Microsatellite instability in sporadic and inherited colon adenocarcinomas from Greek patients : correlation with several clinicopathological characteristics

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Abstract

Background and aims : **Microsatellite instability seems to play a significant role in colorectal carcinogenesis, as it is reported to occur in HNPCC patients as well as in a proportion of sporadic cases. The aim of this study was to examine the presence of microsatellite instability in relation to other commonly observed genetic abnormalities and clinicopathological characteristics of sporadic and inherited colorectal cancers.**

Methodology : **One hundred and three sporadic colorectal adenocarcinomas and 9 adenocarcinomas from HNPCC patients were histologically evaluated. The presence of microsatellite instability was investigated at six loci. *K-ras* and *p53* mutations, *p53* LOH, hMLH1 expression and methylation status were examined as well. Statistical analysis was performed to define possible correlations of the observed genetic alterations with the clinicopathological characteristics of the analysed tumors.**

Results : **High-grade microsatellite instability was found in 14% of sporadic adenocarcinomas and in 78% of adenocarcinomas from HNPCC patients. *K-ras* and *p53* mutations were found in 29% and 28% of sporadic adenocarcinomas respectively and in 0% and 22% of the 9 HNPCC cases. A statistically significant correlation was noticed in sporadic tumors between the presence of MSI-H and tumor location at the proximal colon, as well as with the female gender.**

Conclusions : **Sporadic MSI+ colon adenocarcinomas seem to represent a distinct entity with a unique profile of genetic changes, different from those observed in HNPCC or MSI negative sporadic tumors.** (Acta gastroenterol. belg., **2005**, 68, **294-301**).

Key words : MSI, *K-ras*, *p53*, HNPCC, sporadic colon cancer.

Abbreviations

Hereditary Non-Polyposis Colorectal Cancer (HNPCC), Familial Adenomatous Polyposis (FAP), Chromosomal Instability (CIN), Microsatellite Instability (MSI), High-level Microsatellite Instability (MSI-H), Low-level Microsatellite Instability (MSI-L), Microsatellite Stable (MSS), Mismatch Repair (MMR), Transforming Growth Factor-β Receptor type II (TGF-βRII), Polymerase Chain Reaction (PCR), Loss Of Heterozygosity (LOH), Immunohistochemistry (IHC), Restriction Fragment Length Polymorphisms (RFLPs), Single-Stranded Conformation Polymorphism (SSCP), 5-fluorouracil (5-FU), Tumor Infiltrating Lymphocytes (TIL)

Introduction

Colorectal cancer, apart from sporadic forms that constitute the great majority of tumors (90-95%), includes two well-characterized syndromes : familial adenomatous polyposis (FAP syndrome) and hereditary non-polyposis colorectal cancer (HNPCC syndrome), both inherited in an autosomal dominant mode. Molecular analysis of the responsible genes in each inherited syndrome has provided clues to alternative pathways leading to carcinogenesis in colorectal cancer.

To date, two main distinct pathways leading to the pathogenesis of colon cancer have been identified : chromosomal instability (CIN) and microsatellite instability (MSI). MSI is reported to occur mainly in proximal to the splenic flexure carcinomas and is due to genetic alterations in one of the DNA mismatch repair genes (MMR genes i.e. *hMSH2*, *hMLH1*, *hMSH6*, *hPMS1* and *hPMS2*). The presence of MSI has offered a potential marker for the identification of individuals who probably possess germline mutations in MMR genes. Although MSI characterizes HNPCC syndrome - in 70-90% of HNPCC cases a germline mutation of *hMLH1* and *hMSH2* genes has been reported (1,2)- it has been demonstrated in about 10-15% of sporadic colorectal carcinomas as well (3). It is noteworthy that in these tumors the epigenetic silencing of *hMLH1* gene, due to promoter hypermethylation, constitutes an alternative way to *hMLH1* inactivation by mutation (4).

On the other hand, CIN is reported to occur mainly in distal to the splenic flexure colon carcinomas and involves alterations in both proto-oncogenes and tumor suppressor genes, such as the sequence of mutations in *APC, K-ras, DCC/Smad4* and *p53*, initially proposed by Vogelstein *et al.* (5). The relationship between the MSI phenotype and alterations of the above genes remains to be settled (3,6).

