

Mutations of APC, K-ras, and p53 Are Associated with Specific Chromosomal Aberrations in Colorectal Adenocarcinomas¹

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ABSTRACT

It is widely accepted that both large-scale chromosomal abnormalities and mutation of specific genes, such as *APC*, *K-ras*, and/or *p53*, occur in the majority of colorectal adenocarcinomas. Whether or not a relationship exists between these different forms of genetic abnormalities was previously unknown. Using comparative genomic hybridization and mutational analysis of *APC*, *K-ras*, and *p53* to evaluate 50 colorectal adenocarcinomas, we have shown that mutation of *p53* is significantly associated with gain of 20q, 13q, and 8q and loss of 18q ($P = 0.000, 0.02, 0.044, \text{ and } 0.001$, respectively). Conversely, *APC* mutation did not associate with any of the above-mentioned aberrations but did associate significantly with gain of 7p ($P = 0.01$). Gain of chromosomal arm 12p, although a less common aberration, was significantly associated with *K-ras* mutation ($P = 0.011$). The associations we have described should refine the search for candidate genes underlying chromosomal aberrations and assist in the definition of distinct pathways in colorectal tumorigenesis.

INTRODUCTION

Colorectal cancer is one of the commonest and most extensively studied visceral malignancies, but knowledge of the precise genetic events underlying the formation of these tumors remains incomplete. Most of the published data regarding colorectal tumorigenesis relate to mutation of specific genes and losses of heterozygosity. In 1990, Fearon and Vogelstein (1) proposed a multistage model of colorectal tumorigenesis involving mutation of the *K-ras* oncogene, the *p53* tumor suppressor gene, and allelic loss of 5q and 18q. The gene targeted by allelic loss of chromosome 5q has since been shown to be *APC*³ (2). Although a multitude of studies have provided support for and clarification of this model (3), our group has recently shown that *K-ras* and *p53* mutations are largely mutually exclusive events in colorectal cancers and may define distinct pathways of tumorigenesis (4). Furthermore, an alternative pathway of colorectal tumorigenesis characterized by the presence of microsatellite instability (MSI) has been described in approximately 10% of colorectal tumors. This pathway less frequently involves the aforementioned genes and usually involves loss of function of mismatch repair genes (5, 6). Despite the increased knowledge regarding these key genes, it is recognized that the selective approach of this work has limitations (7), and there is a focus on less reductionist approaches to studying colorectal tumorigenesis. In this respect, there has been a reappraisal of genetic abnormality at the chromosomal level, and the concepts of tumor aneuploidy (altered chromosome complement) and segmental aneu-

ploidy (unbalanced structural rearrangements) are receiving increasing attention.

Chromosomal abnormalities in colorectal tumors were originally detected by conventional cytogenetics (8), but this technique has limitations in its application to solid tumor analysis and has largely been superseded by the advent of new molecular cytogenetic techniques, in particular, CGH (9). Studies of colorectal cancer using CGH have shown the frequent occurrence of chromosomal aberrations such as gain of 20q, 13q, 7p, and 8q and loss of 18q, 17p, and 8p (10–14). Moreover, some chromosomal abnormalities have been reported to occur more frequently than the commonly mutated genes, providing persuasive evidence of their fundamental importance in colorectal neoplasia.

Although extensive knowledge now exists regarding both specific gene mutation and chromosomal aberrations in colorectal tumors, little is known about the relationship between these different forms of genetic alterations. To address this issue, we performed CGH analysis on a series of colorectal cancers for which the mutational status of *APC*, *K-ras*, and *p53* and immunohistochemical status of the mismatch repair proteins hMLH1 and hMSH2 had previously been determined (4). The aims were to define the patterns of chromosomal abnormality in sporadic colorectal cancer and establish whether any associations exist between specific gene mutations and chromosomal aberrations.

MATERIALS AND METHODS

Patient Recruitment and Tumor Material

CGH analysis was performed on tissue from 50 sporadic human colorectal cancers. These cases were the first 50 tumors from a cohort of 106 for which mutational data have recently been reported (4). All patients were Caucasian and were between 45 and 80 years old. In every case, a preoperative histopathological diagnosis of colorectal cancer had been ascertained, and a family history of colorectal cancer had been excluded. All tissue samples were selected by an experienced pathologist (F. A. C.) immediately after surgical resection, snap-frozen in liquid nitrogen, and then stored at -80°C . Age, gender, and Dukes' staging information is given in Table 1.

DNA Extraction

Genomic DNA for CGH and mutational analysis was extracted from each tumor tissue sample using the Wizard Genomic DNA Purification Kit (Promega) according to the manufacturer's instructions. Genomic reference DNA for CGH was extracted from sex-matched venous blood using the Nucleon BACC3 (Amersham Pharmacia Biotech) according to the manufacturer's instructions.

