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Title:

Revisiting the Distinct Histomorphologic Features of Inflammatory Bowel Disease - Associated Neoplastic Precursor Lesions in SCENIC and Post-DALM Era

Short Running Title:
Distinct morphologic features of IBD-associated neoplastic precursors

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Conflict of interest

All authors declare no conflicts of interest

ABSTRACT

Distinct histomorphologic features of colitis-associated dysplasia (CAD) or neoplastic precursors in inflammatory bowel disease (IBD) have never been clearly identified. In this study, we tried to further explore the differentiating morphologic features of CAD by retrospectively reviewing the lesions that were clearly associated with carcinomas (carcinoma-related lesions), and by comparing between the endoscopically nonpolypoid (non-adenoma-like) lesions and polypoid (adenoma-like) lesions and sporadic conventional adenomas found in non-colitic mucosa and in non-IBD individuals. Our study results have revealed that (1) precursor lesions related to IBD-associated colorectal carcinomas were almost always nonpolypoid in macroscopic/endoscopic appearance; (2) nearly half of the carcinoma-related lesions and nonpolypoid lesions were similarly non-adenomatous (non-conventional) lesions, largely serrated type, with no or only mild/focal adenomatous dysplasia, and commonly had mixed adenomatous and non-adenomatous features; (3) carcinoma-related and nonpolypoid adenomatous dysplastic lesions frequently showed some peculiar histocytologic features that we observed and described for the first time, including mixed features of inflammatory pseudopolyp or granulation tissue, pleomorphic and disarrayed nuclei, micropapillary or hobnailing surface epithelial cells, and microvesicular or bubbling cytoplasm of dysplastic cells; and (4) polypoid lesions in colitic mucosa were identical to sporadic adenomas in non-inflamed mucosa and in non-IBD patients, and they lacked the aforementioned features. The seemingly distinctive morphologic characteristics that we proposed here, although still not absolutely specific or unique, can be used as the features of inclusion for identifying the colitis-associated dysplasia on endoscopic biopsies when the endoscopy images are not readily available to the pathologists, and thus to alert clinician for a closer follow-up.

Key words:

Inflammatory bowel disease (IBD), dysplasia, colitis-associated dysplasia (CAD), precancerous lesion, polypoid lesion, nonpolypoid lesion

INTRODUCTION

Longstanding inflammatory bowel disease (IBD), including ulcerative colitis (UC) and Crohn's disease (CD), predisposes to development of colorectal carcinoma (CRC) through inflammation-mediated pathway(s) of carcinogenesis. Identification of IBD/colitis-associated neoplastic precursors (precancerous lesions) has been a challenging task to both clinicians and pathologists.

The conventional precancerous lesion in IBD has been traditionally identified and defined as *dysplasia*, *i.e.*, the lesional mucosa with epithelium showing low- to high-grade cytologic dysplasia, and the lesion is usually elevated/raised (protruding above mucosal surface), although can also be flat or barely visible on colonoscopy. On the other hand, the precursor lesion of CRC in general population is mostly polypoid sporadic conventional adenoma (SCA) which is also histologically defined by cytologic low-grade dysplasia (adenomatous change). Endoscopically, depending on the appearances, the elevated lesions seen in IBD patients have been traditionally subclassified into '*adenoma-like*' (pedunculated or sessile polypoid, with dome-shape and symmetric contour, smooth surface, and well-delineated border) and '*non-adenoma-like*' (multinodular or plaque-like, irregularly-shaped, with ill-defined border) [1]. The latter one has been assumed to represent the true '*colitis-associated dysplasia (CAD)*', *i.e.,* dysplastic lesion being resulted directly from chronic mucosal inflammation and occurring uniquely in IBD. For decades, it was also popularly coded as 'dysplasia-associated lesion or mass (DALM) [2], a term eventually became confusing and misused and now is recommended to abandon [3]. For the adenoma-like lesions, on the other hand, it is still uncertain if they are simply sporadic conventional adenomas in nature but happen to grow in the inflamed mucosa, or if all or some are indeed directly induced and/or promoted by chronic inflammatory environment and thus are

different and specific to individuals with IBD, although a similar lesion occurred in non-colitic area would otherwise be classified as sporadic adenomas.

