Histological and molecular diversity and heterogeneity of precancerous lesions associated with inflammatory bowel diseases

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ABSTRACT

Aims Inflammatory bowel disease (IBD)-associated precancerous lesions may be adenomatous or non-adenomatous with various histomorphologies. We aim to validate the newly proposed classification, to explore the neoplastic nature of the non-adenomatous lesions and to elucidate the molecular mechanisms underlying the different histomorphologies.

Methods 44 background precursor lesions identified in 53 cases of surgically resected IBD-associated colorectal and ileal carcinomas were reviewed for the histomorphological features (classified into adenomatous, mucinous, sessile serrated adenoma (SSA)-like, traditional serrated adenoma-like, differentiated, eosinophilic and serrated not otherwise specified (NOS)) and analysed for a key panel of colonic cancer-related molecular markers.

Results Approximately 60% of the lesions were adenomatous, of which some had mixed serrated, mucinous or eosinophilic changes. The remaining non-adenomatous lesions, including all other types except SSA-like type, mostly showed mixed features and focal adenomatous dysplasia. *KRAS* mutation and p53 mutant-type expression were found in about half cases across all types, while *PIK3CA* mutation only in some of adenomatous and eosinophilic lesions and MLH1/PMS2 loss in a subset of adenomatous, mucinous and eosinophilic but not in differentiated and serrated lesions. SAT-B2 or PTEN loss and IMP3 overexpression were seen in a small subset of lesions. No *BRAF*, *NRAS* or *EGFR* gene mutation was detected in any type. Certain molecular-morphological correlations were demonstrated; however, no single or combined molecular alteration(s) was specific to any particular morphological type.

Conclusions IBD-associated precancerous lesions are heterogeneous both histologically and molecularly. True colitis-associated adenomatous lesions are unlikely conventional adenomas. Non-adenomatous lesions without frank cytologic dysplasia should also be regarded as neoplastic.

INTRODUCTION

Longstanding inflammatory bowel diseases (IBD), including ulcerative colitis (UC) and Crohn's disease (CD), increase the risk of colorectal carcinoma (CRC) development. IBD/colitis-associated colorectal carcinoma (IBD-CRC or CACRC) is believed to develop through an 'inflammation-dysplasia-carcinoma' pathway, which is different from the 'adenoma-carcinoma' sequence in sporadic CRC in general population. The precancerous dysplastic lesions are therefore the target of early detection and elimination in IBD endoscopic surveillance.

The traditional concept of the precancerous dysplastic lesions in IBD is the elevated and non-elevated mucosal abnormalities with histologically unequivocal neoplastic alterations of epithelium showing classic cytologic low-grade dysplasia (adenomatous changes) characterised by nuclear elongation, hyperchromasia and pseudostratification. Depending on the endoscopic appearances, the elevated lesions were subclassified as 'adenoma-like' (polypoid with dome-shaped and symmetric contour, smooth surface and well-delineated border) and 'non-adenoma-like' (irregularly-shaped, multinodular or plaque-like, with ill-defined border). The latter one is readily categorised as 'colitis-associated dysplasia (CAD)', and for decades it has also been popularly called 'dysplasia-associated lesion or mass (DALM)',[1] a term eventually became confusing and misused and no longer recommended.[2] For the adenoma-like lesions, it is still uncertain if they are simply conventional adenomas in nature but just happen to occur in the inflamed mucosa, or if some are indeed induced and/or promoted by mucosal inflammation and thus are different and special to individuals with IBD.

In addition to the adenomatous lesions as CRC precursors, the serrated lesions, characterised by a saw-tooth glandular contour and usually lack of classic cytologic dysplasia, including sessile serrated adenoma (SSA) (sessile serrated lesion is now the recommended term in the latest WHO classification) and traditional serrated adenoma (TSA), known as *serrated pathway* in colorectal carcinogenesis, also occur in colitic mucosa, and their prevalence, anatomic distribution and rate of cytologic dysplasia development are similar between general population and individuals with IBD.[3–5] However, some other forms of serrated lesions that do not meet the histomorphological criteria of SSA or TSA have been frequently observed in patients with IBD and their association with IBD-CRC have been noticed.[6–13] Moreover, several less common non-adenomatous and non-serrated epithelial changes/lesions have been increasingly found

prior or adjacent to IBD-CRC, and they have been considered to be putative neoplastic changes and likely variants of CAD, including *villous hypermucinous,*[13–17]*goblet cell-depleted* or *eosinophilic* changes/lesions, in which no frank adenomatous cytologic dysplasia is appreciated. Furthermore, in some apparently cancer-related background lesions, the epithelium shows nearly normal maturation with only minimal cytologic atypia. All of these non-classic lesions may explain some puzzling IBD-CRC cases in which no apparent dysplasia in the background is readily identified.

With these new observations, our concept about CAD has evolved. An international group of IBD pathology experts has recently proposed a novel histological classification of IBD-associated precancerous/dysplastic lesions,[18 19] which covers the entire known morphological spectrum and classifies the lesions into seven subtypes: (1) conventional adenoma-like (*adenomatous**), (2) hypermucinous (*mucinous**), (3) sessile serrated adenoma (SSA)-like, (4) traditional serrated adenoma (TSA)-like, (5) dysplasia with terminal epithelial differentiation (*differentiated**), (6) goblet cell deficient/depleted (*eosinophilic**) and (7) serrated NOS (*serrated**) (*short terms used in this study). This new classification aims to update and standardise the CAD classification, on which all future pathological and biological studies can be based using a uniformed language. More recently, these non-adenomatous lesions have been included, although only briefly described, in the latest WHO classification of IBD-associated dysplastic lesions.[20]

We highly appreciate and recognise the appropriateness and value of the new classification with strong agreement based on our own experience in a large IBD centre. In attempt to support and validate the new classification and further demonstrate the neoplastic nature of the non-adenomatous lesions, we retrospectively reviewed all of the IBD-CRC cases that we have encountered at our institution over the last two decades, and we applied the updated classification to recategorise the background lesions. Furthermore, we tested a panel of key molecular markers to compare between some of the morphological types, in attempt to elucidate the molecular mechanisms underlying different histomorphologies.

MATERIALS AND METHODS

Patients and study design

We retrieved all of the cases of surgically resected colorectal and ileal invasive carcinomas in patients with IBD encountered in Calgary over 17 years (2000–2017) by searching the Anatomic Pathology database of the Calgary Laboratory Services (currently a part of Alberta Public Laboratory), the sole pathology laboratory serving the greater Calgary region since 1986. Fifty-five cases (total colectomy ×49, right hemicolectomy ×6) in total were retrieved. In two cases, the carcinomas occurred in bowel regions that had never been involved by colitis, so these two cases, presumably unrelated to IBD, were excluded from further study.

Subsequently, clinical, endoscopic and pathological data of the remaining 53 patients were reviewed, as summarised in table 1. The pathology reports on all of the colectomy specimens as well as the searchable colonoscopic biopsies prior to the diagnosis of carcinoma were fully reviewed, and the glass slides of the biopsies with reported dysplasia or suspicious/indefinite for dysplasia were also reviewed.

Furthermore, the archived glass slides of all of the resection specimens and the positive endoscopic biopsies immediately prior to or led to the diagnosis of carcinomas were reviewed by a single experienced gastrointestinal pathologist (XG), which ensured uniform application of the criteria in the pathological evaluation.

The study was approved by the Conjoint Health Research Ethics Board of the University of Calgary.

Histological study of cancerous and precancerous lesions

For each patient, in addition to assessing the characteristics of the invasive carcinomas, the originally sampled bowel mucosa surrounding and adjacent (within 10 cm) to the carcinoma was carefully examined to identify the presence and characterisation of any possible background precursor lesions of any type as described in the abovementioned newly proposed classification. In some cases, the classification was made in conjunction with the prior biopsies, if there were any.

In 41 of the 53 cases, concurrent precursor lesions in the background were identified. In eight of them, additional (one to three) lesions were identified distant from the cancer sites. In the remaining 12 cases, no precursor lesion was identified in the originally sampled tissue. The 41 cases with precancerous lesions identified became the subjects that our further study focused on. Their demographic, clinical and pathological data were summarised in table 2.

The architecture (growth pattern) and cytological features of the lesions were analysed and reclassified according to the novel classification,[18] as described in table 3 and illustrated in figure 1.

Targeted molecular biomarker testing on different subtypes of precancerous lesions

Certain types of the dysplastic lesions, including adenomatous, mucinous, differentiated, eosinophilic and serrated, were further studied on their expression of some biomarkers that are known to be common in colorectal carcinogenesis. The SSA-like and TSA-like lesions were not included for this part of study due to limitation of case/lesion numbers and in consideration that the molecular alterations in these classic serrated lesions have already been well studied.

