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BRIEF ARTICLE

Repeated anastomotic recurrence of colorectal tumors: Genetic analysis of two cases

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Abstract

AIM: To investigate genetics of two cases of colorectal tumor local recurrence and throw some light on the etiopathogenesis of anastomotic recurrence.

METHODS: Two cases are presented: a 65-year-old female receiving two colonic resections for primary anastomotic recurrences within 21 mo, and a 57-year-old female undergoing two local excisions of recurrent anastomotic adenomas within 26 mo. A loss of heterozygosity (LOH) study of 25 microsatellite markers and a mutational analysis of genes *BRAF*, *K-RAS* and *APC* were performed in samples of neoplastic and normal colonic mucosa collected over the years.

RESULTS: A diffuse genetic instability was present in all samples, including neoplastic and normal colonic mucosa. Two different patterns of genetic alterations (LOH at 5q21 and 18p11.23 in the first case, and LOH at 1p34 and 3p14 in the second) were found to be associated with carcinogenesis over the years. A role for the genes *MYC-L* (mapping at 1p34) and *FIHT* (mapping at 3p14.2) is suggested, whereas a role for *APC* (mapping at 5q21) is not shown.

CONCLUSION: The study challenges the most credited intraluminal implantation and metachronous carcinogenesis theories, and suggests a persistent, patient-specific alteration as the trigger of colorectal cancer anastomotic recurrence.

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Key words: Anastomotic recurrence; Colorectal cancer; Allelic loss; Genetic alterations

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INTRODUCTION

Local recurrences (LRs) from colorectal cancer are often inoperable and have poor prognoses, with an estimated 5-year survival of 10 percent and a median survival of 16 mo[1]. LRs are defined as being perianastomotic (when

rising in the extramural tissue) or primitively anastomotic[2]. These latter may be due to implantation of exfoliated cancerous cells in the suture line[3,4] or to metachronous carcinogenesis[5].

We report two singular cases of patients repeatedly developing recurrent tumors (adenocarcinoma, adenoma) at the suture line and/or in the contiguous colonic mucosa within 21 and 26 mo of left hemicolectomy and anterior rectal resection for colorectal adenocarcinoma, respectively. To clarify the molecular mechanism(s) implicated in such a singular feature and, more in general, the development of anastomotic recurrence, we performed an extended genetic analysis of patients' tumor tissues and colonic mucosa obtained from surgical specimens and follow up endoscopy. The investigation was focused on the chromosomal alterations most frequently associated with colorectal cancer development, including mutational analysis of *BRAF*, *K-RAS* and *APC* genes and loss of heterozygosity (LOH) analysis of 25 chromosomal sites known to be involved in colonic carcinogenesis. This is the first genetic study performed on anastomotic recurrence of colorectal cancer.

MATERIALS AND METHODS

Case 1

In November 1998, a 65-year-old woman underwent a left hemicolectomy with a stapled colorectal anastomosis for a 4.5 cm × 3 cm fungating tumor of the sigmoid colon, 32 cm from the anal verge. Preoperative workup did not show any local infiltration or liver/pulmonary metastases. Histological examination showed a moderately differentiated adenocarcinoma infiltrating the whole colonic wall up to the pericolic fat tissue with uninvolved mucosa 23 cm proximal and 20 cm distal to the tumor edges, and 20 tumor-free lymph nodes (pT2N0M0).

In accordance with our follow-up policy[6], the patient underwent clinical and ultrasound evaluation and circulating carcino-embryonic antigen (CEA) determination every three months, as well as computed tomography (CT) scan and colonoscopy one year postoperatively. The latter procedure identified a recurrence involving half the circumference of the colorectal anastomosis. No local or distant metastases were disclosed by a CT scan, and CEA level was normal. A colorectal resection with mesorectal excision and stapled colorectal anastomosis by the double-stapling technique[7] was performed 6 cm from the anal verge. The resected specimen showed a moderately differentiated adenocarcinoma infiltrating the muscle layer with 12 tumor free lymph nodes (pT2N0M0) and normal mucosa, 9 cm proximal and 6 cm distal to the tumor.

The patient presented nine months later with rectal bleeding. At colonoscopy the anastomosis showed a circumferential tumor recurrence and five polyps. The CEA level was normal and a CT scan of the abdomen and thorax did not disclose distant metastases. A colorectal resection with double-stapled coloanal anastomosis 2 cm from the anal verge was performed with a defunctioning ileostomy which was later closed. The patient received adjuvant radiotherapy (45 G) to the pelvis three weeks later. The histopathologic examination showed a moderately differentiated adenocarcinoma infiltrating the muscle layer (pT2N0M0) and five adenomas (3 located proximal and 2 distal) within 3 cm of the suture line, < 1 cm in size, with severe dysplasia.

