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MISMATCH REPAIR PROTEINS AND SURVIVIN IN ADENOMATOUS COLON POLYPS WITH LOW GRADE AND HIGH GRADE DYSPLASIA: AN IMMUNOHISTOCHEMICAL STUDY

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ABSTRACT

Objective: The aim of our study was to observe the immunohistochemical expression pattern of mismatch repair proteins (MMRP) MLH1, MSH2, MSH6 and PMS2, as well as survivin, in colon polyps. Methods: We assessed above mentioned proteins in a unified group of 124 tubular adenomatous colon polyps with regard to the presence of dysplastic abnormalities in order to explore their relationship. Furthermore, we studied their relation to such clinic-morphological parameters as the age of patients, size of adenoma, degree of dysplastic changes and localization of the lesion. Results: Survivin was expressed in 97 cases (78.2%), MLH1 was found in 111 cases (89.5%), MSH2 in 115 cases (92.7%), MSH6 in 118 cases (95.2%) and PMS2 in 105 cases (84.7%). The majority of absent MMRP cases was detected where the adenoma size was less than 10 mm with LGD (low-grade dysplasia). Survivin expression significantly correlated with the adenoma size and dysplasia grade. Subcellular survivin compartmentalization was statistically associated with the adenoma size, dysplasia grade and adenoma localization. Furthermore, we confirmed a significant relation between survivin expression and MMRP. In general, the intensity of immunoreaction was stronger in the MMRP than in survivin. Conclusions: Our recent results suggest that MMRP may suppress the antiapoptotic activity of survivin in LGD and HGD (high grade dysplasia) colon adenomas.

Key words: *mismatch repair proteins, survivin, colon adenoma*

RESUMEN

Antecedentes: Las proteínas de reparación de desajustes (MMRP) y survivin representan señales diametralmente opuestas que pueden controlar las vías apoptóticas. Además, se sabe que tanto MMRP como survivin son poderosos parámetros pronósticos. Material y métodos: El objetivo de nuestro estudio fue observar el patrón de expresión inmunohistoquímica de MMRP MLH1, MSH2, MSH6 y PMS2, y survivin en un grupo unificado de 124 pólipos adenomatosos tubulares de colon con respecto a la presencia de anomalías displásicas para explorar sus relaciones. Además, estudiamos su relación con los parámetros clinicomorfológicos, como la edad de los pacientes, el tamaño del adenoma, el grado de cambios displásicos y la localización de la lesión. Resultados: Survivin se expresó en 97 casos (78,2%), MLH1 se encontró en 111 casos (89,5%), MSH2 en 115 casos (92,7%), MSH6 en 118 casos (95,2%) y PMS2 en 105 casos (84,7%). La mayoría de los casos ausentes de MMRP se detectaron en adenomas de tamaño inferior a 10 mm, con displasia de bajo grado. La expresión de survivin se correlacionó significativamente con el tamaño del adenoma y el grado de displasia. La compartimentalización de survivin subcelular se asoció estadísticamente con el tamaño del adenoma, el grado de displasia y localización del adenoma. Además, confirmamos una relación significativa entre la expresión de survivin y el MMRP. En general, la intensidad de la inmunoreacción fue más fuerte en MMRP en comparación con survivin. Conclusiones: Nuestros resultados recientes sugieren que el MMRP puede suprimir la actividad antiapoptótica del survivin en los adenomas de colon con displasias de bajo y alto grado.

Palabras clave*: reparación de desajustes, survivin, pólipo del colon, displasia*

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INTRODUCTION

The mismatch repair system (MMR) is utilized by proliferating cells to correct errors (mutations) that may develop during DNA replication (Jover et al., 2004; Vilar and Gruber, 2010; Hassen et al., 2012). DNA MMR also controls cell cycle checkpoints and thus genomic stability, and play an important role in apoptosis in response to DNA damage (Cejka et al., 2003). MMR genes are ubiquitous genes encoding mismatch repair proteins (MMRP). There is a tendency to heterodimerization of MMRP to functional doublets. MSH2 forms a heterodimer with MSH6 (or MSH3), which is involved in the diagnostic recognition of misaligned nucleotides and mispaired insertion-deletion loops. Furthermore, MSH2-MSH6 (MSH3) doublet recruits and activates proteins MLH1 and PMS2 (Peltomäki, 2003). Subsequently, the MLH1-PMS2 protein complex recruits nucleases, polymerases and other assorted proteins, which initiate downstream repair functions including the excision of the mismatched DNA strands or microsatellite instable (MSI) sequence repeats (Scherer et al., 2005; Vilar and Gruber, 2010). Defects in the MMR cause an increased spontaneous mutation rate known as the "mutator phenotype" (Charames and Bapat, 2003). In addition to elevated mutation rate, loss of MMR function may also lead to instability in simple sequence repeats (microsatellites). During mono-, di-, tri-, and tetranuclolide repeats replication, a newly formed strand may slip along the original template, thus leading to a bulged mispaired insertion-deletion loop. MMR mutations predominantly in MLH1, MSH2, MSH6, and PMS2 may cause the production of inactivated or abnormally short proteins that cannot perform their normal functions. In the light of all this, loss of protein expression suggests a defective MMR.

