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Morphological, immunohistochemical and molecular features of inflammatory bowel disease associated colorectal carcinoma and associated mucosal lesions – Single institution experience

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ABSTRACT

Background: Patients with inflammatory bowel disease (IBD) – ulcerative colitis (UC) and Crohn's disease (CD) have an elevated risk of developing colorectal carcinoma (CRC). Major risk factor in IBD patients is the continuous chronic inflammation leading to development of dysplasia and carcinoma. Nevertheless, other types of non-conventional but suspicious mucosal changes serrated change/dysplasia, NOS and villous hypermucinous change, have also been reported in IBD patients. Preneoplastic potential of these lesions is still not well elucidated.

Aims: The aim of this study was identification of IBD-associated CRCs focusing on finding related precursor lesions in the surgical specimen or in archival biopsy samples followed by a detailed morphological, immunohistochemical and molecular evaluation. For the purpose of the study the mucosal lesions were divided into conventional IBD-associated dysplasia and non-conventional lesions that were merged under a provisory term of putative preneoplastic lesions (PPL).

Methods: A total of 309 consecutive IBD colectomy specimens diagnosed during a 10-year period were reviewed. Detailed morphological evaluation, immunohistochemical analysis of mismatch repair (MMR) proteins, p53 and O^6-methylguanine DNA methyltransferase (MGMT) expression and molecular analysis for *KRAS*, *NRAS* and *BRAF* gene mutation were performed in the retrieved CRC cases as well as in the detected dysplasia and PPLs of these patients.

Results: We identified 11 cases of morphologically heterogenous IBD-associated CRCs, occurring in 5 males and 6 females, aged 26–79 years (mean 44 years). A total of 22 mucosal lesions were revealed in 8 CRC patients comprising conventional IBD-associated dysplasia (4 lesions), PPLs as serrated change/dysplasia NOS (11 lesions), villous hypermucinous change (5 lesions), and two true serrated lesions (one sessile serrated adenoma and one traditional serrated adenoma). More than one type of lesion was found in 6 patients. Seven CRC cases harbored mutation of *KRAS/NRAS* and one case of *BRAF*. Two patients with *KRAS*-mutated CRC showed the same mutation in PPL in the same specimen (one serrated change NOS and one TSA with high-grade dysplasia). Similarly, one *BRAF*-mutated carcinoma case presented the same mutation in serrated change/dysplasia, NOS in the same specimen. Of the CRCs, two showed deficient MMR system profile, six presented with loss of MGMT expression, and six showed aberrant p53 expression. PPLs showed deficient MGMT expression (14 cases) and aberrant p53 (10 cases) as well.

Conclusion: IBD-associated CRCs are very heterogeneous entities. Besides conventional IBD-related dysplasia, other types of mucosal lesions may be associated with long lasting IBD and CRC e.g. villous hypermucinous change and serrated change/dysplasia, NOS. Since these lesions share certain genetic or immunohistochemical changes with the related CRC, a suspicion is raised that these lesions may also have preneoplastic potential. Awareness of these changes is necessary to prevent their missing and under-reporting, and further studies of these lesions should be carried out.

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1. Introduction

Patients with inflammatory bowel disease (IBD) – ulcerative colitis (UC) and Crohn's disease (CD) have an increased risk of developing colorectal carcinoma (CRC). The risk increases with the duration of the disease and with anatomical extent of the disease [1,2]. Carcinoma development is principally a result of a pro-neoplastic effect of chronic intestinal inflammation leading to dysplastic changes in the epithelium. Traditionally, it is classified as colitis- or IBD-associated dysplasia (intraepithelial neoplasia) and fulfilling yet established histological criteria of conventional dysplasia and divided into three categories – negative for dysplasia, indefinite for dysplasia and positive for dysplasia (low-grade and high-grade) [3]. Recently, new approach has been adopted by the gastroenterologists dividing mucosal lesion into endoscopically visible (polypoid/elevated or non-polypoid/flat/sessile/depressed) and invisible. Invisible lesions are diagnosed by a pathologist microscopically in otherwise endoscopically unsuspicious mucosa. Type of lesion and grade of dysplasia then influences surveillance or treatment options according to the valid guidelines [4–7]. Probably due to improving detection of the IBD-associated dysplasia and surveillance programs the annual incidence of adenocarcinoma in IBD patients is decreasing [8–10]. Nevertheless, there are still patients who develop adenocarcinoma in the setting of IBD without previous signs of conventional IBD-related dysplasia. Certain mucosal lesions that can represent a potentially pre-neoplastic change for CRC have been currently reported in several studies under variable terms including villous hypermucinous change [11], hyperplastic-like change [12] or serrated (epithelial) change, NOS and serrated dysplasia [12–17]. These lesions in general does not fulfil histological criteria of established diagnostic entities as hyperplastic polyp (HP), sessile serrated adenoma/polyp (SSA/P) or traditional serrated adenoma, however, these true serrated lesions are described in IBD-patients as well suggesting the possibility of serrated pathway involvement in IBD-associated CRC development [14–16].

