Hyperplastic polyps and serrated adenomas: precancerous lesions with mixed immunophenotype

ZSUZSANNA PAP1), Z. PÁVAI1), L. DÉNES1), KLARA BRÎNZANIUC1), I. JUNG2)

1)Department of Anatomy
2)Department of Pathology
University of Medicine and Pharmacy of Targu Mures

Abstract
Our immunohistochemical study wants to be a contribution to clarifying the adenoma-carcinoma sequence and serrated pathway of colorectal carcinogenesis. Thus, we performed immunohistochemical analysis of hyperplastic polyps (HP), serrated adenomas (SA), and classical adenomas (tubular adenomas – TA and tubulovillous adenomas – TVA) and carcinomas developed from adenomas (CA) using expression of p53, Ki-67, c-myc, APC, MSH2 and Ets-1 proteins. Because of correlation of the expression of these proteins, we propose several immunophenotypes, which show modifications along the known carcinogenetic mechanisms. Along the adenoma-carcinoma sequence we noted an increase in the expression of p53, Ki-67, c-myc and Ets-1, and a decrease in APC expression. The majority of TAs and TVAs are characterized by p53+/Ki-67+, p53+/c-myc+, p53+/APC+, and Ets-/p53+, Ets-/Ki-67+ immunophenotypes. The majority of HPs and SAs are Ets-/p53-, Ets-/Ki-67+, Ets-/c-myc+, APC+/MSH2-. In approximately 1/3 of the hyperplastic polyps and serrated adenomas, we noted that the decrease in expression of MSH2 is associated with an increase in the expression of p53, c-myc, Ki-67, and Ets-1. Thus, we can conclude that a group of hyperplastic polyps and serrated adenomas display similar immunohistochemical characteristics to tubular and tubulovillous adenomas, which delineates a group of precancerous lesions that can develop via mixed carcinogenic pathways.

Keywords: colon, carcinogenesis, immunophenotypes, hyperplastic polyps, serrated adenomas, classical adenomas.

Introduction
According to literature data, the incidence of ex adenoma CCRs varies between 5% [1] and 20–30% [2]. Ex adenoma carcinomas evolve through two largely independent pathways: (1) the traditional adenoma-carcinoma sequence associated with chromosomal instability (CIN) characterized by bi-allelic inactivation of APC, followed the mutation of KRAS, DCC and TP53 genes; (2) the serrated pathway characterized by DNA microsatellite instability (MIS), BRAF mutation and DNA methylation [3].

KRAS and APC mutations develop more frequently in classical adenomas (tubular – TA, villous and tubulovillous – TVA), whereas BRAF gene mutations and epigenetic modifications occur more frequently in hyperplastic polyps (HP) and serrated adenomas (SA). However, in some HP and SA cases KRAS and APC gene mutations have also been identified [4].

APC is a tumor suppressor gene (5q21-22) involved also in the Wnt signal pathway. APC mutations are present in 30–70% of sporadic adenomas and carcinomas, so it has been proposed that this mutation is an early event in colorectal carcinogenesis [5, 6]. TP53 is the most commonly mutated tumor suppressor gene in various kinds of malignant tumors. TP53 mutations are generally considered to occur at the time of the transformation from the adenoma to carcinoma [6, 7]. Ki-67 is a proliferation-associated protein, which is expressed during all phases of cell cycle except for the resting of cells in G0 phase [8]. The c-myc protein is involved in the cell cycle progression, and is expressed by cells in phase G1 of the cell cycle [7]. hMSH2 is a DNA mismatch repair enzyme (MMR), and together with hMLH1, hPMS1, hPMS2 they are involved in DNA repair. Because of MMR gene mutations, DNA microsatellite instability develops [6].

In our previous publications, we described the changes in expression of E-cadherin, syndecan-1 and Ets-1 protein expression, thus defining different immunophenotypes possibly contributing to colorectal carcinogenesis.

