Rom J Morphol Embryol 2021, 62(4):971–979 ISSN (print) 1220–0522, ISSN (online) 2066–8279 doi: 10.47162/RJME.62.4.09

ORIGINAL PAPER

RJME Romanian Journal of Morphology & Embryology http://www.rjme.ro/

MLH1, BRAF and p53 – searching for significant markers to predict evolution towards adenocarcinoma in colonic sessile serrated lesions

DIANA RĂDUŢĂ[1)], OCTAVIAN MARIUS DINCĂ[2)], GIANINA VIORICA MICU[1)], LUCIANA NICHITA[1,3)], MIRELA DANIELA CIOPLEA[1)], RADU MIHAI BUŞCĂ[1)], RALUCA ARDELEANU[1)], RADU BOGDAN MATEESCU[4,5)], ANDREEA BENGUŞ[5)], SABINA ANDRADA ZURAC[1,3)], CRISTIANA GABRIELA POPP[1)], GEORGE CRISTIAN VLĂDAN[2)]

[1)]*Department of Pathology, Colentina University Hospital, Bucharest, Romania*

[2)]*Department of Oral and Maxillofacial Surgery, Carol Davila University of Medicine and Pharmacy, Bucharest, Romania*

[3)]*Department of Pathology, Carol Davila University of Medicine and Pharmacy, Bucharest, Romania*

[4)]*Department of Internal Medicine, Carol Davila University of Medicine and Pharmacy, Bucharest, Romania*

[5)]*Department of Gastroenterology, Colentina University Hospital, Bucharest, Romania*

Abstract

Background and Aim: Colonic serrated lesions are premalignant lesions, using an alternative malignization pathway, including multiple genetic and epigenetic alterations, as: mismatch repair deficiency due to MutL homolog 1 (*MLH1*) promoter methylation, tumor protein p53 (*TP53*) mutations, activating mutations of v-Raf murine sarcoma viral oncogene homolog B (*BRAF*) and Kirsten rat sarcoma viral oncogene homolog (*KRAS*). Our study aims to evaluate MLH1, BRAF and p53 immunohistochemical (IHC) status in sessile serrated lesions (SSLs), with and without dysplasia. *Materials and Methods*: This is a retrospective case-control study including 20 SSLs with dysplasia and 20 SSLs without dysplasia (matching sex and age). IHC expression of MLH1, BRAF and p53 was evaluated as the percent of nuclear loss of MLH1, cytoplasmic positivity of BRAF and nuclear positivity of p53. Data concerning age, sex, localization of the lesion, dysplasia and IHC results were statistically processed using Microsoft Excel. *Results*: We had very polymorphous patterns of IHC expression for BRAF, MLH1 and p53, especially in the dysplastic group. Thus, two patients were BRAF+/MLH1-/p53+, three were BRAF+/MLH1-/p53-, one was BRAF+/MLH1+/p53- and six were BRAF+/MLH1+/p53+. Dysplastic lesions without *BRAF* mutation exhibited the following phenotype: one case BRAF-/MLH1-/p53+, four BRAF-/MLH1-/p53- and three BRAF-/MLH1+/p53+. In the control group (SSLs without dysplasia), there was a more homogenous distribution of cases: eight cases BRAF+/MLH1+/p53-, seven BRAF-/MLH1+/p53-, one BRAF-/MLH1-/p53+, two BRAF-/MLH1-/p53- and two BRAF-/MLH1+/p53+. *Conclusions*: There are more routes on the serrated pathway, with different mutations and time of acquisition of each genetic or epigenetic lesion with the same morphological result. These lesions should be stratified according to their risk to poor outcome and their need to further surveillance.

Keywords: colonic adenocarcinoma, sessile serrated lesions, dysplasia, BRAF.

Introduction

Colonic serrated lesions, defined by epithelial proliferations with saw-tooth or stellate architecture, are one of the most challenging diagnoses in digestive pathology. They include from benign hyperplastic polyps (HPs) to dysplastic lesions with high potential of evolution towards invasive carcinoma (IC). From their inclusion in 2010 in *World Health Organization* (WHO) Classification of digestive tumors, many researchers offered their attention to serrated lesions of the large bowel, describing pathways of proliferation and evolution towards malignancy, information that led, in 2019, to a new classification [1–3]. Serrated colonic proliferative lesions are now classified by the *WHO* as HPs, sessile serrated lesions (SSLs) with or without dysplasia, traditional serrated adenomas (TSAs), and serrated adenoma, unclassified [2]. Some lesions, previously diagnosed as HPs are, according to this classification, in fact, SSLs [1, 2, 4].

SSLs are, usually, asymptomatic lesions, frequently found incidentally, but some of them are rapidly progressive premalignant lesions, involved in up to 35% of colorectal carcinoma (CRC) [5]. From molecular point of view, SSLs share a distinct genotype with microsatellite instability (MSI), v-Raf murine sarcoma viral oncogene homolog B (*BRAF*) mutation and nuclear hypermethylation [2, 6].

From the pathologist point of view, the criteria to diagnose SSL have never been simpler: for SSLs without dysplasia – one single crypt with unequivocal serrated architecture: asymmetrical proliferation with horizontal growth along the *muscularis mucosae*, dilation of the basal third of the crypt and serrations present into the crypt base [2, 7, 8]. The cytological aspects vary from small basally located nuclei to occasional larger nuclei with inconspicuous nucleoli [2, 7].

Dysplasia in SSLs is a focal change that appears on the pathway to carcinoma. SSLs with dysplasia have complex crypt architecture (crowding, complex branching, cribriform or villous architecture) and cytological atypia with various patterns: intestinal dysplasia (resembling sporadic dysplasia or the dysplasia in conventional adenoma), serrated dysplasia (eosinophilic cytoplasm and round atypical nuclei, prominent nucleoli, and numerous mitoses [1, 2, 9, 10]. Liu *et al.* [9] described a new category of dysplasia in SSLs, called minimal deviation dysplasia, characterized by minimal cytological and architectural abnormalities, accompanied by loss of MutL homolog 1 (*MLH1*) expression. There still are a lot of controversies about the proper manner to report dysplasia (especially low grade) in SSLs and the importance of immunohistochemical (IHC) tests for reporting this feature [11] (Figure 1, a and b).

The serrated pathway to invasive malignancy is an alternative pathway to the more known and documented adenoma–carcinoma pathway, including multiple genetic and epigenetic alterations, the most frequent being mismatch repair deficiency due to *MLH1* promoter methylation, extensive methylation of various CpG islands [2, 12, 13], tumor protein p53 (*TP53*) mutations, activating mutations of *BRAF* and Kirsten rat sarcoma viral oncogene homolog (*KRAS*), mutations that involve WNT signaling pathway [2, 5].

Usually, the first genetic mutation involves mitogen-activated protein kinase (MAPK) pathway (*KRAS*, *BRAF*) [7, 14] Next step in SSLs is hypermethylation of *MLH1* promoter, which is accompanied by the occurrence of dysplasia [9]. Activation of the WNT signaling pathway is frequently found in SSLs progression towards carcinoma, usually occurring later than in adenomas [15], usually through mutations of ring finger protein 43 (*RNF43*)–zinc and ring finger 3 (*ZNRF3*) complex [16].

