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Original Paper

Microsatellite Instability is Rare in Rectal Carcinomas and Signifies Hereditary Cancer

M. Nilbert, M. Planck, E. Fernebro, Å. Borg and A. Johnson

Department of Oncology, University Hospital, S-221 85 Lund, Sweden

We analysed microsatellite instability (MSI) in a consecutive series of 165 rectal carcinomas. Data on a personal and/or family history of cancer were collected from all patients and revealed metachronous cancer in 9 patients, 2 of whom had developed colorectal cancer, and a suspected familial aggregation of colorectal cancer in three families. Only three of the 165 (2%) rectal cancers showed MSI. The patients whose tumours displayed MSI had clinical histories suggesting hereditary cancer—a family history of colorectal cancer and/or synchronous colorectal cancers. Denaturing gradient gel (DGGE) analysis was used to screen the MSI+ patients for mutations in the *hMLH1* and *hMSH2* genes and revealed two new germline mutations; a 1 bp deletion in exon 10 of *hMSH2* creating a premature stop-codon and a splice donor site mutation in intron 16 of *hMLH1*. Considering colorectal carcinomas as a group, MSI has been reported to occur in approximately 10–20% of the tumours and thus can not, *per se* be used for clinical detection of hereditary tumours. This study shows, however, that MSI is rare in rectal carcinomas and when present strongly suggests a hereditary predisposition for colorectal cancer development. © **1999 Elsevier Science Ltd. All rights reserved.**

Key words: rectal cancer, hereditary nonpolyposis colorectal cancer, microsatellite instability, DNA-repair gene mutations, *hMLH1*, *hMSH2*
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INTRODUCTION

COLORECTAL CANCER is one of the commonest malignancies in the Western world. At least 10% is estimated to be hereditary and approximately half of these represents hereditary nonpolyposis colorectal cancer (HNPCC), which is the most common familial cancer syndrome [1, 2]. HNPCC is defined clinically by the occurrence of early onset colon cancer and an increased risk of developing extra-intestinal tumours such as endometrial, hepatobiliary, gastric, urothelial and ovarian cancer. HNPCC patients more often have mucinous and poorly differentiated colorectal carcinomas and a prediliction for proximal tumours; 60–70% of the HNPCC carcinomas are located proximal to the splenic flexure, compared with 30% among the sporadic cases [2]. Approximately 35% of the sporadic adenocarcinomas in the large intestine are situated in the rectum. Although the incidence of rectal cancer in HNPCC is unknown, the fraction of rectal tumours is lower than among the sporadic cases [2].

Correspondence to M. Nilbert, e-mail: mef.nilbert@onk/lu.se
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Widespread subtle alterations of the cancer cell genome was first identified in a subset of sporadic colorectal tumours [3]. These alterations are collectively referred to as microsatellite instability (MSI) and represent insertions and deletions in a variety of repeated sequences, particularly microsatellites representing mononucleotide, dinucleotide or trinucleotide repeats, which occur on average once per 100 000 bases in genomic DNA. Presence of MSI, thus indicates defective mismatch-repair. The MSI+ phenotype can be demonstrated in more than 90% of HNPCC patients fulfilling the Amsterdam criteria [4] and in 10–20% of 'sporadic' colorectal cancers, i.e. tumours occurring in patients who are unlikely to have HNPCC on the basis of age and family history [1, 5–10]. MSI is caused by underlying mutations in genes responsible for DNA-repair. The two major HNPCC suceptibility loci are *hMSH2* on chromosome 2p and *hMLH1* on chromosome 3p [11, 12]. We investigated the frequency of MSI in a consecutive series of 165 rectal carcinomas. Data on familial cancer were available from all cases and were correlated with the MSI data. Patients whose tumours displayed MSI were analysed for mutations in the *hMSH2* and the *hMLH1* genes by denaturing gradient gel electrophoresis (DGGE) analysis and DNA sequencing.

PATIENTS AND METHODS

We analysed 165 consecutive pre-operative biopsies from rectal cancers in patients referred to our regional hospital during 1996–1998. Rectal cancer was defined as an adenocarcinoma located within 15 cm from the anal canal. The median age of the patients was 65 years (range 24–92); 5 of the 165 patients were below 40 years of age. Family history was obtained through verbal questioning of all patients.

Microsatellite markers from different chromosomes were used to evaluate the tumour specimens for MSI. BAT25, BAT26 and BAT40 were used as mononucleotide markers and D2S123, D10S197, D17S787 and BAT34C4 as dinucleotide markers. The primers sequences for BAT25, BAT26 and for BAT40 were as described by Parsons and colleagues [13], for D2S123 as described by Dib and colleagues [14], for D10S197 and D17S787 the sequences were obtained from Weissenbach and colleagues [15] and for BAT34C4 from Zhou and colleagues [16]. Primers for each specific locus were used to amplify the repeat and flanking sequences from blood and tumour DNA using PCR technique. The products were labelled with ^{32}P-dATP during the amplification reactions, followed by electrophoretic separation in 6% denaturing polyacrylamide gels and autoradiography. The MSI analysis has previously been described in detail [17]. We required information from at least four informative loci, always including BAT25, BAT26 and BAT40, in order to score MSI.