Materials and Methods
The study group comprised three hyperplastic polyps (HP), 31 adenomas (five serrated – SA, eight tubular – TA, 18 tubulovillous adenomas – TVA) and four carcinomas developed from adenomas (CA) (of which one carcinoma developed from a SA). The
biopsy and resection material has been processed using histopathology and immunohistochemistry methods at the Pathology Department of the Clinical County Hospital of Târgu Mureș, Romania. 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: p53 (DakoCytomation, Denmark, clone DO-7) in 1:200, MSH2 (LabVision Fremont, CA, USA, clone 25D12) in 1:10, c-myc (LabVision Fremont, CA, USA, clone 9E10.3) in 1:250; and rabbit monoclonal antibodies for Ki-67 (LabVision Fremont, CA, USA, clone SP6) in 1:200 and APC (LabVision Fremont, CA, USA, clone epitope: C-terminal) in 1:75. Ultravision Labeled Polymer system (LabVision, Fremont, CA, USA) with DAB development was used for detecting primary antibodies. Negative controls were performed by omitting the primary antibody.

In order to quantify and compare immunohistochemistry data, we determined the percent of positive tumor cells. We considered a positive reaction whenever over 20% of cells were labeled by p53, Ki-67 and c-myc, and over 10% in case of the Ets-1 antibody, while expression was considered to be negative (decreased or absent) if less than 80% of cells showed positive labeling for APC and MSH2. Changes in immunohistochemical expression of these proteins are presented in Figure 1, according to histologic type of adenomas, and presence or absence of dysplastic and carcinomatous changes.

![Figure 1](image)

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

Results

Classical clinico-pathologic parameters

Mean age of patients was 61.5±3.7 years (min. value 29 years and max. value 79 years), with 60.5% of the tumors occurring in males. Majority of the lesions (80%) were localized to the left colon. Dysplasia was absent in HPs and present in one third of TAs, the majority of TVAs and in all SAs.

Mean age of patients with HP and SA was lower (55.8±12.1 years) compared to that of patients with TA and TVA (63.7±3.8 years).

Immunoexpression of p53, Ki-67, c-myc, APC, and MSH2 proteins

Expression of the analyzed proteins is listed in Table 1. Expression of p53, Ki-67 and c-myc proteins was increased in over 2/3 of adenomas and polyps (A/P), as well as in the majority of carcinomas (CA). The reactions were more pronounced in A/P with dysplasia. P53 hyperexpression showed the highest frequency in TAs and TVAs, while Ki-67 and c-myc protein expression was more intense in TVAs and SAs (Table 1).

APC expression was conserved in HPs and TAs, while being decreased in a quarter of SAs and in 50% of TVAs. APC immunoexpression decreases significantly in A/P with dysplasia \( (p=0.01) \). On the contrary, MSH2 immunoexpression was more frequently decreased in CAs compared to A/P, and in all HPs and SAs \( (p<0.0001) \) (Table 1).
Correlations

Correlations of p53, Ki-67, c-myc, APC, and MSH2 immunoprogression

Incidence of relevant immunophenotypes is presented in Table 2, according to grade of dysplasia, and in Figure 2, according to histological type. The frequency of p53+/Ki-67+ and p53+/c-myc+ cases increases along the adenoma-carcinoma sequence, from A/P without dysplasia, through those with dysplasia, and to CAs (Table 2).

In case of TAs and TVAs p53+/Ki-67+, and p53+/c-myc+ immunophenotypes are more frequent, as compared to HPs and SAs, which are more frequently p53+/c-myc+, and p53+/Ki-67+ (Figure 2). We noted in our patient population that p53 and Ki-67, as well as p53 and c-myc expressions are correlated positively (p<0.0001 and p=0.02, respectively).

We noted a significant increase in the incidence of the p53+/APC+ immunophenotype along the adenoma-carcinoma sequence (p=0.004) (Table 2). The majority of HPs and SAs are p53-/APC+, as opposed to TAs and TVAs where the p53+/APC+ immunophenotype is dominant (Figure 2). In CA developing from SA, APC expression was decreased, and p53 expression was increased. As far as the p53/MSH2 immunophenotype is concerned, we noted a progressive increase in the number of cases with p53+/MSH2- along the adenoma-carcinoma sequence (Table 2). The majority of HPs and SAs are p53-/MSH2-, while the rest are p53+/MSH2-; the majority of TAs and TVAs are p53+/MSH2+ (p=0.002) (Figure 2).