The identification of the underlying pathway leading to colon cancer seems to be of great importance since patients with tumors developed through the MSI pathway are reported to have a better prognosis compared to patients with tumors displaying CIN. Another important clinical difference related to the two pathways emerges in the therapeutic confrontation, with regard to both radiotherapy and chemotherapy ; for instance, it has been suggested that MSI positive cancers are insensitive to 5-fluorouracil (5-FU) and that patients with MSI

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tumors exhibit a better overall survival if they don't receive 5-FU (7). Furthermore, MSI positive cells are highly tolerant to several methylating chemotherapeutic drugs (8). It is thought that in the future clinical decision-making, regarding the selection of appropriate chemotherapeutic agents, could be based on the MSI status of cancers, as a slight difference in the response of MSI positive cancers to chemotherapy may greatly affect their clinical outcome (7,8,9).

In the present study we examined the presence of MSI in parallel with alterations of *K-ras* and *p53* genes in a series of sporadic and inherited colon carcinomas from Greek patients, aiming to investigate any possible correlation between molecular alterations and clinicopathological features of the analyzed tumors.

Methodology

Tumor specimens

One hundred and three sporadic colorectal adenocarcinomas and 9 adenocarcinomas from HNPCC patients were available for examination. HNPCC patients were selected according to the Amsterdam criteria (10) and were divided in two subgroups : group 1, meeting all the Amsterdam criteria (e.g. at least three relatives with histologically proved colorectal cancer, one of them being a first degree relative, at least two successive generations affected and, in one of the affected relatives, colorectal cancer being diagnosed under 50 years) and group 2, meeting all the Amsterdam criteria apart from the age when colorectal cancer is diagnosed, which is over 50 years in all relatives. Seven HNPCC patients satisfied all the Amsterdam criteria for HNPCC (group 1) and the rest two were patients meeting the group 2 criteria. All patients were of Greek descent.

After resection, the neoplastic tissues were fixed in 10% buffered formalin and processed to paraffin wax by routine methods. In most cases, the proportion of neoplastic cells was more than 75% in each block, as estimated using histopathological analysis. In HNPCC patients and in 23 of the sporadic patients, both tumor specimens and blood or normal fresh tissue were available.

Pathologic examination

Formalin-fixed and paraffin embedded tissue sections were routinely processed. Tumors were typed as adenocarcinomas. The evaluated clinicopathological variables are demonstrated in Tables I and II. In detail as far as histologic grading is concerned, grade I (well differentiated) tumors showed well-formed glands in over 75%, grade II (moderately differentiated) in 25% to 75% and grade III (poorly differentiated) in 25%. The Crohn's-like lymphoid reaction was evaluated following the criteria of Graham and Appelman (11) and was classified as conspicuous (intense) and inconspicuous (12).

Table I. — **Clinicopathological features and MSI status in 103 sporadic colorectal adenocarcinomas**

Variables	Total	MSI-H	MSI-L / MSS	p-value
Age (yr)	64.79 +/- 11.38			
Gender	N = 103			
Male	59	4	55	0.019
Female	44	10	34	
Tumoral Location	N = 103			
Right	36	9	27	0.013
Left	67	5	62	
Dukes' stage	N = 103			
A + B (without metastases)	43	6	37	0.584
C (nodal metastases)	52	6	46	
D (distant metastases)	8	2	6	
Grade	N = 103			
I or II	78	11	67	0.790
III	25	3	22	
Extracellular mucin production	N = 79			
Present at 30% of the tumor	24	2	22	0.727
Absent	55	6	49	
Crohn's-like lymphoid reaction	N = 79			
Conspicuous	5	1	4	–
Inconspicuous	74	7	67	
Tumor infiltrating lymphocytes	N = 62			
Present	45	6	39	0.667
Absent	17	3	14	
Vein invasion	N = 62			
Present	12	1	11	0.499
Absent	50	8	42	
Pattern of growth	N = 62			
Expanding	11	2	9	0.704
Infiltrative	51	7	44	
Residual adenomatous tissue	N = 62			
Present	18	2	16	0.627
Absent	44	7	37	
Serrated architecture	N = 62			
Present	10	1	9	0.658
Absent	52	8	44	