Mutation of *p53*, found in different cancers including sporadic and polypoid CRCs, is also described in serrated lesions, although it is conspicuous and usually associated with *KRAS*-mutated lesions [17–19].

Adenomatous polyposis coli (*APC*) truncating mutations are rare and tardive in SSL and their role in carcinogenesis is unclear. They are found more frequently in *BRAF*-mutated lesions, but *APC* mutations seems to be missense and not involved in activation of the WNT signaling pathway [15, 18] (Figure 2).

Serrated adenocarcinoma, an entity first described by *WHO* in 2010 [1], is the final stage of serrated-neoplasia pathway. For its diagnosis, besides characteristic morphological features (saw-toothed architecture), the presence of an associated SSL or TSA is needed [20] (Figures 3 and 4).

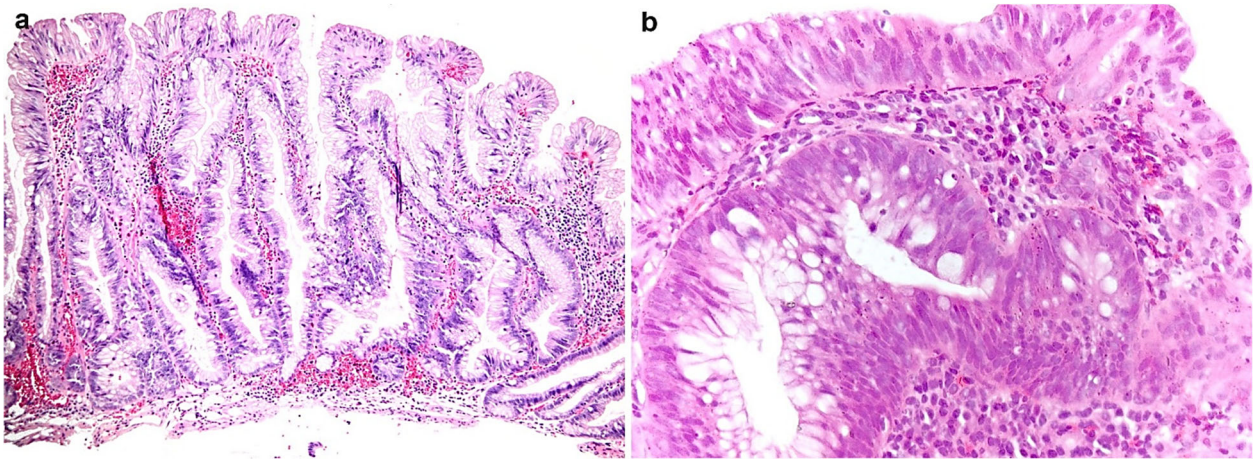

Figure 1 – ***SSLs with LGD. Note the branching crypts with dilated base (a) and the hyperchromatic nuclei with minimal atypia and pseudostratification (b). HE staining: (a) ×100; (b) ×200. HE: Hematoxylin–Eosin; LGD: Low-grade dysplasia; SSLs: Sessile serrated lesions.***

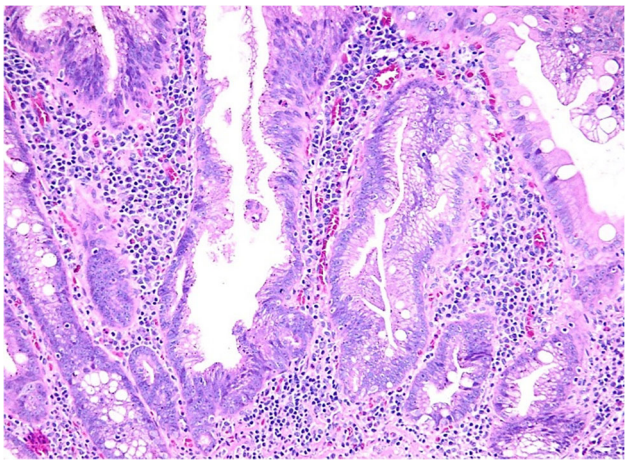

Figure 2 – ***SSL with HGD. Although architectural distortion is not severe, there are significant cellular atypia: large irregular nuclei with visible nucleoli and abnormal nuclear/cytoplasmic ratio. HE staining, ×200. HGD: High-grade dysplasia.***

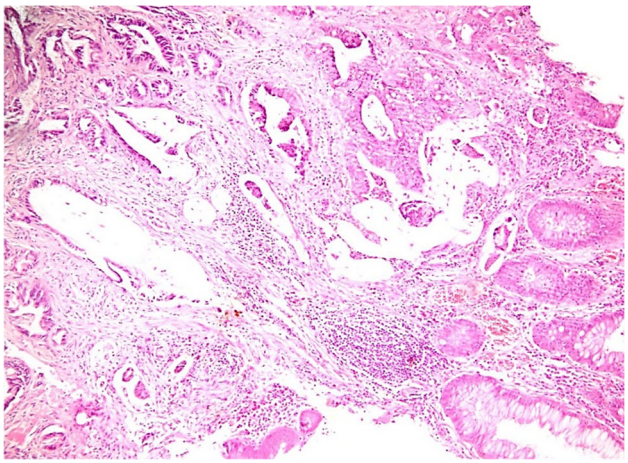

Figure 3 – ***Invasive serrated carcinoma developed in a SSL with dysplasia (LGD and HGD) (left inferior corner). HE staining, ×100.***

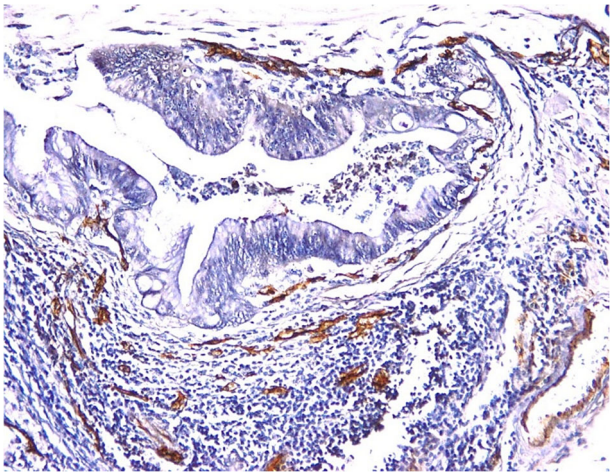

Figure 4 – ***Invasive serrated adenocarcinoma arising from a SSL with dysplasia. Anti-CD34 antibody immunostaining highlighting capillaries in the immediate vicinity of an invasive saw-toothed gland, ×200. CD34: Cluster of differentiation 34.***

Since the classification of colonic serrated lesions had been changed recently and there still are many unanswered questions about the serrated pathway of carcinogenesis [21], there is an important need to report molecular alteration and cellular phenotype in these lesions to have a complete and comprehensive characterization of the serrated pathway to CRC.