The regular yearly follow up revealed no further sign of local recurrence or distant metastases and the patient is in good health 11 years after the initial resection. In January 2010, the patient underwent endoscopic exploration with biopsy.

Case 2

In May 2006, a 57-year-old woman underwent anterior rectal resection with coloanal anastomosis and ileostomy for a 4 cm polypoid lesion of the lower rectum (5 cm from the anal verge); on histological examination of endoscopic biopsies, this proved to be an adenocarcinoma arising in a villous adenoma. Neither regional nor distant spread was present at preoperative CT scan. In the resected specimen the histological diagnosis of adenocarcinoma developing from a high grade villous adenoma was confirmed, with initial invasion of the submucosa (early colorectal cancer), and free lymph nodes (n = 27) and surgical margins (pT1N0M0). After ileostomy closure, an anastomotic substenosis was easily resolved by 2 mechanical dilatations.

The patient was submitted to regular follow-up[6]. Twenty-two months after surgery, a colonoscopy revealed an asymptomatic anastomotic 3 cm polyp, which was completely removed by transanal resection. Histological examination showed a tubulo-villous adenoma with high grade dysplasia.

At the subsequent colonoscopy, 4 mo later, a second anastomotic 2 cm polyp was removed by transanal resection, again revealing an adenoma with high grade dysplasia. Neither local recurrences nor distant metastasis were detected at further follow-up. In September 2009, the patient underwent endoscopic exploration with biopsy.

Tissue processing and genetic analysis (Table 1)

In case 1 the LOH study (see Table 1) and the mutational analysis for *BRAF*, *KRAS* and *APC* (see below) were performed on the following samples: (1) primary adenocarcinoma and the corresponding peritumoral, distal and proximal mucosa; (2) first recurrence and peritumoral and distant mucosa (12 mo postoperatively); (3) second recurrence and adenoma (21 mo after initial surgery); and (4) anastomotic and distant colorectal mucosa (134 mo after initial surgery).

In case 2 the LOH study (see Table 1) and the mutational analysis for *BRAF* and *KRAS* were performed on: (1) primary tumor and peritumoral mucosa; (2) villous adenoma (22 mo postoperatively); (3) recurrent anastomotic adenomas (26 mo after initial surgery); and (4) anastomotic mucosa (40 mo after initial surgery).

Table 1 Microsatellite markers used in the loss of heterozygosity study, with relevant cytogenetic locations, putative genes involved and their function, and references to papers describing a role for colonic carcinogenesis

Microsatellite Markers	Cytogenetic band	Gene	Function	Ref.
BAT40	1p13.1			[8]
MYC-L	1p34			[8]
BAT 26	2p16.3	*hMSH2*	Mismatch repair enzyme	[8]
D2S123	2p16			[8]
D3S1481	3p14	*FHIT*	Histidine triad gene family (purine metabolism)	[9]
D4S2397	4p15.2			[10]
D5S346	5q21	*APC*	Antagonist of the Wnt signaling pathway	[8]
D10S1671	10q25			
D10S169	10q26.3	*MGMT*	DNA defense *vs* O6-methylguanine	[11]
D10S1765	10q23.3	*PTEN*	Protein tyrosine phosphatase	[12]
D16S421	16q22	*CDH1*	Ca++ dependent cell-cell adhesion glycoprotein	
D16S402	16q23-q24			
D16S507	16q23.2			
D17S250	17q21			[8]
TP53ALU	17p13.1	*TP53*	Tumor protein "guardian of the genome"	
TP53	17p13	*TP53*	Tumor protein "guardian of the genome"	[13]
D18S452	18p11.23			[14]
D18S53	18p11.22-p11			[13]
D18S64	18q21	*DCC*	Receptor for netrin 1	[15]
D18S857	18q22.1	*DCC*	Receptor for netrin 1	
DXYS233	Xp22.32-Yp11.3			[16]
SHOX	Xp22.3			[16]
DXYS154	Xqter-Yqter			[16]
DXS8009	Xq25-q26			
DXS8098	Xq24-q25			

Using 5 μm haematoxylin stained sections of tissue specimens routinely formalin fixed and paraffin embedded, DNA was isolated by manual microdissection and extracted using the QIAamp Tissue Kit (Qiagen GmbH, Hilden, Germany). Only tumor samples containing more than 70% tumor cells were included in the study. All microdissection were conducted in close collaboration with the pathologist to ensure consistency with histological diagnoses and accurate dissection for tumor cell enrichment. For each patient DNA extracted from normal lymphocytes was used as reference DNA. DNA quality was assessed by polymerase chain reaction (PCR) amplification of the human beta-globin gene.

