MICROSATELLITE INSTABILITY AND LOSS OF HETEROZYGOSITY OF TUMOR SUPPRESSOR GENES IN BOSNIAN PATIENTS WITH SPORADIC COLORECTAL CANCER

Vesna Hadžiavdić[1,2], Nada Pavlović-Čalić[3], Izet Eminović*[1,2]

[1] Medical Centre for Molecular Biology, Institute of Biochemistry, Faculty of Medicine, University of Ljubljana, Vrazov trg 2, 1000 Ljubljana, Slovenia

[2] Department of Biology and Human Genetics, Faculty of Medicine, University of Tuzla, Univerzitetska 1, 75000 Tuzla, Bosnia and Herzegovina

[3] Gastroenterology Clinic, University Clinics Centre, Trnovac bb, 75000 Tuzla, Bosnia and Herzegovina

* Corresponding author

Abstract

Considering its frequency, high mortality rate as well as many etiological mysteries colorectal cancer is a challenge to contemporary science. In our study we analyzed RER + and RER – phenotypes and their relations with clinical-pathological characteristics of sporadic colorectal cancers. We also analyzed genetic alterations of tumor suppressor genes as well as their relation with microsatellite instability. The study was based on 54 tumor samples and 54 samples of the surrounding healthy tissue of patients with colorectal cancer. According to Amsterdam Criteria and Bethesda Criteria 35/54 or 64,81% belonged in the group of sporadic colorectal cancer. Mononucleotide marker Bat 25 showed instability in 48,57%; Bat 26 in 45,71% and Bat 40 in 29/35 82,86% of tumor samples. Considering dinucleotide markers, TP 53 showed instability in 54,29% and DS123 in 37,14% of tumor samples. Genetic alterations in tumor suppressor genes were found in tumor tissue: NM 23 in 54,29% samples, p53 in 51,43%, APC in 51,43%, DCC2 in 34,29%, RB1 in 22, 86% and DCC 1 in 28,57%. Our studies confirmed that genetic instability had an important role in the development of tumor type. Our results showed that mononucleotide marker Bat 40 might be used for an easy and fast screening procedure in Bosnian population, because it exhibited high percent of microsatellite instability and was in relation with RER+ phenotype. This investigation showed that different genetic alterations may occur during cancer development in each individual patient's tumor. These changes result in MMR inactivation, which causes RER+ phenotype. Our results suggest a connection between alteration in some tumor suppressor genes and MSI phenotype of sporadic colorectal cancer in Bosnian population.

KEY WORDS: microsatellite instability, RER phenotype, sporadic colorectal cancer, loss of heterozygosity

Introduction

Through its frequency, high mortality rate as well as with its many etiological unknowns, colorectal cancer is a challenge to contemporary science. In frequency, it follows right after lung cancer in men and breast cancer in women. The latest genetic information could be used in the near future for the prevention of colorectal cancer, its early diagnosis and for the selection of patients for the most suitable treatment (1). Mutations in genes may stimulate early tumor development and cause multiple mutations in tumor tissues. Mutations in genes of DNA repair system discovered thus far, as well as the expansion of DNA repeating sequences may serve as early indicators for most of mutations that characterize tumor phenotype (2). Cloning and molecular analysis provided proofs that the inactivation of one of MMR genes causes microsatellite instability (MSI) as well as positive replication error (RER +). Most authors set microsatellite instability in at least 40 % of analyzed loci as criteria for RER+ phenotype (3). RER+ phenotype is present in about 90% at hereditary non-polyposis colorectal cancer and in about 15-28% of sporadic colorectal cancer patients. In HNPCC, mutations are usually found in hMLH1 and hMSH2 genes, which result in loss of heterozygosity that inactivates MMR genes. They were also found in sporadic colorectal cancers with RER+ phenotype. Mutations are present in hMSH2, hMLH1, hMSH3 and GTBP genes in majority of HNPCC patients, and in hPMS1 and hPMS2 genes in minority (4,5,6). International Collaborative Group (ICG) published criteria for determination of microsatellite instability in colorectal cancer (7). When the panel of 5 microsatellite loci is used in analysis, the tumor has microsatellite instability if two or more markers are unstable. Microsatellite panel must include mononucleotide markers: Bat 25 and Bat 26, as well as DS123 from the group of dinucleotide markers. These are Bethesda Criteria that determine which colorectal cancers may be tested for microsatellite instability (8). According to these criteria, tumors are classified with regard to microsatellite instability:

- MSI – L low rank tumors that show MSI in less than 30-40% loci;
- MSI-H high rank tumors that show MSI in over 40% loci and have positive replication error - RER+, and
- MSS- tumors with stable microsatellite loci.