The aim of our study was retrieval of cases of IBD-related CRC from our institution archives and apart from reviewing their morphology and prognostic factors the main focus was put on finding any possible precursor lesions in direct association with the tumor in surrounding mucosa as well as in additional samples from surveillance endoscopy.

2. Materials and methods

2.1. Clinicopathological data

A systematic review of the surgical pathology files at The Fingerland Department of Pathology (University Hospital Hradec Králové, Czech Republic) revealed a total of 309 IBD colectomy specimens diagnosed between January 2006 and December 2016. The in-house index system combining organ specific location and diagnosis including combinations was used to retrieve the files. Diagnosis of IBD was established either in previous endoscopical biopsies or from the colectomy specimen itself based on valid histopathological criteria and clinical correlation. For the purpose of this study, only cases with IBD-associated CRC were selected. In every patient, gender, age at the time of CRC diagnosis, type (ulcerative colitis vs. Crohn´s disease) and duration of IBD, tumor localization, grade and pathological TNM (according to AJCC 7th edition TNM staging) were retrieved from the request form and clinical information system and recorded [18]. Morphological characteristics of CRC together with histological prognostic factors including invasion to lymphatic and blood vessels, perineural invasion, type of tumor border configuration and tumor budding were documented as well. Tumor budding was evaluated according to the recommendations of International Tumor Budding Consensus Conference (ITBCC), except in one patient (case 11) who underwent neoadjuvant chemoradiotherapy [19]. The number of sampled and available blocks for each CRC specimen was recorded. All biopsies from IBD-associated CRC patients' history were also reviewed and searched for precursor dysplastic lesions or other mucosal changes, with particular focus on serrated change/dysplasia, NOS, and villous hypermucinous change. Presence of IBD-associated dysplasia and other mucosal changes was evaluated separately depending on the location of the lesion either in adjacency to the CRC, usually in the same slide as cancer, or distant from the CRC in sections of non-neoplastic mucosa or in other archival samples and biopsies. Paraffin blocks for further analyses were available in all cases. Samples containing carcinoma and other mucosal lesions were submitted for immunohistochemical and molecular analysis.

2.2. Immunohistochemical staining

Immunohistochemical staining of MLH1, PMS2, MSH2, MSH6, p53, and MGMT was performed and evaluated (Table 1). Immunohistochemistry was performed from a representative formalin fixed paraffin embedded (FFPE) tissue blocks. For immunohistochemical staining, 2 µm sections were deparaffinized and rehydrated. After antigen retrieval, the sections were incubated with primary antibody (MLH1, PMS2, MSH2, MSH6, and p53) using automated staining system (BenchMark Ultra, Ventana Medical Systems, Tuscon, AZ, USA). The detection of MGMT was processed manually-antigen retrieval was performed using the target retrieval buffer at pH 9.0 (buffer K8007; DAKO Denmark, Glostrup, Denmark) in a water bath for 40 min at 97 °C. Endogenous peroxidase activity was inhibited by immersing the sections in Envision FLEX peroxidase-blocking reagent (RTU, K8010, DAKO-Denmark, Glostrup, Denmark). The slides were incubated with primary antibody (MGMT, dilution 1:50, 32 min). Finally, the sections were incubated with EnVision FLEX/HRP (RTU, Dako Denmark, Glostrup, Denmark) and the reaction was visualized using EnVision FLEX DAB (diaminobenzidine, Dako-Denmark, Glostrup, Denmark). The slides were counterstained with hematoxylin.

Nuclear expression of all 4 MMR markers (MLH1, PMS2, MSH2, MSH6) was considered an MMR proficiency. Loss of any of the 4 MMR markers in the nuclei of the neoplastic cells was considered as MMR deficiency. MGMT nuclear expression was evaluated and scored for the intensity of staining (0 – no staining, 1 – weak staining, 2 – moderate staining, 3 – strong staining). MGMT expression was evaluated as

Table 1
List of antibodies and suppliers.

Primary antibody	Supplier	Species/type	Dilution	Refference/Clone
MLH1	Zytomed	mouse/monoclonal	ND	G168-15
PMS2	Roche/Ventana	rabbit/monoclonal	ND	EPR 3947
MSH2	Cell Marque	mouse/monoclonal	1:100	G219-1129
MSH6	Cell Marque	rabbit/monoclonal	1:100	SP93
p53	Roche/Ventana	mouse/monoclonal	ND	Bp53-11
MGMT	ThermoFischer	mouse/monoclonal	1:50	MT3.1

Abbreviations: ND - not diluted.

present (score 2 or 3) or lost (0 or 1) as reported in the study of Svrcek et al. [20]. Loss of MGMT expression was assigned if at least 50% of neoplastic cells were scored 0/1. The p53 expression was interpreted as normal (corresponding to the wild-type status of the gene) when weak or moderate unevenly distributed positivity was present vs. mutation-type when strong diffuse positivity or complete loss of staining was seen. All staining results were compared with external control tissue on slide or with internal control staining pattern in surrounding tissues.