Protein survivin is a unique member of the inhibitor of apoptosis protein (IAP) family (Amiri and Richmond, 2005). IAP proteins play a key role in the negative regulation of apoptosis (type of programmed cell death spectrum). The multifunctional survivin protein possesses a number of distinct features not shared with the other IAP family members, it is involved in the regulation of cell cycle, inhibits the apoptotic cascade and stimulates angiogenesis. Survivin is highly expressed in embryonic and fetal tissues as well as in human malignancies (Li, 2005; Li and Brattain, 2006), while being almost undetectable in most terminally differentiated normal cells. Furthermore, survivin appears to be localized in different subcellular compartments: in nucleus, in cytoplasm, or as combined immuno-histochemical positivity in both nucleus and cytoplasm (Brennan et al., 2008; Oh et al., 2009). Due to large quantitative differences in the degree of survivin expression in normal adult tissues and in corresponding malignant tumors as well as different subcellular compartment-alization, survivin appears to represent a promising tumor biomarker and prognostic factor (Piras et al., 2007; Ge et al., 2013).

Basically, MMRP and survivin are known to be diametrically opposing signals able to direct the apoptotic pathways. Both MMRP and survivin represent powerful prognostic markers. Therefore, we analyze the immunohistochemical expression of these proteins in sporadic colorectal adenomas with respect to the degree of dysplastic changes in order to explore their relationship. Moreover, we correlate these proteins with clinicomorphological parameters.

Both MMRP and survivin are involved in apoptotic cascades. Abnormalities in the regulation of apoptosis may influence the development of early and developed malignant tumors (Ioana et al., 2010; Yurgelun et al., 2012; Ge et al., 2013). Although main functions of survivin and MMRP are well described in numerous papers (Duffy et al., 2007; Brennan et al., 2008; Li, 2008; Sun et al., 2014), their relation is studied very rarely. To the best of our knowledge, the relation between MMRP and survivin has not yet been elucidated in dysplastic colon adenomas.

MATERIALS AND METHODS

Formalin-fixed paraffin-embedded tissue samples from 124 cases of sporadic colon adenomatous polyps were enrolled in the present study. Pathology reports from all patients were reviewed, and their age, sex, localization as well as size of polyps were recorded. The hematoxylin and eosin – stained slides from each patient were then reviewed to confirm the degree of dysplasia. The cecum, ascending and transverse colon were regarded as the right or proximal colon, whereas the descending colon, sigmoid and rectum were referred to as the left or distal colon (Pino et al., 2009).

Our adenoma group included 87 males (70.2%) and 37 females (29.8%) (average age of males was 60.9 ±10.1 years and the average age of females was 68.7 ±9.1 years). Of all of the male/female patients, the polyps were located in 33/13 (26.6% / 10.5%) cases in the right colon and in 54/24 (43.6% / 19.3%) cases in the left colon.

survivin expression	negative	positive	subcellular localization		intensity of immunoreactivity	
			C	NC/N	+	++/+++
age						
≤ 50	1	10	5	5	8	2
51-70	20	59	30	29	51	8
> 70	6	28	15	13	23	5
comparison – negative vs positive	p=0.375					
correlation with subcellular localization			p=0.730			
trend test in the intensity of immunoreactivity					p=0.919	
size						
≤ 5 mm	17	26	23	3	25	1
6-10 mm	9	43	21	22	34	9
> 10 mm	1	28	6	22	23	5
correlation with survivin expression (negative vs positive)	p=0.0008					
correlation with subcellular localization			p<0.0001			
trend test in the intensity of immunoreactivity					p=0.165	
dysplasia grade						
low	24	44	42	2	41	3
high	3	53	8	45	41	12
correlation with survivin expression (negative vs positive)	p<0.0001					
correlation with subcellular localization			p<0.0001			
trend test in the intensity of immunoreactivity					p=0.032	
localization						
proximal	9	37	10	27	27	10
distal	18	60	40	20	55	5
correlation with survivin expression (negative vs positive)	p=0.692					
correlation with subcellular localization			p=0.0006			
trend test in the intensity of immunoreactivity					p=0.013	

Table 1 - Relationship between survivin expression and clinicomorphological parameters in colon adenomas. C – cytoplasmic, N – nuclear, NC – combined cytoplasmic and nuclear. + weak intensity, ++/+++ moderate / strong intensity

Each paraffin block was cut into three-micrometer thick sections and subjected to immunohisto-chemical staining, three sections for each primary antibody using Thermo Scientific Microm HM430. In order to achieve greater adherence of the tissue sections to the glass surface, we used Flex slides (Dako, Glostrup, Denmark), which had been baked for two hours in an oven at 59°C. The slides were then treated in a PT Link System (Dako). The endogenous peroxidase activity was quenched with 3% hydrogen peroxide for ten minutes. Immunohistochemical reactions for MMRP were performed using Flex monoclonal mouse anti-human MLH1 and MSH2 antibodies (Dako, Clone ES05 and Clone FE1, respectively), and Flex monoclonal rabbit anti-human PMS2 and MSH6 antibodies (Dako, Clone EP51 and Clone EP49, respectively). For survivin, the immunohistochemical staining was performed using monoclonal mouse anti-survivin antibody (Dako, Clone 12C4, dilution 1:50). For MMRP immunoreactions, the sections were incubated for 20 minutes with the primary antibody at room temperature, and sections for MSH2 reaction

were treated by Linker / Mouse for 20 min. The MLH1, MSH2, PMS2 and MSH6 proteins were visualized by means of the EnVision™ Flex / HRP System (Dako) using 3.3´ - diamino-benzidine (DAB) chromogen as substrate. After a one hour-long incubation with the primary antibody and Linker / Mouse treatment for 20 minutes, survivin was visualized by means of the EnVision™ Flex / HRP System using 3-amino-9-ethylcarbazole (AEC) chromogen as substrate, according to the manufacturer´s instructions. All sections were counterstained with Mayer´s hematoxylin (Dako). Negative controls were obtained by omitting the primary antibody.

Analyzed data: The expression and staining intensity of mismatch repair proteins and antiapoptotic protein survivin in 124 cases of sporadic colorectal adenomas.

