adenoma
† specimen which was examined for N-ras Codons 12 and 13

Table 3. Ras-gene mutations in colorectal cancer

tumor No.	age (yr)	sex*	location of tumor*	differentiation**	Dukes' category**	ras mutations	nucleotide and amino acid sequence
1	84	M	S	M	A	(-)	
2			R	M	B	(-)	
3	55	M	R	W	B	(-)	
4	82	F	S	W	B	K12	GAT (Asp)
5	63	F	A	W	B	(-)	
6	78	F	S	W	B	(-)	
7	76	M	C	W	B	(-)	
†8	68	M	S	W	B	(-)	
†9			D	W	B	K12	GAT (Asp)
10	63	M	A	W	B	(-)	
11	71	M	S	W	B	(-)	
12	74	M	R	W/M	B	(-)	
13	73	M	S	M	B	K12	GTT (Val)
14	64	F	R	M	B	(-)	
15	66	F	R	M	B	(-)	
16	59	M	S	W	C	(-)	
†17	57	M	S	W	C	K12	AGT (Ser)
						N12	AGT (Ser)
18	47	F	S	W	C	(-)	
19	72	M	R	W	C	K12	GAT (Asp)
20	71	M	A	W	C	(-)	
21	41	M	R	W	C	(-)	
22	78	M	S	M	C	(-)	
23	65	M	R	M	C	K12	GTT (Val)
†24	59	M	R	M	C	(-)	

* M male F female
** C cecum A ascending T transverse D descending S sigmoid R rectum
** W well differentiated M moderately differentiated
** A confined to the muscularis propria
B extended through the muscularis propria
C metastatic to regional lymphnodes
† specimen which was examined for N-ras Codons 12 and 13

Table 4. Ras-gene mutations in colorectal tumor

ras mutations	nucleotide and amino acid sequence	group 3a adenoma (22specimens)	group 3b adenoma (21specimens)	group 3c adenoma (2specimens)	cancer in adenoma (10specimens)	cancer (24specimens)
		number of specimens with mutation				
K12	GTT (Val)	1	4	0	5	2
K12	GAT (Asp)	2	2	1	1	3
K12	AGT (Ser)	1	2	0	1	1
K12	TGT (Cys)	0	1	0	0	0
K12	GCT (Ala)	0	1	0	0	0
N12	AGT (Ser)	0	0	0	0	1
total number of specimens with mutation		3	8	1	6	6
percent of specimens with mutation		13.6	38.1	50.0	60.0	25.0

Table 5. Relation between ras-gene mutation and clinicopathological features in colorectal adenoma and 'cancer in adenoma'

	group 3a adenoma (22 specimens)	group 3b adenoma (21 specimens)	group 3c adenoma (2 specimens)	cancer in adenoma (10 specimens)	total (55 specimens)
histology	number of specimens with mutation(percent)				
tubular	3/22 (13.7)	8/19 (42.1)	1/2 (50.0)	6/9 (66.7)	18/52 (34.6)
villous	0	0/2 (0)	0	0/1 (0)	0/3 (0)
size (in short diameter)					
< 10mm	1/17 (5.9)	6/12 (50.0)	0/1 (0)	2/3 (66.7)	9/33 (27.3)
10 ≦ ···<20mm	2/5 (40.0)	2/8 (25.0)	0	2/4 (50.0)	6/17 (35.3)
≧ 20mm	0	0/1 (0)	1/1 (100.0)	2/3 (66.7)	3/5 (60.0)
total	3/22 (13.7)	8/21 (38.1)	1/2 (50.0)	6/10 (60.0)	