The areas with specific types of lesional tissues in the corresponding blocks/areas were separately punched to obtain the tissue cores for the following further studies, as long as there was sufficient lesional tissue for that case. For the non-adenomatous lesions, any focus of putative mixed adenomatous epithelium was avoided to the best we could in the tissue collection. The acquired tissue cores were used and split for two further studies on underlying molecular alterations. One was to construct a tissue microarray (TMA) for immunohistochemical study on certain biomarkers. The other was to extract DNA to detect some colon cancer-related gene mutations. For each type of lesion in each case, at least two cores were obtained for TMA and two cores were pooled for DNA extraction. In most cases, for both tests multiple cores of tissue with the same type of lesion were taken from more than one tissue blocks.

In total, 41 most representative microscopic lesions of the five types (adenomatous ×8, mucinous ×8, differentiated ×7, eosinophilic ×9 and serrated ×9) from 56 blocks of 26 patients were included and were designated as five groups (A–E). A TMA was constructed with 100 cores (each in 1.0 mm diameter) containing the 41 lesions. Molecular testing was performed on 61 samples containing the same 41 lesions, including some being pooled with more than one sample on testing, in order to ensure a sufficient quantity of extracted DNA.

Immunohistochemistry and interpretation

With the constructed TMA, immunohistochemistry was performed using Leica Bond II or DAKO Omnis platform on

Table 1 53 cases of IBD-related carcinomas

	UC	CD	Total
Number of cases	32	21	53
Gender			
Male	23 (71.87%)	13 (61.90%)	36 (67.92%)
Female	9 (28.13%)	8 (38.10%)	17 (32.08%)
Age (years)	60.78±9.91 (41–79)	62.81±15.21 (34–89)	61.58±14.70
IBD			
Length of disease prior to CRC (years)	12.90±9.45 (4-34)	7.71±3.39 (3-13)	11.61±6.50 (3-34)
Extent of enterocolitis	E1: 0	L1: 3 (14.28%)	
(E1/E2/E3 for UC, L1/L2/L3 for CD)*	E2: 1 (3.12%)	L2: 9 (42.86%)	
	E3: 31 (96.88%)	L3: 9 (42.86%)	
Disease activity in region of cancer			
Active	25 (78.13%)	19 (90.48%)	44 (83.02%)
Quiescent	7 (21.88%)	2 (9.52%)	9 (16.98%)
Surveillance for dysplasia			
Yes	8 (25%)	4 (19.05%)	12 (22.64%)
No	24 (75%)	17 (80.95%)	41 (77.36%)
Lesions detected near cancer site in history	12 (37.50%)	7 (33.33%)	19 (35.85%)
Indefinite for dysplasia	5 (15.63%)	2 (9.52%)	7 (13.21%)
Low-grade dysplasia/'DALM'	1 (12.50%)	3 (19.05%)	8 (15.09%)
Flat dysplasia		1	
Adenoma, tubular	3	0	
Serrated polyp	1	0	
Inflammatory polyp(s)	3 (9.38%)	1 (4.76%)	4 (7.55%)
Precursor undetected in history	20 (62.50%)	14 (66.67%)	34 (64.15%)
Carcinomas			
Number of cancer			
1	28 (87.50%)	17 (80.95%)	45 (84.91%)
2	3 (9.38%)	4 (19.05%)	7 (13.21%)
3	1 (3.13%)	0	1 (1.88%)
Locations			
Terminal ileum	0	4† (16.67%)	4 (6.90%)
Cecum	5 (14.71%)	4 (16.67%)	9 (15.52%)
Ascending colon	3 (8.82%)	5 (20.83%)	8 (13.79%)
Transverse colon	2 (5.88%)	2 (8.33%)	4 (7.55%)
Descending colon	1 (2.94%)	4 (16.67%)	5 (8.62%)
Sigmoid colon	4 (11.76%)	1 (4.17%)	5 (8.62%)
Rectosigmoid colon	7 (20.59%)	3 (12.50%)	10 (17.24%)
Rectum	12 (35.29%)	1 (4.17%)	13 (22.41%)
Differentiation			
Well	8 (25%)	5 (23.81%)	13 (24.53%)
Moderate	16 (50%)	13 (61.90%)	29 (54.72%)
Poor	8 (25%)	3 (14.29%)	11 (20.75%)
Mucinous features			
Yes	16 (50%)	7 (33.33%)	23 (43.40%)
No	16 (50%)	14 (66.67%)	30 (56.60%)
T stage (depth of invasion)			
T1	3 (9.38%)	3 (14.29%)	6 (11.32%)
T2	8 (25%)	1 (4.76%)	9 (16.98%)
T3	17 (53.12%)	12 (57.14%)	29 (54.72%)
T4	4 (12.50%)	5 (23.81%)	9 (16.98%)
Nodal metastasis			
Positive	14 (43.75%)	5 (23.81%)	19 (35.85%)
Negative	18 (56.25%)	16 (76.19%)	34 (64.15%)
Metastasis at diagnosis			
Yes	1 (3.12%)	2 (9.52%)	3 (5.66%)
No	31 (96.88%)	19 (90.48%)	50 (94.34%)

Continued

Table 1 Continued

	UC	CD	Total

*E1: proctitis, E2: left-sided colitis, E3: pancolitis; L1: ileal, L2: colonic, L3: ileocolonic (Montreal classification).
†1 case of cancer arose in pouch.
CD, Crohn's disease; DALM, dysplasia-associated lesion or mass; IBD, inflammatory bowel disease; UC, ulcerative colitis.

4-µm sections to detect the expression of p53, MLH1, MSH6, PMS2, SAT-B2, ARID1A, PTEN, IMP3 and RB1. The antibodies used were all commercially available and widely used. A corresponding H&E stained section was also reviewed to confirm the histomorphology and appropriateness of each of the corresponding tissue cores.

The immunostaining results of the different markers were read and scored as described previously.[21–25] For all of the markers, except PTEN and IMP3, a nuclear stain was expected to demonstrate their expressions. p53 immunostain was scored as 0 (complete absence of staining), 1 (wild type/normal expression—patchy and weak to moderate staining), 2 (overexpression—diffuse and strong staining) and 3 (cytoplasmic expression). The absence (0), overexpression (2) and cytoplasmic expression (3) are all known to be mutant-type patterns of p53 protein expression, i.e., to indicate *TP53* gene mutation (0—stopgain/indel/splicing/nonsense mutation leading to formation of a truncated, non-immunoreactive protein, 2—non-synonymous/missense mutation, 3—stopgain/indel/splicing mutation with disruption of the nuclear localisation domain).[21 26 27] Mismatch repair (MMR) proteins (MLH1, PMS2, MSH6) were read as 0 (absent/loss/deficient), 1 (weak/heterogeneously loss) and 2 (intact). Only complete loss (score 0) of any of the three proteins was considered significant (indicative of MMR deficiency due to hypermethylation or loss of function mutation of the gene). SAT-B2 was read as 0 (absent/loss), 1 (focal, in 1% to <50% lesional epithelial cells), 2 (diffuse, in >50% cells) and 3 (>90%). PTEN was scored as 0 (absent/loss), 1 (reduced/borderline) and 2 (positive/retained, i.e., clearly visible cytoplasmic staining). ARID1A and RB1 were both read as 0 (absent/loss) and 1 (retained). For all of these four markers, loss of expression was considered significant (indicative of loss of function mutation of genes) in this study. IMP3 was read as 0 (absent/negative), 1 (focal, in 1% to <50% of lesional cells), 2 (diffuse, in >50% of cells) and 3 (diffuse,>95%, so-called block staining). Scores 2 or 3 was arbiturally defined as significantly positive in this study. The immunostains were read by three pathologists (XG, MK, PR) to reach consensus.