In 1990 in Amsterdam, International Collaborative Group established Amsterdam Criteria that are applied in diagnosing HNPCC families (9). These criteria were also used in our study. Generally, when the other allele of tumor suppressor gene is lost or inactivated in a somatic cell, it is regarded as loss of heterozygosity (LOH), which presents a marker for localization and identification of tumor suppressor gene (10). Authors established around 29 % loss of heterozigosity in DCC genes in colorectal cancers, while other reports state 33% LOH (11). Loss of DCC gene in gastric cancers is about 60% (12). Loss of heterozygosity in p53 in colorectal cancer is about 48% (13), and LOH in APC gene is about 31% (14). In our study we analyzed RER + and RER – phenotype and their relation with clinicopathological characteristics in sporadic colorectal cancers. Also, we analyzed genetic alterations of tumor suppressor genes as well as their relation with microsatellite instability.

Materials and Methods

Our study was based on 54 samples of tumor and surrounding healthy tissue of patients with colorectal cancer. The samples were collected at Gastroenterological and Surgical Clinic of University Clinics Center in Tuzla (Bosnia and Herzegovina). Both tumor and healthy surrounding tissue was formalin fixed, and thereafter embedded in paraffin blocks. Method of genomic DNA isolation is based on de-paraffinisation of tissue sections and cell proteolyses with proteinase K (4). Fluorescent chain synthesis of DNA is a method with very broad application in tumor detection, and it is especially important in determination of microsatellite instability (MSI) and loss of heterozygosity (LOH) of tumor suppressor genes. We used mononucleotide and dinucleotide microsatellite markers in the detection of microsatellite instability. In the group of mononucleotide markers we used BAT25, BAT26 and BAT40, and in the group of dinucleotide markers: DS123 and TP 53. For LOH detection, we used intragene markers for the following tumor suppressor genes: NM23, p53, APC, RB1, DCC1 and DCC2 (10). Amplification reactions contained: 2,5 µl 10 x buffer; 1µl DNA (5-10 ng); 2 µl $MgCl_2$ (25mM); 0,5 µl of each dNTP (2,5 mM); 0,1µl AmpliTaq Gold polymerase (0,5 U; Perkin-Elmer); 1µl forward (12,5 pmol) and 1µl reverse primer (12,5 pmol) and sterile water until final volume of 25µl. PCR was performed in a PCR Thermocycler 9600 (Perkin-Elmer). PCR conditions included an initial denaturation step at 94°C for 2 min, 30 cycles of denaturation at 94°C for 10 s, annealing at 55°C for 30 s and elongation at 72°C for 30 s, and final extension at 72°C for 7 min. Amplified PCR products were separated in an automated sequencer 310 ABI PRISM, Genetic Analyser 310 (Perkin Elmer), which enabled separation and quantification of DNA fragments according to the principles of

capillary electrophoresis. Microsatellite analysis comprises of comparison between healthy and tumor tissue of the same patient using Genescan program package for analysis. Software detects fluorescence peaks and shows them in the form of electropherograme. Each fluorescent peak is automatically quantified for size in bp, height and peak field. All samples were tested twice for the confirmation of results. Loss of heterozygosity was calculated mathematically (10) In heterozygous cases, allele ratio was calculated for each pair of normal and tumor tissue according to formula T1:T2/N1:N2 , where T1 and N1 are field values of shorter allele; T2 and N2 are field values of longer allele in tumor (T) and healthy (N) samples. The results were assigned rank from 0,00 to 1,00. If the result was lower or equal to 0,50 the loss of heterozygosity of longer allele was considered significant. Homozygous cases cannot be included in calculation. We used χ^2 test and Fisher's exact test (Arcus Quickstat biomedical for Windows) in statistical data processing.