For decades the distinction between CAD and sporadic adenomas has been considered to be critical, since the diagnosis of CAD (or DALM as the widely used term then) would mandate total colectomy. However, it is no longer the current standard of practice. With the progress of endoscopic techniques, gastroenterology guidelines have also changed the recommendations about the management of IBD-associated dysplastic lesions, in both detection and management. Most of the elevated lesions are now simply be removed endoscopically, as long as they are amenable, and the patients then continue to be followed up by colonoscopic surveillance. On the other hand, with the popular use of chromoendoscopy and high-definition endoscopy, more subtle non-adenoma-like lesions are detected and more targeted biopsies are obtained. The changes in the clinical and endoscopical practice are largely reflected by the development of SCENIC (**S**urveillance for **C**olorectal **E**ndoscopic **N**eoplasia Detection and Management in Inflammatory Bowel Disease Patients: **I**nternational **C**onsensus Recommendations) classification of the IBD dysplastic lesions in 2015, based on the consensus reached by an international group, in which the terms *adenoma-like* and *non-adenoma-like* are abandoned and the classification of the suspicious lesions are simplified into *visible* versus *invisible*, and the visible lesions are further subclassified into *polypoid* (pedunculated or sessile) and *nonpolypoid* (superficial elevated, flat, or depressed) [4].

While the recognition of true IBD-associated neoplastic precursors, particularly the distinction between CAD and sporadic adenoma, may no longer affect the individual patient's clinical management, the distinction is still of clinical and scientific interest, from the prospective of

better defining CAD histologically, understanding the CAD-specific biologic behaviors and underlying the molecular mechanisms as compared to sporadic adenoma.

It has been a common experience and stigma to the pathologists that CAD and sporadic adenoma are histomorphologically so similar that to distinguish between the two on histology alone is almost impossible, especially on the fragmented endoscopic biopsies and in the absence of endoscopic correlation. Specific and convincing differentiating histomorphologic features have been lacking, although a few subtle features have been recommended to be relatively suggestive of one over the other [5-11]. Specific differentiating biomarker of CAD has also been lacking, due to no success in discovering unique molecular alterations in CAD, which was partly complicated by the poor histopathological definition of the true CAD.

Meanwhile, in addition to the conventional adenomatous dysplastic lesions, several other forms of colitis-associated intestinal epithelial changes/lesions and their association with enterocolonic carcinomas in IBD patients have also been increasingly noticed by the pathology community. In these less common lesions, the lesional epithelium appears prominently serrated but not exactly the same as sessile serrated adenoma/lesion (SSA/L) or traditional serrated adenoma (TSA), or hypermucinous and villiform, or deficient in goblet cell differentiation, while it shows no or only minimal to mild cytologic atypia. The neoplastic nature of these non-conventional lesions has been recognized and are considered the variants of CAD [12-16].

In this study, we tried again to further explore the differentiating morphologic features of CAD by retrospectively reviewing the lesions that are clearly associated with carcinomas, and by comparing between the endoscopically nonpolypoid (non-adenoma-like) and polypoid (adenoma-like) lesions and the sporadic adenomas found in non-IBD individuals.