Table 2 Colitis-associated dysplastic lesions adjacent to carcinomas

	UC	CD	Total (%*)
Architecture/Growth pattern			
Tubular	12	9	21 (47.73)
Tubulovillous	10	5	15 (34.09)
Villous	1	1	2 (4.55)
Serrated	3	3	6 (13.64)
Characteristic morphological features			
Serration	10	5	15 (34.09)
Hypermucinous	6	5	11 (25.00)
Serration and hypermucinous	4	3	7 (15.91)
Goblet cell loss (Eosinophilic changes)	5	5	10 (22.73)
Primary classification of lesions			
Adenomatous	19	10	29 (65.91)
Mucinous	2	1	3 (6.82)
SSA-like	0	0	0
TSA-like	1	0	1 (2.27)
Differentiated	3	2	5 (11.36)
Eosinophilic	1	3	4 (9.09)
Serrated, NOS	1	1	2 (4.55)
Mixed (major/minor)			
Adenomatous/Mucinous	0	1	1 (2.27)
Adenomatous/TSA-like	1	0	1 (2.27)
Adenomatous/Differentiated	2	0	2 (4.55)
Adenomatous/Differentiated/Eosinophilic	1	0	1 (2.27)
Adenomatous/Eosinophilic	1	1	2 (4.55)
Adenomatous/Mucinous/Eosinophilic	2	0	2 (4.55)
Mucinous/Serrated/Adenomatous/Differentiated	1	1	2 (4.55)
Differentiated/Adenomatous	0	1	1 (2.27)
Differentiated/Serrated/Mucinous	2	0	2 (4.55)
Eosinophilic/Adenomatous	1	3	4 (9.09)
Serrated/Differentiated/Adenomatous	0	1	1 (2.27)

*Percentage out of 44 lesions in total.
CD, Crohn's disease; SSA, sessile serrated adenoma; TSA, traditional serrated adenoma; UC, ulcerative colitis.

Genetic mutations testing

DNA was extracted from the punched tissue cores from the circled areas of lesions by using the Qiagen extraction kit according to the manufacture's protocols. With the DNA samples, molecular analysis was performed by mass spectrometry using the Agena MassArray kit to detect the colonic cancer panel (iPLEX HS Colon Panel) of five common gene mutations including *BRAF* (G469E, D594G, V600E), *KRAS* (47 mutation hotspots), *NRAS* (30 mutations), *PIK3CA* (E542K, H1047R, H1047L) and *EGFR* (S492R).[28]

The expression patterns of the above biomarkers in the different types of lesions were compared, and the morphological-molecular correlations were ultimately analysed.

Statistical analysis

χ^2 test of independence was performed using Microsoft Excel to compare the difference of the detection rate of each biomarker abnormality and the rate of mucinous carcinoma between the different morphological groups/types. $P<0.05$ was considered to be statistically significant.

RESULTS

Cases overview

Of the 53 patients (with 58 cancers) in total, 12 (22.64%) patients had gone through regular colonoscopic surveillance that followed the popular guideline(s) then and 10 others (18.87%) had irregularly intermittent endoscopic-histological follow-up, prior to the diagnosis of cancer, whereas the rest of the 31 patients did not have histological follow-up on records. Overall, in the patients who were followed up in some way, 26 accumulated lesions were reported on the prior colonic biopsies (not all limited to the areas where the carcinomas developed), including 'DALM'(accepted terminology then) (×5), 'tubular adenoma' (×3), 'flat dysplasia' (×2), 'indefinite for dysplasia'

Table 3 Subtyping variants of colitis-associated precancerous neoplastic lesions

Name	Cytoarchitectural features
Adenomatous (conventional adenoma-like)	Tubular, tubulovillous, or villous architecture. Adenomatous epithelial cells showing classic cytologic dysplasia featured by enlarged, hyperchromatic, elongated or pencillate and stratified nuclei, with no top-down pattern. Crypts are irregular in shape, size and distribution.
Mucinous (hypermucinous and villous)	Villous or villiform architecture. Columnar cells with mucin-rich cytoplasm (microvesicular or/and foveolar-like mucin cap) and nuclei being small or slightly enlarged and basally oriented, with no or mild stratification. The degree of nuclear atypia decreases towards the tip of villi.
SSA-like	Serrated architecture, with crypts slightly distorted and basally dilated, resembling SSA. Cells with basally located small round-to-oval or slightly elongated and slightly stratified nuclei, with surface maturation.
TSA-like	Serrated architecture. Cells with enlarged or slightly elongated, hyperchromatic and slightly stratified nuclei at the base and with abundant eosinophilic cytoplasm and variable cytoplasmic mucin.
Differentiated (dysplasia with terminal epithelial differentiation)	Tubular or slightly tubulovillous architecture. Enterocyte-type cells and goblet cells (slightly less in number and irregular in size) with nuclei being small, round-to-oval, slightly irregular, hyperchromatic, with occasional inconspicuous nucleoli and mostly non-stratified. The nuclear atypia may be limited to lower crypts in some cases.
Eosinophilic (goblet cell- deficient/depleted)	Tubular architecture. Goblet cells are completely or near completely absent. Enterocyte-type cells with eosinophilic cytoplasm and oval-to-slightly enlarged or elongated and hyperchromatic nuclei, with no or mild stratification at the base. Eosinophilic secretion within cryptal lumen may present.
Serrated (serrated, NOS)	Serrated architecture, involving upper portion or full thickness. Serrated crypts distorted, with no characteristic features of SSA. Enterocyte-type cells with slightly eosinophilic and/or slightly mucin-rich cytoplasm and mildly enlarged and mostly non-stratified vesicular nuclei.

SSA, sessile serrated adenoma; TSA, traditional serrated adenoma.

(×8), 'serrated polyp' (×1) and 'inflammatory (pseudo)polyp' (×7). In 2 (3.77%) patients, a conventional-type adenomatous polyp (tubular adenoma) appeared to be the precursor in which carcinoma arose, including an apparent malignant polyp (i.e., focal invasive carcinoma within the adenoma) removed prior to resection in one patient.

Of the invasive carcinomas, nearly half (50% in UC and 33.33% in patients with CD) were mucinous carcinoma or showed significant mucin production.

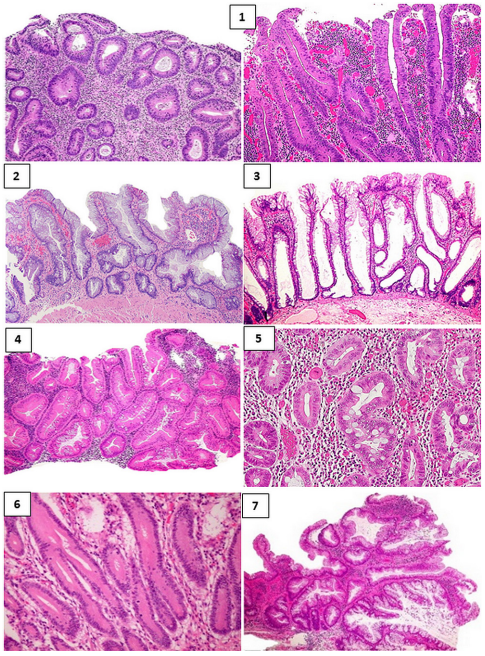

Figure 1 Representative micrographs of the seven types of colitis-associated precancerous lesions in the recently proposed novel classification.

Histomorphological features of different types of precancerous lesions

As shown in table 2, of the 41 cases in which the precancerous lesions (×44) were identified, 21 were tubular in architecture, 15 tubulovillous, 2 villous and 6 serrated. On resubtyping the lesions with the novel classification, more than 60% of the lesions were adenomatous but they showed some features that are not or rarely seen in conventional/sporadic adenomas, as illustrated in figure 2, including high-grade nuclear atypia and pleomorphism, peculiar micropapillary surface epithelium and mixed component of inflammatory (pseudo)polyp (e.g., the dysplastic epithelium appeared to be the minimal residual epithelium residing in or on the surface of granulation tissue). There was only one exception, in which a classic tubular adenoma as precursor was

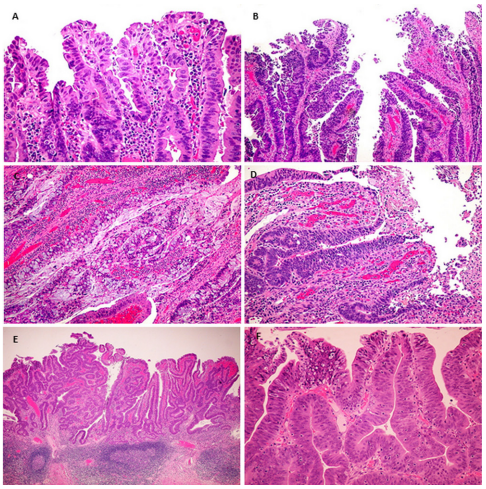

Figure 2 Unusual features seen in colitis-associated adenomatous lesions. (A) A higher grade of nuclear atypia and pleomorphism. (B) Peculiar micropapillary surface epithelium. (C) Dysplasia with dystrophic goblet cells. (D) Dysplastic epithelium appeared to be the residual mucosal epithelium on the surface of granulation tissue. (E/F) Representative micrographs of another case of adenomatous lesion (case ID#40).

determined on reviewing the combined endoscopic and histological features of the previously removed overlying polyp.