Aim

The aim of this study was to evaluate, using immunohistochemistry, MLH1, BRAF and p53 status in SSLs, presenting a comparison between SSLs without dysplasia and SSLs that harbor dysplasia or IC.

Materials and Methods

We designed a retrospective case-control study, including 20 consecutive SSLs with dysplasia (13 low-grade, five high-grade and two high-grade with invasive pT1 carcinoma). For each patient with dysplasia, we included a patient with SSL without dysplasia matching sex and age.

All lesions were colorectal SSLs (diagnosed according to 2019 *WHO* criteria), endoscopically resected and/or biopsied. Tissue samples were immediately immersed in 10% neutral buffered formalin, fixed for 6–24 hours, and then routinely processed using an automatic tissue-processor for paraffin-embedding. From every paraffin block, there were obtained at least four sections from two different levels for routine Hematoxylin–Eosin (HE) staining and supplemental sections for IHC assays (MLH1, BRAF and p53) (Table 1).

Table 1 – ***Immunohistochemistry data***

Primary antibody	Clone	Host	Pretreatment	Dilution
MLH1	E505	Mouse	Citrate	RTU-BOND
BRAF	VE1	Mouse	EDTA	2/200
p53	D07	Mouse	Citrate	1/200

BRAF: v-Raf murine sarcoma viral oncogene homolog B; EDTA: Ethylenediaminetetraacetic acid; MLH1: MutL homolog 1; RTU: Ready-to-use.

Immunostaining of MLH1, BRAF and p53 was done on all 40 cases utilizing constant protocols and timings, on a Leica Bond Max automated immunostainer (Leica Biosystems, IL, USA), and Novocastra diagnostic-certified primary antibodies and detection kits (Leica Biosystems). Basically, after blocking the endogenous peroxidase and the unspecific antigenic sites, the tissue was incubated for one hour with the primary antibodies, then thoroughly washed, and the signal amplified with a species-specific Bond Polymer Refine detection kit. The signal was visualized with 3,3'-Diaminobenzidine (DAB), slides were counter-stained with Hematoxylin, and finally coversliped with a xylene-based medium (Micromount, Leica Biosystems).

Images have then been captured on a Nikon 80i automated microscope, equipped with a 5 MP CCD camera, and the Nikon NIS-Elements software, and allowed us to evaluate the percent of nuclear loss of MLH1, cytoplasmic positivity of BRAF and nuclear positivity of p53. Samples with over 50% of the tumoral nuclei negative for MLH1 were considered as microsatellite instable, the rest were considered microsatellite stable (MSS). Also, there were considered *BRAF*-mutated samples with >50% of the cells positive for BRAF. For p53, we evaluated the percent of nuclear positivity in both dysplastic and non-dysplastic crypts (≥10% – mutated).

Data concerning age, sex, localization of the lesion, dysplasia and IHC results were statistically processed using Microsoft Excel. A Fisher's two-tailed test was performed to evaluate the homogeneity of binary responses grouped as contingency tables, $p<0.05$ was considered statistically significant in all cases.

The study has been approved by the Ethics Committee of Carol Davila University of Medicine and Pharmacy, Bucharest, Romania.

Results

SSLs with low-grade dysplasia (LGD) were predominantly located in the right colon, while those with high-grade dysplasia (HGD) and IC on the left colon and rectum (Figure 5). In the control group (without dysplasia), SSLs were more frequently located in the right colon, and in fact accounted for more than half of the cases for both the right and left colon areas, while clearly representing less than half of the lesions for the rectum and being completely unaccounted for in the cecum and transverse colon.

The median age in the dysplastic group was of 64.45 years (age range between 43 and 78 years), while the HGD +/- IC group showed a median age of 62.46 years (age range between 55 and 78 years) (Figure 6). Although the second group showed a small reduction in the age of the patients, the difference did not attain statistical significance (*t* test, $p=0.1893$).

Patients with LGD were 10 men and three women, while patients with HGD +/- IC were three women and four men, thus the male/female ratio showed a three folds higher preponderance of men for the LGD group (3.333) compared to the HGD +/- IC group (1.333).

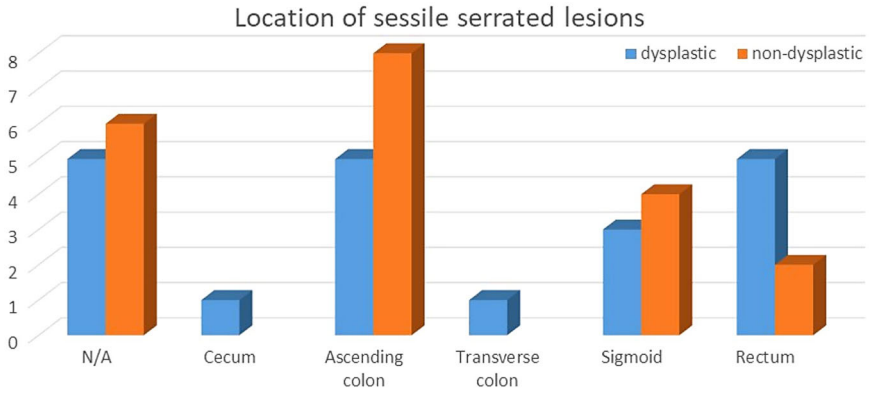

Figure 5 – ***Distribution of SSLs in case-group (dysplastic lesions) and in control group (non-dysplastic lesions). N/A/: Not available.***

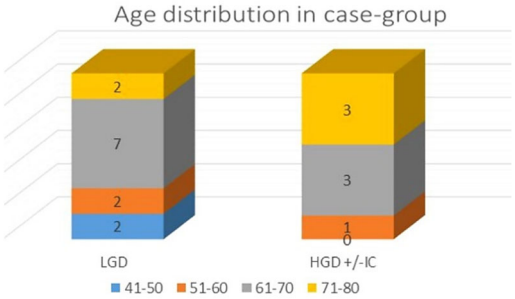

Figure 6 – ***Age distribution of patients with dysplastic lesions. IC: Invasive carcinoma.***

BRAF positivity was found in over 50% of the cells in eight out of 13 cases of LGD and in four out seven cases with HGD +/- IC (Figures 7 and 8). In the control group, only eight out 20 cases had BRAF positivity in over 50% of the cells, and the overall proportions of reactivity differences could not be deemed significantly different between groups (Fisher's two-tailed test, p=0.3431). Although plotting the data revealed a clear inverse proportion for BRAF positive/negative ratio in control *versus* both pathological entities, the relatively small number of cases, especially for the two pathological groups, would explain the fail to attain statistical significance.