Mutational Detection and hMSH2/hMLH1 Immunohistochemistry

Mutational analyses of *APC*, *K-ras*, and *p53* and immunohistochemistry for hMLH1 and hMSH2 for each tumor were performed as recently reported (4).

CGH

Slide Preparation. Metaphase cells for CGH were prepared according to routine protocols from peripheral blood lymphocytes. Cell suspensions were

Received 12/12/02; accepted 6/2/03.

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¹ Supported by the Scottish Hospital Endowment Research Trust, The Royal College of Surgeons of Edinburgh, Tayside University Hospital Trust, Tayside Area Oncology Fund, The Anonymous Trust, Tayside, The Food Standards Agency, and the Medical Research Council.

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³ The abbreviations used are: APC, adenomatous polyposis coli; CGH, comparative genomic hybridization.

Table 1 Summary of patient characteristics and site, stage, total number of chromosomal aberrations, and immunohistochemical and mutational data of each tumor

Tumor	Sex	Age at diagnosis (yrs)	Site	Dukes' stage	Total gains and losses	hMLH1/hMSH2	p53	K-ras	APC
1	F	67	R ^a	B	16	Normal	+ ^b	- ^b	- ^b
2	M	77	S	A	8	Normal	+	-	-
3	M	59	S	B	11	Normal	-	-	-
4	F	67	S	A	13	Normal	+	-	+
5	M	48	C	C	7	Neg hMLH1 ^c	-	-	-
6	F	70	R	C	9	Normal	+	-	-
7	M	77	R	A	19	Normal	+	-	+
8	M	74	S	B	12	Normal	+	-	+
9	F	64	R	C	11	Normal	+	+	+
10	F	66	R	B	14	Normal	+	-	-
11	M	66	S	A	11	Normal	+	-	-
12	M	68	S	B	16	Normal	+	-	-
13	F	74	D	B	13	Normal	+	-	+
14	F	58	R	C	25	Normal	+	-	-
15	M	56	R	A	5	Normal	-	+	+
16	M	68	A	B	15	Normal	+	-	+
17	F	55	S	C	11	Normal	+	-	+
18	F	76	R	C	5	Normal	+	-	-
19	M	75	S	C	11	Normal	-	-	+
20	F	46	R	C	3	Normal	+	-	-
21	F	61	A	B	2	Neg hMLH1	-	-	-
22	M	70	R	C	18	Normal	-	+	+
23	F	59	R	A	18	Normal	-	-	-
24	M	64	S	C	7	Normal	+	-	+
25	F	69	R	C	10	Normal	-	+	+
26	M	67	R	C	8	Normal	-	+	-
27	M	76	S	B	17	Normal	+	-	+
28	F	80	C	B	7	Normal	+	-	+
29	M	68	R	B	14	Normal	+	-	-
30	M	76	C	B	10	Normal	-	+	+
31	F	80	R	C	5	Normal	+	-	-
32	F	67	S	C	8	Normal	+	-	+
33	M	67	S	C	12	Normal	+	-	+
34	M	65	C	A	10	Normal	-	+	+
35	M	76	C	C	6	Normal	-	-	+
36	F	64	S	B	22	Normal	+	-	+
37	M	61	R	B	13	Normal	+	-	+
38	M	76	R	C	10	Normal	+	-	+
39	F	79	C	C	0	Normal	-	-	-
40	M	61	S	C	23	Normal	+	-	+
41	M	57	D	C	10	Normal	+	-	-
42	F	75	C	C	6	Normal	-	+	+
43	F	69	R	B	22	Normal	+	-	+
44	M	71	S	A	34	Normal	+	-	+
45	M	73	S	C	25	Normal	+	-	+
46	M	59	R	C	17	Normal	-	+	-
47	M	76	S	C	17	Normal	-	-	+
48	M	64	C	C	14	Normal	+	+	-
49	M	71	R	C	0	Normal	-	-	-
50	F	75	R	B	21	Normal	+	-	-

^a R, rectum; C, cecum; A, ascending colon; D, descending colon; S, sigmoid colon.

^b + signifies gene mutated, - signifies no mutation detected.

^c Neg hMLH1, negative for hMLH1.

dropped onto glass slides that were air-dried and aged overnight at 37°C on a heating block. Denaturation was performed for 4 min in a 70% formamide, 2× SSC solution at 74°C, followed by graded dehydration in 70%, 85%, and 100% cold ethanol.

DNA Labeling by Nick Translation. DNA was fluorochrome-labeled with spectrum green (tumor DNA) or spectrum red (normal DNA) by nick translation using a nick translation kit according to the manufacturer's instructions (Vysis Ltd., Richmond, Surrey, United Kingdom). The reaction time for nick translation was adjusted to produce probe fragment sizes of 500–2000 bp as determined by gel electrophoresis.