Of the other nearly 40% of the non-adenomatous lesions, most showed mixed morphologies with various combinations (of 2–4 different types) and also commonly mixed with focal adenomatous cytological dysplasia. Up to a third of the lesions showed mixed features of serrated and hypermucinous epithelium. About a quarter showed loss of goblet cells ('eosinophilic changes') or features of inflammatory pseudopolyp. Of the mixed lesions, five were predominantly differentiated, four were predominantly eosinophilic and three were predominantly mucinous. Only one case was entirely mucinous, one entirely TSA-like and two entirely serrated. The examples of various types of the lesions are shown in figure 1.

With regard to the possible morphological association between the type of precancerous lesion and that of invasive carcinoma, we found that the rate of mucinous carcinoma between each type of precancerous lesions showed no significant difference ($p>0.05$), although table 4 seemed to show serrated dysplasia being slightly more associated with mucinous carcinoma, whereas goblet cell depleted dysplasia less associated with mucinous carcinoma. Certain type of association was clearly noted in a few unusual cases though.

Of note, the various variables in tables 1 and 2 showed no statistically significant difference between UC and CD.

Molecular alterations and morphological-molecular correlations

The genetic testing and immunohistochemical staining results of the 41 lesions are shown in details in the tables 4 and 5.

Genetic mutations among what have been detected were seen only in *KRAS* and *PIK3CA*. *KRAS* mutation was found across all of the five morphological types. No statistically significant difference existed between any of the groups.

PIK3CA mutation was detected in only adenomatous and eosinophilic lesions, with a positive rate of 25% and 55.6%, respectively, with the detection rate being significantly higher in eosinophilic type ($p=0.0018$).

BRAF mutation was surprisingly not detected in any of the lesions, even in serrated type. Also, neither *NRAS* nor *EGFR* gene mutation was involved in any type of lesions.

p53 was found to show mutant-type expression with a similar rate (around 50%, $p>0.05$ in between) across all types, including mostly overexpression, and loss of expression as well as cytoplasmic expression.

Both MLH1 and PMS2 were lost together in a small percentage (12.5%–33.3%) of lesions in adenomatous, mucinous and eosinophilic types with no significant difference in between, but no loss in differentiated and serrated lesions, while MSH6 was intact in all types of lesions. The identical loss of both MLH1 and PMS2 is apparently indicative of *MLH1* gene inactivation and most likely the result of *MLH1* hypermethylation or somatic mutation. A genomic mutation of *MLH1* gene is very unlikely in this setting. MMR deficiency and mutant p53 were mutually exclusive.

Loss of SAT-B2 expression was seen in 10%–20% of lesions in all of the non-adenomatous lesions with no significant difference in between, but in none of the adenomatous lesions.

Loss of PTEN expression was seen in 1/4 of adenomatous and 1/8 of mucinous lesions, but not in any other types. Interestingly, in the two cases of adenomatous lesions (ID#28 and #31) with loss of PTEN, the adenomatous changes were both present on the surface of necroinflammatory granulation tissue and showed relatively unusual morphology featured by bizarre-looking highly atypical and pleomorphic nuclei and dystrophic goblet cells, as exampled in figure 2A–D.

IMP3 overexpression in a block-staining pattern was seen in only one case of adenomatous lesion (ID#13, as shown in figure 3). In this case, interestingly, an eosinophilic lesion was also admixed and both types had identical *PIK3CA* mutation. A patchy expression of IMP3 was seen in only adenomatous and mucinous lesions (12.5% and 50%, respectively, $p>0.05$).

ARID1A and RB1 both showed retained expression in all of the lesions, indicative of no mutation of these two genes involved in the IBD-associated precancerous lesions.

As shown in figure 4, two different hotspots of *KRAS* mutation (G12D versus G13D) were detected in two different cases (ID#6 and #35) of serrated lesions with similar but slightly different morphological features.

Uncommon *KRAS* and *PIK3CA* mutations were detected in rare and unusual adenomatous lesions. In case ID#28, the only case with a peculiar micropapillary surface (as shown in figure 2B), a combination of a non-hotspot *PIK3CA* mutation (H1047R) and *KRAS* mutation (G13D) was detected, with no abnormal expression of p53, MMR proteins and SAT-B2. In case ID#40, as shown in figure 2E/F), which was clearly not a conventional adenoma on morphology, a non-hotspot *KRAS* mutation (A146T) was detected, in combination with loss of MLH1/PMS2. The *KRAS* A146T mutation was not found in any other lesions in the study.

Further analyses on lesions with mixed morphological types with focus on morphological-molecular correlations

Eleven of the 26 (42%) cases had mixed morphological types of lesions, either in different areas of the lesion or in the neighbouring lesions. Further molecular analyses in each morphological component of the same lesion from the same individuals, as shown in table 4 (cases marked with *) as well as exampled in figures 4–6, are in our opinion particularly interesting and helpful for us to understand the molecular-morphological correlation.

In case ID#7, as shown in figure 5, both differentiated and serrated lesions were seen in a same tissue block, and both lesions shared mutant-type p53 overexpression. The difference was the *KRAS* mutation, which was detected in serrated but not in differentiated lesion, suggestive of a link between *KRAS* mutation (G13D) and the serrated morphology.

In case ID#13, as shown in figure 3, eosinophilic and adenomatous lesions coexisted in multiple blocks, and even a clear transition from eosinophilic to adenomatous changes was noticed. Both shared the same *PIK3CA* mutation (E542K), whereas adenomatous type had additional *KRAS* mutation (G12V) and IMP3 overexpression.

In case ID#51, as shown in figure 6, in association with a mucinous carcinoma, three types of non-adenomatous (mucinous, differentiated, serrated) lesions were identified in different regions of colon. Both mucinous and differentiated lesions also showed superficial and mild serration. Only slight difference in molecular profiles was identified between the different lesions. A combination of mutant-type p53 overexpression and SAT-B2 loss was seen across the three types. An identical *KRAS* mutation (G12V) was detected in both mucinous and serrated lesions, but not in differentiated type. Additionally, focal adenomatous cytology was seen in all types, although the adenomatous component was not separately sampled for further study since it was too limited.

Table 4 Genetic mutations and biomarker expression abnormalities in different types of precancerous lesions

			Carcinoma		Genetic mutation					Immunohistochemical expression (Scores)									Mixed morphological features
Group*	Patient ID#	IBD type	Location	? Mucinous	KRAS	PIK3CA	BRAF	NRAS	EGFR	P53	MLH1	PMS2	MSH6	SATB2	PTEN	IMP3	ARID1A	RB1	(focal)
A (n=8)	13†	UC	Sigmoid	Yes	G12V	E542K	ND	ND	ND	1	2	2	2	2	2	**3**	1	1	Eosinophilic
	31†	UC	Rectal stump		G12D	ND	ND	ND	ND	2	1	1	1	2	**0**	0	1	1	
	16†	UC	Ascending colon		ND	ND	ND	ND	ND	**0**	2	2	2	2	2	1	1	1	
	40†	UC	Rectal stump		A146T	ND	ND	ND	ND	1	**0**	**0**	2	2	2	0	1	1	
	38	CD	Splenic flexure		G13D	H1047R	ND	ND	ND	1	1	1	2	2	2	0	1	1	
	28	CD	Ileal pouch	Yes	ND	ND	ND	ND	ND	**3**	1	1	2	1	**0**	0	1	1	
	9	UC	Sigmoid	Yes	ND	ND	ND	ND	ND	**3**	2	2	2	2	2	0	1	1	
	49†	CD	Terminal ileum		ND	ND	ND	ND	ND	1	**0**	**0**	2	1	1	0	1	1	Eosinophilic
B (n=8)	34	CD	Cecum		ND	ND	ND	ND	ND	**2**	2	1	2	2	2	2	1	1	Adenomatous (TVA-like)
	31†	UC	Rectal stump		G12D	ND	ND	ND	ND	**2**	1	1	2	1	2	2	1	1	Adenomatous (TVA-like)
	51†	CD	Hepatic flexure	Yes	G12V	ND	ND	ND	ND	**2**	1	1	2	**0**	2	2	1	1	Adenomatous (TVA-like)
	41†	CD	Terminal ileum		G12D	ND	ND	ND	ND	1	2	2	2	1	2	0	1	1	
	15†	UC	Cecum	Yes	G12A	ND	ND	ND	ND	1	**0**	**0**	1	1	**0**	0	1	1	Adenomatous (TVA-like)
	37	UC	Rectosigmoid	Yes	ND	ND	ND	ND	ND	**0**	2	2	2	2	2	2	1	1	Adenomatous (TVA-like)
	52†	UC	Descending colon		ND	ND	ND	ND	ND	1	2	2	2	2	2	0	1	1	Adenomatous (TVA-like)
	48	UC	Rectosigmoid	Yes	ND	ND	ND	ND	ND	1	2	2	2	2	1	1	1	1	Adenomatous (TVA-like)
C (n=7)	7†	UC	Transverse colon	Yes	ND	ND	ND	ND	ND	**2**	2	2	2	2	2	0	1	1	Serrated
	12†	UC	Cecum		ND	ND	ND	ND	ND	1	1	2	1	2	2	0	1	1	
	27	UC	Ascending & Sigmoid	Yes	ND	ND	ND	ND	ND	**2**	2	2	2	2	2	1	1	1	Adenomatous
	51†	CD	Hepatic flexure	Yes	ND	ND	ND	ND	ND	**2**	2	2	2	**0**	2	0	1	1	
	36†	CD	Ascending & Transverse	Yes	ND	ND	ND	ND	ND	1	2	2	2	2	2	1	1	1	
	52†	UC	Descending colon		ND	ND	ND	ND	ND	1	2	2	2	2	2	1	1	1	Adenomatous
	16†	UC	Ascending colon		G13D	ND	ND	ND	ND	1	2	2	2	1	2	0	1	1	Adenomatous
D (n=9)	13†	UC	Sigmoid	Yes	ND	E542K	ND	ND	ND	1	2	2	2	2	2	0	1	1	
	33	CD	Rectal stump		ND	ND	ND	ND	ND	**2**	2	2	2	**0**	2	0	1	1	Adenomatous
	41†	CD	Terminal ileum		ND	E542K	ND	ND	ND	1	0	0	2	1	2	0	1	1	Adenomatous
	46	CD	Terminal ileum		G12D	E545K	ND	ND	ND	1	0	0	2	**0**	2	1	1	1	Adenomatous
	44†	CD	Transverse colon		ND	E542K	ND	ND	ND	**2**	2	2	2	2	2	0	1	1	Adenomatous
	3	UC	Rectosigmoid		ND	ND	ND	ND	ND	**2**	2	1	2	2	2	0	1	1	Adenomatous
	49†	CD	Terminal ileum		ND	H1047R	ND	ND	ND	1	**0**	**0**	2	1	1	1	1	1	
	16†	UC	Ascending colon		Q61H	ND	ND	ND	ND	**2**	2	2	2	1	2	1	1	1	Adenomatous
	37†	UC	Rectosigmoid	Yes	ND	ND	ND	ND	ND	1	2	2	2	2	2	0	1	1	Adenomatous