Mutation screening of *hMLH1* and *hMSH2* was performed using denaturing gradient gel electrophoresis (DGGE) analysis as previously described [18]. A 40-mer GC-clamp was added to the 5′ end of one of the primers, which were designed to encompass the entire exon. The DGGE primer sequences were for *hMLH1* exon 16 5′-CATTTG-GATGCTCCGTTAAAG-3′ (forward) and 5′-GCGGCC-GCCCGTCCCGCCGCCCCCGCCCCGCCGCGGCCG-CACAACAGAAGTATAAGAATGGCTGTC-3′ (reverse) and for *h*MSH2 exon 10 5′-CTTTTTCTTTTCTTCTTG-3′ (forward) and 5′-GCGGCCGCCCGTCCCGCCGCCC-CCGCCCCGCCGCGGCCGCAATAAAGGGTTAAAAA-TA-3′ (reverse). PCR amplification was performed using AmpliTaq gold and the times and temperatures used were 93° for 12 min followed by 35 cycles of 93° for 30 sec, 55° for 45 sec and 72° for 45 sec, followed by an extension period of 72° for 5 min. Gradient gels were made by mixing 100% denaturant (7 M urea and 40% formamide) with 0% denaturant stock solutions in 7% acrylamide gels in a gradient maker. The gels were run for 4–4.5 h at 130 V, stained with ethidium bromide and photographed. Exons displaying aberrant bands on DGGE were sequenced using specially designed sequencing primers containing an M13 sequence at the 5′end of the forward primer, but were otherwise identical to those used in the DGGE analysis, as were also the reverse primers. Sequencing was performed on an Applied Biosystems 373 sequencer using cycle sequencing with AmpliTaq polymerase FS and using the reagents supplied by Applied Biosystems (Perkin Elmer-Roche, Branchburg, New Jersey, U.S.A.).

RESULTS

Family histories from the 165 rectal cancer patients revealed that 55 patients had one, 18 had two and 14 patients had three or more first-degree relatives affected by any type of cancer. The family histories from three families revealed three or more relatives with colorectal cancer. However, because of late (>50 years) onset tumours or tumours occurring in only one generation, none of these families fulfilled the Amsterdam criteria for the classification of HNPCC. Of these three, the rectal tumour from 1 patient (case 1) displayed MSI, whereas the other 2 patients had MSI-negative rectal carcinomas. Except for colon cancer in case 2 and metachronous endometrial cancer in case 3, other HNPCC related cancer types—gastric, ovarial, urothelial or hepatobiliary cancer—were not seen in any of the patients or their first-degree relatives. Metachronous malignancies had developed in 9 patients; 2 with previous colon cancer, 4 with breast cancer, 1 with an endometrial cancer, 1 with multiple myeloma and 1 patient who had developed a laryngeal cancer.

MSI was present in 3 of the 165 rectal tumours and all three tumors had widespread instability affecting all loci investigated. One additional tumour displayed instability of only one locus and was classified as MSI-negative. The patients with MSI + tumours presented a personal and/or family history suggesting hereditary cancer (Table 1). Case 1 was a 64-year-old man who disclosed that 5 of his 9 siblings had developed colorectal cancer, the youngest at the age of 34 years, and one sister with rectal cancer had also developed an endometrial carcinoma. Case 2 was a man who had developed a right-sided colon cancer at the age of 46 years, a left-sided colon cancer at the age of 54 years and a rectal cancer at the age of 75 years. Case 3 was a woman who had developed an endometrial carcinoma at the age of 43 years and a rectal cancer at the age of 55 years. Neither of the latter 2 patients had a family history suggesting hereditary colorectal cancer. None of these patients fulfilled the Amsterdam criteria for the diagnosis of HNPCC, but all complied with the recent Bethesda guidelines for MSI testing of colorectal tumours [4, 19].

Blood samples from the 3 patients with MSI + tumours were further investigated for germline mutations in the DNA-repair genes *hMLH1* and *hMSH2*. Cases 1 and 2 revealed novel constitutional DNA-repair gene mutations; a truncating mutation in *hMSH2* exon 10 and a splice donor mutation in *hMLH1* intron 16 (Table 1, Figure 1).