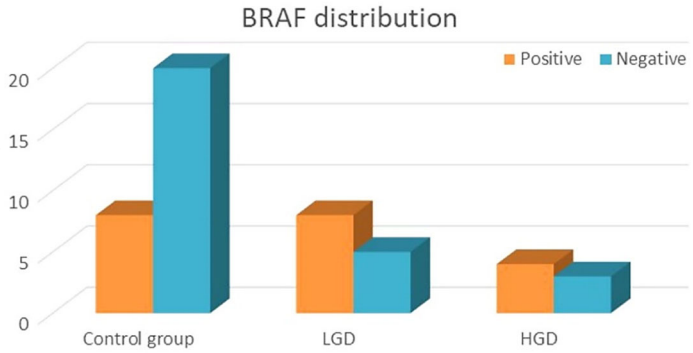

Figure 7 – ***Mutation of BRAF is becoming increasingly frequent as the degree of dysplasia is rising. BRAF: v-Raf murine sarcoma viral oncogene homolog B.***

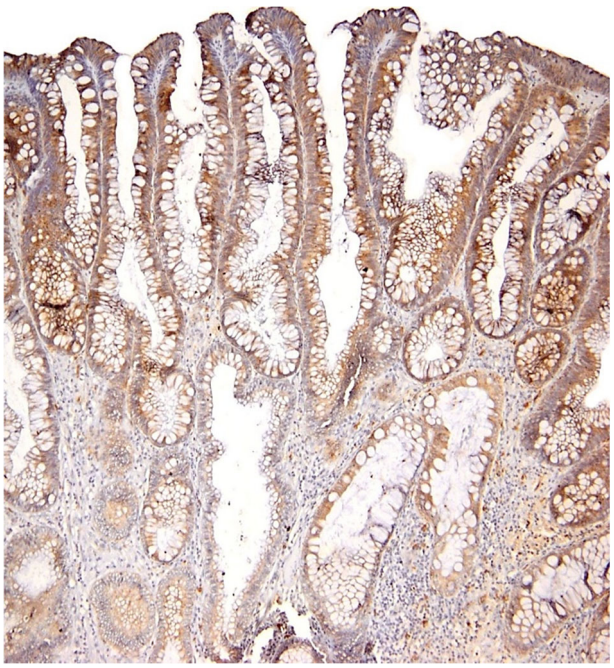

Figure 8 – ***Anti-BRAF antibody immunostaining revealing intense positivity in epithelial cells in a LGD SSL, ×100.***

Only three patients from the control group had MutS homolog 1 (MSH1) loss (MSI) (Figure 9; Figure 10, a and b), while in the case-group 10 patients had loss of MLH1 expression (six out seven cases with HGD +/- IC and four patients with LGD). Thus, there was a clear-cut gradual increase in the lost/preserved ratio from control to LGD and HGD +/- IC groups, and the ratios showed a statistically significant difference between the three groups (Fisher's two-tailed test, p=0.0407).

p53 aberrant expression was noted in three cases without dysplasia and in 12 cases with dysplasia (without any correlation with the severity of dysplasia) (Figures 11 and 12). Since LGD cases showed a much more predominant positive/negative ratio, the overall inter-group differences were highly significant (Fisher's two-tailed test, p=0.0079).

All *BRAF*-mutated SSL without dysplasia were MSS, while in the dysplastic group seven out of 12 *BRAF*-mutated cases were MSS (Figure 13).

Practically, we had a very polymorphous pattern of IHC expression of BRAF, MLH1 and p53, especially in the dysplastic group. Thus, two patients were BRAF+/MSI/p53+ (with LGD), three were BRAF+/MSI/p53- (all with HGD +/- IC), one patient was BRAF+/MSS/p53- (with LGD) and six patients were BRAF+/MSS/p53+ (five with LGD and one with HGD).

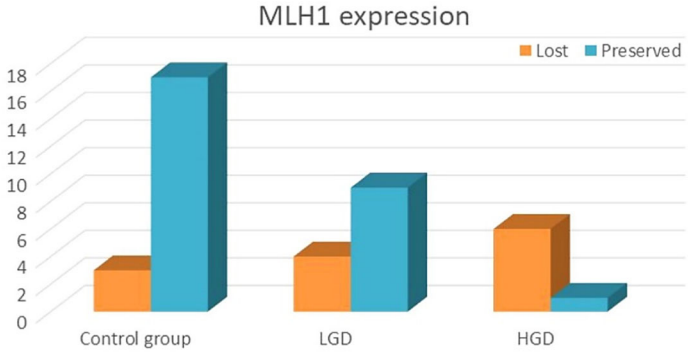

Figure 9 – *MLH1 loss revealed in all SSLs, with a strong tendency to dysplastic lesions. MLH1: MutL homolog 1.*

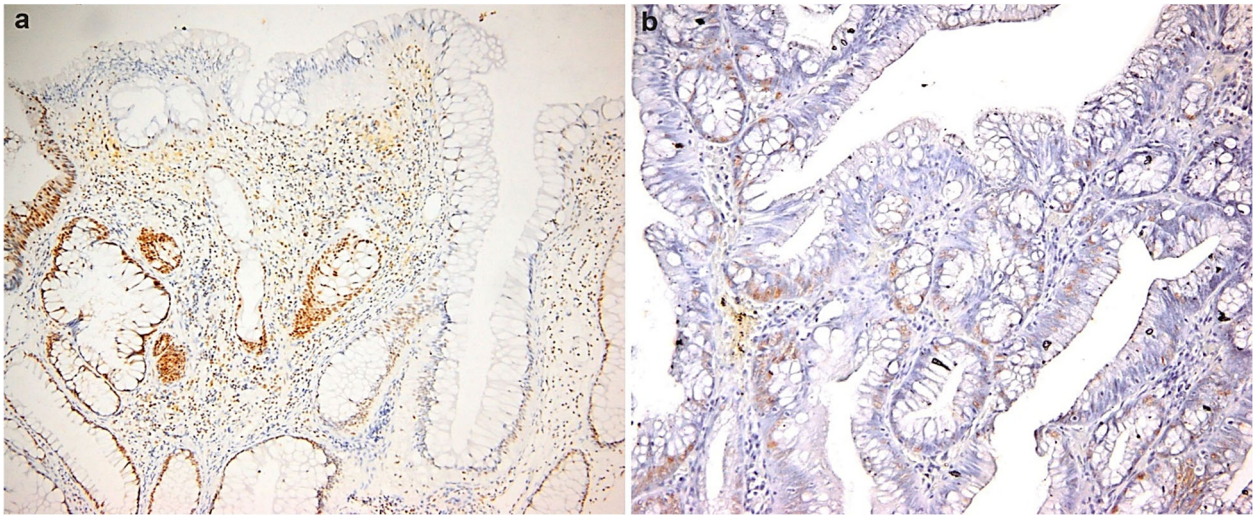

Figure 10 – *MLH1 loss in a non-dysplastic SSL (a) and in a HGD SSL (b). There is a higher percent of negative cells in the dysplastic lesion. Anti-MLH1 antibody immunostaining: (a) ×100; (b) ×200.*