2.3. Molecular genetic analysis

All samples with CRC as well as with precursor lesions were tested for *KRAS*, *NRAS* and *BRAF* mutational status. For molecular testing, 5 µm thick tissue sections from FFPE blocks were prepared. DNA was isolated using MagCore Genomic DNA FFPE One-Step Isolation Kit (Rbc Bioscience, New Taipei, Taiwan) and automated nucleic acid extractor MagCore HF 16 according to the manufacturer's manual. The amount of extracted DNA was measured with dsDNA Broad Range Assay Kit (Invitrogen Qubit, ThermoFisher Scientific, Waltham, MA, USA).

For *KRAS* and *NRAS* mutation detection, AmoyDx® KRAS and NRAS Mutation Detection Kit (Amoy Diagnostics) was used and DNA samples were then analyzed with real-time PCR system Cobas Z 480 (Cobas/Roche Molecular Diagnostics). Using these kits, we were able to examine KRAS exon 2 (G12D, G12 A, G12 V, G12S, G12R, G12C, G13D, G13C), exon 3 (A59 T, Q61 K, Q61 L, Q61R, Q61 H) and exon 4 (K177 N, K177 A, A146 T, A146 V and A146 P) mutations and NRAS exon 2 (G12D, G12S, G12C, G12 V, G12 A, G13D, G13R, G13 V), exon 3 (A59D, Q61R, Q61 K, Q61 L, Q61 H) and exon 4 (K117 N(G > C/T, A146 T) mutations. Analytical sensitivity of the RAS tests is the detection of 2% mutant alleles on the wild-type background.

For *BRAF* mutation detection, *BRAF* StripAssay (ViennaLab Diagnostics, Vienna, Austria) PCR amplification method with reverse hybridization of the product was used. Results for hybridization on a strip was evaluated by naked eye according to the manufacturer's protocol. This test covers *BRAF* V600 A, V600D, V600E (c.1799 T > A), V600E (c.1799_1800TG > AA), V600 G, V600 K, V600 M, V600R and K601E mutations. Analytical sensitivity of the test is the detection of 1% mutant alleles on the wild-type background.

3. Results

3.1. Patient characteristics

We have identified 11 cases of IBD-associated CRC in a cohort of 309 IBD patients with surgical specimen available, diagnosed during a 10-year period. The patients were 6 females and 5 males, aged 26–79 years (median 42 years; mean 45 ± 15 years). The youngest patient with carcinoma was 26 years old and 6 other patients were younger than 45 years.

Ulcerative colitis was diagnosed in 8 patients and Crohn's disease in 3 patients. Mean age of patients at the time of the IBD diagnosis was 33 ± 16 years (range 7–79 years, median 24 years). There was a total of 4 patients diagnosed in the pediatric age group (below 18 years of age) and a total of 7 patients diagnosed with IBD below the age 40 years (Montreal classification A2).

3.1.1. IBD-associated carcinoma characteristics – time to diagnosis, location and staging

Duration of the IBD preceding development of CRC ranged 0–31 years (median 15 years; mean 15 ± 10 years). The carcinoma developed over a long period of time following the diagnosis of IBD in most patients. In two patients (cases 4 and 11) the diagnosis of IBD and carcinoma was concurrent and in two patients (cases 1 and 10) IBD was diagnosed only 10 and 13 months before the carcinoma occurred. The data are summarized in Table 2.

Most of the tumors (7 out of 11) were localized in the left colon and rectum, followed by transverse colon, caecum and ascending colon. Regarding IBD type, in 5 UC patients the carcinoma affected recto-sigmoid area and in one each caecum, transverse colon, and ascending colon, respectively. One CD-related CRC was localized in transverse colon and two cases occurred in splenic flexure. Regarding the clinical staging, 2 cases were stage I, 4 cases stage II, and 5 cases stage III. Four cases showed extensively infiltrative disease pT4 and 5 CRCs developed lymph node metastases.

3.1.2. Morphological characteristics of the IBD-associated carcinomas (main histomorphological characteristics are summarized in Table 3)

While most of the cases were adenocarcinomas, NOS, there was a significant intratumoral heterogeneity in individual cases. Tumor grade according the WHO histological criteria could be evaluated in ten out of 11 cases [21]. One case could not be graded due to previous neoadjuvant chemoradiotherapy (case 11) and unavailable pretreatment endoscopical biopsy sample. Most of the carcinomas were grade 2 (6 out of 10) with variable proportion of tubular and cribriform growth pattern. There were two well differentiated CRC and two high-grade (grade 3) CRC cases. Two grade 1 carcinomas (case 1 and 5) shared certain morphological patterns both being composed of well differentiated glands with cuboid or cylindric cells with pale cytoplasm reminding of gastric-like foveolar appearance. Gastric differentiation was proved by at least partial immunohistochemical expression of MUC5AC and MUC6 in neoplastic cells.[1] Mucinous carcinoma was present in 3 cases as a minor portion (20–30%), and in one case as a major component (over 60%). One case (case 3) showed poorly cohesive/signet ring cell carcinoma component, combined with mucinous and cribriform pattern with comedo-type necrosis.