Probe Mix Preparation and Hybridization. Spectrum green-labeled tumor DNA (200 ng) and spectrum red-labeled normal DNA (200 ng) were mixed with 10 µg of COT1 DNA (Life Technologies, Inc.). The probe mixture was precipitated using 0.1 volume of sodium acetate and 2.5 volumes of cold ethanol and centrifuged at 14000 rpm for 30 min. DNA pellets were air-dried, redissolved in 10 µl of Hybrisol (Appligene Oncor), and then denatured in a 73°C water bath for 10 min. The probe mix was added to the slide, a coverslip was applied, and hybridization was allowed to occur for 48–72 h in a humidified chamber at 37°C. Nonhybridized DNA was washed off in 0.4× SSC/0.3% NP40 at 73°C for 2 min followed by 1 min in 2× SSC/0.1% NP40 at room temperature. The chromosomes were counterstained with 4',6-diamidino-2-phenylindole/Antifade solution (Appligene Oncor).

Fluorescence Microscopy and Digital Image Analysis. Images of 8–10 metaphase spreads were captured using a cooled charge-coupled device camera (SenSys; Photometrics) mounted on an epifluorescence microscope (Olympus BX60) equipped with three single excitation filters, a multiband-pass emission filter, and a dichroic mirror. Digital image analysis was performed using Vysis Quips software on a MacIntosh G3 workstation. All karyotyping was checked by a qualified cytogeneticist (M. S.). Increased DNA sequence copy number was defined as a green:red fluorescence ratio of 1.15 or greater, and a decreased DNA copy number was defined as an average ratio of 0.85 or less. Normal genomic DNA was used as a negative control for each experiment.

Statistical Analysis. All statistical analyses were performed using SPSS statistical software (SPSS Inc., Chicago, IL). The Mann-Whitney *U* test was used to assess differences between two samples for a specific parameter, and Fisher's exact test was used to check for associations between two parameters.

RESULTS

Numerical Chromosomal Aberrations and Clinicopathological Associations. Of the 50 tumors analyzed by CGH, 48 (96%) were found to be aneuploid, showing at least one chromosomal aberration

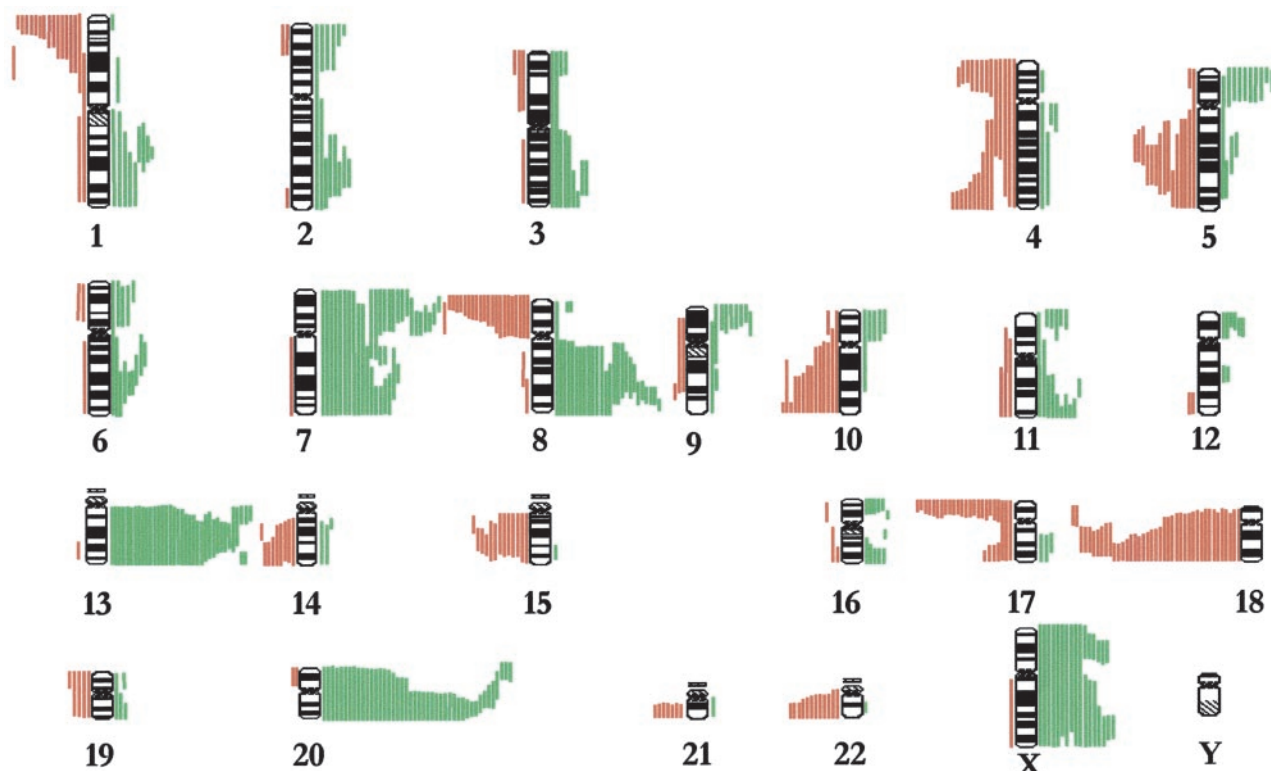


Fig. 1. Summary of CGH results for 50 tumors. Green bars to the right of ideogram represent regions of chromosomal gain, and red bars to the left represent regions of loss.