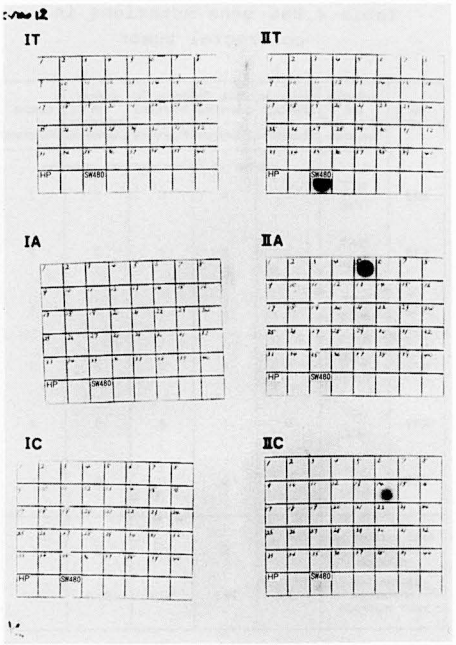

Fig. 2 Detection of K-ras Codon 12 Mutations

HP is the DNA obtained from a cell line of human placenta, and SW480 is that obtained from a cell line of colorectal cancer known to have a mutation from guanine to thymine in the second base of the K-ras Codon 12. The left row is the results showing the lesion where the first base of the K-ras Codon 12 had mutated to thymine, adenine, and cytosine, in this order from the top and the right row is the results showing the lesion where the second base had mutated to thymine, adenine, and cytosine from the top in this order.

SW480 had the mutation to GTT. Tumor No. 5, a colorectal cancer specimen, had the mutation to GAT, and Tumor No. 14, a gastric cancer specimen, had the mutation to GCT. That is, the second base of Codon 12 was considered to have the mutation from guanine to adenine in the colorectal cancer specimen, and to cytosine in the gastric cancer specimen.

Table 6. Chromosome aberrations and ras-gene mutations in colorectal adenoma

tumor No.	age (yr)	sex	size (mm)	location #1	histology #2	histological grade of atypia	chromosome: period of cultivation (day)	chromosome: cell form #3	chromosome: karyotype #4	chromosome: analysis	ras: mutations	ras: nucleotide and amino acid sequence
1	52	M	16X10	R	T.A.	group 3a	13	E・Fi	N	N	not done	
2	55	M	10X6	T	T.A.	group 3a	13	U	A	43, 44/−Y, −16, −17, −17, −18, +20, 1p+, 13p+, i(13q), 1～2 mar & N	(−)	
3	65	M	20X17	R	T.A.	group 3b	3	Fl	A	46, XY, 5q− & N	(−)	
4	49	M	22X17	R	T.A.	group 3b	6	E・Fl	A	52, XY, +7, +9, +13, +18, +21, +21 & N	not done	
5	47	M	15X12	S	T.A.	group 3b	4	E・Fi・Fl	A	48, XY, +7, +16 & N	(−)	
6			20X15	R	T.A.	group 3b	12	E・Fi	A	48, XY, +7, +13/+9, +20 & N	(−)	
7	59	M	20X16	T	T.A.	group 3c	3	E・Fl	A	47, XY, +13 & N	not done	

#1 T : transverse S : sigmoid R : rectum
#2 T.A. : tubular adenoma
#3 E : epithelioid cell Fi : fibroblastoid cell Fl : floating cell U : unclassified
#4 N : normal karyotype A : abnormal karyotype supposed to originate from tumor cell

Table 7. Chromosome aberrations and ras-gene mutations in 'cancer in adenoma'

tumor No.	age (yr)	sex	size of their adenomas (mm)	#1 location	#2 histology of their adenomas	histological grade of atypia of their adenomas	chromosome: period of cultivation (day)	chromosome: #3 cell form	chromosome: #4 karyotype	chromosome: analysis	ras: mutations	ras: nucleotide and amino acid sequence
8	54	F	25X20	C	T.A.	group 3b	10	E	A	44, 45/−12, −17, −18, −21, −22, 1p⁻, 3p⁻, inv(9p⁺ q⁻), 3～4 mar & N	K12	GTT (Val)
9	55	M	18X17	R	T.A.	group 3c	5	e・Fi	A	48, XY, +7, +9 & N	not done	
10	41	M	25X23	R	T.A.	group 3c	11	E・Fi	N	N(Ab1)	(−)	

#1 C : cecum R : rectum

#2 T.A. : tubular adenoma

#3 E : epithelioid cell e : a few epithelioid cell Fi : fibroblastoid cell

#4 N : normal karyotype A : abnormal karyotype supposed to originate from tumor cell