Results

Analysis of genetic instability at microsatellite loci and RER phenotype of sporadic colorectal cancers

In the total sample of 54 patients 35 or 64,81% belong to sporadic colorectal cancer, according to Amsterdam Criteria and Bethesda Criteria. Mononucleotide marker Bat 25 showed instability in 17/35 (48,57%); Bat 26 in 16/35 (45,71%) and Bat 40 in 29/35 (82,86%) of tumor samples (Figure 1). In the group of dinucleotide markers, TP 53 showed instability in 19/35 (54,29%) and DS123 in 13/35 (37,14%) of tumor samples. With regard to instability among mononucleotide markers the difference was found significant ($p<0,01$), however, the difference between dinucleotide markers was not ($p>0,05$). Significance test showed significant differences between mononucleotide and dinucleotide markers with regard to microsatellite status ($p<0,01$). Number of unstable microsatellite loci in certain sample defines its RER phenotype as either RER+ or RER-. The study showed that 19/35 (54,29%) of tumor samples belong to RER+ phenotype, and 16/35 (45,71%) belong to RER- phenotype. There is a significant difference between RER+ and RER- tumor phenotype in frequency of unstable microsatellite loci ($p<0,01$). In the group of tumors with RER+ phenotype microsatellite instability was established at mononucleotide marker Bat 40 in 18/19 (94,74%), and dinucleotide marker TP53 in 14/19 (73,68 %) of samples. In the group of tumors with RER- phenotype, mononucleotide marker Bat 40 showed instability in 11/16 (68,75%) cases, and dinucleotide marker TP 53 as well as Bat 26 showed instability in 5/16 (31,25%) tumor tissues. There is a significant difference between mononucleotide and dinucleotide markers with regard to RER status ($p<0,01$). Statistically significant differences were also found among dinucleotide markers with regard to RER phenotype ($p=0,01$). Analysis of some clinicopathological parameters (sex, ages, tumor localization, hystopathological classification) showed that colorectal

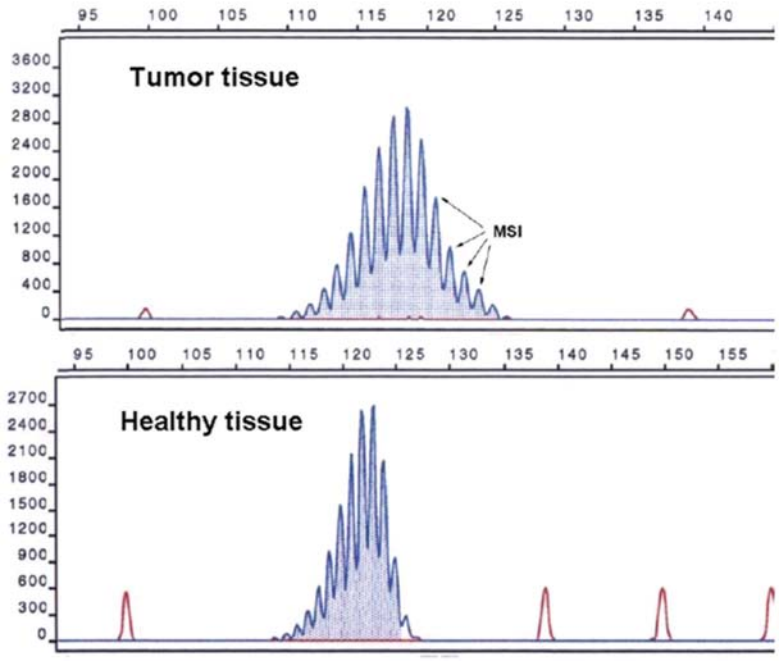

FIGURE 1. Microsatellite instability at Bat 40

Clinicopathological characteristics	Total	RER + tumor phenotype		RER – tumor phenotype	
		No.	%	No.	%
Sex					
male	26	14	53,85	12	46,15
female	9	5	55,56	4	44,44
Age category					
> 50	24	12	50,00	12	50,00
< 50	11	7	63,64	4	36,36
Tumor localization					
Left-side	24	15	62,50	9	37,50
Right-side	11	4	36,36	7	63,64
Histopathological classification					
Mucion	5	4	80,00	1	20,00
adenocarcinoma	30	15	50,00	15	50,00