(Table 1). The median number of total aberrations per tumor was 11.0 (range, 0–34). Gains occurred more frequently than losses, with a median number of gains of 7.0 (range, 0–14), and a median number of losses of 4.0 (range, 0–20). Gains and losses were usually large and often involved entire chromosomal arms or whole chromosomes (Fig. 1). The level of gain shown in Fig. 1 was at least a single copy gain. However, many tumors had regions showing greatly increased green: red fluorescence ratios, suggesting multiple copy gain (data not shown). The single most frequent aberration was gain of 20q, which occurred in 40 tumors (80%). Chromosomal aberrations were defined as recurrent if they occurred in at least 20 cases ($\geq 40\%$), and there were six such regions of gain and three such regions of loss (Table 2). Recurrent gains were 20q (80%), 13q (74%), 7p (54%), 8q (50%), 20p (48%), and 7q (44%). Recurrent losses were 18q (76%), 17p (46%), and 8p (42%).

The median numbers of total aberrations in male ($n = 29$) and female patients ($n = 21$), 12 and 11, respectively, were not significantly different ($P = 0.4$). A significant difference was seen between the median numbers of aberrations in tumors from the right side (median, 7; range, 0–15) of the colorectum compared with those from the left (median, 12.5; range, 0–34; $P = 0.01$). If, however, the tumors were divided into colon and rectal tumors, no significant difference was detected (13.5 for rectal tumors and 11.0 for colon tumors, $P = 0.3$). The median numbers of aberrations in Dukes' stage A, B, and C tumors were 12, 14, and 9.5, respectively. The differences between Dukes' stage A and stage B tumors and between Dukes' stage A and stage C tumors were not statistically significant ($P = 0.67$ and $P = 0.21$). However, Dukes' stage C tumors were found to have significantly fewer aberrations than Dukes' stage B tumors ($P = 0.04$). If tumors with no nodal involvement (Dukes' stage A and B) were compared with those with nodal involvement (Dukes' stage C), the latter group had significantly fewer aberrations than the former ($P = 0.034$). Two tumors, cases 39 and 49, showed no chromosomal

aberrations or mutations of the genes analyzed and were normal for hMLH1 and hMSH2 immunohistochemistry. Frozen sections of tissue from each case were assessed, which confirmed that the proportion of invasive adenocarcinoma within each sample was in excess of 70%.

Associations of Number of Chromosomal Aberrations with Tumor Genotype. The gene mutation data have been reported previously as part of a larger study (4). In this cohort of 50 cases, of the genes studied, *p53* was most frequently mutated, with 33 of 50 (66%) tumors containing a mutation. *APC* mutations were found in 28 of 50 cases (56%), and *K-ras* mutations were found in 10 of 50 cases (20%).

Table 3 summarizes the differences in the number of aberrations in tumors with a mutated gene compared with tumors without that mutation. Only *p53*-mutated tumors compared with wild-type *p53*

Table 2 Chromosomal aberrations occurring in $\geq 40\%$ of tumors

Recurrent chromosomal aberration	No. of tumors with aberration (%)
20q gain	40 (80)
18q loss	38 (76)
13q gain	37 (74)
7p gain	27 (54)
8q gain	25 (50)
20p gain	24 (48)
17p loss	23 (46)
7q gain	22 (44)
8p loss	21 (42)

Table 3 Median numbers of aberrations in tumors with mutated gene compared with tumors with wild-type (wt) gene (significant differences are shown in bold)

Groups analyzed	No. of gains	No. of losses	Total no. of aberrations
Mutant <i>p53</i> vs. wt <i>p53</i>	8.0 vs. 6.0	5.0 vs. 3.0^a	13.0 vs. 10.0^b
Mutant <i>K-ras</i> vs. wt <i>K-ras</i>	6.5 vs. 7.0	3.5 vs. 5.0	10.0 vs. 12.0
Mutant <i>APC</i> vs. wt <i>APC</i>	8.0 vs. 6.5	4.5 vs. 4.0	12.0 vs. 10.0

^a $P = 0.006$.

^b $P = 0.02$.