MLH1 expression	**negative**	**positive**	**intensity of immunoreactivity**	
			+	++/+++
age				
≤ 50	0	11	3	8
51-70	8	71	22	49
>70	5	29	8	21
comparison - negative vs positive	p=0.378			
trend test in the intensity of immunoreactivity			p=0.900	
size				
≤5mm	6	37	18	19
6- 10 mm	4	48	14	34
>10 mm	3	26	1	25
correlation with MLH1 expression (negative vs positive)	p=0.611			
trend test in the intensity of immunoreactivity			p=0.0001	
dysplasia grade				
low	11	56	27	29
high	2	55	6	49
correlation with MLH1 expression (negative vs positive)	p=0.019			
trend test in the intensity of immunoreactivity			p<0.0001	
localization				
proximal	4	42	6	36
distal	9	69	27	42
correlation with MLH1 expression (negative vs positive)	p=0.618			
trend test in the intensity of immunoreactivity			p=0.005	

Table 2 - Relationship between MLH1 expression and clinicomorphological parameters in colon adenomas. + weak intensity, ++ / +++ moderate / strong intensity

Compared parameters: In all of the cases, the immunoreaction intensity for survivin and MMRP as well as the subcellular localization of survivin were assessed semi-quantitatively by two independent observers (MA, SD) (Adamkov et al., 2014). For assessment, we used microscope Zeiss, AXIO, Scope A.1. The expression and staining intensity of MMRP MLH1, MSH2, MSH6, and PMS2 vs. age of patients, size of adenomas, degree of dysplasia and colon localization; relation between MMRP and survivin.

Statistical analysis: The χ^2test was used for the statistical analysis regarding whether the survivin, MLH1, MSH2, MSH6 and PMS2 expression correlates with age, tumor size, dysplasia grade and tumor localization as well as for comparison

of mutual relations between the above-mentioned proteins. The Cochran-Armitage test for trend was used to evaluate whether the intensity of survivin, MLH1, MSH2, MSH6 and PMS2 immunoreactivity correlates with the tumor size, dysplasia grade and tumor localization. Mutual comparison of protein expression intensity was also analyzed using Cochran-Armitage test. This statistical analysis was performed using Microsoft® Excel 2010/XLSTAT©-Pro (Addinsoft, Inc., Brooklyn, NY, USA); the significance level was set at $p<0.05$.

MSH2 expression	**negative**	**positive**	**intensity of immunoreactivity**	
			+	++/+++
age				
≤ 50	0	11	1	10
51-70	6	73	19	54
>70	3	31	6	25
comparison - negative vs positive	p=0.607			
trend test in the intensity of immunoreactivity			p=0.854	
size				
≤5mm	4	39	14	25
6- 10 mm	3	49	11	38
>10 mm	2	27	1	26
correlation with MSH2 expression (negative vs positive)	p=0.801			
trend test in the intensity of immunoreactivity			p=0.002	
dysplasia grade				
low	7	60	21	39
high	2	55	5	50
correlation with MSH2 expression (negative vs positive)	p=0.138			
trend test in the intensity of immunoreactivity			p=0.001	
localization				
proximal	2	44	4	40
distal	7	71	22	49
correlation with MSH2 expression (negative vs positive)	p=0.337			
trend test in the intensity of immunoreactivity			p=0.006	

Table 3 - Relationship between MSH2 expression and clinicomorphological parameters in colon adenomas. +weak intensity, ++ / +++ moderate / strong intensity

RESULTS

In our panel of 124 colorectal adenomas, survivin was expressed in 97 cases (78.2%) (Table 1), MLH1 in 111 cases (89.5%) (Table 2), MSH2 in 115 cases (92.7%) (Table 3), MSH6 in 118 cases (95.2%) (Table 4), and PMS2 in 105 cases (84.7%) (Table 5).

In the “Dysplasia” parameter, absent cases were mainly associated with low grade dysplasia: 11/13 cases (84.6%) for MLH1 (Table 2), 7/9 cases (77.8%) for MSH2 (Table 3), 5/6 cases (83.3%) for MSH6 (Table 4), and 15/19 cases (78.9%) for PMS2 (Table 5). In colon localization, absent cases were more frequent in distal colon: 9/13 cases (69.2%) for MLH1 (Table 2), 7/9 cases (77.8%) for MSH2 (Table 3), 5/6 cases (83.3%) for MSH6 (Table 4), and 15/19 cases (78.9%) for PMS2 (Table 5).

MSH6 expression	negative	positive	intensity of immunoreactivity	
			+	++/+++
age				
≤ 50	0	11	3	8
51-70	1	78	20	58
>70	5	29	8	21
comparison - negative vs positive	p=0.007			
trend test in the intensity of immunoreactivity			p=0.920	
size				
≤5mm	2	41	19	22
6- 10 mm	2	50	11	39
>10 mm	2	27	1	26
correlation with MSH6 expression (negative vs positive)	p=0.826			
trend test in the intensity of immunoreactivity			p<0.0001	
dysplasia grade				
low	5	62	25	37
high	1	56	6	50
correlation with MSH6 expression (negative vs positive)	p=0.139			
trend test in the intensity of immunoreactivity			p=0.0003	
localization				
proximal	1	45	8	37
distal	5	73	23	50
correlation with MSH6 expression (negative vs positive)	p=0.288			
trend test in the intensity of immunoreactivity			p=0.100	

Table 4 - Relationship between MSH6 expression and clinicomorphological parameters in colon adenomas. + weak intensity, ++ / +++ moderate / strong intensity

Survivin: The χ^2test confirmed that the presence of survivin expression (Table 1) significantly correlated with tumor size (p=0.0008) and dysplasia grade (p<0.0001), while the correlation with age and tumor localization was statistically insignificant (p=0.375, p=0.692 respectively).