Eight CRCs showed invasion into lymphatic vessels, all presenting with the extramural type of invasion and four of them also with intramural lymphatic invasion. Blood vessel invasion was seen in three cases. There were three carcinomas (cases 6, 8 and 9) displaying perineural invasion. All of them showed very prominent infiltration of the myenteric plexus giving the tumor a horizontal spread pattern (Fig. 1).

Eight tumors featured no or low level of tumor budding (Bd1), even when showing an infiltrative type of tumor border configuration. Two cases were classified in intermediate Bd2 and high Bd3 category, respectively (Fig. 2).

3.2. Characteristics of IBD-associated dysplasia and putative preneoplastic lesions (PPL)

Overall, 22 mucosal lesions were found in 8 CRC patients. For the purpose of this study only lesions from CRC patients are described and evaluated. We have found PPLs and IBD-associated dysplasia in non-CRC cases as well, but these results are going to be evaluated and published separately.

Epithelial mucosal changes were noted both in the same slide in mucosa directly adjacent to the adenocarcinoma as well as in areas distant from the CRC sampled primarily for non-neoplastic mucosa assessment. There were 4 cases of CRC with severe ulceration and the adjacent mucosa could not be evaluated for precursor lesions.

Precursor **IBD-associated dysplasia** of conventional type meeting the definition criteria of unequivocal neoplastic alteration of the epithelium [3,16] was found in four cases. Flat dysplasia was more common (3 cases) than elevated dysplasia (1 case) (Fig. 3).

Regarding PPL lesions, these were found directly adjacent to the CRC as well as at the distant mucosa. Most common of these lesions was

[1] Additional immunohistochemical testing of MUC5AC and MUC6 was performed upon request of one of the reviewers. Primary antibody MUC5AC (clone MRQ-19, Ventana, Tuscon, AZ, USA) and MUC6 (clone CLH5, Novocastra, Leica Biosystems, Newcastle upon Tyne, UK) were used. Since these stainings were not part of the study evaluation, they are not included in the methods section.

Table 2
Age of patients at IBD and CRC diagnosis and duration of the IBD.

Age		Mean	Median
at the IBD diagnosis	7–79 years	33 (± 16) years	24 years
at the CRC diagnosis	26–79 years	45 (± 15) years	42 years
Duration of the IBD to the diagnosis of CRC	0–31 years	13 (± 10) years	15 years

Abbreviations: CRC - colorectal carcinoma; IBD - inflammatory bowel disease.

serrated epithelial change/dysplasia, NOS characterized by predominantly superficial mucosal serrations in otherwise flat or slightly elevated mucosa. There was variable loss of mucin in goblet cells, cytoplasmic eosinophilia and bland or mildly enlarged nuclei (Fig. 4). Unfortunately, criteria for dysplasia evaluation in these lesions are missing and thus we have merged all lesions under the descriptive term of serrated change/dysplasia, NOS. Serrated change/dysplasia, NOS was found three times in the mucosa directly neighboring the CRC with direct transition to invasive carcinoma (cases 6, 8 and 9) (Fig. 5). Other 7 lesions were found at the distant mucosa of the same specimen and one lesion in additional endoscopical biopsy.

The second most common PPL lesion was **villous hypermucinous change** with formation of rather sessile and slender villous mucosal projections with abundance of intracellular mucin in enterocytes with variably sized vacuoles giving the lesion a frothy and very pale appearance Nuclei were small, round, commonly vesicular, basally located and without prominent mitotic activity. This lesion was found in 5 samples. In 3 CRC cases it was found in adjacent mucosa with direct transition to invasive carcinoma (case 1, 5 and 7) (Fig. 6). Two other lesions were found in mucosa distant from CRC.

There were also two **true serrated lesions/polyps** present in the chronically inflamed mucosa; sessile serrated adenoma (SSA) in case 6 and traditional serrated adenoma (TSA) with high-grade dysplasia in case 11. Both of these lesions were distinguished from PPLs based on histology fulfilling current diagnostic criteria for SSA and TSA respectively. Both of the lesions were sharing the same *KRAS* mutation with the carcinoma.

More than one type of mucosal lesion was found in 6 patients combining IBD-associated dysplasia with serrated change/dysplasia (4 cases) or with serrated and villous hypermucinous change (2 cases).

3.3. Immunohistochemical staining – MMR status, p53 and MGMT expression in carcinoma, IBD-associated dysplasia, and PPLs

Immunohistochemical staining for DNA mismatch repair proteins showed 2 CRCs with defective **MMR status**. One patient (case 1) presented with isolated loss of MSH6 in CRC as well as in villous hypermucinous change adjacent to the carcinoma. One patient (case 2) showed concurrent loss of MLH1 and PMS2 staining in CRC. Ten PPL samples, 9 with serrated dysplasia/change, NOS and one with villous hypermucinous change showed downregulation of MLH1 expression in the superficial serrated area of the lesion, while in the basal cells of the crypts the staining was retained.