Table 4 Associations of recurrent chromosomal aberrations with clinicopathological features and genotypes (significant Ps are shown in bold)

Recurrent chromosomal aberration	Association with gender (P)	Association with left-sided tumors (P)	Association with rectal tumors (P)	Association with nodal status (P)	Association with APC mutation (P)	Association with K-ras mutation (P)	Association with p53 mutation (P)
20q gain	1.00	0.02	1.00	1.00	0.73	1.00	0.000
13q gain	0.35	0.10	1.00	0.53	0.20	1.00	0.02
18q loss	0.53	0.001	0.51	0.37	0.32	1.00	0.001
8q gain	0.78	0.01	0.39	0.78	1.00	0.042^a	0.044
7p gain	0.25	1.00	0.78	0.27	0.01	1.00	1.00
20p gain	0.025^b	1.00	0.17	0.79	0.17	0.29	0.24
17p loss	0.047^b	0.74	0.58	0.046^c	0.577	0.74	0.77
7q gain	0.78	0.73	0.78	0.73	0.16	0.73	1.00
8p loss	0.77	0.03	0.39	1.00	1.00	0.72	0.07
12p gain	0.38	1.00	1.00	0.67	0.21	0.01	0.16

^a Wild-type K-ras tumors are associated with 8q gain.

^b 20p gain and 17p loss are associated with male gender.

^c 17p loss is associated with no nodal involvement.

tumors had significantly more losses and significantly more total aberrations. Two tumors in this cohort showed loss of hMLH1 by immunohistochemistry, neither of which had mutation of *p53*, *APC*, or *K-ras*. All tumors were normal for hMSH2 immunohistochemistry. Tumors with negative hMLH1 immunohistochemistry had significantly fewer chromosomal losses compared with those with normal hMLH1 immunohistochemistry ($P = 0.025$), but no significant difference in the number of gains or total number of aberrations were observed.

Associations of Recurrent Chromosomal Aberrations with Clinicopathological Features and Tumor Genotypes. The associations of recurrent chromosomal aberrations with patient gender, tumor site, nodal involvement, and *p53*, *K-ras*, and *APC* mutation are summarized in Table 4. Both 20p gain and 17p loss were seen significantly more frequently in male patients ($P = 0.025$ and $P = 0.047$, respectively). Left-sided tumors, compared with right-sided tumors, were found to be associated with 20p and 8q gain ($P = 0.02$ and $P = 0.01$) and 18q and 8p loss ($P = 0.001$ and $P = 0.03$). Rectal tumors compared with colonic tumors, however, were not associated with any of the recurrent chromosomal aberrations. No association was found between the recurrent aberrations and tumors with nodal involvement, but tumors without nodal involvement were found to be associated with 17p loss ($P = 0.046$).

Mutation of *p53* showed a significant association with gain of 20q, 13q, and 8q and loss of 18q ($P = 0.000$, $P = 0.02$, $P = 0.044$, and $P = 0.001$, respectively). Conversely, *APC* mutation did not associate with any of the above-mentioned aberrations but did associate significantly with gain of 7p ($P = 0.01$). Mutation of *K-ras* was not associated with any of the recurrent aberrations, but wild-type *K-ras* was found to be associated with 8q gain ($P = 0.042$). Gain of chromosomal arm 12p, although a less common aberration (occurring in only 12% of cases), was found to be significantly associated with a *K-ras* mutation ($P = 0.011$).

DISCUSSION

To our knowledge, this is the first report to address chromosomal aberration; mutation of *p53*, *K-ras*, and *APC*; and immunohistochemical staining for hMLH1 and hMSH2 in the same series of colorectal cancers. In this analysis of 50 cases, we have found that a number of specific chromosomal aberrations occurred more frequently than mutations of *p53*, *K-ras*, or *APC*. Furthermore, we have shown that the relationship between chromosomal abnormality and gene mutation was not random, with certain highly specific associations emerging.

The recurrent chromosomal gains and losses detected in our study are comparable with those reported in other cytogenetic and CGH studies of colorectal cancer (8, 10–14). Similarly, the mutation rates of *p53*, *APC*, and *K-ras* in this series are consistent with those

reported in the literature (15). There is, however, a paucity of data in the literature regarding the coexistence of chromosomal aberrations and mutation of *p53*, *K-ras*, and/or *APC* in the same colorectal tumors. In this study, we have found that all recurrent chromosomal aberrations detected by CGH (Table 2) occurred more frequently than *K-ras* mutations, and three (+20q, -18q, and +13q) occurred more frequently than either *p53* or *APC* mutation, providing convincing evidence of the crucial role of chromosomal abnormalities in colorectal tumorigenesis. Gain of chromosome 20q is the single most frequent genetic event in our study, and there is evidence to suggest that the level of 20q amplification correlates with the metastatic potential of colorectal cancers (16). However, there is currently no persuasive evidence regarding the underlying molecular pathology of this abnormality, nor is this abnormality included in prevailing models of colorectal tumorigenesis.