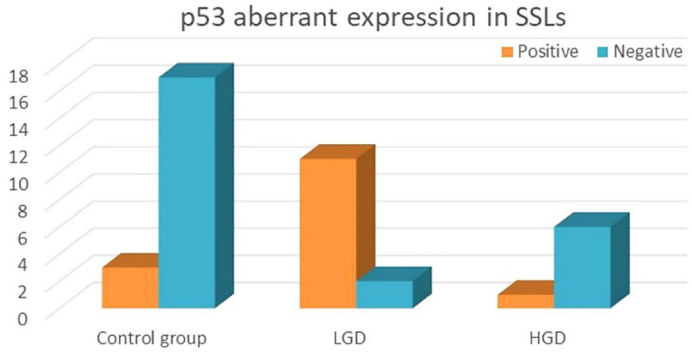

Figure 11 – *Mutation of p53 significantly more frequent in HGD SSLs.*

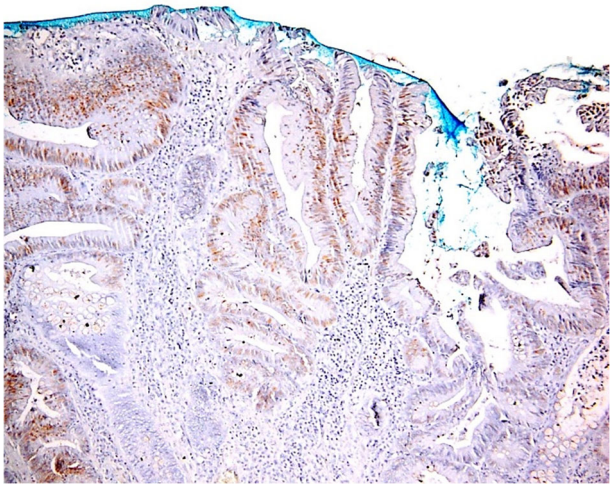

Figure 12 – *p53 aberrant immunoexpression in a SSL with HGD. Anti-p53 antibody immunostaining, ×40.*

Also, dysplastic lesions without *BRAF* mutation exhibited the following phenotype: one case BRAF-/MSI/p53+ (with HGD + IC), four cases BRAF-/MSI/p53- (three with LGD and one with HGD) and three cases BRAF-/MSS/p53+ (two with LGD and one with HGD).

In the control group (SSLs without dysplasia), IHC assays revealed a much more homogenous distribution of cases: eight cases were BRAF+/MSS/p53-, seven cases were BRAF-/MSS/p53-, one case was BRAF-/MSI/p53+, two cases were BRAF-/MSI/p53- and two were BRAF-/MSS/p53+ (Figure 14).

In conclusion, in the non-dysplastic group, all BRAF-mutated lesions were MSS and p53 negative. Also, most BRAF-negative lesions had the same features. Interestingly, patients with dysplasia did not exhibit this pattern of immunostaining (just one case BRAF+/MSS/p53-), probably because they have more mutation in epithelial cells.

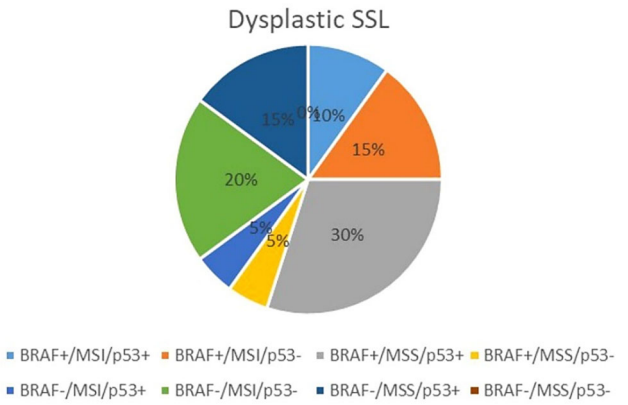

Figure 13 – *High polymorphism of immunophenotype in dysplastic SSLs. MSI: Microsatellite instability; MSS: Microsatellite stable.*

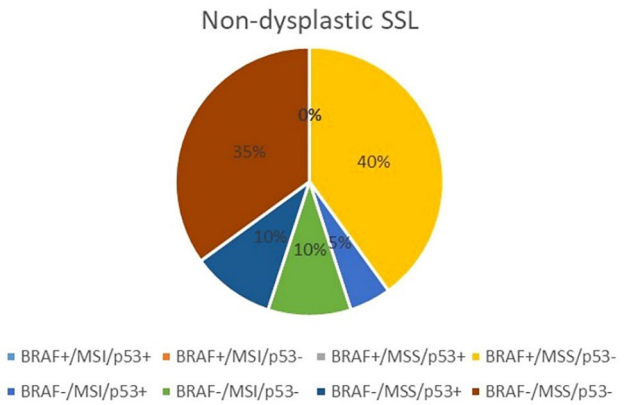

Figure 14 – *Mild polymorphism of immunophenotype in non-dysplastic SSLs.*

Discussions

For gastroenterologists, SSLs, especially dysplastic ones, are considered as "triple jeopardy" precursors for interval colorectal adenocarcinomas (diagnosed in the surveillance period after complete colonoscopy) [22] due to their rapid progression, difficult endoscopically identification and resection and the high incidence of incomplete resection. Nevertheless, pathologists also have their jeopardy to add misdiagnosis, due to the very rapidly changing definitions in this domain. To get a deeper knowledge of how SSLs pathway works, we studied 40 lesions (with and without dysplasia, matched in 20 case-control pairs) and reported their heterogeneous IHC phenotypes. We have chosen p53 because it is used largely to aid the diagnosis of dysplasia in colorectal lesions, and MLH1 and BRAF since they are specifically involved in serrated pathway of carcinogenesis and, also, can be used as prognosis tools in CRC [23].

Our study confirms the preference SSLs have for the right, proximal colon [24], but raised an important problem: lesions in the distal colon and rectum were more advanced. It is very important for endoscopists to know these characteristics, since serrated lesions are frequently small, plat lesions, with Kudo unspecific appearance, difficult to identify and assess endoscopically and usually incompletely resected [25, 26]. There is a continuous search for improving endoscopic diagnosis and management of SSLs with or without dysplasia and emerging data are important steps towards a successful protocol for patients with SSLs [27–29].

Age is not very significant in SSLs, patients are, usually in their 5th or 6th decade of life. In our study, we matched patients according to their age and sex, so there are no differences to analyze between case and control groups. Although, inside the case-group (patients with dysplasia) there is no significant differences between median age of patients with LGD and patients with HGD, confirming the observation that evolution towards invasive neoplasia on the serrated pathway is usually rapid. This is a significant difference of biological behavior comparing to adenomatous pathway, in which evolution from adenoma to IC is slow and patients with high levels of dysplasia are older than patients with LGD lesions [18, 21, 30]. This observation may modify the surveillance schedule for patients with microvesicular HPs or previous diagnosed SSLs.

Males increased prevalence of SSLs is foreseeable since CRC and all its premalignant lesions are more likely found in males [31, 32]. Although a small study, our research confirms this prevalence, in the case-group being included 14 men and six women. Although not statistically significant (*t* test, p=0.4258), median age for men was smaller than median age for women in the case-group (63.36 years *vs* 67 years), confirming the fact that CRC affects younger men [33].