Polymerase chain reaction

The molecular analysis was performed with a panel of 25 polymorphic microsatellite markers located on chromosomal regions potentially involved in colorectal cancer development and progression and listed in Table 1. Primer sequences and amplification conditions were in accordance with the Genome Database information (http://www.ncbi.nlm.nih.gov/genemap99). Forward primers were synthesized with a fluorescent tag (WellRed dyes from Research Genetics, Huntsville, AL, United States).

The target sequences were amplified by PCR in a 25 μL reaction mixture containing 2 μL DNA sample, 10x buffer (10 mmol/L Tris-HCl pH 9.0, 50 mmol/L KCl, 0.1% Triton X-100), 1.5 mmol/L $MgCl_2$, 0.2 mmol/L of each dNTP (Promega, Madison, WI), 0.4 μmol/L of each primer and 1.25 U Taq Polymerase (Promega, Madison, WI). Microsatellites were submitted to 35-40 cycles of amplification at different annealing temperatures (range 57 ℃-61 ℃). The presence and correct size of amplimers were evaluated by 2% agarose gel electrophoresis. The fluorescently labelled PCR products were subjected to electrophoresis on an automated DNA sequencer CEQ 8000XL (Beckman Coulter Inc., Fullerton, CA), and the fluorescent signals from the different sized alleles were recorded and analyzed using CEQ 10000XL analysis software (Beckman Coulter).

Definition of LOH and allelic imbalance

The LOH was defined as the ratio of relative allelic peak height in the tumor DNA to relative allelic peak height in the corresponding normal DNA. The formula employed for the calculation was T2: T1/N2: N1, where T1 and N1 are the height values for the smaller allele and T2 and N2 are the height values for the larger allele of the tumor (T) and normal (N) samples respectively. For informative markers LOH was scored when the signal reduction for one allele was of 40%. This degree of allelic imbalance (AI) indicates that a substantial proportion of the cells within a sample contains the same DNA abnormality and likely represents the presence of a clonal population. Abnormal results were demonstrated at least twice with equivalent results. At certain loci AI probably reflects increased copy number rather than loss of an allele. Distinguishing between these possibilities is important conceptually, but would not change data analysis. Therefore, all AIs were labelled as LOH.

The same areas of chromosomal regions showing LOH were repeated in an independently microdissected sample from different paraffin blocks when sufficient tissue was available.

Microsatellite instability

The novel appearance in the tumor DNA of one or more alleles, i.e. new peaks in the electropherogram, not present in its paired normal DNA, was considered as an indicator of microsatellite instability (MSI). Samples were classified as microsatellite stable or unstable according the revised Bethesda Criteria[8].

BRAF, KRAS and APC mutation analysis

Direct sequencing was performed to identify *BRAF* V600E mutations, *KRAS* codon 12/13 mutations and *APC* exon 15. Primer sequences for *BRAF* and *KRAS* were: *BRAF-F* (5'TGCTTGCTCTGATAGGAAAATGA-3'), *BRAF-R* (5'-TGGATCCAGACAACTGTTCAAA-3'), *KRAS-F* (5'-GCCTGCTGAAAATGACTGAA-3') and *KRAS-R* (5'-AGAATGGTCCTGCACCAGTAA-3'), which generated fragment lengths of 165 and 167 bp respectively. *APC* mutation analysis was performed using three sets of primers, amplifying two overlapping portions of exon 15 in accordance with Su *et al*[17]: *APC-1F* (5'-CATCAGCTGAAGATGAAATAGGA-3') and *APC-1R* (5'-GCAATCGAACGACTCTCAAA-3'), codons 1281–1402, 364 bp; *APC-2F* (5'-ATGTTCAGGAGACCCCACTC-3') and *APC-2R* (5'-CACTCAGGCTGGATGAACAA-3'), codons 1376–1508, 396 bp; and *APC–3F* (5'-GGGTCCAGGTTCTTCCAGAT-3') and *APC-3R* (5'-TTGCCACAGGTGGAGGTAAT-3'), codons 1478-1607, 387 bp.

