Correlation of APC and MLH1/MSH2 Expression in Colon Adenomas/Polyps

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Background: Adenomatous polyposis coli (APC) gene is thought to play a key role in the majority of sporadic colorectal cancers developed through the chromosomal instability pathway. In 10–15% of CRC the defect of the mismatch repair genes (MLH1, MSH2), the microsatellite instability is the underlying mechanism for carcinogenesis. The aim of this study was the correlation of APC, MLH1 and MSH2 immunoexpression in different types of colon adenomas/polyps (A/P).
Materials and methods: We processed biopsies and surgical pieces of colon A/P and carcinoma developed in adenoma (CC). The APC, MLH1, MSH2 expression were graded, and used for establishing different immune phenotypes that have been compared by statistical tests.
Results: The majority of tubular and tubulovillous adenomas have the MLH1+/MSH2+/APC+ immune phenotype, and the ratio of MLH1–/MSH2–/APC+ cases increases in case of hyperplastic polyps and serrated adenomas. A/P developing in the right colon and in patients below 40 years were more frequently MLH1–/MSH2–/APC+.
Conclusions: APC immunoexpression decreases in adenomas/polyps with dysplasia, and MLH1 and MSH2 expression is altered especially in hyperplastic polyps and serrated adenomas.

Keywords: colon adenoma, immunohistochemistry, APC, MLH1, MSH2

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Introduction

The majority of colorectal cancers (CRC) are sporadic and develop through the chromosomal instability pathway. 10–15% of CRCs develop through another underlying mechanism for carcinogenesis: microsatellite instability (MSI) [1].

Chromosomal instability leads to early mutation of the Adenomatous Polyposis Coli (APC) (5q21-22) gene, which is present in 30–70% of adenomas/polyps (A/P) and sporadic CRCs [2]. The APC gene is considered a "gatekeeper" gene that maintains the integrity of colon epithelium, and its mutation leads to dysplastic abnormalities in adenomas [3].

Microsatellite instability (MSI) becomes apparent by the change in the number of DNA sequences (microsatellites) repeating in the genome. Correction of these errors is performed partly by the Mismatch Repair (MMR): MutL Homolog 1 (MLH1) and MutS Homolog 2 (MSH2) enzymes [4]. Metilation of the MLH1 gene is the most frequent cause of MSI in CRC, and it is associated with the mutation of the BRAF gene [4]. MSI was identified in 29% of HPs and 53% of SA [5], while it is very rare in adenomas and polyps (A/P) with dysplastic abnormalities [4], which are associated especially with the mutation of the APC gene [6]. For MSI screening there has been a recommendation to perform immunohistochemistry testing of MMR proteins expressed by the nuclei of the normal epithelial cells of the colon. Lack of nuclear expression in precancerous lesions and in CRCs suggests inactivation of these proteins [1].

The aim of our study was to compare immunoexpression of MLH1, MSH2 and APC proteins in colon adenomas/polyps, and correlation with clinico-pathological factors in order to find out their role in colorectal carcinogenesis.

Material and methods

We studied 17 hyperplastic polyps (HP), 42 adenomas (16 serrated – SA, 8 tubular – TA, 18 tubulovillous adenomas – TVA) and 7 carcinomas developed from adenomas (CC) of the colon from the archived biological material of the Pathology Department of the County Emergency Clinical Hospital of Tîrgu Mureş, Romania. Mean age of patients was 59.8±2.89 years. The 3 μm thick sections obtained from the formalin fixed and paraffin embedded resection tissue specimens were routinely dewaxed and rehydrated. Antigen retrieval was performed by pressurized steam cooking (citrate solution, pH=6) followed by endogenous peroxidase blocking. We used the following mouse monoclonal antibodies for APC (LabVision Fremont, CA, USA, clone Epitop: C-terminal) in 1:75, MSH2 (Diagnostic BioSystem, Pleasanton, USA, clone 25D12) in 1:25, MLH1 (Abcam Biochemicals, Cambridge UK, clone G168-15) in 1:25. Ultravision Labeled Polymer system (LabVision, Fremont, CA, U.S.A), and DAB developing was used for detecting primary antibodies. Negative controls were performed by omitting the primary antibody.