BRAF mutation is a precocious event on the serrated pathway [34], being associated by various studies with an increased aggressiveness and rapid evolution. In our case-group, more than half of the patients had *BRAF*-mutated lesions (12 out of 20). Moreover, BRAF expression was found also in SSLs without dysplasia (eight out of 20), practically similar with the incidence of *BRAF* mutation in LGD group, which raise an alarm about the possibility of rapid progression to carcinoma of although bland-looking, non-alarming lesions. In the previous mentioned context of frequent incomplete resection of SSLs, this observation raises the idea that BRAF immunostaining can be useful for further therapeutical decision in incompletely resected SSLs, with or without dysplasia. A further study for identifying the relationship of *BRAF* and *KRAS* mutations is needed, *KRAS* being an alternative mutation to activate MAPK pathway [14, 35, 36].

MSI by hypermethylation of *MLH1* promoter is a frequent, but somehow later step in serrated carcinogenesis. In our study, there is a significant difference between the case-groups and the control-one. *MLH1* loss is much more frequent in the dysplastic SSLs included, confirming the fact that acquisition of this mutation is the hallmark of visible dysplasia [9, 11]. Still, three cases from the control group exhibited *MLH1* loss indicating that, at least in some cases, *MLH1* loss precedes histologically dysplasia, and some lesions are more evolved than others on the pathway to malignancy [37]. Also, interesting is the observation that half of dysplastic SSLs are, still, MSS (no *MLH1* loss). Kane *et al.* recently described a subgroup of serrated CRCs *BRAF*-mutated and MSS with a dismal prognosis [38]. In our study, seven out of 10 MSS dysplastic SSLs were *BRAF*-mutated. For these lesions, it was described

an alternative pathway of carcinogenesis involving WNT/*β*-catenin signaling path and transforming growth factor-beta (TGF-*β*) upregulation [16, 38, 39].

The most significant difference between the two groups is the presence of p53 mutation, indicating the fact it is a late mutation on the carcinogenesis pathway, p53-mutated lesions being more probable the ones which will rapidly evolve towards invasive and metastatic carcinoma. Some studies observed a strong inverse correlation between *TP53* mutation and MSI phenotype in CRC [40, 41]. In this study, 11 out of 27 lesions with MSS harbored a *TP53* mutation, whereas only four out 13 MSI lesions were *p53* mutated (weak correlation, not statistically significant). There is also a correlation between *p53* and *BRAF* mutation, especially in dysplastic SSLs (eight out 12 *BRAF*-mutated had *p53* mutation), and while in non-dysplasia group only three out of eight *BRAF*-mutated lesions harbored *p53* mutation.

An observation about the control group showed that all *BRAF*-mutated SSLs without dysplasia were MSS. It is interesting to know if the evolving lesions in this group will suffer further mutations or if these lesions are going to evolve towards a *BRAF*-mutated MSS carcinoma, entity that has, usually, as precursor, a TSA [42].

In the end, the most interesting and intriguing observation of our study is the polymorphism of mutations in SSLs. Although this is a small study, including only 40 lesions and evaluating IHC expression of only three mutation-related markers, we had a very interesting distribution of cases. Practically, there are eight possible combinations (four *BRAF*-mutated: BRAF+/MSI/p53+, BRAF+/MSI/p53-, BRAF+/MSS/p53+, BRAF+/MSS/p53- and four BRAF negative: BRAF-/MSI/p53+, BRAF-/MSI/p53-, BRAF-/MSS/p53+, BRAF-/MSS/p53-) and dysplastic SSLs were distributed in seven categories (2; 3; 6; 1; 1; 4; 3; 0). Non-dysplastic SSLs were less polymorphous, being restrained in five categories (0; 0; 0; 8; 1; 2; 2; 7). This observation is interesting for diagnosis, classification, and treatment [42, 43].

Conclusions

Our study raises the idea that there are more routes on the serrated pathway, with different mutations and time of acquisition of each genetic or epigenetic lesion with the same morphological result. Probably these lesions have a different biological behavior and should be stratified according to their risk to poor outcome and their need to further surveillance. Also, some of these mutations are therapeutical targets (*BRAF*, *KRAS*), potentially useful for treatment of malignant serrated lesions. Although there are a lot of recent data in literature about serrated lesions and their evolution towards malignancy, still there are controversies and questions about the real malignant potential of each serrated lesions and the appropriate clinical management of these patients. Emerging data suggest that IHC studies are necessary to obtain surveillance guides based on strong medical proofs.

Conflict of interests

None of the authors have no conflict of interests to disclose.

Ethics approval

Colentina University Hospital's Ethics Committee approved this research. The research complied with acceptable international standards mentioned in the Declaration of Helsinki.

Patient consent

All included patients signed and informed consent, permitting the use of their data to research.

Data availability

All data are available on request.

Authors' contribution

Diana Răduţă & Octavian Marius Dincă are first authors in equal proportion.

References

[1] Bosman FT, Carneiro F, Hruban RH, Theise ND (eds); World Health Organization (WHO) & International Agency for Research on Cancer (IARC). WHO Classification of tumours of the digestive system. 4th edition, vol. 3, IARC Press, Lyon, France, 2010, 160–165. https://publications.iarc.fr/Book-And-Report-Series/Who-Classification-Of-Tumours/WHO-Classification-Of-Tumours-Of-The-Digestive-System-2010

[2] World Health Organization (WHO) Classification of Tumours Editorial Board. WHO Classification of tumours. Digestive system tumours. 5th edition, vol. 1, International Agency for Research on Cancer (IARC) Press, Lyon, France, 2019, 163–169. https://publications.iarc.fr/Book-And-Report-Series/Who-Classification-Of-Tumours/Digestive-System-Tumours-2019

[3] Niv Y. Changing pathological diagnosis from hyperplastic polyp to sessile serrated adenoma: systematic review and meta-analysis. Eur J Gastroenterol Hepatol, 2017, 29(12): 1327–1331. https://doi.org/10.1097/MEG.0000000000000994 PMID: 29049128

[4] Anderson JC, Lisovsky M, Greene MA, Hagen C, Srivastava A. Factors associated with classification of hyperplastic polyps as sessile serrated adenomas/polyps on morphologic review. J Clin Gastroenterol, 2018, 52(6):524–529. https://doi.org/10.1097/MCG.0000000000000840 PMID: 28723863

[5] Cassese G, Amendola A, Maione F, Giglio MC, Pagano G, Milone M, Aprea G, Luglio G, De Palma GD. Serrated lesions of the colon–rectum: a focus on new diagnostic tools and current management. Gastroenterol Res Pract, 2019, 2019: 9179718. https://doi.org/10.1155/2019/9179718 PMID: 30774654 PMCID: PMC6350577

[6] Sweetser S, Smyrk TC, Sugumar A. Serrated polyps: critical precursors to colorectal cancer. Expert Rev Gastroenterol Hepatol, 2011, 5(5):627–635. https://doi.org/10.1586/egh.11.67 PMID: 21910580

[7] Crockett SD, Nagtegaal ID. Terminology, molecular features, epidemiology, and management of serrated colorectal neoplasia. Gastroenterology, 2019, 157(4):949–966.e4. https://doi.org/10.1053/j.gastro.2019.06.041 PMID: 31323292