Table 8. Chromosome aberrations and ras-gene mutations in colorectal cancer

tumor No.	age (yr)	sex	#1 location	#2 differentiation	Dukes' category	chromosome: period of cultivation (day)	chromosome: #3 cell form	chromosome: #4 karyotype	chromosome: analysis	ras: mutations	ras: nucleotide and amino acid sequence
11	73	M	S	M	B	14	e・Fi	A	72/3 copy=2, 3, 19, 4 copy=6, 7, 2 copy=X & N	K12	GTT (Val)
12	82	F	S	W	B	17	Fi	B	random clone 46/−8, −15, +20, 6q⁺, 4q⁻, 17q⁺=2 & N	K12	GAT (Asp)
13	71	M	S	W	B	11	Fi	B	N & −Y=4	(−)	
14	63	F	A	W	C	4	U	N	N	not done	
15	72	M	R	W	C	15	Fi	N	N & −Y=2	K12	GAT (Asp)
16	47	F	S	W	C	5	E	A	46, X, −X, −4, +7, −8, −10, −13, −18, −19, 2q⁺, 9p⁺, 10q⁻, 12p⁺, 15p⁺, 17p⁺, 18q⁺, +6 mar	(−)	
17	71	M	A	W	C	3	E・Fi・Fl	A	86/1p⁻, 3p⁻, 5q⁻, 8q⁺, 1copy=5, 21, 3copy=2, 3, 4, 10, 11, 12, 4copy=6, 19, 22, 5copy=7, 2copy=X & N	(−)	
18	41	M	R	W	C	4	U	B	N & −13=2, Ab=4	(−)	

#1 A : ascending S : sigmoid R : rectum

#2 W : well differentiated M : moderately differentiated

#3 E : epithelioid cell e : a few epithelioid cell Fi : fibroblastoid cell Fl : floating cell U : unclassified

#4 N : normal karyotype A : abnormal karyotype supposed to originate from tumor cell B : clone of −Y or non-clonal abnormality

DISCUSSION

The ras gene was first identified as the transforming ability in murine sarcoma virus (MSV), a retrovirus. Thereafter, the gene was found to exist even in normal cells. About a decade ago, studies using the NIH3T3 cell transfection method revealed that the DNA of many human cancer cells has malignant transformation activity. When the properties of these oncogenes with transformation activity were studied, 90% or more of the genes were found to be ras family genes. The detection rate of K-ras gene is high in pancreatic cancer, colorectal cancer, and lung cancer[23]. Various reports have shown that mutation of the gene is observed in 40-70% of cases of colorectal cancer.

Our findings suggest that the ras gene mutation is often a relatively early event in colorectal tumorigenesis. This is in agreement with the findings reported by various investigators on tumors in experimental animals[1),7)], human pre-leukemic state[6),9)], and human keratoacanthoma[8)]. However, our findings suggest that the ras gene mutation is not always the first genetic change, because the frequency of this mutation was low in colorectal adenoma in group 3a that is considered to be at an early developmental stage of colorectal tumor. Another interesting fact is that the frequency of the ras gene mutation was not high in colorectal cancer. On the other hand, in the two colorectal adenomas No.33 and 34 and in the two colorectal cancers No.8 and 9, each pair of which were obtained from the same patient at the same time, one of each pair was mutation positive and the other negative. Therefore, it is impossible to explain the occurrence and development of a colorectal tumor by a single factor, namely, ras gene mutation, and investigations of other genetic changes must be made.

In 1971, Knudson presented a hypothesis that retinoblastoma, a hereditary malignant neoplasm, occurs when both alleles of a particular gene undergo mutations, giving rise to the concept of recessive oncogene. Today, the multi-step carcinogenesis of human cancer and changes in the degree of its malignancy are considered to be explained by various combinations of dominant oncogenes and recessive tumor suppressor genes[20)].

The numerical excess of chromosome 7 is assumed to be attributable to amplification of the EGF receptor gene because this gene is present in the chromosome, as has already been shown[21)]. With amplification of the EGF receptor, p21 with activated mutation may remain at as the p21·GTP type and continue to give signals of proliferation[16),17)]. This chromosome is thus suggested to be involved in the occurrence and development of colorectal tumor. Various investigators have already reported the roles of EGF in

carcinogenesis[18], increased expression of the EGF receptor in human colorectal cancer[3], and relationship between the EGF receptor and mutation-inducing ras·p21 in human colorectal cancer[13),14)].

We also focused our attention to these three chromosomes 5, 17, and 18 and found that the aberrations of chromosomes 17 and 18 often occur simultaneously. The mutual effect as a tumor suppressor gene of p53 gene located on 17p and DCC gene located on 18q was suggested to be more important than the APC gene located on 5q. However, we could not determine at what stage of colorectal tumor these genes were expressed. We speculate that the genetic changes including the mutation of the ras gene occur in combination with the progression of the tumor. This point requires further study.

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