MATERIALS and METHODS:

Study Cohorts

Five groups of lesions were enrolled into the study:

(1) Carcinoma-related lesions, *i.e.*, the background lesions at the periphery of or/and adjacent to invasive carcinomas occurred in bowel region(s) involved by chronic colitis: 64 cases of surgically resected IBD (UC or CD)-associated colorectal invasive adenocarcinomas encountered at our institutions in Calgary and Seattle and retrieved through the Anatomic Pathology database. All archived slides of the surgical resection specimens and their associated pre-operative endoscopic biopsies that led to the diagnosis of carcinoma were reviewed. The putative precancerous lesions in the background of carcinomas and/or in proximity to carcinomas were searched and analyzed. 52 lesions were identified in 50 of the 64 cases. None except one of the lesions were polypoid lesions upon review of the gross description of the surgical specimens and the preoperative endoscopy reports (if available). 28 lesions were identified in the regions where the mucosa was described to be nodular, rough, slightly elevated, or depressed on the original gross descriptions. The rest of the lesions were identified on the slides and appeared to be flat; however, the exact macroscopic appearance of the original mucosal sites was not known. In the only one exception a classic tubular adenoma as the precursor was determined upon reviewing the combined endoscopic and histologic features (morphologic contour of lesion and nuclear features) of the previously removed overlying polypoid lesion (not shown here). This case was removed from further comparative analysis.

(2) Nonpolypoid (non-adenoma-like) lesions, *i.e.*, the elevated lesions that did not appear like conventional adenomatous polyps on endoscopy, characterized by mildly and irregularly

elevated, multinodular, plaque-like, or villous/villiform, broad-based, mostly with indistinct boundaries. The lesions were all detected during surveillance colonoscopies in longstanding UC and CD patients and located within the regions with chronic active or inactive colitis. All lesions were detected and were all removed or biopsied endoscopically.

(3) Polypoid (adenoma-like) lesions, i.e., the elevated lesions that looked like adenomatous polyps on endoscopy, characterized by single, well circumscribed, dome-shaped or pedunculated, adenoma-looking polyps seen in the bowel regions involved by chronic active or quiescent colitis. 5 lesions were identified on carcinoma-related colectomy specimens, which were located in different regions of bowel far away from (>10 cm) the carcinoma and were classified based on the gross appearance. 55 lesions were all removed endoscopically during colonoscopic surveillance and were selected based on their endoscopic appearance upon reviewing the corresponding colonoscopic images, and the colitic mucosa background was confirmed by chart review including previous colonoscopic and biopsy histologic findings.

(4) Sporadic conventional adenomas in IBD patients, i.e., elevated lesions that looked like adenomatous polyps, characterized by single, well circumscribed, dome-shaped sessile or pedunculated, adenoma-looking polyps seen in colorectal mucosa uninvolved by colitis. 32 cases were selected by correlating colonoscopic reports and images with pathologic reports, and the colitis-free background was confirmed by chart review including previous colonoscopic and biopsy histologic findings.

(5) Sporadic conventional adenomas in non-IBD patients, i.e., the non-syndromic conventional adenomas in non-IBD patients. 60 lesions were detected in patients who received colonoscopy for colon cancer screening and otherwise had no colorectal disease, and these lesions were partly

randomly selected from the Anatomic Pathology database and partly collected on purpose through the first author's daily sign-out work to include some tubulovillous adenomas and some adenomatous polyps with certain unusual/atypical features (*e.g.*, severely inflamed, with bottom-up pattern of dysplasia, with marked nuclear pleomorphism, with mixed mucin-rich foci, etc.). All of the lesions were further confirmed by correlating with colonoscopic images.

The demographic details of the study subjects are shown in Table 1.

The study was approved by Institutional Review Board (IRB) at both University of Calgary and University of Washington.

Study Design

All of the lesions were further analyzed for their morphologic features, including the glandular architecture and epithelial cytologic changes. The lesions in the groups (1) to (3) were firstly subclassified into adenomatous lesion (*i.e.,* with adenomatous cytologic low-grade dysplasia), sessile serrated adenoma (SSA, recently termed sessile serrated lesion /SSL in the latest WHO classification), traditional serrated adenoma (TSA), and four other types of non-adenomatous (or non-conventional) lesions (*i.e.,* with no conventional adenomatous cytologic dysplasia) according to a recently proposed classification of IBD-associated dysplasia and the latest WHO classification [12-14], including at least mucinous/hypermucinous lesion (*mucinous**), goblet cell-deficient (*eosinophilic**) lesion, dysplasia with terminal epithelial differentiation (*differentiated* dysplasia*), and serrated not otherwise specified (NOS) (*serrated**) (* modified terms used the authors in this article and our previous reports [15,16]). Some of the serrated lesions were classified as SSA-like or TSA-like types when the lesion has the characteristic

features. The detailed descriptions of the four major types of the non-conventional patterns are summarized in Table 2.