[8] Hu K, Shen S, Zhang L. Herniation of crypts in hyperplastic polyp and sessile serrated adenoma: a prospective study. Am J Cancer Res, 2018, 8(1):144–153. PMID: 29416927 PMCID: PMC5794728

[9] Liu C, Walker NI, Leggett BA, Whitehall VL, Bettington ML, Rosty C. Sessile serrated adenomas with dysplasia: morphological patterns and correlations with MLH1 immunohistochemistry. Mod Pathol, 2017, 30(12):1728–1738. https://doi.org/10.1038/modpathol.2017.92 PMID: 28752838 PMCID: PMC5719122

[10] Cenaj O, Gibson J, Odze RD. Clinicopathologic and outcome study of sessile serrated adenomas/polyps with serrated *versus* intestinal dysplasia. Mod Pathol, 2018, 31(4):633–642. https://doi.org/10.1038/modpathol.2017.169 PMID: 29271414

[11] Kim JH, Kang GH. Evolving pathologic concepts of serrated lesions of the colorectum. J Pathol Transl Med, 2020, 54(4): 276–289. https://doi.org/10.4132/jptm.2020.04.15 PMID: 32580537 PMCID: PMC7385269

[12] Nguyen LH, Goel A, Chung DC. Pathways of colorectal carcinogenesis. Gastroenterology, 2020, 158(2):291–302. https://doi.

org/10.1053/j.gastro.2019.08.059 PMID: 31622622 PMCID: PMC6981255

[13] Sacco M, De Palma FDE, Guadagno E, Giglio MC, Peltrini R, Marra E, Manfreda A, Amendola A, Cassese G, Dinuzzi VP, Pegoraro F, Tropeano FP, Luglio G, De Palma GD. Serrated lesions of the colon and rectum: emergent epidemiological data and molecular pathways. Open Med (Wars), 2020, 15(1): 1087–1095. https://doi.org/10.1515/med-2020-0226 PMID: 33336065 PMCID: PMC7718641

[14] O'Brien MJ, Yang S, Clebanoff JL, Mulcahy E, Farraye FA, Amorosino M, Swan N. Hyperplastic (serrated) polyps of the colorectum: relationship of CpG island methylator phenotype and *K-ras* mutation to location and histologic subtype. Am J Surg Pathol, 2004, 28(4):423–434. https://doi.org/10.1097/00000478-200404000-00001 PMID: 15087661

[15] Borowsky J, Dumenil T, Bettington M, Pearson SA, Bond C, Fennell L, Liu C, McKeone D, Rosty C, Brown I, Walker N, Leggett B, Whitehall V. The role of *APC* in *WNT* pathway activation in serrated neoplasia. Mod Pathol, 2018, 31(3):495–504. https://doi.org/10.1038/modpathol.2017.150 PMID: 29148535

[16] Hashimoto T, Yamashita S, Yoshida H, Taniguchi H, Ushijima T, Yamada T, Saito Y, Ochiai A, Sekine S, Hiraoka N. *WNT* pathway gene mutations are associated with the presence of dysplasia in colorectal sessile serrated adenoma/polyps. Am J Surg Pathol, 2017, 41(9):1188–1197. https://doi.org/10.1097/PAS.0000000000000877 PMID: 28614199

[17] Murakami T, Akazawa Y, Yatagai N, Hiromoto T, Sasahara N, Saito T, Sakamoto N, Nagahara A, Yao T. Molecular characterization of sessile serrated adenoma/polyps with dysplasia/carcinoma based on immunohistochemistry, next-generation sequencing, and microsatellite instability testing: a case series study. Diagn Pathol, 2018, 13(1):88. https://doi.org/10.1186/s13000-018-0771-3 PMID: 30458818 PMCID: PMC6247685

[18] De Palma FDE, D'Argenio V, Pol J, Kroemer G, Maiuri MC, Salvatore F. The molecular hallmarks of the serrated pathway in colorectal cancer. Cancers (Basel), 2019, 11(7):1017. https://doi.org/10.3390/cancers11071017 PMID: 31330830 PMCID: PMC6678087

[19] Ogino S, Cantor M, Kawasaki T, Brahmandam M, Kirkner GJ, Weisenberger DJ, Campan M, Laird PW, Loda M, Fuchs CS. CpG island methylator phenotype (CIMP) of colorectal cancer is best characterised by quantitative DNA methylation analysis and prospective cohort studies. Gut, 2006, 55(7):1000–1006. https://doi.org/10.1136/gut.2005.082933 PMID: 16407376 PMCID: PMC1856352

[20] Pai RK, Bettington M, Srivastava A, Rosty C. An update on the morphology and molecular pathology of serrated colorectal polyps and associated carcinomas. Mod Pathol, 2019, 32(10): 1390–1415. https://doi.org/10.1038/s41379-019-0280-2 PMID: 31028362

[21] Nakanishi Y, Diaz-Meco MT, Moscat J. Serrated colorectal cancer: the road less travelled? Trends Cancer, 2019, 5(11): 742–754. https://doi.org/10.1016/j.trecan.2019.09.004 PMID: 31735291 PMCID: PMC6894428

[22] Burgess NG, Tutticci NJ, Pellise M, Bourke MJ. Sessile serrated adenomas/polyps with cytologic dysplasia: a triple threat for interval cancer. Gastrointest Endosc, 2014, 80(2):307–310. https://doi.org/10.1016/j.gie.2014.03.050 PMID: 24890425

[23] Lochhead P, Kuchiba A, Imamura Y, Liao X, Yamauchi M, Nishihara R, Qian ZR, Morikawa T, Shen J, Meyerhardt JA, Fuchs CS, Ogino S. Microsatellite instability and *BRAF* mutation testing in colorectal cancer prognostication. J Natl Cancer Inst, 2013, 105(15):1151–1156. https://doi.org/10.1093/jnci/djt173 PMID: 23878352 PMCID: PMC3735463

[24] Sano W, Hirata D, Teramoto A, Iwatate M, Hattori S, Fujita M, Sano Y. Serrated polyps of the colon and rectum: remove or not? World J Gastroenterol, 2020, 26(19):2276–2285. https://doi.org/10.3748/wjg.v26.i19.2276 PMID: 32476792 PMCID: PMC7243646

[25] Murakami T, Sakamoto N, Ritsuno H, Shibuya T, Osada T, Mitomi H, Yao T, Watanabe S. Distinct endoscopic characteristics of sessile serrated adenoma/polyp with and without dysplasia/carcinoma. Gastrointest Endosc, 2017, 85(3):590–600. https://doi.org/10.1016/j.gie.2016.09.018 PMID: 27663716

[26] Tate DJ, Jayanna M, Awadie H, Desomer L, Lee R, Heitman SJ, Sidhu M, Goodrick K, Burgess NG, Mahajan H, McLeod D, Bourke MJ. A standardized imaging protocol for the endoscopic prediction of dysplasia within sessile serrated polyps (with video). Gastrointest Endosc, 2018, 87(1):222–231.e2. https://doi.org/10.1016/j.gie.2017.06.031 PMID: 28713060