Mutations of *p53* and *APC* have both been implicated in the development of aneuploidy, although the precise relationship between altered chromosome number and these mutations remains poorly defined (15, 17, 18). Our results (Table 3) suggest that *p53* mutation, but not *K-ras* or *APC* mutation, is associated with a more deranged karyotype. However, the groups of tumors compared in Table 3 are likely to be heterogeneous with regard to unidentified genetic abnormalities and, therefore, may not be directly comparable. Indeed some unidentified genetic abnormalities may themselves be responsible for the development of aneuploidy. Perhaps of greater significance is the observation that wild-type *p53*, *APC*, and *K-ras* tumors did contain chromosomal gains and losses, providing convincing evidence that aneuploidy can occur in the absence of these mutations.

As seen in this and other studies (10–14), specific, clonally selected, chromosomal gains and losses in colorectal cancer are not only common but exhibit a nonrandom pattern (Fig. 1). Whether or not this pattern of chromosomal aberrations is related to clonally selected, coexisting gene mutations was previously unknown. We have shown that mutation of *p53* is significantly associated with four recurrent chromosomal aberrations (Table 4). Because 20q gain, 13q gain, 18q loss, and 8q gain and mutation of *p53* are all common events, it is conceivable that they cosegregate simply as a result of their frequency. Mutation of *APC* in this series, however, occurs at a similar frequency to *p53* mutation, but *APC* does not cosegregate significantly with these chromosomal aberrations. Conversely, *APC* mutation is significantly associated with gain of 7p, an aberration with which mutant *p53* is not associated. Recently, our group has shown that mutation of *p53* and mutation of *K-ras* are rarely found in the same colorectal tumor (4), suggesting that mutation of each gene is a component of two separate tumorigenic pathways. The finding in this study that gain of chromosomal arm 8q is significantly associated with both wild-type *K-ras* and *p53* mutation provides alternative evidence

that these events are mutually exclusive. Because *K-ras* mutation only occurred in 20% of tumors, a significant association between the recurrent chromosomal abnormalities and this mutation would not be expected. However, *K-ras* mutation was found to significantly associate with gain of 12p, an aberration observed in only 12% of cases. We believe these results demonstrate interdependence between two types of genetic abnormality, gene mutation and specific chromosomal abnormality.

In a previous study of 45 colorectal cancers, De Angelis *et al.* (11) also found *p53* mutation to be significantly associated with 8q gain and 18q loss. Although mutation of *p53* appeared to correlate with 20q and 13q gain, this did not reach statistical significance. These discrepancies may be due to the higher number of tumors that had no chromosomal aberrations (11%) in their study compared with ours (4%). In their study, no significant association was reported between *K-ras* mutation and specific chromosomal abnormalities, and *APC* mutations were not studied. In another recent study of colorectal adenomas, malignant polyps, and adenocarcinomas (14), an inverse correlation was found between *K-ras* mutation and 8q gain, a finding that is consistent with our results. In their study, mutations of the *APC* gene did not correlate with any CGH abnormality, and *p53* mutations were not studied.

Höglund *et al.* (19), by statistical analysis of 531 tumor karyotypes, have recently described several different cytogenetic pathways in colorectal tumorigenesis. One of these pathways appears to be initiated by +7p, and another appears to be initiated by +12p. The chromosomal imbalances that followed each of these aberrations differed with +8q, +9, +17p, -17p, and +20q being common after +7p and +2, +5p, +9, and +14q frequently occurring after +12p. It is interesting that +7p and +12p were two of the earliest aberrations because we have found these aberrations to associate with *APC* and *K-ras* mutations, respectively, and both *APC* and *K-ras* mutations have been shown to exist in the earliest neoplastic colorectal lesions (20). Furthermore, the aberrations +8q and +20q, which we have found to associate with a *p53* mutation, commonly occurred in the +7p group, but not the +12p group. This observation is consistent with our current finding that *K-ras* mutation is associated with +12p but correlates negatively with +8q and, by extension, supports our previous observation that *K-ras* and *p53* mutations are mutually exclusive events (4).

It is generally accepted that chromosomal losses correspond to loss of tumor suppressor genes and that chromosomal gains result in additional copies of oncogenes. Several candidate genes mapping to common regions of gain and loss in colorectal tumorigenesis have been postulated, *e.g.*, the oncogenes *c-src* on 20q (21, 22), *c-myc* on 8q (23, 24), and epidermal growth factor receptor on 7p (25, 26) and the tumor suppressors *SMAD2* and *SMAD4* on 18q (27, 28). However, there is currently insufficient evidence to firmly implicate these genes in colorectal tumorigenesis, and the number of potential alternative genes remains large. It is possible that the chromosomal aberration(s) that we have shown to associate with each mutated gene constitutes a genetic environment that is advantageous for the selection of the gene mutation or, alternatively, the gene mutation provides a favorable environment for selection of the chromosomal abnormalities. Thus, if functional relationships are found between *p53*, *K-ras*, or *APC* and candidate genes on the associated chromosomal regions, our findings may lend support to the potential role of such candidate genes in colorectal tumorigenesis. In this regard, it is interesting that epidermal growth factor receptor (7p12.3-12.1), which is overexpressed in the majority of colorectal neoplasms (26), has been shown, when defective or inhibited, to lead to a 90% reduction in intestinal polyp number in the *APC* (Min) model (29).