Correlating APC and MSH2 expression we noted that all HPs and most of SAs are APC+/MSH2-, while all TAs and 50% of TVAs and CAs are APC+/MSH2+ (in CA developed from an SA, APC expression was moderately, and MSH2 expression was markedly decreased) (p<0.0001). In approximately 20% of SAs and TVAs we noted the APC+/MSH2- immunophenotype (Figure 2).

Table 2 – Correlation of the analyzed markers, according to grade of dysplasia

<table>
<thead>
<tr>
<th>Marker Combination</th>
<th>HP</th>
<th>SA</th>
<th>TA</th>
<th>TVA</th>
<th>Fisher test</th>
</tr>
</thead>
<tbody>
<tr>
<td>p53+/Ki-67+</td>
<td>41%</td>
<td>60%</td>
<td>87%</td>
<td>75%</td>
<td>p=0.3</td>
</tr>
<tr>
<td>p53+/c-myc+</td>
<td>33%</td>
<td>70%</td>
<td>62%</td>
<td>75%</td>
<td>p=0.5</td>
</tr>
<tr>
<td>p53+/APC+</td>
<td>25%</td>
<td>70%</td>
<td>62%</td>
<td>0</td>
<td>p=0.004</td>
</tr>
<tr>
<td>p53+/MSH2-</td>
<td>8%</td>
<td>10%</td>
<td>12%</td>
<td>25%</td>
<td>p=0.5</td>
</tr>
</tbody>
</table>


Figure 2 – Correlation of p53 expression with Ki-67, c-myc, APC, and MSH2 expression, and the evolution of APC/MSH2 immunophenotypes according to adenoma type (HP – hyperplastic polyps, SA – serrated adenomas, TA – tubular adenomas, TVA – tubulovillous adenomas).

Correlation of Ets-1 expression with expression of p53, Ki-67, c-myc, APC, MSH2 proteins

Incidence of relevant immunophenotypes is presented in Figure 3, according to histological type. The majority of HPs and SAs are Ets-/p53-, while TAs and TVAs are Ets+/p53+. In approximately 20% of SAs and TVAs we noted the Ets+/p53+ immunophenotype (Figure 3).

A/P with dysplasia are more frequently Ets+/Ki67+ (p=0.02) and Ets+/c-myc+ (p=0.03). A small portion of HPs are Ets-/Ki-67-, while the majority of them, along with SAs are Ets-/Ki-67+; in 20% of SAs there is a transition to the Ets+/Ki-67+ phenotype. This transition from Ets-/Ki-67- through Ets-/Ki-67+ into Ets+/Ki-67+ can be tracked from TAs, through TVAs to CAs.
Similar results have been obtained with the Ets/c-myc immunophenotype (Figure 3).

A/P with dysplasia are more frequently Ets-/APC−, and those without dysplasia are Ets-/APC+ (p<0.001). All HPs and TAs, the majority of SAs, and approximately 50% of TVAs and CAs are Ets-/APC+. All HPs and the majority of SAs are Ets-/MSH2−, while all TAs and the majority of TVAs and CAs are Ets+/MSH2+ (p=0.004). In approximately 20% of SAs we noted the presence of Ets+/APC- and Ets+/MSH2- immunophenotypes (Figure 3).

Discussion

Our immunohistochemical study wants to be a contribution to clarifying the adenoma-carcinoma sequence and serrated pathway of colorectal carcinogenesis. Thus, we studied HP, SA, classical adenoma (TA, TVA) and CA cases, and we delineated several immunophenotypes, which show modifications along the known carcinogenic pathways.

Classically, colorectal adenocarcinoma was seen to arise from sporadic precursor lesions: TA, VA, TVA along the adenoma-carcinoma pathway. HP have been viewed as clinically innocuous lesions, but more recently the concept of an alternative hyperplastic polyp-serrated adenoma–carcinoma pathway has been suggested, based on the common molecular biology changes described in relation to these lesions. Our observation related to the younger age of patients with HP as compared to age of those with SA has also been confirmed by other authors. Supposedly HPs transform into SAs [10, 11]. Base on the studied immunophenotypes, we noted that SAs display an intermediate immunohistochemical configuration between HPs and TAs. SAs are more frequently p53+, Ki-67+, and c-myc+ compared to HPs, and less frequently p53+ and c-myc+ compared to TAs and TVAs. According to Cunningham and Riddell [10], SAs may be classified between TAs and TVAs, as far as cell proliferation/apoptosis ratio is concerned. The malignant potential of the SA is also suggested by the dysplastic modifications present in all SA cases, confirmed by other studies as well [12].