Continued

Table 4 Continued

Group*	Patient ID#	IBD type	Carcinoma		Genetic mutation				Immunohistochemical expression (Scores)								Mixed morphological features (focal)
			Location	? Mucinous	KRAS	PIK3CA	BRAF	NRAS	EGFR	P53	MLH1	PMS2	MSH6	SATB2	PTEN	IMP3	ARID1A	RB1	
E (n=9)	7†	UC	Transverse colon	Yes	G13D	ND	ND	ND	ND	**2**	2	2	2	2	2	0	1	1	Adenomatous
	32	UC	Rectum	Yes	ND	ND	ND	ND	ND	1	2	2	2	2	2	0	1	1	Inflammatory polyp
	52†	UC	Descending colon		G12D	ND	ND	ND	ND	1	2	2	2	2	2	0	1	1	
	42	CD	Rectum		ND	ND	ND	ND	ND	1	2	2	2	2	2	0	1	1	
	36†	CD	Ascending and Transverse	Yes	G12D	ND	ND	ND	ND	1	2	2	2	2	2	1	1	1	
	6†	UC	Sigmoid	Yes	G12D	ND	ND	ND	ND	1	2	1	2	2	2	1	1	1	Adenomatous
	12	UC	Cecum		ND	ND	ND	ND	ND	1	1	1	2	2	2	1	1	1	Mucinous
	35†	UC	Hepatic flexure	Yes	G13D	ND	ND	ND	ND	1	2	2	2	2	2	0	1	1	Adenomatous
	51†	CD	Hepatic flexure	Yes	G12V	ND	ND	ND	ND	**2**	2	2	2	**0**	2	0	1	1	Adenomatous (TVA-like)

*Group A: Adenomatous, Group B: Mucinous, Group C: Differentiated, Group D: Eosinophilic, Group E: Serrated.
†Cases with mixed types; p53: 1 wild type, 0/2/3 mutant; MLH1/PMS2/MSH6: 0 MMR deficient, 1/2 MMR proficient; SAT-B2/PTEN: 0 loss, 1/2 retained; IMP3: 0/1 negative, 2/3 positive; ARID1A/RB1:1 retained.
CD, Crohn's disease; IBD, inflammatory bowel disease; UC, ulcerative colitis.

Similar mixed and heterogeneous features were also seen in some other cases (eg, #16, #31, #41), as shown in table 4.

Overall, it is our strong impression that two important points can be made based on our findings. One, in general none of the molecular alterations nor a recognisable panel from the limited biomarkers that we studied was specific to any particular morphological type. Two, although a minimal adenomatous dysplasia is commonly mixed in all of the other morphological types, additional heterogeneous molecular alterations truly existed and may be responsible for the morphological diversity.

DISCUSSION

Based on analysing the apparently cancer-related background lesions found in resected bowel with IBD-CRC, the present study further demonstrates the histological variety of IBD-associated precancerous lesions, preliminarily discloses the underlying molecular alterations and echoes the newly proposed updated classification. Meanwhile, our data show that these lesions are mostly heterogeneous in morphology while share many of the common CRC-related molecular alterations, and that it is not uncommon to have more than one type of lesions occurring in an individual patient, either in mixed/composite fashion or in different temporal-spatial distribution. Of note, it is interesting that almost none of our cases of IBD-CRCs arose from classic conventional/sporadic adenomas or typical SSAs or TSAs.

Adenomatous lesion with widespread classic cytologic dysplasia is obviously the most common type, accounting for more than 60% of our cases. However, a number of important points were noted from the study. First, these adenomatous lesions did not appear to be the conventional-type adenomas. In none except one of our cases did the lesions resemble a classic adenomatous polyp. Second, these lesions showed some unusual cytoarchitectural features, mainly high-grade nuclear atypia and pleomorphism, peculiar micropapillary surface and mixed component of inflammatory (pseudo)polyp with dysplastic epithelium mounted on granulation tissue. To our observation, these three features seem to be quite unique to CAD, although not sensitive, and may help us distinguish CAD from conventional adenoma in the biopsy diagnosis. Third, mixed focal adenomatous cytologic dysplasia, more or less, is common in the non-adenomatous lesions. From this point of view, almost none of the non-adenomatous lesions is absolutely free of cytological dysplasia. It is therefore our postulation that the development of adenomatous dysplasia is the necessary step for any type of CAD lesions to progress to carcinoma, as seen in the case of SSA in general population.

Serrated pathway is another known mechanism in the carcinogenesis of CRC. Other than typical SSA and TSA, some atypical serrated lesions that do not show characteristic morphology of SSA are seen in patients with IBD and their precancerous nature has been increasingly noticed,[6 11] including frequent *KRAS* mutation.[11 12] Additionally, various hyperplastic-like serrated changes, mostly with serration being focal and/or superficial, are sometimes seen in IBD surveillance biopsies taken from mostly sessile polypoid or multinodular mucosa or even mucosa without visible lesion. Their biological nature and pathological significance are not yet determined. They have commonly been presumed to be regenerative hyperplasia and are even classified as hyperplastic polyps.[8] Recently, these atypical serrated lesions in IBD were designated as 'serrated epithelial change (SEC)' and suspected to be related to adjacent or subsequent dysplasia,[9 10] although it is still poorly defined and has not been fully validated. A subset of SEC was found to contain aneuploidy, which raises a concern

Table 5 Molecular alteration detection rate (%)

Lesion type	KRAS	PIK3CA	p53	MLH1	SATB2	PTEN	IMP3
Adenomatous	50 (4/8)	25 (2/8)	50 (4/8)	25 (2/8)	0	25 (2/8)	12.5 (1/8)
Mucinous	50 (4/8)	0	50 (4/8)	12.5 (1/8)	12.5 (1/8)	12.5 (1/8)*	50 (4/8)
Differentiated	14.3 (1/7)	0	42.9 (3/7)	0	14.3 (1/7)	0	0
Eosinophilic	22.2 (2/9)	55.6 (5/9)*	44.4 (4/9)	33.3 (3/9)	22.2 (2/9)	0	0
Serrated	66.7 (6/9)	0	22.2 (2/9)	0	11.1 (1/9)	0	0

*P<0.005, as compared with adenomatous type.

for the possibility of SEC developing neoplasia via the chromosomal instability pathway,[21] and it was also found to harbour *KRAS* mutation in about half cases and *BRAF* mutation and/or *TP53* mutation in a subset of cases.[12] In comparison, typical SSAs have less *KRAS* mutation but more *BRAF* mutation[12] and no aneuploidy.[29] In our series, we failed to identify any typical SSAs as precursor of our IBD-CRC cases. Whether this negative finding is suggestive of a low risk of malignant transformation of SSAs in IBD may be a question. On the other hand, the serrated lesions found in our cases all fit the class as serrated NOS CAD. Noticeably, all of the serrated lesions showed lack of *BRAF* mutation, a major molecular driver in colonic SSA,[7] which seems to support the notion that these lesions are indeed different from SSA in nature. This finding is in agreement with that reported by Srivastava *et al*,[7] but is different from two reports in which *BRAF* mutation was detected.[30 31] The different results may be related to the test methodology (DNA-based assay versus immunohistochemistry using anti-V600E antibody) as well as to the diagnostic criteria being used. It was also known that *BRAF* mutation and *KRAS* mutation are mutually exclusive and SSA with dysplasia had *KRAS* but no *BRAF* mutation.[32] In line with most published reports, the majority of our serrated lesions had *KRAS* mutation and some had mutant-type p53 expression but no MMR deficiency, which again supports the neoplastic nature of such serrated lesions.