TABLE 1. Relations between RER tumor phenotype and clinicopathological characteristics

cancer was found mostly in males 26/35 (74, 78%) and in the age group over 50 24/35 (68,57%), tumors were located on the left-side (rectum and sigma region) in 24/35 (68,57%), and 30/35 (85,71%) were adenocarcinomas. Analysis of microsatellite instability of individual markers and clinicopathological characteristics showed no statistically significant difference (p>0,05). Analysis of RER tumor phenotype and clinicopathological characteristics showed that RER+ phenotype was more frequent in females (5/9 or 55,56%). In the age group under 50 it was present in 7/11 (63,64%) of samples, and according to the tumor localization RER+ phenotype was more frequent in the left-side (15/24 or 62,5%). RER- phenotype was more frequent in males (12/26 or 46,15%); in the age group over 50 in 12/24 (50%) and they belong to the group of right-side tumors in 7/11 or 63,64% (Table 1). Statistic analysis showed no significant differences between RER+ and RER- phenotype with regards to clinicopathological parameters (p>0,05).

Genetic alterations in tumor suppressor genes were found in NM 23 in 19/35 (54,29%) samples, in p53 in 18/35 (51,43%), in APC in 18/35 (51,43%), in DCC2 in 12/35 (34,29%), in RB1 in 8/35 (22,86 %) and DCC 1 in 10/35 (28,57%) of tumor tissues. The highest frequency of homozygous samples were found for DCC 1 tumor suppressor gene in 14/35 (40,00%). DCC 2 was homozygous in 11/35 (31,42%) and p53 locus in 1/35 or 2,86%. There is a significant difference between tumors with allele loss and tumors without allele loss (p<0,05). Microsatellite instability of marker Bat 25 was found in tumor tissues with loss of heterozygosity at locus NM23 in 12/19 (63,15%). Microsatellite instability in Bat 26 was found in tumor tissues with LOH in RB 1 in 5/8 (62,5%) and marker Bat 40 was unstable in tumors with LOH in APC tumor suppressor gene in 16/18 (88,89%) samples. There is no significant difference (p>0,05) in the occurrence of microsatellite instability of mononucleotide markers in tumor tissues with loss of heterozygosity. Microsatelite instability of dinucleotide marker TP53 was found in tumor tissues with loss of heterozygosity at RB1 in 5/8 (62,5%), and marker DS123 was present in tumors with loss of heterozygosity at APC tumor suppressor gene in 8/18 (44,44%) samples. There is no significant difference (p>0,05) in the occurrence of microsatellite instability of dinucleotide markers in tumor tissues with loss of heterozygosity. Comparison between RER phenotype and genetic alterations showed that loss of heterozygosity at tumor suppressor gene APC was pronounced in tumors belonging to RER+ phenotype (in 11/19 or 57,89%), while the smallest percent of LOH was found at RB1 and DCC1 (in 4/19 or 21,05%). Loss of heterozygosity at tumor suppressor gene p53 was found in tumors with RER- phenotype in 10/16 or 62,5%, while the smallest frequency was found at RB1 in 4/16 (25%)(Table 2).

There is no significant difference between RER+ phenotype and RER- phenotype of tumors with regards to the frequency of LOH at tumor suppressor genes (p>0,05). In RER+ tumor phenotype, allele loss most frequently occurred at two loci simultaneously (7/19 or 36,84%); which is also the case in tumors with RER- phenotype

Genetic alteration	RER + tumors N=19		RER – tumors N=16	
	No.	%	No.	%
NM23				
AI	10	52,63	9	56,25
P53				
AI	8	42,11	10	62,50
APC				
AI	11	57,89	7	43,75
RB1				
AI	4	21,05	4	25,00
DCC1				
AI	4	21,05	6	37,50
DCC2				
AI	6	31,58	6	37,50