There were 6 CRCs with either complete loss (cases 3, 4) or overexpression of **p53** (cases 7, 8, 9, 11) and 5 cases with normal expression pattern. Out of all 22 precursor lesions a total of 11 samples showed an abnormal expression of p53 including IBD-associated dysplasia (3 out of 4). Two cases of serrated change/dysplasia, NOS shared the aberrant expression of p53 with adjacent carcinoma.

There were six CRC cases (cases 1, 2, 5, 6, 7, 9) with loss of **MGMT expression**, while the remaining tumors showed strong diffuse expression. Three CRC cases with MGMT loss showed mutation in *KRAS gene* (cases 1, 2, 6) and one in *BRAF* gene (case 7). MGMT was evaluated as lost in 14 PPLs and seen only in the patients with lost MGMT expression in the carcinoma. Loss of MGMT expression was seen in 9 serrated change/dysplasia, NOS lesions, in all 5 villous hypermucinous changes, and in 2 flat IBD-associated dysplasias. Two cases of serrated change/dysplasia, NOS with MGMT loss showed either *BRAF* mutation (case 7) or *KRAS* mutation (case 6) as well. Data are summarized in Table 4.

3.4. Molecular analysis of KRAS, NRAS and BRAF

Molecular analysis was performed in all CRC samples as well as in all precursor lesions samples. Overall, 6 cases of CRC harbored mutation of *KRAS* only (cases 1, 2, 4, 8, 10, 11), one case of both *KRAS* and *NRAS* (case 6) and one case of *BRAF* only (case 7). Three cases were wild-type in all examined genes (cases 3, 5, 9).

Three mutated carcinoma cases with *KRAS* (cases 6, 11) and *BRAF* (case 9) mutations harbored identical mutation in other sample taken from the same resection specimen (cases 6, 9, 11). Morphologically, these were serrated change, NOS (case 6, 9), and TSA with high-grade dysplasia (case 11). In two patients (cases 7, 11), there were also other samples with wild-type status, both representing serrated change/dysplasia, NOS.

Three patients presented with mutation in carcinoma only (all *KRAS*) without mutation in another sample from the same specimen (cases 1, 4, 8). These samples included villous hypermucinous change (case 1) and serrated change/dysplasia, NOS (cases 4, 8).

Interestingly, there was one patient (case 5) in whom carcinoma showed wild-type profile but an additional sample from the same specimen with serrated change, NOS, harbored *BRAF* V600E mutation. Comparison of mutational status in CRC, PPLs and dysplasia is summarized in Table 5.

4. Discussion

We identified 11 cases of IBD-associated CRC in a period of 10 years among 309 retrieved resected IBD cases. In general, we have observed morphological heterogeneity in the CRC morphology as deceptively bland appearing CRC with gastric differentiation (case 1 and 5), mucinous differentiation or unusual form of perineural spread of the tumor within a myenteric plexus.

Most interesting feature revealed in our series was direct association of CRC not only with conventional IBD-dysplasia but also with other PPLs overall in 7 patients. Despite the fact, that **IBD-associated dysplasia** (intraepithelial neoplasia) has been regarded as the main precursor lesion for CRC in IBD patients [4–7] we have identified only three flat and one elevated IBD-associated dysplasia in our cases. Moreover, none of these lesions were actually detected during the surveillance endoscopy and were revealed at the time of CRC diagnosis in the resection specimen.

We have seen three cases of **villous hypermucinous change** in the mucosa adjacent to the CRC (case 1, 5, and 7). Two of the associated CRCs were morphologically very similar with gastric-like appearance. CRC in case 1 shared a loss of MGMT expression with the associated PPL. CRC in case 5 was wild-type for *KRAS*, *NRAS* and *BRAF* but the associated villous hypermucinous lesion exhibited *BRAF* V600E mutation. Carcinoma from case 7 shared same *BRAF* mutation with adjacent PPL.

The possible link of villous hypermucinous change and UC was suggested already in 1999 by Andersen et al. [11]. They evaluated 161

Table 3
Clinical and histomorphological characteristics of IBD-associated CRC.