As for survivin distribution, results revealed that survivin subcellular localization (Table 1) significantly correlates with the size of tumor (p<0.0001), dysplasia grade (p<0.0001) and tumor localization (p=0.0006).

Based on the Cochran-Armitage test for trend (Table1), we confirmed a statistically significant trend between survivin intensity of immuno-reactivity (weak/strong) (Figures 1a and 1b) and dysplasia grade (p=0.032) as well as the localization of the tumor (p=0.013).

MLH1: In evaluation of absent cases, majority of them (10/13 cases, 76.9%) were found in adenoma size <10 mm (Table 2).

MLH1 expression significantly correlated with dysplasia grade (p=0.019). High-grade adenomas expressed MLH1 in 96% of cases, low grade adenomas only in 83% of cases. Intensity of immunoreactivity of MLH1 significantly increased with the increasing tumor size and higher dysplasia grade (p=0.0001, p<0.0001 respectively). Interestingly, proximally located adenomas were statistically associated with stronger immunoreaction intensity (p=0.005) (Figure 2a).

MSH2: Table 3 shows MSH2 expression in adenomatous polyps. Most absent cases (7/9, 77.8%) were associated with adenomas <10 mm.

PMS2 expression	negative	positive	intensity of immunoreactivity	
			+	++/+++
age				
≤ 50	2	9	3	6
51-70	11	68	34	34
>70	6	28	13	15
correlation - negative vs positive	p=0.848			
trend test in the intensity of immunoreactivity			p=0.742	
size				
≤5mm	9	34	20	14
6- 10 mm	7	45	24	21
>10 mm	3	26	6	20
correlation with PMS2 expression (negative vs positive)	p=0.420			
trend test in the intensity of immunoreactivity			p=0.008	
dysplasia grade				
low	15	52	35	17
high	4	53	15	38
correlation with PMS2 expression (negative vs positive)	p=0.018			
trend test in the intensity of immunoreactivity			p<0.0001	
localization				
proximal	4	42	14	28
distal	15	63	36	27
correlation with PMS2 expression (negative vs positive)	p=0.116			
trend test in the intensity of immunoreactivity			p=0.017	

Table 5 - Relationship between PMS2 expression and clinicomorphological parameters in colon adenomas. + weak intensity, ++ / +++ moderate / strong intensity

The comparison between MSH2 positive and negative adenomas did not reveal any significant relation to the observed clinicopathological parameters (p>0.05). However, the intensity of immunoreactivity in MSH2 positive samples significantly increased with an increasing tumor size and higher dysplasia grade (p=0.002, p=0.001 respectively). Additionally, the intensity of immunoreactivity was significantly higher in adenomatous polyps with proximal localization (p=0.006) (Figure 2b).

MSH6: MSH6 protein was absent in 6 cases, 4/6 cases (66.7%) were found in adenoma size <10 mm (Table 4).

MSH6 expression showed similar characteristics as MSH2. We confirmed a relation between MSH6 expression and age (p=0.007), but the other clinicopathological parameters did not reveal any significant relation (p>0.05). Nonetheless, the intensity of immunoreactivity in MSH6 positive samples significantly increased with the increasing tumor size and higher dysplasia grade (p<0.0001, p=0.0003, respectively).

PMS2: Majority of absent cases (16/19, 84.2%) were detected in adenomas <10 mm (Table 5).

PMS2 expression significantly correlated with dysplasia grade (p=0.018). PMS2 positivity was increased with higher dysplasia grade. Moreover, the intensity of immunoreactivity in PMS2 positive samples also significantly increased with the increasing tumor size and higher dysplasia grade (p=0.008, p<0.0001, respectively). Also, while 66% of adenomatous polyps with proximal localization demonstrated strong intensity of immunoreactivity, it was only 43% of polyps with distal localization that demonstrated the same (p=0.017).

Relation between survivin and mismatch proteins (Table 6).