TABLE 2. Relations between RER phenotype and genetic alterations

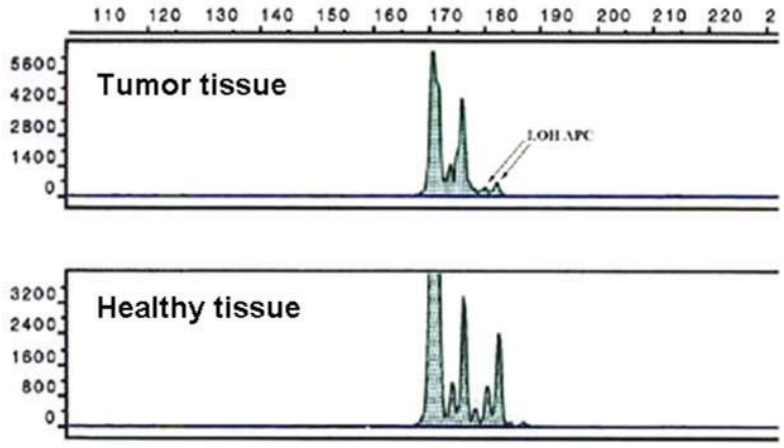

FIGURE 2. Loss of heterozygosity in APC tumor suppressor gene

(6/16 or 37,5%). There is no significant difference between RER+ and RER- phenotypes with regards to the number of loci with loss of heterozygosity (p>0,05). Analysis of genetic alteration and clinicopathological characteristics showed that loss of heterozygosity at gene p53 was more frequent in females (7/9 or 77,78%), while in males allele loss was found at NM23 gene in 15/26 (57,69%). In the age group under 50, loss of heterozygosity was found at p53 gene in 8/11 (72,73%) samples, while in the age group over 50 loss of heterozygosity was found at NM 23 gene as well as in APC in 13/24 (54,17%). Regarding tumor localization, in left-side tumors loss of heterozygosity was more frequent in APC gene with 14/24 (58,33%); while in the right-side tumors, allele loss was more frequent in p53 gene in 16/30 (53,33%) (Figure 2). In mucionus adenocarcinoma loss of heterozygosity was more frequent at locus TP53 in 4/5 (80%), in adenocarcinoma allele loss was present at NM23 in 9/11 (81,82%) (Table 3). No significant difference between loss of heterozygosity in tumor suppressor gene and clinicopatholoical characteristics was established (p>0,05).

Discussion

There is a belief that changes in the system of MMR genes cause not only HNPCC development, but sporadic collorectal cancer as well (15).Analysis of MSI status in Japanese patients (16) showed microsatellite instability in 51% tumors while 26% showed that they belong to MSI-H tumors. Other authors (17) confirmed presence of microsatellite instability in 89% patients with multiple primary cancers in different organs. At the same time, there are reports of 32,1% tumor tissues with unstable microsatellite loci (18). According to the results of authors (7,19-20) who analyzed microsatellite alterations in certain markers, we also selected five microsatellite markers and divided them into MSI-H and MSI-L group. Our results suggested 54,29% frequency of MSI-H status markers while the frequency of MSI-L was 45,71%. The differences between mononucleotide and dinucleotide markers with regards to their microsatellite status are significant (p<0.01). Microsatellite instability of mononucleotide markers Bat 26 and Bat 25 are highly sensitive and they represent specific instability indicator (21). Microsatellite instability of Bat 26 marker in identification of sporadic forms of collorectal cancers may become useful for identification of new HNPCC cases. Microsatellite instability was found in 86% samples. Correlation between MSI status and clinicopathological parameters is significant. Tumors appear in the younger group of patients with predominant location in the right-side, and tissues had more mucinous components. Analysis shows that 33% tumor tissues with MSI positive status at Bat 26 were new germline mutations in MLH1 gene. Authors consider that mononucleotide marker Bat 26 may serve as an easy and fast screening procedure preceding mutation analysis (22). Previous studies confirmed Bat 26 and Bat 40 to be highly sensitive (in 75% and 63% cases respectively) for the detection of microsatellite instability in adenomas and cancers (23). In our study mononucleotide marker Bat 25 showed instability in 48,57% cases; Bat 26 in 45,71% and Bat 40 was highly unstable in as many as 82,86% of tumor samples (Figure 1). Among dinucleotide markers, TP53 showed instability in 54,29% and DS123 in 37,14% of tumor samples. Study of Hong Kong patients showed 10% frequency of RER+ phenotype in sporadic collorectal cancers (24). Some studies found that 27,8% tumors belong to RER + phenotype (25) or that RER + phenotype ranked between 11% and 28% (26). Also, replication errors were found in