All of the adenomatous lesions were also further analyzed for several unreported peculiar histocytologic features noticed by the first author (XG) based on his own observations, as listed in Table 4.

Statistics

Chi Square test of independence was performed using Microsoft Excel to compare the difference of the detection rate of each morphologic feature. A p value < 0.05 was considered to be statistically significant.

RESULTS

As shown in Table 3, for both of the carcinoma-related lesions and the endoscopically nonpolypoid lesions, approximately 40% were non-adenomatous/non-conventional lesions in different histomorphologies, including mucinous, differentiated, eosinophilic, and serrated lesions, as described in Table 2 and illustrated in Figures 1. The serrated lesions were particularly common, consisting in 13% of the two groups of lesions combined, and they could be prominent and large, as demonstrated by an unusual case shown in Figure 3. It was a large multinodular and villous appearing lesion in sigmoid in a patient with longstanding UC, and it has been gradually enlarged, up to 6 cm in length and be circumferential, during 4 years of follow-up prior to surgical resection. The index biopsy and multiple subsequent biopsies were interpreted as hyperplastic polyp and inflammatory polyp. Upon review all samples, the lesion was recognized as a serrated lesion, NOS. On immunohistochemistry, the lesion showed no BRAF V600E mutation or mismatch repair deficiency, while the lesional epithelial cells showed absence of p53 expression, probably suggestive of *TP53* mutation (stopgain/indel/splicing mutation).

The adenomatous dysplastic lesions, still the majority in the carcinoma-related and nonpolypoid lesions, also had mixed foci of various non-adenomatous morphologic components in a small number (ranging approximately from 2% to 15%) of cases, as shown in Table 3 and illustrated in Figure 4, including serration (sawtoothed configuration of cryptal and surface epithelium), which was most common, hypermucinous epithelium, and eosinophilic change (loss of goblet cells and eosinophilic cytoplasm).

In comparison, none of the endoscopically polypoid lesions showed any of the non-adenomatous features, as demonstrated in Figure 5, except rare lesions (less than 7%) met the criteria of sessile serrated adenomas/lesions (with no cytologic dysplasia). Similarly, the sporadic conventional adenomas, both of those occurred in bowel regions uninvolved by colitis in IBD patients and those in non-IBD individuals, only occasionally showed a very focal mucin-rich or eosinophilic or serrated epithelial changes (in less than 6% of adenomatous polyps).

Furthermore, as shown in Table 3 and illustrated in Figures 6, the IBD-associated adenomatous dysplastic lesions in carcinoma-related and nonpolypoid lesions frequently showed some peculiar histocytologic features that were not or only occasionally and focally seen in polypoid lesions and sporadic adenomas. These features, especially collectively, seemed to nicely distinguish the polypoid and nonpolypoid dysplastic lesions. One is the presence of mixed features (or as a background) of inflammatory pseudopolyp characterized by considerable composition of necroinflammatory tissue, often as granulation tissue, and dropout of glands. In some lesions, the dysplastic epithelium was the only surviving epithelium in otherwise a background of necroinflammatory tissue, which may produce a picture of strip(s) of dysplastic epithelium mounting on the top of granulation tissue or an isolated dysplastic island in the middle of granulation tissue. The second is the higher grade nuclear features as compared to conventional adenoma, including the significant nuclear pleomorphism (variable size of nuclei) and sometimes disarrayed nuclear arrangement within the dysplastic epithelium, while they are different from the usual pattern of high-grade dysplasia in non-IBD settings with regards to the absence of glandular architectural complexity. The other peculiar features include the micropapillary or hobnailing surface epithelial cells and microvesicular or bubbling cytoplasm of dysplastic cells. These features was seen in about 20% to 40% of the nonpolypoid or carcinoma-

related adenomatous dysplastic lesions. Additionally, mixed focal or patchy non-adenomatous epithelial changes are common, as described above.