DNA sequencing was performed using EurofinsMWGOperon/M-Medical (Milano, Italy). Sequencing results were verified in our laboratory in both directions using DNA STAR PC software (Lasergene, Madison, WI, United States). The presence of mutations was determined through alignment with normal sequences as reported in NCBI/Blast Human Genome database (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

RESULTS

LOH analysis

The results of LOH analysis for all 25 chromosomal markers investigated are reported in Table 2.

Case 1

A generalised genetic instability at various sites in tumoral and non-tumoral, histologically normal mucosal samples was seen. Chromosomes 5q, 16q and 18 presented with the highest frequency of LOH. In particular, LOH at 5q21 and 18p11.23 loci was consistently found in all tumor samples, including primary and recurrent adenocarcinomas and the late occurring adenoma, but was not found in any sample of non-tumoral mucosa. In contrast, LOH at 16q23-24 was consistently present in all extratumoral mucosa samples (except one showing MSI), being absent in all tumor specimens. LOH at 10q26.3 and 18q21 loci was also found to be restricted to non-tumoral mucosal samples, but only in those collected at the time of the initial surgery. The primary tumor, but not the neoplasms observed at the time of recurrences, showed LOH at 1p13.1 and 1p34.

When investigated for MSI, all samples showed a stable phenotype (in accordance with the Bethesda revised criteria[8]) except for one individual (proximal) sample of normal mucosa at the initial surgery, which was characterized by MSI in 5 of the chromosomal markers analyzed (low-MSI). The mutational analysis of exon 15 of *APC* gene demonstrated the presence of a single nucleotide polymorphism in the codon 1493 ACG > ACA (T1493T) in all tumoral and non-tumoral samples.

Case 2

LOH at 3p14 was found to be a consistent, specific tumor change occurring in all neoplastic specimens but not in samples of non-neoplastic mucosa. Furthermore, allelic loss was seen at the locus 1p34 of tumor specimens (except for the third adenoma) but was also observed in the peritumoral non-neoplastic mucosa, whereas LOH at 10q23.3 was restricted to the primary tumor and corresponding peritumoral mucosa. Scattered LOH changes were also found in homologous pseudo-autosomal regions (DXYS233, DXYS154, SHOX) of the sex chromosomes X-Y in the normal mucosa and the third adenoma. No evidence for MSI was yielded by any of the samples analyzed in this case.

Mutation analysis

The sequence analyses for *K-RAS* and *BRAF* mutation performed in tumor and normal tissues of both cases showed a wild type phenotype in all samples. Mutation analyses for exon 15 of *APC*, performed in Case 1, in which LOH was present at the gene locus in 5q21, yielded negative results.

DISCUSSION

Sixteen percent of patients undergoing colorectal resection for colon cancer present with a local recurrence[18], and, since in 12% of cases[19] the recurrences occur primarily at the site of the anastomosis, it may be estimated that roughly 2% of patients undergoing a colorectal resection for cancer will eventually develop an anastomotic recurrence. The mechanism(s) involved in the development of anastomotic recurrence are poorly understood. The present study has focused on genetic alterations occurring in primary and recurrent tumors as well as in the extra-tumoral colonic mucosa of two patients with repeated and rather early recurrence of anastomotic/perianastomotic neoplasms. To this end, a search was carried out for allelic losses at 25 chromosomal sites known to be involved in colonic carcinogenesis (Table 1) and for mutational events in three genes (*K-RAS*, *BRAF* and *APC*) commonly altered in colorectal cancer. This extensive genetic analysis included the normal mucosa at the time of the resection of the primary tumor ("genetic predisposition") and the potential changes in the genetic pattern of recurrent tumors and/or colonic mucosa pos-

Table 2 Results of loss of heterozygosity analysis with 25 microsatellite markers amplified in the present cases