10% of collorectal cancers (30). Our results suggest rather high frequency of RER+ phenotype of 54,29%, which is higher than previously reported. Also, there is significant difference between mononucleotide and dinucleotide markers with regard to RER+ phenotype ($p<0,01$). Our studies confirmed that genetic instability plays an important role in the development of this type of tumor. According to previous reports, many patients with sporadic collorectal cancers may develop familial history of collorectal cancer. The percentage of CRC was the highest in families with MSS-phenotype tumors (88%), followed by MSI-L phenotype (78%) and MSI-H (67%) phenotype tumors. MSS tumors were preferentially localized in the distal colon (27). Studies of MSI+ phenotype of colorectal cancers as determined by the analysis of Bat 26 mononucleotide marker established significant difference between males and females with regard to tumor location: MSI+ tumors were more frequent in females (27,83%) in the left segment of colon. Age differences were not significant (age of tumor appearing was between 60 and 65) (28). Further studies showed that in 35 % cases microsatellite instability was registered at all of the five tested dinucleotide markers (DS123,etc.) Frequency of MSI positive status in proximal colon was 44,4%, in distal 37,2% and in rectum 23,8%. High MSI-H showed a significant correlation with proximal tumor localization. All the tumors found in rectum had MSI-L status. Also, the analysis showed no correlation between clinicopathological characteristics and MSI tumor status (29). Results of our studies are in agreement with previous studies. Analysis of some clinicopathological parameters (sex, ages, tumor localization, histo-pathological classification) showed the dominance of males with 74,78% and persons over 50 years of age with 68,57%. Tumors are located in the left-side (rectum and sigma region) 68,57%, and 85,71% of samples were adenocarcinomas. Analysis showed no significant difference between RER+ and RER- tumors with regard to age. In RER- group the average age was about 68,2 years and 55,2 years in RER+ group (25). Further studies established a relation between RER phenotype and histo-pathological variables in patients from Hong Kong. There is no significant difference between RER+ phenotype and the localization of tumor which is usually right-sided. There is a significant difference with regards to sex and age. The data suggest that RER+ phenotype is more frequent in males. Also, the data show that younger patients under 50 years of age had RER+ tumor phenotype and that the difference between RER+ and RER- tumor phenotype is significant (24). RER+ tumors were found in patients 60 years old, while RER- tumors were found in older females about 65 years old. These studies showed a significant relation between RER+ tumors and tumor localization in proximal colon. There is no significant difference between these two groups of tumors and RER- tumor localization (30). Results of our studies (Table 1) concur with previous reports that RER+ phenotype was more frequent in age group under 50 with 63,64%. Majority of RER+ tumors were left-side tumors 62,5%, and these appear in females in 55,56%. RER- phenotype was more frequent in males in 46,15%; in the age group over 50 in 50% and in 63,64% those tumors were located in the right side. Statistical analysis showed no significant difference between RER+ and RER- tumor phenotypes with regard to clinicopathological parameters ($p>0,05$). Previous studies have shown an inverse relation between RER phenotype and loss of heterozygosity at chromosomes 5q, 17p and 18q (31). In Hong Kong patients, RER+ tumor phenotype, which was compared with RER- tumor phenotype, showed low level of mutations in the form of LOH at tumor suppressors as well as p53 in 20%, MCC in 0%, DCC in 20% and APC in 33%. High frequencies of allele loss of over 30 % (33) were found at DCC and APC locus, but at RB 1 locus the frequency was exceptionally low, less than 20%. In Caucasian and Japanese patients, loss of heterozygosity at 18q21 chromosome was over 70%, and loss of heterozygosity at APC was between 23% and 48% (33). Our studies showed the highest frequency of genetic alterations in tumor suppressor gene NM 23 which were identified in 54,29% samples. Then, tumor suppressor p53 was identified in 51,43%, APC in 51,43%, DCC2 tumor suppressor gene in 34,29%, DCC 1 in 28,57% samples of tumor tissue. The lowest percent of LOH in a tumor suppressor gene was found at RB1 in 22,86 % samples. Loci homozygosity was found at DCC 1 tumor suppressor gene in 40,00%, at DCC 2 in 31,42% and at p53 locus in 2,86% samples. There is a significant difference between tumors with allele loss and tumors without allele loss ($p<0,05$). Results of a study (34) confirmed that replication error and loss of heterozygosity at 5q, 8p, 17p, 18q and 22q chromosomes were exceptional pre-metastatic events in tumor genesis of colorectal cancer. Studies of genetic instability at tumor suppressor genes p53 and NM 23 as well as clinicopathological forms at rectal cancers established alterations in NM 23 in all RER+ tumors, while p53 tumor suppressor was altered in one case. There is no statistically significant difference between clinical parameters and RER status (25). Loss of heterozygosity was confirmed at 18q chromosome in 53,8% samples and RER+ phenotype was found in 17,85% cases. There is no significant difference between LOH at 18q chromosome and clinicopatho-