We considered a decreased or absent reaction if less than 99% of cells showed positive labeling for APC, MLH1 and

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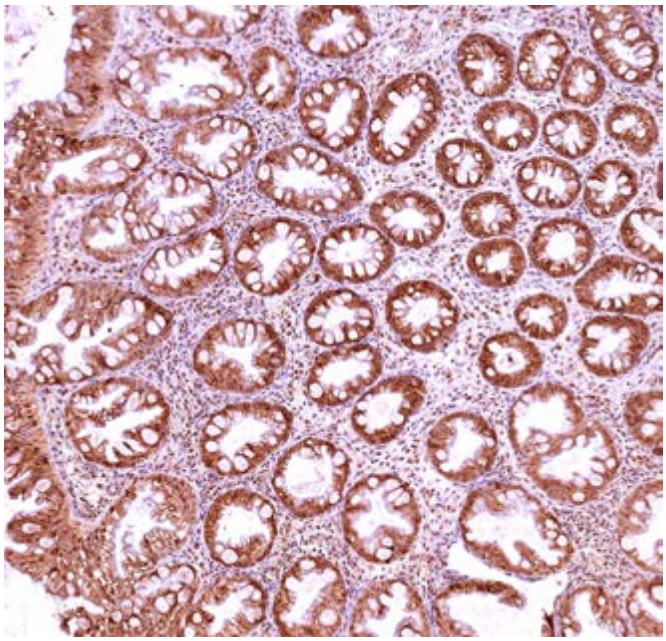

Fig. 1. Maintained APC expression in a hyperplastic polyp without dysplasia, 13x

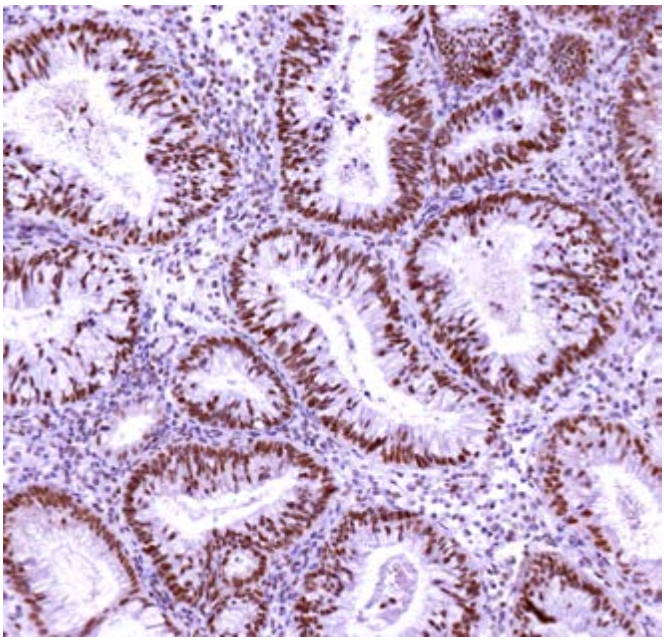

Fig. 2. Maintained MSH2 expression in a tubular adenoma with moderate dysplasia, 20x

MSH2. Results were analyzed using the Graph Pad In Stat 3, version 3.06 statistic calculation software (GraphPad Software Inc., San Diego, U.S.A.). We considered the association significant when $p<0.05$, with 95% confidence interval.

Results

1. Immunoexpression of APC, MLH1 and MSH2 proteins

APC expression was noted in 83% (49/59) of A/P and in all CCs (Figure 1). The ratio of cases with low APC expression increased with the grade of dysplasia, and it was more frequent in TVAs. MLH1 and MSH2 expression was decreased 40.6%, and 45.7% of the cases, respectively, in A/P, and in 85.7%, and 71.4% respectively in CCs. This decrease was more pronounced in HP and in SA, as compared to TA and TVA (Figure 2, 3, 4). All A/P cases developed under 40 years of age, and the majority of the lesions developed in the right colon maintained their APC expression, and showed decreased MLH1 and MSH2 expression. Localization of the lesions, gender and age of the patients do not show statistically significant correlations with APC, MSH1 and MLH2 expression (Table I).

2. Correlations

MLH1 expression correlates with MSH2 expression ($p<0.05$). The ratio of MSH2+/MLH1+ cases decreases, and the ratio of MSH2–/MLH1– cases increases starting from TA, through TVA, HP, SA until CC ($p<0.05$).

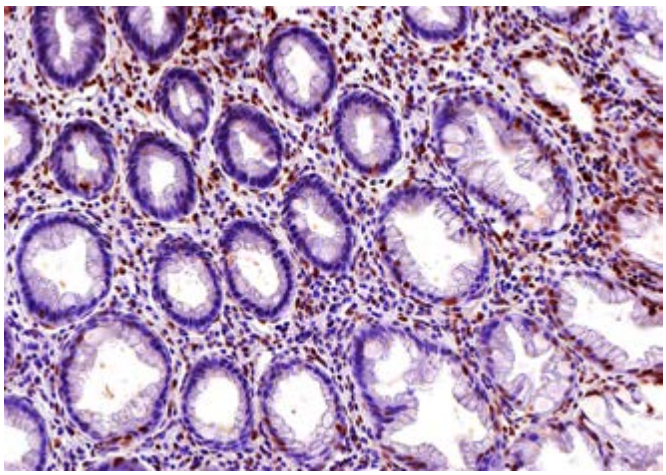

Fig. 3. Decreased MSH2 expression in a serrated adenoma with low-grade dysplasia, 20x