Similarly, a less common hypermucinous lesion has been noticed in IBD setting. It was first described by Morson *et al* as villous or flat epithelium with abundant, elongated and distended goblet cells but no nuclear atypia.[14] Anderson *et al* further demonstrated the precancerous nature of this type of 'hypermucinous and villous epithelial changes' by showing *KRAS* mutation in more than 60% of such lesions seen in patients with longstanding UC.[15] Similar lesions were later reported in CD as well, associated with up to 30% of enteric carcinomas in patients with CD.[16 17] This type of lesion is now included into the latest WHO classification and is renamed as 'mucinous dysplasia'.[20] Our study again showed *KRAS* mutation in half of the mucinous lesions as well as frequent aberrant p53 and IMP3 and sometimes loss of MLH1/PMS2, which are strong evidence of the neoplastic nature of this type of lesion.

Contrary to the mucin-rich lesion, a type of mucin-lacking epithelial changes featured by absence of goblet cells has also drawn attention. Loss/depletion of mucin-containing goblet cells is a phenomenon commonly seen in reactive intestinal epithelium during mucosal injury, particularly inflammatory condition such as infection and IBD.[31] It was considered the glandular epithelium immaturation due to failure of goblet cell differentiation from the progenitor cells. Interestingly, the goblet cell-lacking phenotype is also seen in a rare form of apparently

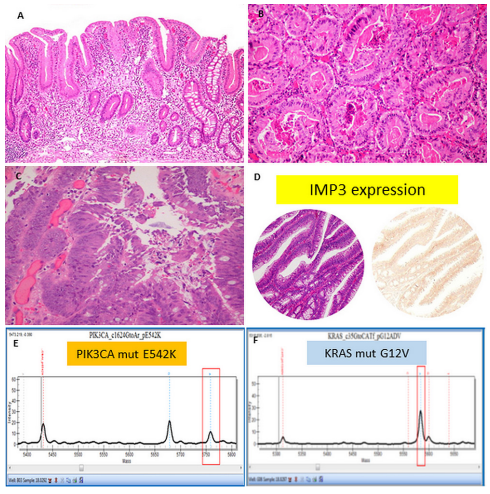

Figure 3 A case of mixed eosinophilic and adenomatous lesions: Case ID#13 (a 81-year-old woman with UC developed a non-mucinous adenocarcinoma in sigmoid colon). (A) Adjacent eosinophilic lesion with absence of goblet cells and lack of frank cytologic atypia (50×). (B) High-grade eosinophilic dysplasia, with eosinophilic secretion in the lumen of glands. (C) Mixed area of adenomatous changes (200×). (D) The adenomatous lesion represented on TMA and immunohistochemical stain showing IMP3 overexpression (not in eosinophilic type). (E) Identical *PIK3CA* E542K mutation shared by both eosinophilic and adenomatous lesions. (F) *KRAS* G12V mutation detected in adenomatous lesion only. UC, ulcerative colitis,

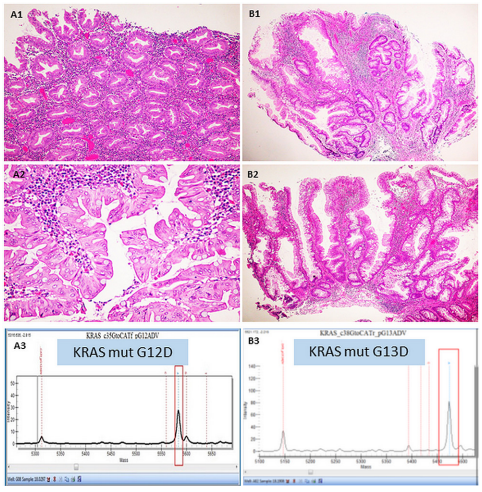

Figure 4 Comparison of two cases of serrated lesion: Case A (ID#6, a 41-year-old man with UC developed a mucinous carcinoma in sigmoid) and Case B (ID#35, a 50-year-old man with UC developed a non-mucinous carcinoma in hepatic flexure). (A1–A2) Serrated NOS lesion with no adenomatous changes (100× and 200×). (A3) *KRAS* mutation (G12D) detected. (B1–B2) Similar serrated NOS lesion with no adenomatous changes (100× and 200×). (B3) *KRAS* mutation (G13D) detected. UC, ulcerative colitis.

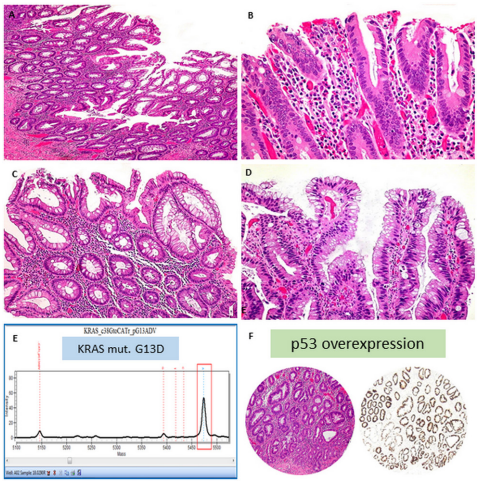

Figure 5 Mixed serrated and differentiated lesions: Case ID#7 (a 44-year-old man with UC developed a mucinous carcinoma in transverse colon). (A/C) Serrated lesion (100× and 200×). (B/D) Differentiated lesion (100× and 200×). (E) *KRAS* mutation (G13D) detected in serrated lesion, but not in differentiated type. (F) Both types of the lesions shared mutant-type p53 overexpression (corresponding tissue core in TMA on H&E stain and p53 immunohistochemical stain). TMA, tissue microarray; UC, ulcerative colitis.

neoplastic changes in which mild nuclear atypia is accompanied. The lesional epithelial cells also have ample cytoplasms that appear eosinophilic on H&E stain, for which this type of changes was initially designated as *eosinophilic dysplasia*, a term also coded for a rare variant of dysplasia found in cervix

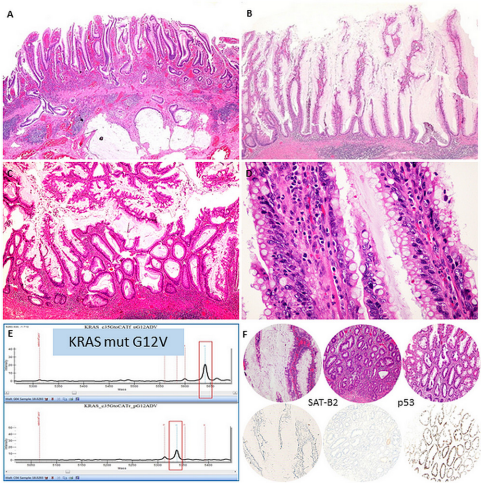

Figure 6 Mixed mucinous, differentiated and serrated lesion: Case ID#51 (a 43-year-old woman with CD developed a mucinous carcinoma in hepatic flexure, with at least three types of non-adenomatous lesions identified). (A) A panoramic view of the carcinoma (20×), including invasive mucinous carcinoma and overlying mucosa showing mucinous changes with mild cytological atypia. (B) Adjacent mucosa with villiform mucinous changes without frank cytological atypia. (C) Adjacent serrated lesion (100×). (D) Adjacent differentiated lesion (200×). (E) Identical *KRAS* mutation (G12V) detected in both mucinous and serrated lesions but not in differentiated lesion. (F) The same lesions represented on TMA (left - mucinous lesion with loss of SAT-B2; middle - differentiated lesion with loss of SAT-B2 and p53 overexpression (not shown); right - serrated lesion with p53 overexpression, also with loss of SAT-B2 (not shown)). CD, Crohn's disease.

and gallbladder.[33 34] It was also known as 'impaired or incomplete goblet cell maturation'. This type of changes in IBD has been interpreted by many as indefinite for dysplasia. Recently, it has been recognised as another variant of CAD, and *goblet cell-depleted* or *goblet cell-deficient dysplasia* has become the favoured term. However, this type of dysplasia in IBD has never been fully described in details but only been mentioned briefly by some experts (personal communication as well as lectures and review articles of Noam Harpaz and Robert Riddell[19 35 36]) until it is included into the latest WHO classification recently.[20] In the cases we present here, mild nuclear atypia and mixed focal adenomatous dysplasia were common, and sequential progress from low-grade to high-grade dysplasia and to invasive carcinoma was clearly appreciated. Furthermore, several molecular abnormalities including *KRAS*, *PIK3CA*, p53 and MLH1/PMS2, similar to that in adenomatous dysplasia, have been detected. Taken together, the neoplastic nature of this type of lesion is doubtless.