Genomic DNA isolation

20 μm sections were used for DNA extraction. The samples were digested for 3 hours at 55°C using 200 μl of digestion buffer consisting of 50 mM Tris, 1 mM

Table II. — **Clinicopathological features and MSI status in 9 HNPCC adenocarcinomas**

Variables	Total (N = 9)	MSI-H	MSI-L / MSS
Age (yr)	52.44 +/- 10.89		
Gender	N = 9		
Male	3	2	1
Female	6	5	1
Tumoral Location	N = 9		
Right	2	2	0
Left	7	5	2
Dukes' stage	N = 9		
A + B (without metastases)	5	3	2
C (nodal metastases)	4	4	0
D (distant metastases)	0	0	0
Grade	N = 9		
I or II	6	4	2
III	3	3	0
Extracellular mucin production	N = 9		
Present at 30% of the tumor	0	0	0
Absent	9	7	2
Crohn's-like lymphoid reaction	N = 9		
Conspicuous	3	3	0
Inconspicuous	6	4	2
Tumor infiltrating lymphocytes	N = 4		
Present	4	4	0
Absent	0	0	0
Vein invasion	N = 4		
Present	0	0	0
Absent	4	4	0
Pattern of growth	N = 4		
Expanding	1	1	0
Infiltrative	3	3	0
Residual adenomatous tissue	N = 4		
Present	0	0	0
Absent	4	4	0
Serrated architecture	N = 4		
Present	0	0	0
Absent	4	4	4

EDTA, 0.5% SDS and 200 µg/ml Proteinase K. Genomic DNA was extracted with phenol-chloroform and precipitated in ice-cold ethanol. The DNA was redissolved in distilled water and quantitated by spectrophotometry at 260 nm under UV light.

PCR

500 ng of total DNA were amplified in a 50 µl reaction mixture containing 25 pmoles of each primer, 25 mM each dNTP, 1.5 mM $MgCl_2$, 1 mM KCl, 0.1% gelatin and 1.5 U Taq DNA polymerase (BD Biosciences, Palo Alto, CA, USA). The profile used in the Progene Techne thermal cycler was : 5 min at 95°C once ; 30 sec at 95°C, 40 sec at 52-56°C, 1 min at 72°C for 40 cycles ; 7min at 72°C once. Sequences of the primers used are according to previous studies (13,14, 15).

RFLP analysis

5-10 µl aliquots of the PCR product were digested with the appropriate restriction enzyme to reveal the presence of mutations. The enzyme used for mutation detection in codon 12 of the *K-ras* gene was BstNI and incubation was performed at 60°C overnight. The products were analyzed by ethidium bromide staining under UV light after electrophoresis on a 4% (3 Nusieve : 1 agarose) gel.

SSCP analysis

PCR products were screened for mutations in exons 5-8 of the *p53* gene. Firstly, PCR products were diluted in 10 µl formamide-dye solution (95% formamide, 20 mM EDTA, 0.05% bromophenol blue, 0.05% xylene cyanol), denatured for 7 min at 95°C and kept on ice until loaded onto a 0,5X MDE gel (BMA, USA). Electrophoresis was carried out at 3 W for 16-18 hours at 4°C. After electrophoresis, the gel was silver stained and examined for abnormal band patterns.

Microsatellite instability analysis

Five microsatellite markers previously proposed (16) (BAT25, BAT26, D5S346, D2S123 and D17S250), as well as a specific sequence of the *TGF-β RII* gene encompassing a 10 poly(A) tract at codons 125-128 were analyzed. The PCR products were loaded on a 25% non-denaturing polyacrylamide gel and run at 100-200V for 20 hours. Silver staining was then used to visualize the bands. Human colon cancer cell line HCT116, which is known to express microsatellite instability phenotype was used as a positive control. Tumors exhibiting MSI with at least 2 of the 6 microsatellite markers altered were classified as MSI-high (MSI-H). Tumors demonstrating instability at a single locus were classified as MSI-low (MSI-L) and tumors with no alterations in the markers examined were classified as stable (MSS) (16). The analysis of MSI+ cases was repeated twice by independent PCR reactions.

Sequencing

For *Tgf-βRII* A_{10} tract mutation analysis the Big Dye terminator cycle sequencing kit was used. The products were analyzed on an ABI prism 310 Genetic analyzer

(Perkin-Elmer, California, USA). PCR primers were used for sequencing as well.