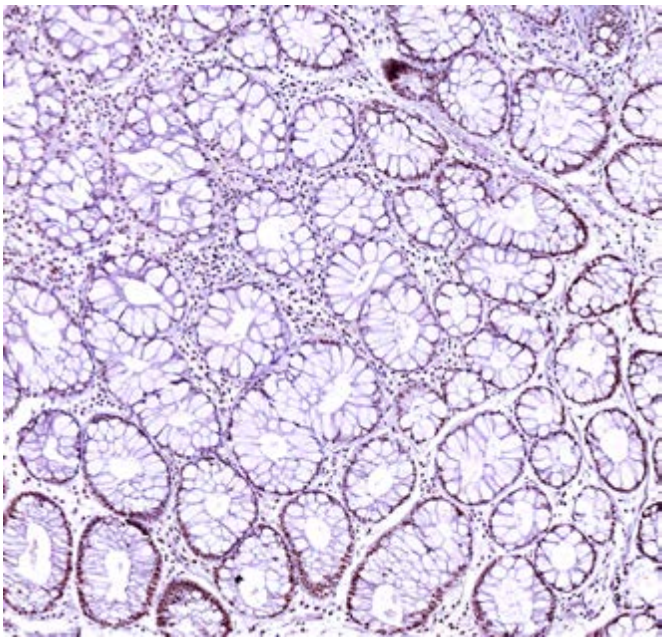

Fig. 4. Decreased MLH1 expression in a hyperplastic polyp without dysplasia, 13x

Table I. Correlation of clinico-pathological factors with APC, MSH2, MLH1 immunoexpression

	APC–	APC+	p	MLH1–	MLH1+	p	MSH2–	MSH2+	p
Histological type									
HP	0	17		6	11		11	6	
SA	2	14		10	6		11	5	
TA	0	8	<0.05	2	6	=0.05	1	7	<0.05
TVA	8	10		6	12		4	14	
CC	0	7		6	1		5	2	
Dysplasia									
w/o D	2	23		11	14		12	13	
LD	2	17	<0.05	9	10	>0.05	10	9	>0.05
MoD	2	7		3	6		3	6	
HD	4	2		1	5		2	4	
Localization									
Right colon	1	13	>0.05	8	6	>0.05	9	5	>0.05
Left colon	4	30		15	19		15	19	
Age (years)									
<40	0	3	>0.05	3	0	>0.05	3	0	>0.05
>41	10	53		27	36		29	34	
Gender									
Female	4	28	>0.05	13	19	>0.05	15	17	>0.05
Male	6	28		17	17		17	17	

HP – hyperplastic polyps, SA – serrated adenomas, TA – tubular adenomas, TVA – tubulovillous adenomas; CC- carcinomas; w/o D – adenomas without dysplasia, LD – low-grade dysplasia, MoD – moderate dysplasia, HD – high-grade dysplasia;

Thus the majority of TA and TVA are characterized by the MSH2+/MLH1+ immune phenotype, and the majority of HP and SA by mixed immune phenotypes, where one or both markers have decreased or absent expression. In case of CC the MSH2–/MLH1– immune phenotype is predominant (Figure 5).

75% of TA were MSH2+/MLH1+/APC+, while this immune phenotype was noted in just 33% of TVA. Otherwise, in case of TVA we noted mixed immune phenotypes. The ratio of MSH2+/MLH1+/APC+ cases decreases in case of SA (12.5%), as compared to HP (35.3%), while the ratio of MSH2–/MLH1–/APC+ cases was about similar in case of HP (35.3%) and SA (37.5%). The MSH2–/MLH1+/APC+ immune phenotype was detected only in case of HP (29.4%), and SA (18.7%). Most of the examined CC (71.4%) were MSH2–/MLH1–/APC+ (p<0.05) (Figure 6).

Most of the A/P without dysplasia (56%) show mixed MSH2/MLH1 immune phenotypes with APC+ expression, and only 36% are MSH2+/MLH1+/APC+. 32.3% of A/P with dysplasia were MSH2+/MLH1+/APC+, and 44% show mixed MSH2/MLH1 immune phenotypes with APC+ expression, and in 22.8% with APC– expression (p<0.05).

In the right colon 50% of A/P are MSH2–/MLH1–/APC+, and in the left colon 38% of the cases are MSH2+/MLH1+/APC+, and only 26.4% are MSH2–/MLH1–/APC+.

All A/P developed under 40 years of age are MSH2–/MLH1–/APC+, and the ratio of this immune phenotype decreases with age (25.4% over 40 years). 33.3% of A/P developed over 40 years of age are MSH2+/MLH1+/APC+.