Our study reinforces the importance of both molecular and genome-

wide approaches to the analysis of solid tumors. We believe that the frequency of chromosomal aberrations demonstrated in this and other studies necessitates their inclusion in future models of colorectal tumorigenesis. However, the question of whether the chromosomal events precede or follow the gene mutations remains unanswered and will be clarified by studies on adenomas with varying degrees of dysplasia. This work is currently being performed in our department. In addition, we intend to further characterize the tumors in the present study by clinical follow-up and by analyzing other important tumor markers. It is likely that the diversity of genetic profiles within colorectal cancers underpins the distinct differences seen with regard to their clinical behavior and therapeutic response. Knowledge of the associations between genetic alterations may assist in the assessment of a patient's response to therapy and may ultimately lead to improved therapeutic strategies.

REFERENCES

1. Fearon, E. R., and Vogelstein, B. A genetic model for colorectal tumorigenesis. *Cell*, 61: 759-767, 1990.
2. Groden, J., Thliveris, A., Samowitz, W., Carlson, M., Gelbert, L., Albertsen, H., Joslyn, G., Stevens, J., Spirio, L., Robertson, M., *et al.* Identification and characterization of the familial adenomatous polyposis coli gene. *Cell*, 66: 589-600, 1991.
3. Arends, J. W. Molecular interactions in the Vogelstein model of colorectal carcinoma. *J. Pathol.*, 190: 412-416, 2000.
4. Smith, G., Carey, F. A., Beattie, J., Wilkie, M. J., Lightfoot, T. J., Coxhead, J., Garner, R. C., Steele, R. J., and Wolf, C. R. Mutations in APC, Kirsten-ras, and p53: alternative genetic pathways to colorectal cancer. *Proc. Natl. Acad. Sci. USA*, 99: 9433-9438, 2002.
5. Ionov, Y., Peinado, M. A., Malkhosyan, S., Shibata, D., and Perucho, M. Ubiquitous somatic mutations in simple repeated sequences reveal a new mechanism for colonic carcinogenesis. *Nature (Lond.)*, 363: 558-561, 1993.
6. Olschwang, S., Hamelin, R., Laurent-Puig, P., Thuille, B., De Rycke, Y., Li, Y. J., Muzeau, F., Girodet, J., Salmon, R. J., and Thomas, G. Alternative genetic pathways in colorectal carcinogenesis. *Proc. Natl. Acad. Sci. USA*, 94: 12122-12127, 1997.
7. Schreiber, S., Hampe, J., Eickhoff, H., and Lehrach, H. Functional genomics in gastroenterology. *Gut*, 47: 601-607, 2000.
8. Bardi, G., Pandis, N., Mitelman, F., and Heim, S. Karyotypic characteristics of colorectal tumors. In: S. R. Wolman and S. Sell (eds.), *Human Cytogenetic Cancer Markers*, 1st ed., pp. 151-168. Totowa, NJ: Humana Press Inc., 1997.
9. Kallioniemi, A., Kallioniemi, O. P., Sudar, D., Rutovitz, D., Gray, J. W., Waldman, F., and Pinkel, D. Comparative genomic hybridization for molecular cytogenetic analysis of solid tumors. *Science (Wash. DC)*, 258: 818-821, 1992.
10. Ried, T., Knutzen, R., Steinbeck, R., Blegen, H., Schrock, E., Heselmeyer, K., du Manoir, S., and Auer, G. Comparative genomic hybridization reveals a specific pattern of chromosomal gains and losses during the genesis of colorectal tumors. *Genes Chromosomes Cancer*, 15: 234-245, 1996.
11. De Angelis, P. M., Clausen, O. P., Schjølberg, A., and Stokke, T. Chromosomal gains and losses in primary colorectal carcinomas detected by CGH and their associations with tumour DNA ploidy, genotypes and phenotypes. *Br. J. Cancer*, 80: 526-535, 1999.
12. Curtis, L. J., Georgiades, I. B., White, S., Bird, C. C., Harrison, D. J., and Wyllie, A. H. Specific patterns of chromosomal abnormalities are associated with RER status in sporadic colorectal cancer. *J. Pathol.*, 192: 440-445, 2000.
13. Paredes-Zaglul, A., Kang, J. J., Essig, Y. P., Mao, W., Irby, R., Wloch, M., and Yeatman, T. J. Analysis of colorectal cancer by comparative genomic hybridization: evidence for induction of the metastatic phenotype by loss of tumor suppressor genes. *Clin. Cancer Res.*, 4: 879-886, 1998.
14. Hermesen, M., Postma, C., Baak, J., Weiss, M., Rapallo, A., Sciotto, A., Roemen, G., Arends, J. W., Williams, R., Giaretti, W., De Goeij, A., and Meijer, G. Colorectal adenoma to carcinoma progression follows multiple pathways of chromosomal instability. *Gastroenterology*, 123: 1109-1119, 2002.
15. Leslie, A., Carey, F. A., Pratt, N. R., and Steele, R. J. The colorectal adenoma-carcinoma sequence. *Br. J. Surg.*, 89: 845-860, 2002.
16. Hidaka, S., Yasutake, T., Takeshita, H., Kondo, M., Tsuji, T., Nanashima, A., Sawai, T., Yamaguchi, H., Nakagoe, T., Ayabe, H., and Tagawa, Y. Differences in 20q13.2 copy number between colorectal cancers with and without liver metastasis. *Clin. Cancer Res.*, 6: 2712-2717, 2000.
17. Kaplan, K. B., Burds, A. A., Swedlow, J. R., Bekir, S. S., Sorger, P. K., and Nathke, I. S. A role for the adenomatous polyposis coli protein in chromosome segregation. *Nat. Cell Biol.*, 3: 429-432, 2001.
18. Fodde, R., Kuipers, J., Rosenberg, C., Smits, R., Kielman, M., Gaspar, C., van Es, J. H., Breukel, C., Wiegant, J., Giles, R. H., and Clevers, H. Mutations in the APC tumour suppressor gene cause chromosomal instability. *Nat. Cell Biol.*, 3: 433-438, 2001.
19. Höglund, M., Gisselsson, D., Hansen, G. B., Sall, T., Mitelman, F., and Nilbert, M. Dissecting karyotypic patterns in colorectal tumors: two distinct but overlapping pathways in the adenoma-carcinoma transition. *Cancer Res.*, 62: 5939-5946, 2002.