Case No.	Sex	Type of IBD	Age (at CRC, yrs)	Carcinoma localization	CRC pTNM and clinical stage group	Grade	Carcinoma histomorphology and grade	Cytological features - cytoplasm, nuclei, nucleoli	Morphological markers - tumor border configuration, Bd, LyA, Ai, Pn
case 1	F	CD	29	lienal flexure	pT3 pN1b IIIb	grade 1	large irregular glands, pale, gastric foveolar appearance	pale cytoplasm, oval and vesicular nuclei, inconspicuous nucleoli	infiltrative border, Bd1 (0), LyA ext, Ai 0, Pn 0
case 2	F	UC	26	descending colon	pT3 pN0 IIA	grade 2	large cribriform glands mucinous component (30%)	dark eosinophilic or amphophilic cytoplasm, apical snouts, large nuclei with prominent nucleoli pale cytoplasm, smaller nuclei	infiltrative border, Bd2 (5), LyA ext, Ai 0, Pn 0
case 3	M	UC	36	rectum	pT4a pN2b IIIC	grade 3	solid-cribriform growth, comedo-necrosis mucinous component (30%) poorly cohesive (signet ring cell) component (30%)	dark eosinophilic cytoplasm, neuroendocrine features, large nuclei with nucleoli pale cytoplasm, smaller nuclei pale cytoplasm, vacuoles with mucin, small nuclei	infiltrative border, Bd3 (20), LyA int/ext, Ai int/ext, Pn ext
case 4	F	UC	57	transverse	pT4 pN1b IIIB	grade 2	large cribriform formations, comedo-necrosis	cylindric cells with eosinophilic cytoplasm, large nuclei, smudgy chromatin, some cells with large nucleolus	infitrative border, vertical growth, Bd2 (6), LyA ext, Ai 0, Pn 0
case 5	F	UC	42	ascending colon	pT2 N0 I	grade 1	tubular growth, foveolar gastric appearance mucinous component (20%)	darker cytoplasm in tubular areas, pale cytoplasm with mucin, vesicular nuclei with nucleoli pale cytoplasm	pushing border, Bd1 (0), LyA 0, Ai 0, Pn 0
case 6	F	UC	48	rectum	pT2 pN0 2014 I	grade 2	serrated, tubulo-papillary, confluent cribriform growth, horizontal growth, myenteric spread	abundant dark eosinophilic cytoplasm, large nuclei with nucleoli	infiltrative border, horizontal pattern, Bd1 (0), LyA 0, Ai 0, Pn int ext myenteric
case 7	M	UC	42	ceacum	pT1 N1 IIIA	grade 2	mucinous carcinoma (over 60%) tubular component	pale cytoplasm, small nuclei darker cytoplasm, small nuclei	pushing border, Bd1 (0), LyAi ext, direct LN infiltration, Ai ext, Pn 0
case 8	F	CD	31	transverse	pT3 pN0 II	grade 2	smaller dark cribriform formations, tubular, horizontal growth, myenteric spread	light eosinophilic cytoplasm, smaller dark nuclei without visible nucleoli	infiltrative border, Bd1 (0), LyA int/ext, Ai 0, Pn int/ext - myenteric
case 9	M	CD	55	lienal flexure	pT4a N2b IIIC	grade 2 grade 3 solid	darker and paler cribriform structures, large pale solid areas, horizontal growth, myenteric spread	eosinophilic cytoplasm and vesicular nuclei with nucleoli in cribriform areas, pale cytoplasm in solid areas, histiocytoid appearance, vesicular/crushed nuclei	infiltrative border, Bd1 (2), LyA int/ext, Ai ext, Pn int/ext - myenteric
case 10	M	UC	52	rectum	pT3 N0 IIA	grade 2	tubular and cribriform, comedo necrosis	dark pink cytoplasm, dark nuclei, inconspicuous nucleoli	infiltrative border, Bd1 (0), LyA int/ext, Ai 0, Pn0
case 11	M	UC	79	rectum	pT4a pN0 IIB	grade N/E	small cribriform formations, single cells - regressive changes, Dworak score 2	abundant eosinophilic cytoplasm, large nuclei with nucleoli	infiltrative border, Bdx (postRT), LyA 0, Ai 0, Pn 0

Abbreviations: Ai – angioinvasion; Bd – budding; CD - Crohn's disease; ext – extramural; IBD - inflammatory bowel disease; int – intramural; LN - lymph node; LyA – lymphangioinvasion; N/E - not evaluated; Pn - perineural spread; UC - ulcerative colitis.

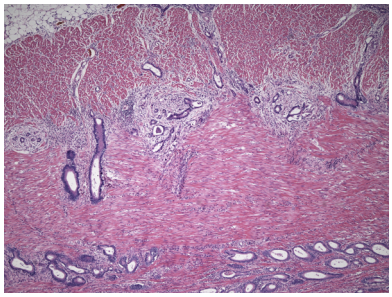

Fig. 1. Myenteric perineural spread with prominent horizontal growth pattern, HE 40x.
HE – haematoxylin-eosin.

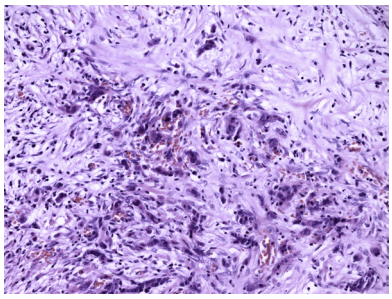

Fig. 2. Hotspot of tumor budding with 20 buds in field area of 0,785 mm^2 belonging to Bd3 category, HE 200x.
HE – haematoxylin-eosin; IBD – inflammatory bowel disease.

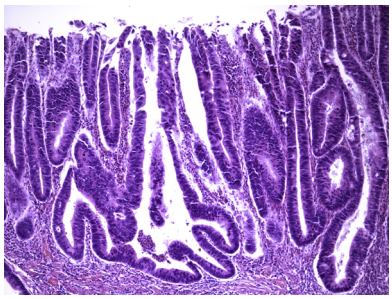

Fig. 3. Flat IBD-related high-grade dysplasia, HE 100x.
HE – haematoxylin-eosin; IBD – inflammatory bowel disease.