Table 1. *Clinical data and DNA-repair gene mutations*

Case	Age (years)/ sex	Metachronous cancer/ age (years)	Family history of colorectal cancer	Gene and location of mutation	Nucleotide change	Type of mutation
1	64/M	–	6/10 Siblings colorectal cancer	*hMSH2* exon 10	Deletion A	Deletion A at codon 529, termination at codon 542
2	75/M	Colon/46 and 54	–	*hMLH1* intron 16	GAGgtg→GAGttg	g→t in splice donor site
3	55/F	Endometrial/43	–	–	–	

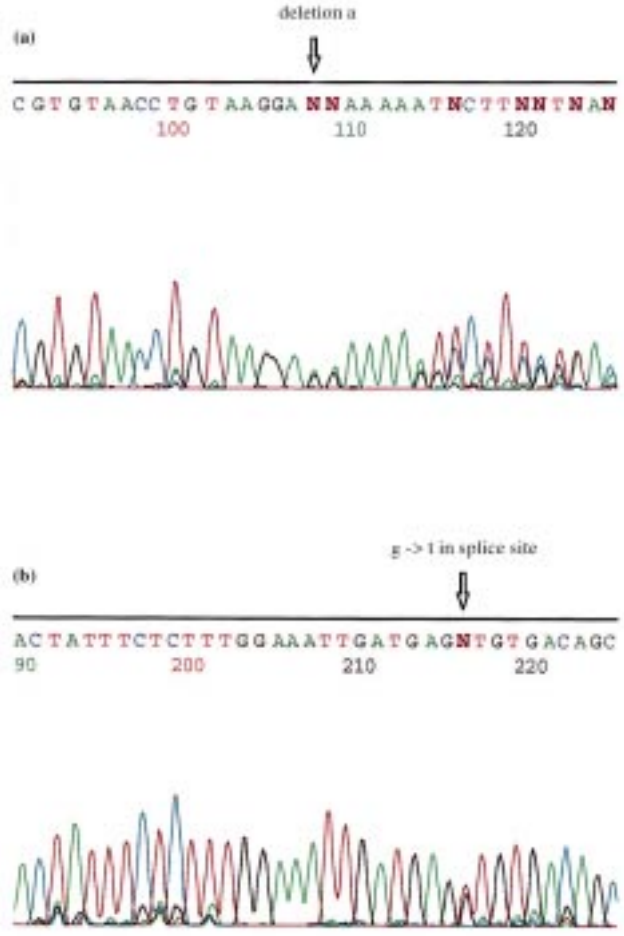

Figure 1. (a) 1 bp deletion in codon 529 of *hMSH2* exon 10 creating a premature stop-codon in codon 542. (b) g→t mutation in the splice donor site of *hMLH1* intron 16.

DISCUSSION

Clinically, colon carcinomas and rectal carcinomas differ concerning treatment, risk for local recurrence and prognosis. Most of the impressive amount of genetic data in this field, is based on studies including both tumour entities. In our series of 165 consecutive rectal cancer patients, genealogical data was collected from all patients. Previous studies including patients with carcinomas of the colon and the rectum have detected hereditary patterns suggesting autosomal dominant inheritance in at least 10% of the cases [1]. We found 14 patients with three or more first-degree relatives with various types of cancer, 3 individuals with a suspected familial aggregation of colorectal cancer and 9 patients who had developed metachronous cancer, 2 of which were colorectal. No patient fulfilled the Amsterdam criteria for the classification of HNPCC [4], but 5 patients, including the 3 who later turned out to have MSI + tumours and to carry germ-line mutations in DNA-repair genes, fullfilled the recent Bethesda guidelines [19]. Thus, hereditary patterns based on family history, as well as on the low frequency of MSI + tumours in our series, seem to be low in rectal carcinomas.

In studies comprising carcinomas from the colon and from the rectum MSI occur in 10–20% of the tumours [3, 5–10]. Thus, considering colorectal cancers as a group, detection of MSI is not *per se* a clinically useful parameter for detection of hereditary tumours, since MSI occurs also in a subset of non-HNPCC colorectal cancers [20]. We studied MSI and DNA-repair gene mutations in a consecutive series including only rectal carcinomas and detected MSI in a considerably lower frequency, 2%. Our findings are in accordance with Lothe and colleagues [5], who found MSI in more than two loci in only 1/105 rectal cancers. Furthermore, among the previously reported sporadic distal colorectal cancers, which has been classified as MSI +, almost half of the patients have displayed either a low age of onset, metachronous tumours or a family history of cancer, suggesting hereditary colorectal cancer [3, 7, 17, 21]. Although not fulfilling the Amsterdam criteria for the classification of HNPCC, all 3 patients with MSI + rectal carcinomas in our study had personal and/or family histories suggesting hereditary colorectal cancer, and germ-line mutations in *hMSH2* and *hMLH1* were identified in 2 of these 3 patients. Neither of these mutations have, to our knowledge, previously been reported. The phenotypic consequence of these specific mutations and any possible association with rectal cancer is unknown.

Data on metachronous tumour development and family history of cancer can together with MSI analysis help identify patients carrying constitutional DNA-repair gene mutations. Such mutations have implications for follow-up for the individual because of the increased risk for metachronous colorectal cancer and development of other HNPCC-associated tumour types. Also other family members may be at risk of hereditary cancer and should be offered genetic counselling and inclusion in adequate screening programmes. Our findings show that MSI is a rare phenomenon in rectal cancers, but when present strongly suggests that the individual has a genetic predisposition for colorectal cancer development.

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