[27] McCarthy AJ, O'Reilly SM, Shanley J, Geraghty R, Ryan EJ, Cullen G, Sheahan K. Colorectal serrated neoplasia: an institutional 12-year review highlights the impact of a screening programme. Gastroenterol Res Pract, 2019, 2019:1592306. https://doi.org/10.1155/2019/1592306 PMID: 30881445 PMCID: PMC6381559

[28] Burgess NG, Pellise M, Nanda KS, Hourigan LF, Zanati SA, Brown GJ, Singh R, Williams SJ, Raftopoulos SC, Ormonde D, Moss A, Byth K, P'Ng H, McLeod D, Bourke MJ. Clinical and endoscopic predictors of cytological dysplasia or cancer in a prospective multicentre study of large sessile serrated adenomas/polyps. Gut, 2016, 65(3):437–446. https://doi.org/10.1136/gutjnl-2014-308603 PMID: 25731869

[29] Sano W, Fujimori T, Ichikawa K, Sunakawa H, Utsumi T, Iwatate M, Hasuike N, Hattori S, Kosaka H, Sano Y. Clinical and endoscopic evaluations of sessile serrated adenoma/polyps with cytological dysplasia. J Gastroenterol Hepatol, 2018, 33(8):1454–1460. https://doi.org/10.1111/jgh.14099 PMID: 29377243

[30] Chen CD, Yen MF, Wang WM, Wong JM, Chen THH. A case-cohort study for the disease natural history of adenoma–carcinoma and *de novo* carcinoma and surveillance of colon and rectum after polypectomy: implication for efficacy of colonoscopy. Br J Cancer, 2003, 88(12):1866–1873. https://doi.org/10.1038/sj.bjc.6601007 PMID: 12799628 PMCID: PMC2741116

[31] Chavali LB, Hu K, Sheth A, Gao N, Xiong W, Zhang L. Colorectal sessile serrated lesion with large size or synchronous neoplasm: a prospective study. Eur J Gastroenterol Hepatol, 2020, 32(2):199–204. https://doi.org/10.1097/MEG.0000000000001642 PMID: 31851088 PMCID: PMC7273847

[32] Sandouk F, Al Jerf F, Al-Halabi MHDB. Precancerous lesions in colorectal cancer. Gastroenterol Res Pract, 2013, 2013: 457901. https://doi.org/10.1155/2013/457901 PMID: 23737765 PMCID: PMC3666221

[33] White A, Ironmonger L, Steele RJC, Ormiston-Smith N, Crawford C, Seims A. A review of sex-related differences in colorectal cancer incidence, screening uptake, routes to diagnosis, cancer stage and survival in the UK. BMC Cancer, 2018, 18(1):906. https://doi.org/10.1186/s12885-018-4786-7 PMID: 30236083 PMCID: PMC6149054

[34] Fanelli GN, Dal Pozzo CA, Depetris I, Schirripa M, Brignola S, Biason P, Balistreri M, Dal Santo L, Lonardi S, Munari G, Loupakis F, Fassan M. The heterogeneous clinical and pathological landscapes of metastatic *Braf*-mutated colorectal cancer. Cancer Cell Int, 2020, 20:30. https://doi.org/10.1186/s12935-020-1117-2 PMID: 32015690 PMCID: PMC6990491

[35] Clarke CN, Kopetz ES. *BRAF* mutant colorectal cancer as a distinct subset of colorectal cancer: clinical characteristics, clinical behavior, and response to targeted therapies. J Gastrointest Oncol, 2015, 6(6):660–667. https://doi.org/10.3978/j.issn.2078-6891.2015.077 PMID: 26697199 PMCID: PMC4671844

[36] Phipps AI, Alwers E, Harrison T, Banbury B, Brenner H, Campbell PT, Chang-Claude J, Buchanan D, Chan AT, Farris AB, Figueiredo JC, Gallinger S, Giles GG, Jenkins M, Milne RL, Newcomb PA, Slattery ML, Song M, Ogino S, Zaidi SH, Hoffmeister M, Peters U. Association between molecular subtypes of colorectal tumors and patient survival, based on pooled analysis of 7 international studies. Gastroenterology, 2020, 158(8):2158–2168.e4. https://doi.org/10.1053/j.gastro.2020.02.029 PMID: 32088204 PMCID: PMC7282955

[37] Yozu M, Kem M, Cenaj O, Mino-Kenudson M, Odze RD, Misdraji J. Loss of expression of *MLH1* in non-dysplastic crypts is a harbinger of neoplastic progression in sessile serrated adenomas/polyps. Histopathology, 2019, 75(3):376–384. https://doi.org/10.1111/his.13874 PMID: 30974487

[38] Kane AM, Fennell LJ, Liu C, Borowsky J, McKeone DM, Bond CE, Kazakoff S, Patch AM, Koufariotis LT, Pearson J, Waddell N, Leggett BA, Whitehall VLJ. Alterations in signaling pathways that accompany spontaneous transition to malignancy in a mouse model of *BRAF* mutant microsatellite stable colorectal cancer. Neoplasia, 2020, 22(2):120–128. https://doi.org/10.1016/j.neo.2019.12.002 PMID: 31935636 PMCID: PMC6961716

[39] Yachida S, Mudali S, Martin SA, Montgomery EA, Iacobuzio-Donahue CA. Beta-catenin nuclear labeling is a common feature of sessile serrated adenomas and correlates with early neoplastic progression after *BRAF* activation. Am J Surg Pathol, 2009, 33(12):1823–1832. https://doi.org/10.1097/PAS.0b013e3181b6da19 PMID: 19745699 PMCID: PMC2788075
[40] Ionov Y, Peinado MA, Malkhosyan S, Shibata D, Perucho M. Ubiquitous somatic mutations in simple repeated sequences reveal a new mechanism for colonic carcinogenesis. Nature, 1993, 363(6429):558–561. https://doi.org/10.1038/363558a0 PMID: 8505985
[41] Cottu PH, Muzeau F, Estreicher A, Fléjou JF, Iggo R, Thomas G, Hamelin R. Inverse correlation between RER+ status and *p53* mutation in colorectal cancer cell lines. Oncogene, 1996, 13(12):2727–2730. PMID: 9000147
[42] Satorres C, García-Campos M, Bustamante-Balén M. Molecular features of the serrated pathway to colorectal cancer: current knowledge and future directions. Gut Liver, 2021, 15(1):31–43. https://doi.org/10.5009/gnl19402 PMID: 32340435 PMCID: PMC7817929
[43] Kedrin D, Gala MK. Genetics of the serrated pathway to colorectal cancer. Clin Transl Gastroenterol, 2015, 6(4):e84. https://doi.org/10.1038/ctg.2015.12 PMID: 25856207 PMCID: PMC4459531

Corresponding author
Cristiana Gabriela Popp, MD, Department of Pathology, Colentina University Hospital, 19–21 Ştefan cel Mare Highroad, Sector 2, 020125 Bucharest, Romania; Phone +4021–317 32 45, Fax +4021–316 55 12, e-mail: brigaela@yahoo.com

Received: February 8, 2022

Accepted: May 11, 2022