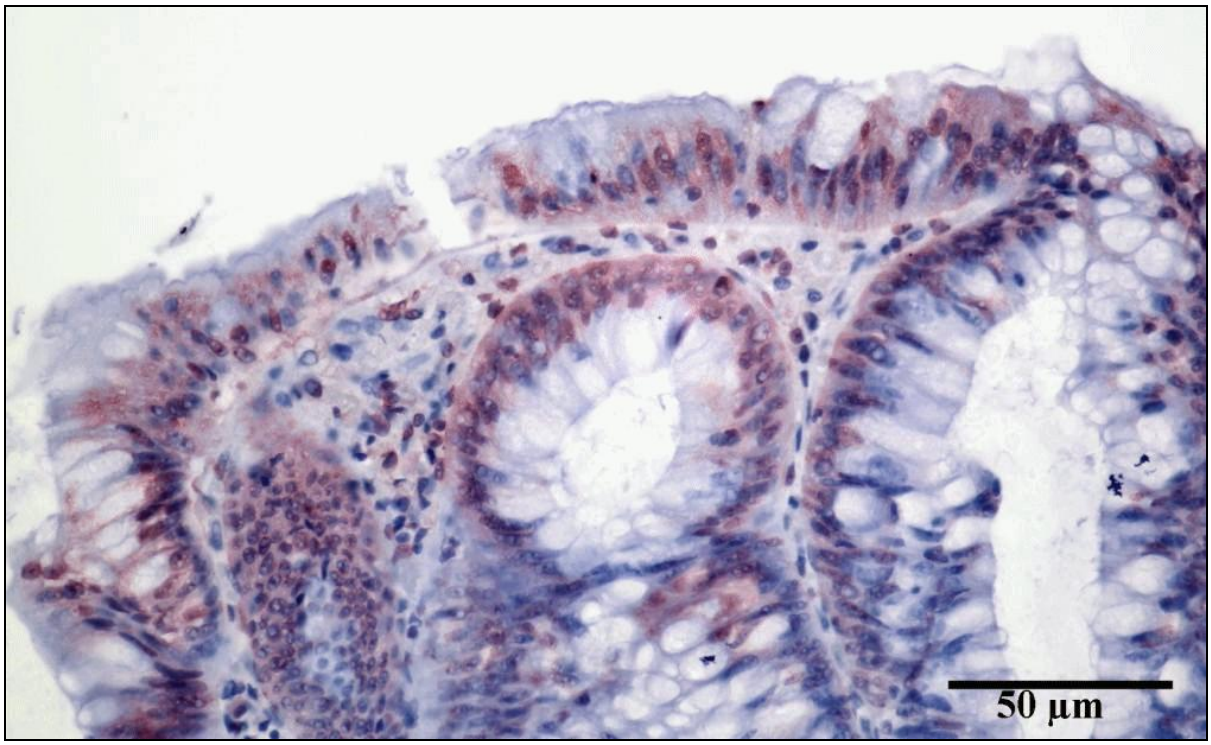

Figure 1a - Week intensity of survivin immunoreaction in LGD proximally located colon adenoma.

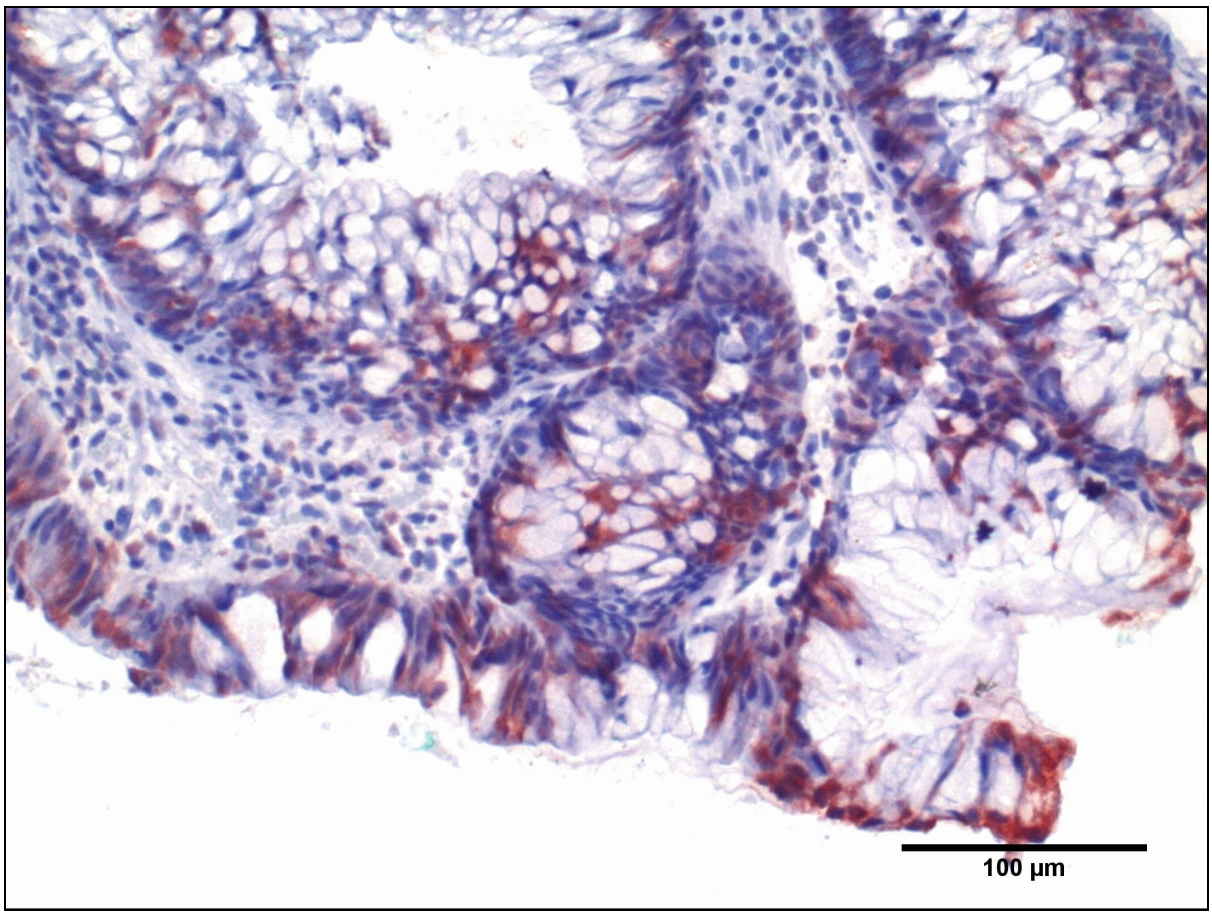

Figure 1b - Strong intensity of survivin immunoreaction in HGD distally located colon adenoma