Clinicopathological characteristics	N	Genetic alterations in tumor suppressor NM 23 No. %	p53 No. %	APC No. %	RB 1 No. %	DCC1 No. %	DCC2 No. %
Sex							
male	26	15 57,69	11 42,3	13 50,00	5 19,23	10 38,46	12 46,15
female	9	4 44,44	7 77,78	5 55,56	3 33,33	0 0	0 0
Age category							
> 50	24	13 54,17	10 41,67	13 54,17	6 25,00	7 29,17	7 29,17
< 50	11	6 54,55	8 72,73	5 45,45	2 18,18	3 27,27	5 45,45
Tumor localization							
Left-side	24	12 50,00	9 37,5	14 58,33	4 16,67	7 29,17	10 41,67
Right-side	11	7 63,64	9 81,82	4 36,36	4 36,36	3 27,27	2 18,18
Histopathological classification							
Mucinous	5	3 60,00	4 80,00	3 60,00	1 20,00	2 40,008	3 60,00
adenocarcinomas	30	16 53,33	14 46,67	15 50,00	7 23,33	26,67	9 30,00

TABLE 3. Relations between genetic alterations in tumor suppressor gene and clinicopathological characteristics

logical characteristics with regard to sex, tumor state and differentiation level. Allele loss was more frequently detected in adenocarcinoma located in distal part of colon than in tumors located in proximal part (35). Loss of heterozygosity was detected in malignant biopsies of gastro-esophagus in 74 % cases. LOH in APC, DCC tumor suppressors as well as in MSH3 loci can be detected in histologically normal tissues and in adenocarcinomas (36). Those are potential markers for early neoplastic progression. Microsatellite instability in three loci simultaneously was found in 31,25% cases, specifically, in the group of patients with average age of 35 years. This finding suggests a very important role of MMR gene in cancer pathogenesis at this age (37). Their research showed loss of heterozygosity in the region of DCC tumor suppressor in 39,1% patients at the age of 35. The analysis of genetic alterations and microsatellite instability revealed no correlation with clinicopathological characteristics. No patient had family history of colorectal cancer and all tumors were located in the left-side. There are two large groups of colorectal cancers which can be individualized and which suggest correlation among molecular, clinical and pathological forms of tumors (38). The first group consists of tumors with LOH in 80% cases, which are located in distal part of colon. The second group includes tumors with pronounced microsatellite instability without allele loss. These tumors make 15% of all colorectal cancers and in 30 % belong to the group of right-side tumors. Authors conclude that these tumors had better prognosis. Our analysis of genetic alterations and clinicopathological characteristics showed loss of heterozygosity at p53 in 77,78% cases, and that allele loss at NM 23 was found in males in 57,69% samples. In the age group under 50, loss of heterozygosity was the most prominent at p53 gene in 72,73% samples, and in the age group over 50 loss of heterozygosity appeared at NM 23 and APC genes in 54,17% samples (Figure 2). With regards to the tumor localization, loss of heterozygosity was more frequent in left-sided tumors at APC gene in 58,33% samples. In right-sided tumors allele loss at p53 was 53,33%. In mucionous adenocarcinoma, loss of heterozygosity was found at locus TP 53 in 80% samples. In adenocarcinoma allele loss was found at NM 23 in 81,82% cases (Table 3). There is no significant difference between loss of heterozygosity in tumor suppressor genes with regard to clinicopathological characteristics ($p>0,05$). In a previous study (40) RER status and loss of heterozygosity at 2p, 3p, 5q, 11p, 17p and 18q