LOH detection in p53

The variable number of tandem repeats (VNTR) segment in intron 1 of the *p53* (17) was amplified using primers previously described (18). PCR products from tumor and matched-normal tissue were run in parallel lanes on a 4% agarose gel stained with ethidium bromide. Only the cases showing two different alleles in the normal tissues were considered as informative for LOH. LOH was determined when loss of one allele was evident in the corresponding tumor tissue (products showing one band).

Methylation analysis

Genomic DNA was modified with sodium bisulfite as described previously (19). Briefly, 1.5 µg of genomic DNA was denatured with 0.3M NaOH for 30 min at 42°C. 10 mM hydroquinone (Sigma) and 3 M sodium bisulfite (Sigma) at pH 5.0 were added and the samples were incubated at 55°C for 17 h. After treatment, the DNA was purified using the Jetquick DNA Clean-Up Spin Kit (Genomed). The DNA was then desulfonated with 0.3M NaOH for 15 min at 37°C, neutralized with Sodium acetate and then precipitated in 100% ethanol overnight at -20°C. The samples were resuspended in distilled water and stored at -20°C. This procedure resulted in the conversion of unmethylated cytosine to thymine, whereas the methylated cytosine remained unchanged.

Methylation Specific Polymerase Chain Reaction (MS-PCR)

Primer sequences were used specific for both methylated and unmethylated forms of *hMLH1* promoter. The primer sequences were as follows : unmethylated reaction : sense primer : 5'-TTTTGATGTAGATGTTTTATTAGGGTTGT – 3', antisense primer : 5'- ACCACCTCATCATAACTACCCACA – 3', methylated reaction : sense primer : 5'- ACGTAGACGTTTTATTAGGGTCGC – 3', antisense primer : 5'- CCTCATCGTAACTACCCGCG – 3'. Amplification were carried out in a thermal cycler (Progene, Techne) for 40 cycles (40sec at 94°C, 45sec at 59-60°C and 50sec at 72°C) followed by a final extension of 7min at 72°C. Samples without DNA (negative controls) as well as methylated DNA (positive controls) were included for each set of PCR. The PCR products were analyzed on a 3% agarose gel and visualized under UV illumination after ethidium bromide staining.

Immunohistochemical analysis

MLH1 immunostaining was performed using hMLH1 mouse monoclonal antibody (G168-15, Pharmingen, San Diego, California). Sections (3 to 4 µm) were deparaffinized, rehydrated, immersed in 3% H_2O_2 for 30 min and microwaved at 750 W in 0.01 M citrate buffer (pH 6.0) for 20 min and left to cool down in TBS. Sections were incubated with hMLH1 antibody at 1:180 dilution overnight. Immunostaining was performed using the standard avidin-biotin complex and visualized with diaminobenzidine tetrahydrochloride solution. According to the percentage of positively stained neoplastic nuclei, each case was considered to display reduced (< 20%) or preserved (> 20%) expression of hMLH1 ; the same cut-off points have been applied elsewhere (20). All immunostained nuclei were scored as positive, irrespective of staining intensity. As proposed elsewhere (21), tumor cells were judged to be negative for expression only if they lacked nuclear staining in a sample in which normal crypt epithelial cells of the proliferative compartment and/or lymphoid cells in the germinal center of lymphoid nodules in each slide were stained (internal positive controls). Absence of staining in the smooth muscle of muscularis propria defined satisfactory negative background.

Results

MSI analysis

A hundred and twelve colon adenocarcinomas including tumors from 9 HNPCC (group 1 and group 2) patients were examined for the presence of MSI. A panel of six mononucleotide and dinucleotide microsatellite markers was analyzed when normal tissue was available ; otherwise the sensitive quasimonomorphic mononucleotide markers BAT26 and TGF-βRII were applied (Fig. 1).

The MSI analysis of 103 sporadic adenocarcinomas revealed 17 cases with MSI. MSI-H was found in 14/103 (14%) sporadic cases as well as in 7 out of 9 (78%) adenocarcinomas from HNPCC patients, all of the latter belonging to group 1. MSI-L was observed in 3 sporadic (3%) and 2 adenocarcinomas from HNPCC "group 2" patients (22%), while the rest 86 sporadic adenocarcinomas were MSS (83%). All MSI-H samples showed alterations in *TGF-βRII* as well.