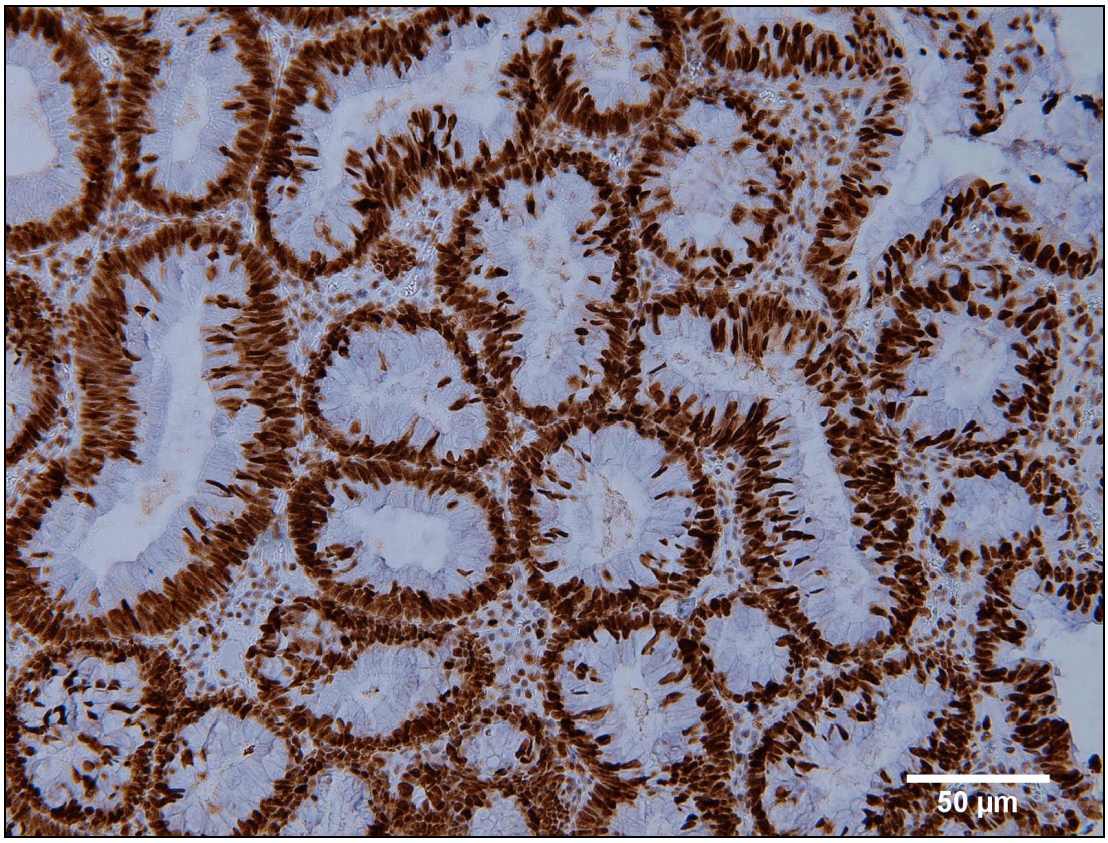

Figure 2a - Strong intensity of MLH1 immunoreaction in LGD proximally located colon adenoma.

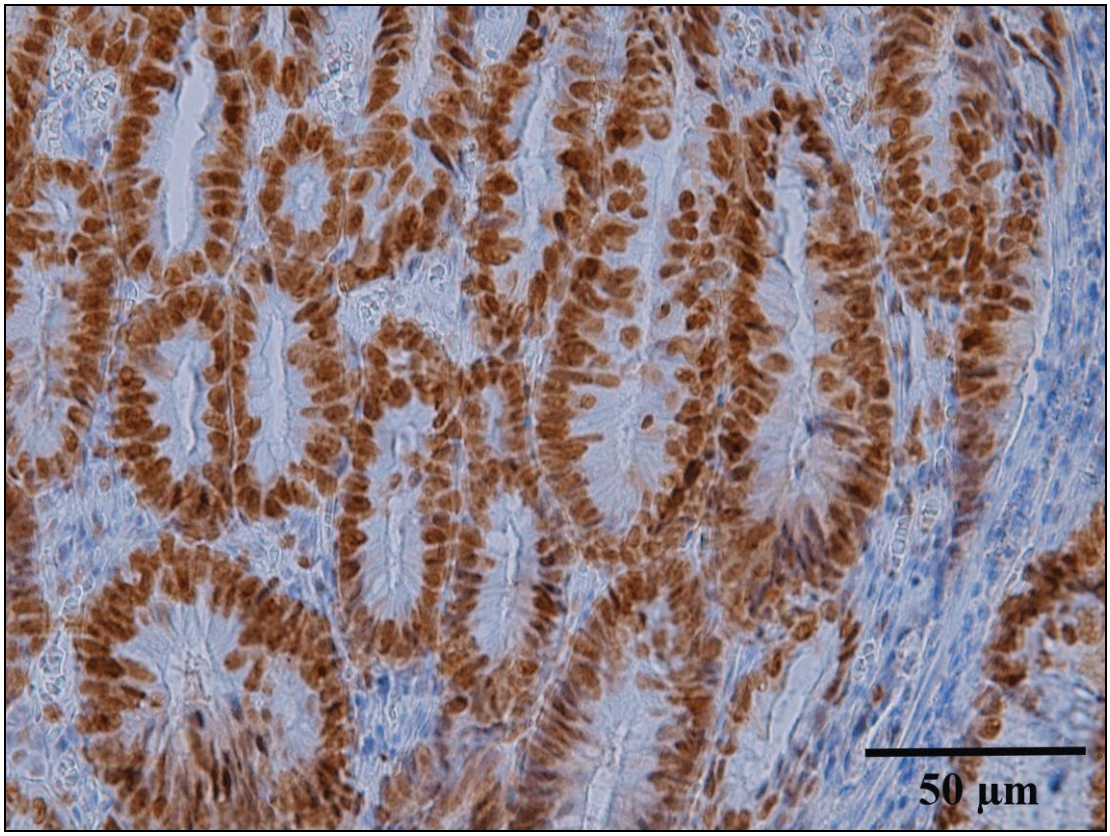

Figure 2b - Week intensity of MSH2 immunoreaction in HGD distally located colon adenoma.