chromosomes were analyzed. The authors used CA repeat motive sequence of dinucleotide markers. They found higher frequency of LOH in RER- tumor phenotype (54,3%) than in RER+ tumor phenotype (22,5%). Analysis of microsatellite instability and loss of heterozygosity of p53 and APC tumor suppressor genes in 83 colon and rectum tumors confirmed that 82 tumors belong to RER+ phenotype (41). Loss of heterozygosity at p53 was found in 59% samples and at APC in 26% samples. Authors established lower frequency of RER+ phenotype in rectal tumors with earlier loss of heterozygosity. In colorectal tumors with the same phenotype, mutations in APC locus were less frequent than mutations in p53. Tumors with RER- phenotype and no loss of heterozygosity can still develop as a result of carcinogenesis model. Some authors found loss of heterozygosity at 17p chromosome in 61% of right–sided and 60% of left-side tumors. RER+ phenotype was found in 43% of right-sided tumors and 24 % of left-sided tumors (40, 41). The heterogeneous pattern of tumor mutations suggests that multiple alternative genetic pathways to colorectal cancer exist and accepted genetic model of cancer development is not representative of major tumors (42). Our results show that the LOH in APC is specially frequent in tumors that belong to RER+ phenotype (57,89%), and that the least frequent were

LOH at RB1 and DCC1 in 21,05% cases. Tumors with RER- phenotype had presented loss of heterozygosity at p53 gene in 62,5% samples. The lowest one was at RB 1 in 25% cases (Table 2). There is no significant difference between RER+ tumor phenotype in occurrence of loss of heterozygosity of tumor suppressor genes (p>0,05).

Conclusion

Our results suggest that mononucleotide marker Bat 40 can be used as an easy and fast screening procedure, because it exhibits high frequency of microsatellite instability and is associated with RER+ phenotype. Mononucleotide and dinucleotide markers exhibit high microsatellite instability in rectum and colon. Poly-A repeat sequences (mononucleotide markers) show higher microsatellite instability than CA repeat sequences (dinucleotide markers). Analysis of loss of heterozygosity in tumor suppressor genes established high frequency of genetic alterations in tumor suppressor gene NM 23 in 54,29% samples, and p53 and APC in 51,43% samples in the group of sporadic tumors. However, it is important to underline that differences in frequencies of microsatellite instability between our study and those of other authors suggest the importance of abundance and type of microsatellite markers analyzed in certain type of cancer. Our study has shown that genetic alterations in tumor may differ at individual level and reflect changes that result from the process of carcinogenesis. Those changes inactivate MMR genes and potentially lead to RER phenotype development. Our results suggest an association between alterations in certain tumor suppressor genes and MSI phenotype of this tumor type.

List of Abbreviations

MMR	-	Mismatch repair genes
MSI	-	Microsatellite instability
RER +, –	-	Replication error positive
hMSH2	-	Human mut S homolog 2
hMSH3	-	Human mutS homolog 3
hPMS1 and hPMS2	-	Human post-meiotic segregation 1 and 2
GTBP	-	G/T mismatch-binding protein
FAP	-	Familial adenomatous polyposis
APC	-	Adenomatous polyposis coli
LOH	-	Loss of heterozygosity
DCC	-	Deleted colorectal carcinoma
MCC	-	Mutated in colorectal cancer

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