Clinicopathological features of MSI-H adenocarcinomas

Twenty-one MSI-H adenocarcinomas (sporadic, n = 14, HNPCC, n = 7) were classified according to gender (male, n = 6, female, n = 15), location (left colon, n = 10, right colon, n = 11), grade (well differentiated, n = 3, moderately differentiated, n = 13, poorly differentiated, n = 5) and Duke's stage (A, n = 2, B, n = 7, C, n = 10, D, n = 2) (Tables I, II). Three of the 14 sporadic MSI-H adenocarcinomas (21%) were *K-ras* mutated and 4 of them (29%) were *p53* mutated. None of the HNPCC adenocarcinomas was *K-ras* mutated, while in 2 of them (29%) a *p53* mutation was demonstrated.

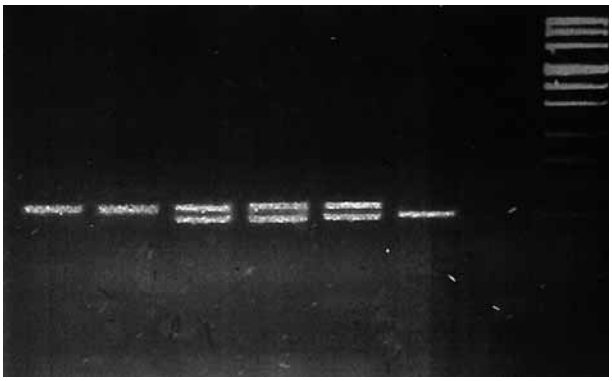

Fig. 1. — BAT26 analysis. Lanes 1,3,4 : MSI- adenocarcinomas ; lane 2 : MSI+ adenocarcinoma ; lane 5 : human colon cancer cell line HCT116.

Clinicopathological features of MSI-L and MSS adenocarcinomas

All 3 MSI-L sporadic cases were female, moderately differentiated and located at the distal colon. Two of them were of Dukes' stage B and one of C. One out of three MSI-L carcinomas had only a *p53* exon 8 mutation, while another one had only a *K-ras* codon 12 mutation and in the last one both *p53* exon 5 and *K-ras* mutations were determined. The 2 MSI-L HNPCC (group 2) cases were of Dukes' stage B, moderately differentiated, located at the distal colon and exhibited no mutations in both *K-ras* and *p53* genes.

MSS adenocarcinomas were observed in 86 out of 103 sporadic cases and were classified according to gender (male = 55, female = 31), location (left colon = 59, right colon = 27), grade (well differentiated = 9, moderately differentiated = 55, poorly differentiated = 22) and Dukes' stage (A = 6, B = 29, C = 45, D = 6) (Tables I, II). *K-ras* and *p53* mutations were determined in 25/86 (29%) and 18/70 (26%) adenocarcinomas respectively.

K-ras and p53 molecular analysis

K-ras mutations at codon 12 were found in 30/103 (29%) sporadic adenocarcinomas but not in any of the 9 specimens from HNPCC patients (groups 1 and 2). SSCP analysis of *p53* exons 5-8 revealed mutations in 24/87 (28%) sporadic adenocarcinomas and in 2/9 (22%) HNPCC adenocarcinomas (group 1).

LOH analysis

A subset of 23 sporadic cases with available matching normal tissue were examined for LOH of *p53.* Twelve cases were scored as informative and in 2 of them LOH was demonstrated (Fig. 2). In one of these two adenocarcinomas a *p53* exon 8 and a *K-ras* codon 12 mutation were found as well. Both of them were MSS adenocarcinomas. Moreover, LOH analysis applied on the 9 HNPCC adenocarcinomas was negative in all 3 informative cases.