20. Smith, A. J., Stern, H. S., Penner, M., Hay, K., Mitri, A., Bapat, B. V., and Gallinger, S. Somatic APC and K-ras codon 12 mutations in aberrant crypt foci from human colons. *Cancer Res.*, *54*: 5527–5530, 1994.
21. Talamonti, M. S., Roh, M. S., Curley, S. A., and Gallick, G. E. Increase in activity and level of pp60c-src in progressive stages of human colorectal cancer. *J. Clin. Investig.*, *91*: 53–60, 1993.
22. Le Beau, M. M., Westbrook, C. A., Diaz, M. O., and Rowley, J. D. Evidence for two distinct c-src loci on human chromosomes 1 and 20. *Nature (Lond.)*, *312*: 70–71, 1984.
23. Taub, R., Kirsch, I., Morton, C., Lenoir, G., Swan, D., Tronick, S., Aaronson, S., and Leder, P. Translocation of the c-myc gene into the immunoglobulin heavy chain locus in human Burkitt lymphoma and murine plasmacytoma cells. *Proc. Natl. Acad. Sci. USA*, *79*: 7837–7841, 1982.
24. Masramon, L., Arribas, R., Tortola, S., Perucho, M., and Peinado, M. A. Moderate amplifications of the c-myc gene correlate with molecular and clinicopathological parameters in colorectal cancer. *Br. J. Cancer*, *77*: 2349–2356, 1998.
25. Carlin, C. R., and Knowles, B. B. Identity of human epidermal growth factor (EGF) receptor with glycoprotein SA-7: evidence for differential phosphorylation of the two components of the EGF receptor from A431 cells. *Proc. Natl. Acad. Sci. USA*, *79*: 5026–5030, 1982.
26. Porebska, I., Harlozinska, A., and Bojarowski, T. Expression of the tyrosine kinase activity growth factor receptors (EGFR, ERB B2, ERB B3) in colorectal adenocarcinomas and adenomas. *Tumour Biol*, *21*: 105–115, 2000.
27. Hahn, S. A., Schutte, M., Hoque, A. T., Moskaluk, C. A., da Costa, L. T., Rozenblum, E., Weinstein, C. L., Fischer, A., Yeo, C. J., Hruban, R. H., and Kern, S. E. DPC4, a candidate tumor suppressor gene at human chromosome 18q21.1. *Science (Wash. DC)*, *271*: 350–353, 1996.
28. Eppert, K., Scherer, S. W., Ozcelik, H., Pirone, R., Hoodless, P., Kim, H., Tsui, L. C., Bapat, B., Gallinger, S., Andrusis, I. L., Thomsen, G. H., Wrana, J. L., and Attisano, L. MADR2 maps to 18q21 and encodes a TGF β -regulated MAD-related protein that is functionally mutated in colorectal carcinoma. *Cell*, *86*: 543–552, 1996.
29. Roberts, R. B., Min, L., Washington, M. K., Olsen, S. J., Settle, S. H., Coffey, R. J., and Threadgill, D. W. Importance of epidermal growth factor receptor signaling in establishment of adenomas and maintenance of carcinomas during intestinal tumorigenesis. *Proc. Natl. Acad. Sci. USA*, *99*: 1521–1526, 2002.