	Case 1 (adenocarcinoma of the sigmoid colon - pT2N0M0)												Case 2 (adenocarcinoma of the rectum - pT1N0M0)					
	Initial surgery (left hemicolectomy)				Second procedure (colorectal resection) 12 mo after initial surgery (a.i.s.)			Third procedure (colorectal resection) 21 mo a.i.s.		Follow up (colonoscopy) 134 mo a.i.s.			Initial surgery (proctocolectomy)			Second procedure (endosc. resection) 22 mo a.i.s.	Third procedure (endosc. resection) 26 mo a.i.s.	Follow up (colonoscopy) 40 mo a.i.s.
	Prim. tumor	Peritu. mucosa	Distal mucosa	Prox. mucosa	First recurr.	Peritu. mucosa	Distant mucosa	Second recurr.	Aden	Anast. mucosa	Rectal mucosa	Colon mucosa	Prim. tumor	Peritu. mucosa	Aden.	Adenoma	Adenoma	Anast. mucosa
1p13.1	LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH
1p34	LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	LOH	LOH	LOH	LOH	NO LOH	NO LOH
2p16.3	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH
2p16	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
3p14	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	LOH	NO LOH	LOH	LOH	LOH	NO LOH
4p15.2	NO LOH	NO LOH	LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	LOH	NI	NI	NI	NI	NI	NI
5q21	LOH	NO LOH	NO LOH	NO LOH	LOH	NO LOH	NO LOH	LOH	LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH
10q23.3	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	LOH	LOH	NO LOH	NO LOH	NO LOH	NO LOH
10q25	NI	NI	NI	MSI	NI	NI	NI	NI	NI	NI	NI	NI	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH
10q26.3	NO LOH	NO LOH	LOH	LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	NI	NI	NI	NI	NI	NI
16q22	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
16q23.2	NO LOH	NO LOH	NO LOH	MSI	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH
16q23-q24	NO LOH	LOH	LOH	MSI	NO LOH	LOH	LOH	NO LOH	NO LOH	LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	
17p13	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	NI	NI	NI	NI	NI	NI
17p13.1	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH
17q21	NO LOH	NO LOH	NO LOH	MSI	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH
18p11.23	LOH	NO LOH	NO LOH	NO LOH	LOH	NO LOH	NO LOH	LOH	LOH	LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH
18p11.22-p11	NO LOH	NO LOH	LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	NI	NI	NI	NI	NI	NI
18q21	NO LOH	LOH	MSI	LOH	NO LOH	NO LOH	NO LOH	NO LOH	LOH	NO LOH	LOH	NO LOH	NI	NI	NI	NI	NI	NI
18q22.1	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH
Xp22.32-Yp11.3	NI	NI	NI	MSI	NI	NI	NI	NI	NI	NO LOH	NO LOH	NO LOH	NO LOH	LOH	NO LOH	NO LOH	NO LOH	NO LOH
Xp22.32-Yp11.3	NO LOH	NO LOH	LOH	NO LOH	NO LOH	LOH	NO LOH	NO LOH	NO LOH	LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	LOH	LOH
Xqter-Yqter	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NO LOH	LOH	NO LOH	NO LOH	NO LOH	NO LOH
Xq24-q25	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
Xq25-q26	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	NO LOH	NI	NI	NI	NI	NI	NI
FAL (%)	23.5	12.5	29.4	13.3	11.1	11.1	5.9	11.1	16.7	16	10.5	5.2	20	26.7	13.3	13.3	13.3	6.25

LOH: Loss of heterozygosity; NO LOH: Retention of heterozygosity; NI: Not informative; MSI: Microsatellite instability; FAL%: Fractional allelic loss (No. of markers with LOH/total No. of informative markers).

sibly involved in tumor progression and recurrences.

The LOH analysis (Table 1) showed in both cases a diffuse genetic instability at various sites both in tumor tissue and in extra-tumoral mucosa, although the affected loci largely differed between neoplastic and non-neoplastic samples as well as between non-neoplastic samples taken from different colonic regions. The fractional allelic loss did not significantly vary in the tumors

as compared to the normal mucosa or in the peritumoral as compared to distant mucosa (Table 2). In both cases a noticeable persistence of genetic changes both in the primary and recurrent tumors was found even though the affected chromosomal loci differed from one case to the other, being 5q21 and 18p11.23 in Case 1 and 1p34 and 3p14 in Case 2. Since these changes (with the exception of 3p14 in the first samples of Case 2) were consistently absent in the extra-tumoral mucosa, they may reasonably be considered as reflecting chromosomal alterations responsible for tumor development. Their consistent appearance in primary and recurrent tumors (with the exception of the third adenoma in Case 2) supports the supposition of an identical genetic mechanism for anastomotic recurrences. The potential involvement of the *APC* gene, which maps at 5q21, in colonic carcinogenesis of Case 1 is not supported by the lack of detectable mutations in the gene exon 15, which is more commonly altered in colo-rectal cancers. In Case 2, the LOH at 1p34 and 3p14.2 sites, the loci of mapping of the *MYC-L* and *fragile histidine triad* (*FHIT*) genes respectively suggest a key role of these two genes in this patient' s tumor development. In this regard it is worth noting that 1p34 LOH occurs in both tumoral and non-tumoral samples, whereas 3p14.2 LOH is absent in normal mucosa. This may suggest that the *MYC-L* alteration may reflect a "mark" of a proliferative instability leading to carcinogenesis, whereas the alteration of the *FHIT* gene may suggest its role in the events occurring at the early phase of carcinogenesis (i.e., transformation from normal mucosa to adenoma).