Dysplasia with terminal epithelial cell differentiation, in short as 'differentiated dysplasia' in our study, is another newly recognised variant of CAD. It was also termed 'crypt dysplasia' in the latest WHO classification.[20] In this type of lesion both nuclear atypia and architectural alteration are only very mild or minimal, which does not reach the degree to be classified as classic low-grade dysplasia by the traditional criteria, so that it may be overlooked and deceivingly gives an impression of no dysplasia in some patients. Although it has been mentioned by some experts (personal communication, and lectures given by Noam Harpaz and Robert Riddell to USCAP[19 35]), no further study on this type of lesion has been reported. Here, we demonstrated molecular evidence suggesting this type of lesions can be regarded as neoplastic, mainly *TP53* and *KRAS* mutations, as commonly seen in the aforementioned other types of CAD.

The general molecular mechanisms underlying the colitis-associated neoplasia are the sequential accumulation of somatic mutations induced largely by inflammatory cytokines, complicated by epithelial cell damage and regeneration/repairing over the long course of disease. Due to the large extent of disease involvement, the mucosal genetic landscape changes are widespread and cause the so-called *field effects* including a variety of genetic abnormalities.[37–41] Considerable mutation burden was detected in the non-dysplastic mucosa adjacent to carcinoma.[41] Moreover, the patchy nature of inflammation and regenerative changes as well as other factors (e.g., intestinal microbiome) would cause heterogeneity of the field effects in different areas of bowel, which lead to development of multifocal and histologically variable preneoplastic lesions in which CRC may arise. Studies on the molecular basis of the different morphological types of CAD have just emerged. Patil *et al* found by immunohistochemistry that adenomatous and hypermucinous dysplasia involve mostly aberrant p53 (in 33% and 75% of cases, respectively), whereas serrated and differentiated dysplasia are associated with combined aberrant expression of p53 and β-catenin (in about 50% of cases for both types).[30] These data are similar to, although not fully consistent with ours. More studies on larger series of cases by comprehensive genetic analysis to further determine the different morphology-specific molecular profiles are expected. In addition to the genetic mechanisms, the possibility of the massive local inflammatory environment in chronic active colitis eliciting a negative or positive selection of antigenicity and hence causes a shift in certain molecular/protein phenotypes of the neoplasm also remains to be addressed.

Whether these lesions are monoclonal or polyclonal is unknown due to lack of direct evidence, although as neoplasia

they are presumed to be resulted from the proliferation of clonal population. Mutant clone evolution and clone expansion, as determined by clonal length altering mutations in polyguanine tracts, have been detected in colonic mucosa with UC, particularly in those with neoplasia developed, with clonal population found in both neoplastic lesion and in distant nonneoplastic tissue.[38 39] When considering the morphological heterogeneity, however, the presence of multiple clones in each lesion is also possible. In fact, an early study on the p53 gene mutation in UC-associated adenomatous low-grade dysplasia revealed polyclonal rather than monoclonal changes, even within the microdissected single crypts.[42]

Whether the different morphological types of the precancerous lesions are correspondingly or preferentially associated with certain histological subtypes or grade/differentiation of carcinomas remains uncertain. In our data, the serrated lesion seems to be slightly more associated with mucinous carcinoma, whereas goblet cell-depleted dysplasia was less associated with mucinous carcinoma; however, the differences did not reach statistical significance. Between cases with different types of CAD, the histological grade of carcinoma was similar; and no any type was related to a specific type or variant of carcinoma. Differentiated dysplasia was not necessarily associated with low-grade and well-differentiated carcinoma such as low-grade tubuloglandular carcinoma,[43] although in one of our cases it was indeed related to a very well differentiated adenocarcinoma. Crolleau *et al* found that the hypermucinous dysplasia was associated with aggressive mucinous or signet-ring cell carcinomas.[17] A group led by Robert Riddell reported an association of villous adenomatous dysplasia with mucinous carcinoma and goblet cell-depleted dysplasia with low-grade tubuloglandular carcinoma.[44] Another recent study from the same group reported loss of SATB2 expression in more than half of IBD-CRCs,[45] which is also different from our precursor lesion study in which SATB2 loss was seen in only 15% of the non-adenomatous lesions in general. Whether the SATB2-negative CADs have a higher risk to progress to carcinoma may be an interesting question. Prospective studies on the risk of cancer arising from different types of dysplasia are needed.

Last, based on our study and others, all of the non-adenomatous lesions should be equally regarded as neoplastic, although it remains to be discussed whether or not we should call them all 'dysplasia', considering the classic cytologic dysplasia is usually missing. In our opinion, it is no longer appropriate and not recommended to designate any of the non-adenomatous lesions as 'indefinite for dysplasia' in pathology report.

Take home messages

- Inflammatory bowel disease (IBD)-associated precancerous lesions, or colitis-associated dysplasia, are heterogeneous in both histological and molecular features.
- Non-adenomatous bowel lesions seen in long-standing IBD may be subclassified as mucinous (hypermucinous and villous), eosinophilic (or goblet cell depleted), differentiated (dysplasia with terminal epithelial differentiation), serrated (serrated NOS), sessile serrated adenoma-like and traditional serrated adenoma-like types.
- Non-adenomatous lesions without frank cytological dysplasia should also be regarded as neoplastic and variants of IBD-associated precancerous lesions.
- Mixed types/features are common in a single lesion or in different areas/regions of bowel in cases of IBD with neoplasia.
- Focal adenomatous cytological dysplasia is commonly present in all types of non-adenomatous lesions, which may suggest that adenomatous change is a necessary step during the progress of the no-adenomatous lesions into carcinoma.
- Molecular alterations in IBD-associated precancerous lesions are heterogeneous. Many colon cancer-related genetic events are shared by all types of the precancerous lesions. No specific molecular-morphological correlation has been identified by studies so far.

Correction notice The paper has been corrected since it appeared Online First. The author Marco Perizzolo's name was mispelled as 'Perrizzolo' and has been corrected.

Handling editor Runjan Chetty.

Contributors XG designed the study, performed the pathology review, selected and collected the tissue samples for study, read the immunohistochemistry, analyse the entire data and wrote the manuscript which was reviewed by all coauthors. MK provided some antibodies and read the immunohistochemistry. PFR read the immunohistochemistry. JF, MI and SG performed clinical and endoscopic data review. SL and YO performed the tissue punching, TMA construction and immunohistochemistry. MP and RJW performed molecular testing and analysed the results. DJD helped study design and reviewed molecular testing results.