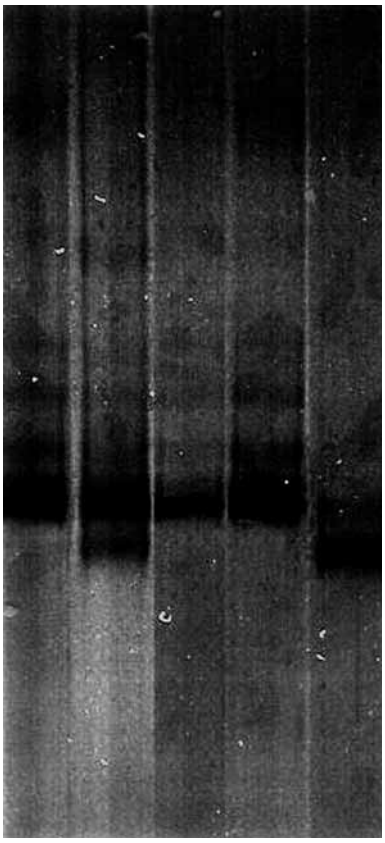

Fig. 2. — LOH analysis of *p53* intron 1. Lanes 1,2 : non-informative case ; lanes 3,4 : normal adenocarcinoma ; lanes 5,6 : adenocarcinoma exhibiting LOH ; lane 7 : negative control ; M : puc mix 8 marker.

hMLH1 methylation and expression analysis

In 23 sporadic adenocarcinomas, with available matching normal tissue, the methylation status of *hMLH1* promoter region was examined. Two out of 3 MSI-H tumors had methylated *hMLH1* promoters. These cases were immunohistochemically negative for hMLH1 protein expression. The third MSI-H tumor that had unmethylated *hMLH1* promoter showed a positive immunoreaction for hMLH1 protein (Fig. 3).

Statistical associations

All the results were evaluated with regard to the clinicopathological characteristics of the patients' tumors, such as age, gender, location, degree of differentiation, stage, mucin production, Crohn's like peritumoral infiltration, tumor infiltrating lymphocytes, pattern of growth, vein invasion, residual adenomatous tissue and serrated architecture. A statistically significant correlation was noticed in sporadic tumors between the presence of MSI-H and tumor location at the proximal colon, as well as with the female gender (Table I).

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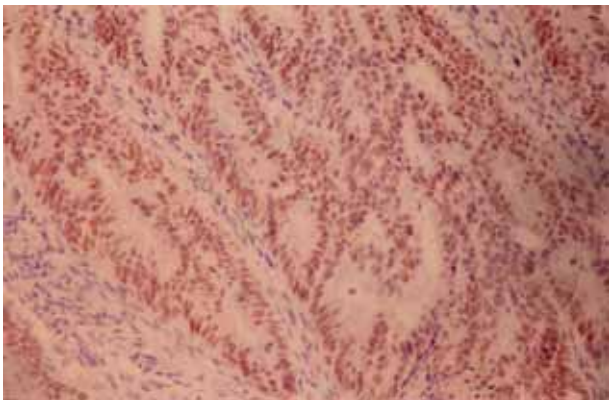

Fig. 3. — Positive hMLH1 immunoreaction in well to moderately differentiated cancerous formations (Immunoperoxidase stain, ×250).

Discussion

Microsatellite instability phenotype is characterized by the accumulation of somatic alterations in the length of simple, repeated sequences called microsatellites due to defects of the DNA mismatch repair system. A panel consisting of five microsatellite markers, as well as a number of alternative loci that should be tested in order to assess microsatellite instability in colorectal tumors has been proposed (16). On this basis, tumors are classified as demonstrating MSI-H (two or more markers unstable), MSI-L (one marker unstable) or microsatellite stability (MSS) (no markers unstable).

To determine the MSI status in sporadic and HNPCC cases we applied the National Cancer Institute workshop panel of five microsatellite markers (BAT25, BAT26, D2S123, D5S346 and D17S250), as well as the proposed alternative marker *TGF-βRII*. In cases where no corresponding normal tissue was available we employed only the sensitive quasimonomorphic marker BAT26 and *TGF-βRII* (22). In particular, BAT26 has proven to be very useful especially for the identification of MSI-H colorectal cancers. On the other hand, the sensitivity of BAT26 in identifying MSI-L tumors on its own has been disputed, as instability at the BAT26 locus appears rarely in these tumors (20).