We confirmed a significant relation between survivin expression and all mismatch proteins ($p<0.05$). Subsequent analysis revealed that adenomatous polyps in which survivin intensity was lower than the intensity of mismatch protein - MLH1, MSH2, MSH6 and PMS2 formed the following percentages of all samples: 64%, 72%, 71% and 52%, respectively. Adenomas with identical intensity of survivin and mismatch protein - MLH1, MSH2, MSH6 and PMS2 formed 24%, 21%, 24% and 29%, respectively.

expression	MLH1			MSH2			MSH6			PMS2		
	A	+	++/ +++	A	+	++/ +++	A	+	++/ +++	A	+	++/ +++
survivin A	1	13	13	1	14	12	0	16	11	1	20	6
survivin +	12	17	53	8	11	63	6	15	61	18	25	39
survivin ++/+++	0	3	12	0	1	14	0	0	15	0	5	10
comparison	survivin vs MLH1 p=0.019			survivin vs MSH2 p=0.0002			survivin vs MSH6 p<0.0001			survivin vs PMS2 p=0.0002		

Table 6 - Relationship between survivin and mismatch proteins expression in colon adenomas. A - absent, + weak intensity, ++ / +++ moderate / strong intensity

DISCUSSION

Many molecular abnormalities have been described in adenomatous polyps, including defects in MMR, causing an increased spontaneous mutation rate, known as the mutator phenotype (Charames and Bapat, 2003). Loss of MMR function may accelerate the development and accumulation of mutations in those genes, which are responsible for controlling cell growth. This fact provides support to a reasonable hypothesis for a rapid enlargement of colon adenomatous polyps and their progressive structural transformation to carcinomas (Molaei et al., 2011).

In our series, we found a vast majority of MMRP absent cases in adenomas <10 mm. In general, size of adenoma is considered to be a valuable prognostic marker, since large adenomas >10 mm are associated with worse histo-morphological features (Toll et al., 2011). On the contrary, Sheridan et al. (2006) suggest that small sized sessile serrated adenomas (SSA) may develop into carcinomas despite their relatively small size. Molecular abnormalities develop through MSI (microsatellite instability). Decreased or absent immunostaining for MMRP was described in many SSA (Lee et al., 2005; Oh et al., 2005). Recently, several studies demonstrated that immunohistochemical detection of abnormal expression of MMRP is capable of identifying defective MMR genes. Molecular testing of MSI status consists of polymerase chain reaction and gel electrophoresis to examine the DNA sequences (Khoo et al., 2013). There is an excellent correlation between immunohistochemical results and MSI analysis. Both of these approaches are recommended to diagnose the abnormal status of MMR (Lanza et al., 2011; Khoo et al., 2013). Based on our recent results, we point out that loss of MMRP expression is also related to small sized sporadic adenomas <10 mm, and that increased risk of malignancy should be taken into consideration in these colon lesions. Data regarding the expression absence of MMRP in large vs. small sporadic colorectal adenomas is scarce. Most papers describe and study these proteins in adenomas associated with Lynch Syndrome (Halvarsson et al., 2005; Pino et al., 2009; Walsh et al., 2012).

Furthermore, in our adenoma group, absent cases were mainly associated with low grade dysplasia. The degree of dysplastic changes is an important histomorphological and prognostic parameter. High grade dysplasia is the strongest predictor for the development of malignant tumor. Interestingly enough, we found a higher prevalence of abnormal staining for four MMRP in low grade adenoma cases in comparison to high grade cases. Currently, there is a heated discussion within literature concerning the expression of MMRP and severity of dysplasia. Some authors concluded that loss of MMRP

expression is also detected in the absence of high grade dysplasia (De Jong et al., 2004). Contrarily, significant correlation was found in adenomas between MSI and high grade dysplasia (linoa et al., 2000). Pino et al. (2009) observed significant association between absent immunohistochemical staining of MMRP and high grade dysplasia. Another study by Walsh et al. (2012) demonstrated mismatch repair deficiency in 12/12 adenomas with high grade dysplasia (100%) and in 60/79 adenomas with low grade dysplasia (76%). However, all of the above mentioned research groups were dealing with hereditary non-polyposis colorectal cancer (HNPCC) adenomas.

In sporadic colorectal adenomas, predominant MMRP loss is MLH1. Silencing of the MLH1 gene by promoter hypermethylation results in partial or complete immunohistochemical absence of the protein in question (Hawkins and Ward, 2001). We found loss of MLH1 protein in 13/124 cases (10.5%). Typically, all absent MLH1 cases were accompanied by loss of immunohistochemical positivity for PMS2. PMS2 protein is probably unstable without its heterodimer twin (Young et al., 2002). The absence of more than one MMRP demonstrates progression via MSI pathway, and this pattern may suggest progressive transformation through adenoma-dysplasia-carcinoma sequence in a portion of assessed cases (Sheridan et al., 2006). Surprisingly, Nakagawa et al. (2001) concluded that normal colonic mucosa may also represent a possible precursor lesion by the spread of MLH1 promoter methylation with subsequent development of sporadic MSI+ colorectal cancer. Methylation of MLH1 promoter was also presented by Kuniyasu et al. (2004) in hyperplastic mucosa adjacent to colon cancer in athymic mice. Thus, our findings indicate that expression abnormalities in MMRP system may play a critical role in the early stages of development of premalignant and malignant colon lesions.