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REFERENCES

1 Blackstone MO, Riddell RH, Rogers BHG, *et al*. Dysplasia-associated lesion or mass (DALM) detected by colonoscopy in long-standing ulcerative colitis: an indication for colectomy. *Gastroenterology* 1981;80:366–74.
2 Chiu K, Riddell RH, Schaeffer DF. DALM, rest in peace: a pathologist's perspective on dysplasia in inflammatory bowel disease in the post-DALM era. *Mod Pathol* 2018;31:1180–90.
3 Lee LH, Iacucci M, Fort Gasia M, *et al*. Prevalence and anatomic distribution of serrated and adenomatous lesions in patients with inflammatory bowel disease. *Can J. Gastroenterol. Hepatol* 2017;2017.
4 Jackson WE, Achkar J-P, Macaron C, *et al*. The significance of sessile serrated polyps in inflammatory bowel disease. *Inflamm Bowel Dis* 2016;22:2213–20.
5 HM K, Harpaz N, McBride RB, *et al*. Serrated colorectal polyps in inflammatory bowel disease. *Mod. Pathol* 2015;28:1584–93.
6 Yamane L*et al*. Serrated pathway in colorectal carcinogenesis. *WJG* 2014;20:2634–40.
7 Srivastava A, Redston M, Farraye FA, *et al*. Hyperplastic/serrated polyps in inflammatory bowel disease: a case series of a previously undescribed entity. *Am. J. Surg. Pathol* 2008;32:296–303.
8 Shen J, Gibson JA, Schulte S, *et al*. Clinical, pathologic, and outcome study of hyperplastic and sessile serrated polyps in inflammatory bowel disease. *Hum Pathol* 2015;46:1548–56.
9 Johnson DH, Khanna S, Smyrk TC, *et al*. Detection rate and outcome of colonic serrated epithelial changes in patients with ulcerative colitis or Crohn's colitis. *Aliment Pharmacol Ther* 2014;39:1408–17.
10 Parian A, Koh J, Limketkai BN, *et al*. Association between serrated epithelial changes and colorectal dysplasia in inflammatory bowel disease. *Gastrointest Endosc* 2016;84:87–95.
11 Setaffy L, Högenauer C, Lemmerer M, *et al*. Large serrated polyp with KRAS mutation in inflammatory bowel disease: a "nondysplastic dysplasia-associated lesion or mass (DALM)"? *Endoscopy* 2013;45:E235–6.
12 Johnson D, Taylor WR, Aboelsoud MM, *et al*. DNA methylation and mutation of small colonic neoplasms in ulcerative colitis and Crohn's colitis: implications for surveillance. *Inflamm. Bowel. Dis* 2016;22:1559–67.
13 Kamarádová K, Vošmiková H, Rozkošová K, *et al*. Morphological, immunohistochemical and molecular features of inflammatory bowel disease

associated colorectal carcinoma and associated mucosal lesions – single institution experience. *Pathol Res Pract* 2019;215:730–7.
14 Morson BC, Jass JR, Sobin LH. *Precancerous lesions of the gastrointestinal tract. A histological classification, volume 1*. London: Balliere Tindall, 1985: 139.
15 Andersen SN, Lovig T, Clausen OPF, *et al*. Villous, hypermucinous mucosa in long standing ulcerative colitis shows high frequency of K-ras mutations. *Gut* 1999;45:686–92.
16 Kilgore SP, Sigel JE, Goldblum JR. Hyperplastic-Like mucosal change in Crohn's disease: an unusual form of dysplasia? *Mod Pathol* 2000;13:797–801.
17 Crolleau C, Pote NM, Guedj N, *et al*. Small bowel adenocarcinoma complicating Crohn's disease: a single-centre experience emphasizing the importance of screening for dysplasia. *Virchows. Arch* 2017;471:611–7.
18 Harpaz N, Goldblum JR, Shepherd N, *et al*. Novel classification of dysplasia in IBD. *Mod. Pathol* 2017;30.
19 Riddell R. Dysplasia (and serrated lesions) in IBD. *Rodger Haggitt Memorial Lecture to Gastrointestinal Pathology Society at USCAP Annual Meeting*, 2017. Available: https://handouts.uscap.org/AN2017/2017_CM16_Ridde_0501_post.pdf
20 Odze RD, Harpaz N. Inflammatory bowel disease-associated dysplasia of the colorectum. In: *Who classification of tumors. digestive system tumors. 5th edition. who classification of tumors editorial board. International research agency on cancer*, 2019: 174–6.
21 Köbel M, Piskorz AM, Lee S, *et al*. Optimized p53 immunohistochemistry is an accurate predictor of TP53 mutation in ovarian carcinoma. *J. Pathol. Clin. Res* 2016;2:247–58.
22 Djordjevic B, Hennessy BT, Li J, *et al*. Clinical assessment of PTEN loss in endometrial carcinoma: immunohistochemistry outperforms gene sequencing. *Mod Pathol* 2012;25:699–708.
23 Alkushi A, Köbel M, Kalloger SE, *et al*. High-Grade endometrial carcinoma: serous and grade 3 endometrioid carcinomas have different immunophenotypes and outcomes. *Int J Gynecol Pathol* 2010;29:343–50.
24 Köbel M, Anglesio MS, Brenton JD. You won't believe this old test … that does cheap single-cell mutation detection. *J Pathol Clin Res* 2018;4:149–53.
25 Baniak N, Fadare O, Köbel M, *et al*. Targeted molecular and immunohistochemical analyses of endometrial clear cell carcinoma show that pole mutations and DNA mismatch repair protein deficiencies are uncommon. *Am J Surg Pathol* 2019;43:531–7.
26 Bennett WP, Hollstein MC, Hsu I-C, *et al*. Mutational spectra and immunohistochemical analyses of p53 in human cancers. *Chest* 1992;101:19S–20.
27 Köbel M, Ronnett BM, Singh N, *et al*. Interpretation of p53 immunohistochemistry in endometrial carcinomas: toward increased reproducibility. *Int J Gynecol Pathol* 2019;38:S123–31.
28 Kriegsmann M, Arens N, Endris V, *et al*. Detection of KRAS, NRAS and BRAF by mass spectrometry - a sensitive, reliable, fast and cost-effective technique. *Diagn Pathol* 2015;10:132–42.
29 Choi W-T, Wen KW, Rabinovitch PS, *et al*. Dna content analysis of colorectal serrated lesions detects an aneuploid subset of inflammatory bowel disease-associated serrated epithelial change and traditional serrated adenomas. *Histopathology* 2018;73:464–72.
30 Patil DT, Goldblum JR, Odze RD. Immunohistochemical and molecular characterization of dysplasia subtypes in ulcerative colitis. *Mod Pathol* 2017;30.
31 Bossard C, Denis MG, Bézieau S, *et al*. Involvement of the serrated neoplasia pathway in inflammatory bowel disease-related colorectal oncogenesis. *Oncol Rep* 2007;18:1093–7.
32 Bergstrom KSB, Guttman JA, Rumi M, *et al*. Modulation of intestinal goblet cell function during infection by an attaching and effacing bacterial pathogen. *Infect Immun* 2008;76:796–811.
33 Ma L, Fisk JM, Zhang RR, *et al*. Eosinophilic dysplasia of the cervix: a newly recognized variant of cervical squamous intraepithelial neoplasia. *Am J Surg Pathol* 2004;28:1474–84.
34 Fadare O, DeMartini SD. Eosinophilic dysplasia of the gallbladder: a hitherto undescribed variant identified in association with a porcelain gallbladder. *Diagn Pathol* 2006;1:15.
35 Harpaz N, Ward SC, Mescoli C, *et al*. Precancerous lesions in inflammatory bowel disease. *Best Pract Res Clin Gastroenterol* 2013;27:257–67.
36 Harpaz N, Polydorides AD. Colorectal dysplasia in chronic inflammatory bowel disease: pathology, clinical implications, and pathogenesis. *Arch Pathol Lab Med* 2010;134:876–95.
37 Rinehart EM, Nowak J, Lindeman NI, *et al*. Molecular profiling of dysplasia subtypes in inflammatory bowel disease: a comparative analysis with sporadic precursors of colorectal carcinoma. *Mod Pathol* 2017;30.
38 Salk JJ, Salipante SJ, Risques RA, *et al*. Clonal expansions in ulcerative colitis identify patients with neoplasia. *Proc Natl Acad Sci U S A* 2009;106:20871–6.
39 Salk JJ, Bansal A, Lai LA, *et al*. Clonal expansions and short telomeres are associated with neoplasia in early-onset, but not late-onset, ulcerative colitis. *Inflamm Bowel Dis* 2013;19:2593–602.
40 Baker KT, Salk JJ, Brentnall TA, *et al*. Precancer in ulcerative colitis: the role of the field effect and its clinical implications. *Carcinogenesis* 2018;39:11–20.
41 Baker A-M, Cross W, Curtius K, *et al*. Evolutionary history of human colitis-associated colorectal cancer. *Gut* 2019;68:985–95.
42 Yoshida T, Mikami T, Mitomi H, *et al*. Diverse p53 alterations in ulcerative colitis-associated low-grade dysplasia: full-length gene sequencing in microdissected single crypts. *J Pathol* 2003;199:166–75.
43 Levi GS, Harpaz N. Intestinal low-grade tubuloglandular adenocarcinoma in inflammatory bowel disease. *Am J Surg Pathol* 2006;30:1022–9.
44 Iwaya M, Ota H, Tateishi Y, *et al*. Relationship between carcinoma subtype and overlying dysplasia in IBD-associated colorectal carcinoma. *Mod. Pathol* 2018;31.
45 Iwaya M, Ota H, Tateishi Y, *et al*. Colitis-Associated colorectal adenocarcinomas are frequently associated with non-intestinal mucin profiles and loss of SATB2 expression. *Mod Pathol* 2019;32:884–92.