In the present study, MSI-H was determined at a percentage of 14% in sporadic adenocarcinomas and in 7 out of 9 HNPCC adenocarcinomas. The 2 patients of the second group that did not fulfill all the Amsterdam criteria probably correspond to the HNPCC-like group which is characterized by a very low incidence of MSI (23). Taking into account that virtually all of HNPCC tumors and about 10-15% of sporadic colorectal tumors display MSI (3), our results seem to be consistent with previous studies. The *TGF-βRII* gene has been previously reported to be inactivated by mutation in 90% of MSI+ colon tumors and *TGF-βRII* alterations were found almost exclusively in MSI+ tumors (24,25). In our study, all MSI+ sporadic and HNPCC adenocarcinomas exhibited *TGF-βRII* alterations as well.

MSI-H tumors are reported to occur slightly more often in women than in men, to localize mainly at the proximal colon, to be poorly differentiated, to produce mucin and to exhibit peritumoral lymphocytic infiltration much more frequently compared with MSI-L and MSS tumors (4,26). Similarly, in our study, MSI-H indeed showed a statistically significant correlation with the female gender ($p = 0.019$) and proximal colon location ($p = 0.013$).

The coexistence of MSI+ phenotype and mutations of *K-ras* and *p53* genes in sporadic and inherited colon tumors remains a subject of considerable debate. Some investigators have identified *K-ras* mutations in a relatively low percentage of MSI+ tumors, whereas others have noted frequencies equivalent to those found in MSS tumors (21,27,28). Certain authors have observed similar prevalence of *K-ras* mutations in both HNPCC and sporadic carcinomas, whereas others have noted that *K-ras* mutations occur more frequently in HNPCC than in sporadic MSI+ cancers (21,29). In our study, however, 21% of the MSI+ sporadic adenocarcinomas were mutated at codon 12 of the *K-ras* gene, but no *K-ras* mutations were detected in adenocarcinomas of HNPCC patients.

Recent research findings have indicated that *p53* mutations seem to occur more often in MSI- tumors compared to MSI+ ones (21,24,27,28). In this study, *p53* mutations were determined in 4 out of 14 (29%) MSI-H sporadic adenocarcinomas examined, in 2 out of 7 (29%) MSI-H HNPCC adenocarcinomas, as well as in 20 out of 73 (27%) MSS and MSI-L sporadic adenocarcinomas. It is obvious that due to the small number of the sporadic and HNPCC MSI-H adenocarcinomas examined, statistically significant conclusions -relatively to the percentage of p53 mutations in these samples- cannot be drawn.

Our results also support the hypothesis that hypermethylation of the *hMLH1* promoter region leading to transcriptional silencing of the *hMLH1* gene is the probable cause of the loss of hMLH1 protein expression in the large majority of *hMLH1* negative MSI-H tumors.

The assessment of MSI status is important for the genetic characterization of colorectal carcinomas. Apart from the clinicopathological features that have been examined in the present study, some other characteristics such as tumor infiltrating lymphocytes (TIL), lack of dirty necrosis and mucinous differentiation are also proposed as predictors of MSI in addition to molecular characteristics of the tumors (30,31). As far as other organ malignancies are concerned, HNPCC–related endometrial carcinomas are characterized by poor differentiation, more frequent Crohn-like lymphoid reaction, lymphangioinvasive growth and more tumor-infiltrating lymphocytes as compared with colonic cases (32).

Recent comparative analyses have shown that overall TIL count is significantly higher in HNPCC/HNPCC-

like group and that mucinous type appears more frequently in HNPCC MSI-H than in sporadic MSI-H colorectal carcinomas (33). In another study, mucin secretion, poor differentiation, tumor heterogeneity and glandular serration are more evident in sporadic MSI-H than in HNPCC colorectal carcinomas (34). Statistical significant correlations between these clinicopathological characteristics and HNPCC or sporadic MSI-H tumors of our study could not be demonstrated, because the number of the HNPCC tumors available for examination was limited.

According to our observations sporadic MSI+ cancers represent a distinct entity with a unique profile of genetic changes (lower prevalence of *ras* and *p53* alterations and common mutations at *TGF-βRII* gene) and differing from MSS cancer in terms of preponderance among female and proximal colon predilection. Since the prognostic significance of the identification of MSI-H tumors as well as the importance in therapeutic application remain extremely high, both molecular and clinicopathological features should be used in order to define MSI-H tumors. Futhermore, all available data including family history, age at onset of malignancy and molecular features should be applied to make the crucial distinction between sporadic and HNPCC MSI-H colon cancers.

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