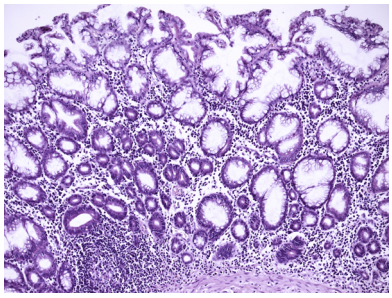

Fig. 4. Serrated change/dysplasia, NOS, distant from CRC, HE 100x.
HE – haematoxylin-eosin; CRC – colorectal carcinoma.

microdissected samples from 13 UC patients who underwent proctocolectomy either for persisting dysplastic changes or CRC. They found 13 such lesions in the set studied. Sixty-one percent of the lesions carried a *KRAS* mutation and were suggestive of potential risk lesion in UC patients. A morphologically very similar lesion was described in 2000 by Kilgore et al. [12] in Crohn's disease patients.

Similarly, we have seen three cases of CRC with adjacent **serrated changes and dysplasia** (case 6, 8 and 9). Two of these lesions shared the same mutation in *KRAS* and *BRAF* with the related CRC, respectively. One lesion shared overexpression of p53 with the CRC.

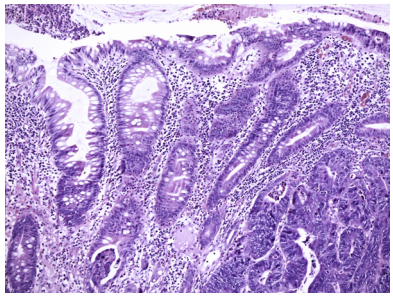

Fig. 5. Serrated change/dysplasia, NOS, adjacent to CRC, HE 100x.
HE – haematoxylin-eosin; CRC – colorectal carcinoma.

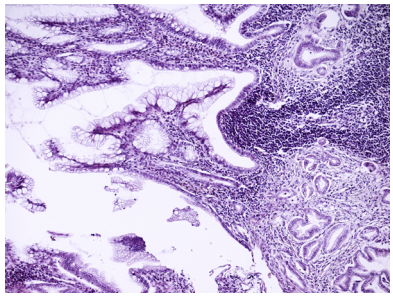

Fig. 6. Villous hypermucinous change, in direct transition to adjacent CRC, HE 100x.
HE – haematoxylin-eosin; CRC – colorectal carcinoma.

Serrated changes and neoplasias were described in UC-related CRC by Rubio [13] as well as by Bossard et al. [14] in 2007. In a set of 96 colectomies, Rubio found serrated growth pattern in 12% of lesions described as UC-adenomatous growth and in 29% of lesions they called it UC-adenomas. Bossard et al. reviewed 36 patients and identified a hyperplastic polyp and traditional serrated adenoma both of them with *BRAF* V600E mutation. Srivastava et al. published in 2008 three patients with multiple serrated lesions in a setting of IBD and compared them with findings in hyperplastic/serrated polyposis syndrome. [15]

Regarding DNA mismatch repair gene status, we have found MMR deficiency in 2 carcinomas (case 1 and 2). MMR deficiency is often discussed in IBD-related carcinomas and by reports it is detected in about 10% of the cases. [22,23]. The mechanisms of underlying MMR deficiency in IBD-associated CRC seems to be different from sporadic CRCs and are more closely related to the type of molecular defects found in hereditary MSI-H tumors associated with Lynch syndrome [20,23–25]. The MSI deficiency in one of our patients (case 2) was actually proved to be of germline origin and the patient fulfilled criteria for **Lynch syndrome** according to the Amsterdam and revised Bethesda criteria and molecular testing in other family members as well [26,27]. Interestingly, the villous hypermucinous lesion related to the CRC in case 1 showed similar MMR deficiency as the related CRC.

Results of **MGMT** expression were eventually rather controversial than expected. MGMT loss of expression seen in 6 cases of IBD-associated CRC and it was also present in 16 precursor lesions mainly in the PPL group. As shown by some authors [15,20,28], loss of MGMT function and expression is seen in sporadic as well as in IBD-related and inherited types of CRC and can be found also in the surrounding non-neoplastic and inflammatory mucosa suggesting that the MGMT methylation can precede other transformational molecular changes. In carcinomas, a strong association between MGMT loss and MSI but not *KRAS* or *BRAF* mutations is reported [20]. Nevertheless, we have seen 3 cases with *KRAS* mutation (cases 1, 2, 6) and one case with *BRAF*

Table 4
Immunohistochemical and molecular results in PPL lesions, IBD-associated dysplasia and serrated adenomas.