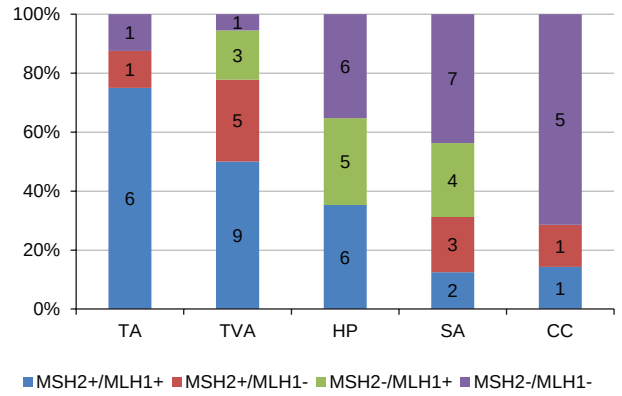

Fig. 5. Ratio of MSH2/MLH1 immune phenotype according to A/P type. HP – hyperplastic polyps, SA – serrated adenomas, TA – tubular adenomas, TVA – tubulovillous adenomas, CC – carcinomas

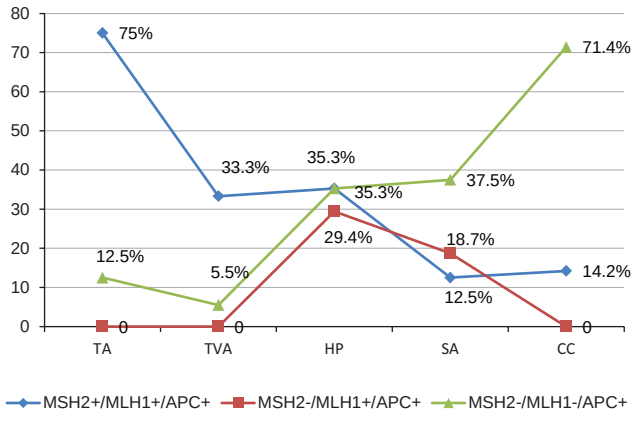

Fig. 6. Evolution of MSH2/MLH1/APC immune phenotypes according to A/P type. HP – hyperplastic polyps, SA – serrated adenomas, TA – tubular adenomas, TVA – tubulovillous adenomas, CC – carcinomas

This immune phenotype is the most frequent in the 41 to 50 years (50%) and 71 to 80 years age groups (53.3%).

Discussions

In our study the APC protein expression was detected in the majority of A/P (83%), in all HP and TA, and in the majority of SA (87.5%) and TVA (55.5%). According to bibliographic data, the A/P ratio with maintained APC expression varies between 71 and 86.7% [7,8,9], which confirms our observations regarding HP [10]. Instead, the APC positive TA, TVA, SA ratio varies between 56 and 76% [7,8,10]. Our results show that APC expression decreases significantly with the grade of dysplasia; similar results have been obtained by Bortlik et al (2006) as well [8]. We noted that A/P developed in young patients (under 40 years), and in the right colon more frequently show decreased APC expression; this also has been noted by other authors [7,8].

MLH1 and MSH2 expression was decreased in 40.6%, and 45.7%, respectively, in A/P and in the majority of CC (85.7%, and 71.4%, respectively). These ratios are lower in other studies, both in case of A/P (3–20%) [11,12,13,14], and CRC (2–33%) [12,14,15,16,17]. Oh et al. 2005 reported a significant decrease of Mismatch Repair proteins expression in CC, as compared to A/P [18]. Unlike other authors, we observed that in case of HP and SA, MMR proteins expression is much more decreased compared to TA and TVA [11,12]. MMR immunoexpression did not correlate with age and gender of the patients, and localization of A/P [11,12]. We observed that A/P developed in the right colon and in young patients, more frequently have altered MMR expression. It is known that CRC with MSI are poorly differentiated, located especially in the right colon, and they are more frequent in women and young people [15,16].

By correlating MMR expression and APC expression, we noticed that the ratio of MSH2+/MLH1+/APC+ cases decreases, and the ratio of MSH2–/MLH1–/APC+ cases increases from TA, through TVA, HP, SA until CC. In about 30% of A/P with dysplasia mixed immune phenotype MMR occur with decreased APC expression, while in case of A/P without dysplasia mixed immune phenotype MMR predominate with retained APC expression. MSH2–/MLH1–/APC+ A/P are more frequent in young patients and the right colon. According to our knowledge there are no bibliographical references related to immune phenotype changes in colon A/P.

Conclusions

APC immunoexpression decreases in adenomas/polyps with dysplasia, and MLH1 and MSH2 expression is altered especially in hyperplastic polyps and serrated adenomas.

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