In the present study, distally located sporadic adenomas displayed loss of immunostaining for four MMRP more frequently. Our results are in concordance with previous observations (Pino et al., 2009). Several research groups revealed that increased risk of malignant transformation of sporadic colorectal adenomas is higher in distal colon than in proximal colon (Nusko et al., 1997; Chaves et al., 2000; Rijcken et al., 2002). By contrast, in patients with HNPCC, abnormal MMRP immunostaining was detected frequently in adenomas of proximal colon (Samowitz et al., 2001; Rijcken et al., 2002). These opposite findings are not yet elucidated.

Upon evaluation of positive cases, we noted a variable intensity of immunoreaction almost in every single case. Therefore, we also assessed the intensity of MMRP reaction in order to find possible correlations with other studied parameters. Due to the fact that adenoma cells frequently expressed heterogenic intensity, the dominant pattern was used for scoring. Statistical analysis revealed significant correlation of moderate and strong immunoreaction with size of adenoma >5 mm and high grade dysplastic changes for MLH1, MSH6, MSH2, and PMS2 proteins. This means that nuclear accumulation of MMRP with its moderate and strong intensity may represent an immunohistochemical indicator of repairing activities in growing high grade dysplasia adenomas.

Taking into account the key role of survivin in the regulation of apoptosis, it is not surprising that elevated survivin levels were described in a wide spectrum of malignant tumors, premalignant lesions and cancer-cell lines. In our series of dysplastic adenomas, we detected survivin in 78.2% of cases. In general, immunohistochemical survivin over-expression in malignant biopsy samples indicates a worse prognosis, relapse and/or decreased response to chemotherapeutic management (Mesri et al., 2001). Further, proliferative fenotype of survivin is associated with poor prognostic histomorphological parameters, such as vascular invasion and tumor grade 3 (Adamkov et al., 2012).

As expected, the presence of survivin expression significantly correlated with adenoma size and degree of dysplasia, statistically significant differences were observed between subcellular localization of survivin and adenoma size, dysplasia grade as well as its localization. Furthermore, there is a significant trend between the intensity of survivin immunoreaction and dysplasia grade and localization. These recent results suggest that survivin expression pattern in colon adenomas is also associated with worse prognostic features. In general, there is a higher incidence of focal malignant changes in larger adenomas and in HGD adenomas (Muto et al., 1975). This may prove a definitive relationship between adenomas and colorectal carcinomas (the adenoma-carcinoma sequence) (Talbot et al., 2006).

Briefly, MMRP are responsible for the correction of mutations during DNA replication, and the antiapoptotic survivin is an ideal protein for the development of premalignant and malignant lesions. Our analysis revealed a significant relation between the expression of MMRP and survivin (Table 6). Likewise, the evaluation of intensity of immunoreaction also resulted in interesting findings. As described in our recent results, a majority of adenomatous polyps demonstrated lower intensity of survivin immuno-

reaction as opposed to the intensity of MMRP. In addition, some percentage of cases revealed identical immunoreaction intensity for both MMRP and survivin. All of this may slightly uncover the relation of these proteins.

MMRP MLH1, MSH2, and PMS2 are also linked with apoptotic cascade via p53 or its homologue p73 (Stojic et al., 2004), e.g. p73 protein is directly stabilized by PMS2 protein and this interaction enhances its proapoptotic function (Shimodaira et al., 2003). Other studies, such as Luo et al., (2004) and Hassen et al., (2012), described the key role of MLH1 and PMS1/PMS2 heterodimerization. Nuclear accumulation of this complex may increase the activation of p53 by ataxia-teleangiectasia mutated (ATM) protein kinase. Köster et al. (2007) reported a significant correlation between immunohistochemical expression for MSH2 and p53, and apoptosis in cervical carcinoma. Zhang et al. (1999) showed that overexpression of MSH2 and MLH1 may induce apoptosis and that MSH2-deficient cells do not display apoptotic features. The expression of functional activity of p53 may regulate the expression of MSH2, since a binding site for p53 was discovered in the promoter region of the MSH2 gene (Scherer et al., 1996a; Scherer et al., 1996b). Thus, it seems that the common denominator between MMRP and apoptosis is the p53 protein. The p53 protein can either activate the apoptotic cascade by up-regulation of several associated genes or suppress those genes with antiapoptotic functions. Survivin is known to be suppressed by wild type p53, because it interacts with the survivin promoter, proven to be the first promoter to confer p53-dependent repression (Hoffman et al., 2002; Mirza et al., 2002). In addition, p53 may interfere with bcl-2 proteins in mitochondria with subsequent release of cytochrome c (Chipuk et al., 2004) and formation of proapoptotic multi-protein complex apoptosome.

Köster et al. (2007) indicate that MMRP system may be more active in the early stages of cancer development. The presence of dysplastic changes in colon adenomas provides evidence for their malignant potential. In our group of colon adenomas, we detected moderate and strong intensity of immunoreaction for MMRP in a majority of positive cases. Additionally, we revealed a statistically significant relation between immunoreactivity of MMRP and survivin. Increasing intensity of MMRP immunoreactivity was accompanied by decreasing intensity of survivin immunoreactivity.

Taking into account our current study results as well as the above-mentioned interactions between the proteins in question we suggest that MMRP may influence the antiapoptotic function of survivin by indirect mechanism via activation of p53 in LGD and HGD colon adenomas.

Conflict of Interest

The authors declared no conflict of interest in relation to the article.

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Ethical Approval

Our study was approved by local Ethics Committee of Jessenius Faculty of Medicine in Martin, registered in Office for Human Research Protection, U.S., Department of Health and Human Services under N°: IORG0004721.

Contributions

M.A.: Substantial contribution to design and conception, acquisition of data, analysis of data, interpretation of data, writing of article, collection of funds.

D.V.: Statistical analysis.

S.D.: Immunohistochemical stainings.

S.G.: Interpretation of data.

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