The two most credited theories to explain the occurrence of anastomotic recurrence are the intraluminal implantation of exfoliated cancerous cells[3,4] and the metachronous carcinogenesis[5], possibly triggered by modifications of the microenvironment around the suture depending on the surgical technique[20,21] or the materials used[22-24]. Both theories fail to satisfactorily explain our findings. Indeed, in both cases the intraluminal implantation theory, though supported by the presence of consistent genetic alterations in primary and recurrent tumors, is contradicted by other genetic alterations (such as LOH at 1p13.1 and 1p34 loci in case 1, and at 10q23.3 in case 2) that are present in the primary but not in the recurrent neoplasms. Moreover, the recurrence of perianastomotic benign tumors (adenomas) with overlapping genetic changes is also in contrast with the implantation theory. On the other hand, the metachronous carcinogenesis theory by itself cannot explain the short time needed to develop new tumors and their location at the suture line or within a short distance from it, since metachronous carcinogenesis *per se* implies the chance onset of a second adenoma/adenocarcinoma in any segment of the colonic remnant at an interval of years. Also the hypothesis that the anastomosis' surgical techniques[20,21] and/or the materials used[22-24] may be implicated in carcinogenesis by altering DNA at specific sites seems to be confounded by our findings, since the same genetic alterations found in recurrent neoplasms were present in the primary tumors, whose development obviously cannot be associated to previous surgical procedures. Moreover, such a hypothesis does not explain why recurrences occur in a very small minority of patients, in spite of the standardized surgical procedures performed (including the materials used) in all patients affected by colorectal cancer.

A genomic instability of DNA in tumor and adjacent tissues has already been described in breast cancer, where independent mutational events were observed[25-27]. Considering colon cancer, Ahlquist *et al*[28] found various epigenetic changes in mucosa surrounding colorectal neoplastic lesions, and hypothesised that the tumor itself may have caused a "field cancerization" of the contiguous mucosa. This phenomenon, in our opinion, is unlikely to have occurred in the present cases, owing to the large discrepancy in genetic changes between the tumors and the normal extratumoral mucosa in spite of a diffuse, tumor-independent genetic instability in the colonic mucosa of our patients. Independently of its aetiology, Umeto *et al*[29] suggested that colonic genetic instability associated with microenvironmental changes may "predispose" to metachronous carcinogenesis by altering several genes implicated in colon cancer development. In our cases, the consistency of genetic alterations among primary and recurrent tumors, even if separated by an interval of years, suggests a persistent, patient-specific alteration rather than a generic, diffuse DNA instability, as the trigger of recurrent carcinogenesis after radical resection of colorectal cancer.

In conclusion, although the mechanism of elective recurrent carcinogenesis in the anastomotic and/or perianastomotic areas still remains unsolved, since genetic alteration patterns differ in the two cases, our study supports a role for the genes found to be altered. Further studies in larger series of patients are warranted for assessing the potential role of these gene changes in the detection of patients at risk of developing an early anastomotic recurrence, and for confirming the patient-specificity of genetic alterations responsible of cancerogenesis, regardless of other genetic alterations occurring in colonic mucosa through years.

COMMENTS

Backgrounds

Local recurrences (LRs) from colorectal cancer are often inoperable and have poor prognoses, with an estimated 5-year survival of 10 percent and a median survival of 16 mo. LRs are defined as being perianastomoticor primitively anastomotic. These latter may be due to implantation of exfoliated cancerous cells in the suture line or to metachronous carcinogenesis.

Innovations and breakthroughs

This is the first genetic study performed on anastomotic recurrence of colorectal cancer.

Applications

Although the mechanism of elective recurrent carcinogenesis in the anastomotic and/or perianastomotic areas still remains unsolved, since genetic alteration patterns differ in the two cases in the study,it supports a role for the genes found to be altered.

Peer review

The conclusion needs to be modified given that the findings are based on a sample of two patients.

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