In our study, p53, Ki-67, and c-myc expression in A/P and CAs showed no statistically significant differences. Most studies emphasize that p53 hyperexpression is significantly more frequent in CRCs than in A/P [7, 13–17], whereas our results pertaining c-myc and Ki-67 proteins are similar to those obtained by other authors [7, 8, 17]. In our cases, hyperexpression of these proteins was more frequent in adenomas showing dysplastic modifications [7, 8, 13, 16, 18]. P53 hyperexpression was more pronounced in most of the classical adenomas [19–21], while Ki-67 hyperexpression was more frequent in TVAs and SAs [21, 22].

P53 expression correlates positively with Ki-67 and c-myc expression, as frequency of p53+/c-myc+ and p53+/Ki-67+ immunophenotypes increases with grade of dysplasia in TAs, TVAs, against HPs and SAs. Literature data show a lack of correlation between p53 and c-myc [17], and p53 and Ki-67 [7, 18] describing a positive correlation between p53 expression and cytoplasmic expression of c-myc protein [7].

Moreover, APC and MSH2 expression shows no significant differences in A/P and CAs, which is most probably due to the small number of CA cases included in the study, and the fact that examined sections contain residual adenoma tissue. Iwamoto M et al. [5] noted that APC expression is significantly more frequently decreased in CCRs compared to A/P, and Yasugi A et al. [23] reported that MSH2 expression is retained in ex adenoma carcinomas as compared to de novo ones. APC immunoeexpression decreases significantly in classical adenomas according to grade of dysplasia, an observation also confirmed by Kim DH et al. [24]. As opposed to the study of Oh K et al. [21], reporting that MSH2 expression is retained both in HPs, SAs, and classical adenomas, but it is decreased in the adenomatous component of mixed (adenomatous and hyperplastic) polyps, we noted a decrease in MSH2 expression of HPs and SAs.

Our observations regarding HPs and SAs with similar immunophenotype features to TAs and TVAs are also supported by studies reporting that the serrated
pathway of colorectal tumorigenesis appears to be heterogeneous. Thus, Sawyer EJ et al. [25] reported that some SAs develop along pathways involving changes in APC/β-catenin, however KRAS mutation was less frequent in SAs than in classical adenomas. Fu X et al. [26] suggested that the complete methylation of APC promoter 1A is present in a subset of SAs. Actually, SAs can be divided into two types: (1) sessile serrated adenomas (characterized by microsatellite instability, mutation of BRAF, extensive DNA methylation) and (2) traditional serrated adenomas (characterized by KRAS mutation, p53 accumulation) [3, 27]. In addition to the two “classical” pathways in colorectal carcinogenesis, Jass JR et al. [3] assumed that there is a number of “fusions” pathways that combine mechanisms associated with both classical and serrated adenomas. Microsatelliteand chromosomal stable colorectal cancer may exhibit more frequently p53 hyperexpression and conserved APC expression, compared to chromosomal unstable CRC [28].

In our study population, adenomas and polyps display increased expression of p53, Ki-67, c-myc, retained expression of APC, and lack of Ets-1 expression. Along the adenoma-carcinoma sequence, we noted an increase in the expression of p53, Ki-67, c-myc and Ets-1, and a decrease in APC expression. In approximately 1/3 of hyperplastic polyps and serrated adenomas, we noted that decreased expression of MSH2 is associated with an increase in expression of p53, c-myc, Ki-67, and Ets-1.

### Conclusions

Based on the above, we concluded that a group of hyperplastic polyps and serrated adenomas display similar immunohistochemical features to tubular and tubulovillous adenomas; as a result, we delineated a group of precancerous lesions of the colon that can develop through mixed carcinogenic pathways.

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Corresponding author
Zsuzsanna Pap, University Assistant, Department of Anatomy, University of Medicine and Pharmacy of Târgu Mureș, 38 Gheorghe Marinescu Street, 540142 Târgu Mureș, Romania; Phone +40740–672 545, e-mail: papzsuzsa@yahoo.com

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