Type of lesion	CRC vicinity	CRC distant	Additional sample	Total	MGMT loss	p53 loss or overexpression	MMRd	MLH1 surface loss	KRAS/ NRAS mutated	BRAF mutated
Villous hypermucinous change	3	2	0	5	5/5 (100 %)	2/5 (40 %)	1/5 (20 %)	1/5 (20 %)	0	1/5 (20 %)
Serrated change/dysplasia, NOS	3	7	1	11	9/11 (82 %)	5/11 (45 %)	0	9/11 (82 %)	2/11 (18 %)	2/11 (18 %)
Flat IBD-related dysplasia	3	0	0	3	2/3 (67 %)	2/3 (67 %)	0	0	2/3 (67 %)	0
Elevated IBD-related dysplasia	1	0	0	1	0	1/1 (100 %)	0	0	1/1 (100 %)	0
Sessile serrated adenoma (SSA)	0	1	0	1	0	0/1	0	0	1/1 (100 %)	0
Traditonal serrated adenoma (TSA)	0	1	0	1	0	1/1 (100 %)	0	0	1/1 (100 %)	0

Abbreviations: CRC - colorectal carcinoma; IBD - inflammatory bowel disease; MMRd - mismatch repair deficient; NOS - not otherwise specified; PPL – putative preneoplastic lesion.

Table 5
Molecular results in colocrectal carcinoma and PPLs.

	Carcinoma			Serrated change/dysplasia, NOS			Villous hypermucinous change			IBD-associated dysplasia			SSA			TSA		
	KRAS	NRAS	BRAF	KRAS	NRAS	BRAF	KRAS	NRAS	BRAF	KRAS	NRAS	BRAF	KRAS	NRAS	BRAF	KRAS	NRAS	BRAF
1*	p.G12D	WT	WT	x	x	x	WT	WT	WT	x	x	x	x	x	x	x	x	x
2	p.G13D	WT	WT	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
3	WT	WT	WT	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
4	p.G12D	WT	WT	WT	WT	WT	x	x	x	x	x	x	x	x	x	x	x	x
5*	WT	WT	WT	WT	WT	V600E	WT	WT	WT	x	x	x	x	x	x	x	x	x
6#	p.G12D	12/13	WT	p.G12D	WT	WT	x	x	x	p.G12D	WT	WT	p.G12D	WT	WT	x	x	x
7$	WT	WT	V600A/E	WT	WT	V600A	WT	WT	V600A	x	x	x	x	x	x	x	x	x
8#	p.G12D	WT	WT	WT	WT	WT	x	x	x	p.G12D	WT	WT	x	x	x	x	x	x
9#	WT	WT	WT	WT	WT	WT	x	x	x	WT	WT	WT	x	x	x	x	x	x
10	p.G12D	WT	WT	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
11	p.G13D	WT	WT	x	x	x	x	x	x	p.G13D	WT	WT	x	x	x	p.G13D	WT	WT

Abbreviations: NOS – not otherwise specified, IBD – inflammatory bowel disease, SSA – sessile serrated adenoma, TSA – traditional serrated adenoma, WT – wild-type.
*cases with two villous hypermucinous changes.
#cases with two serrated changes/dysplasia, NOS (mutated reported).
$case with three serated changes/dysplasia, NOS.

mutation (case 7) within the group of MGMT-negative carcinomas. These results seem to differ compared to previous studies, possibly due to different MGMT status evaluation (promotor methylation evaluation versus loss of expression by immunohistochemistry).

Finally, our findings support the fact of occurrence of PPLs in IBD patients. We have also shown that these lesions can be multifocal, multiple and combined and with direct transition into invasive carcinoma. Serrated change/dysplasia, NOS was described overall 11 times in seven patients and in one case shared similar *KRAS* mutation as the related CRC. Similarly, villous hypermucinous change shared the same *BRAF* V600 A mutation as the associated CRC. PPLs adjacent to the carcinoma also shared similar p53, MGMT and MMR status in some of the cases. All these findings are supporting the hypothesis that these non-conventional lesions may precede and progress into the IBD-associated carcinoma and should be regarded as preneoplastic lesions

Finally, it is necessary to point out various limitations of the study as low case number and retrospective study design using only archival specimen with definite number of paraffin blocks without the possibility of additional specimen sampling. Particularly, in case of this study, specimens with cancer were grossed mainly for the purpose of carcinoma staging and might have led to undersampling of other parts of resected colon and possibly missing more of the preneoplastic lesions including PPLs elsewhere in the specimen

5. Conclusions

Similarly to sporadic cases, IBD-associated colorectal carcinoma shows morphological, immunohistochemical and molecular heterogeneity. Moreover, we have shown that IBD-associated carcinomas may be preceded by precursor lesions different from conventional IBD-associated dysplasia. These putative preneoplastic lesions may include villous hypermucinous change and serrated change/dysplasia, NOS. Interestingly, more than 80% of mucosal changes seen in our patients were PPLs being more common mucosal change then conventional dysplasia, and 27% (6 out of 22) of them were found in the mucosa adjacent to the carcinoma or even in direct transition to the invasive cancer. This raises a suspicion that not only these lesions can be found in IBD patients much more often, but they can also be true precursors for CRC. Moreover, since all of these lesions were detected retrospectively, it is probable that they are often missed in the histology and thus under-reported. Awareness of these new possibly preneoplastic lesions, their recognition and reporting as well as further studies are necessary.

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