Figure 7 showed a good example of typical CAD with conventional dysplasia (colitis-associated adenomatous dysplastic lesion). It was a sessile lesion in rectum stump 3 years in a UC patient status post total colectomy. On both macroscopic and microscopic view, the lesion did not look like a conventional adenoma, although it displayed tubulovillous architecture. Molecular testing (by mass spectrometry using the Agena MassArray™ kit) detected a rare hotspot of *KRAS* mutation (A146T), and it was accompanied by loss of MLH1 and PMS2 (on immunohistochemistry), likely the result of *MLH1* gene promoter methylation, but no aberrant p53 expression.

On the other hand, certain characteristic features commonly seen in conventional adenomatous polyps, including the 'top-down' pattern of cytologic dysplasia and symmetric contour, were never seen in the nonpolypoid or carcinoma-related adenomatous CAD.

Of note, in many pathologists' experience, almost all of the aforementioned features of the non-adenomatous/non-conventional lesions, including serration, increased cytoplasmic mucin, and loss of goblet cells with or without eosinophilic cytoplasm, could be focally seen in inflammatory polyps and even in regenerating status of colitic mucosa, which may sometimes cause overinterpretation and also make people wondering if the non-adenomatous variants of CAD represent the neoplastic transformation of some prominent inflammation-induced transitional epithelial changes that were initially reactive and regenerative in nature.

Table 1. Demographic Data of Study Subjects

Patients & Lesions	Carcinoma-related lesions (Pt/lesion = 50/52) % (n)	Non-polypoid lesions (Pt/lesion = 34/34) % (n)	Polypoid lesions (Pt/lesion = 57/57) % (n)	Sporadic adenomas in IBD (Pt/lesion = 32/32) % (n)	Sporadic adenomas in non-IBD (Pt/lesion = 52/60) % (n)
IBD					
UC	60 (30)	59 (20)	61 (35)	59 (19)	0
CD	40 (22)	41 (14)	39 (22)	41 (13)	0
Gender					
Male	64 (32)	56 (19)	65 (37)	56 (18)	60 (31)
Female	36 (18)	44 (15)	35 (20)	44 (14)	40 (21)
Age	56 ± 12	62 ± 14	59 ± 18	60 ± 15	58 ± 10
Locations of lesions					
Cecum	14 (7)	12 (4)	11 (6)	9 (3)	7 (4)
Right colon	17 (9)	20 (7)	21 (12)	16 (5)	17 (10)
Transverse colon	12 (6)	12 (4)	12 (7)	9 (3)	13 (8)
Left colon	40 (21)	36 (12)	32 (18)	38 (12)	35 (21)
Rectum	17 (9)	20 (7)	24 (14)	28 (9)	28 (17)

Table 2. Non-adenomatous / Non-conventional Variants of Neoplastic Precursor Lesions in IBD

Type	Histocytologic Features
Mucinous (hypermucinous and villous dysplasia)	Villiform (villous or tubulovillous) architecture. Columnar cells with mucin-rich cytoplasm (or microvesicular or foveolar-like mucin cap) and nuclei being small or slightly enlarged with inconspicuous nucleoli, and basally oriented, with no or mild stratification. The degree of nuclear atypia decreases towards the tip of villi.
Differentiated (dysplasia with terminal epithelial differentiation)	Tubular or tubulovillous architecture. Enterocyte-type cells and goblet cells (slightly less in number, but not absent, and variable in size) with nuclei being mildly to markedly enlarged, round-to-oval, slightly irregular, hyperchromatic, with inconspicuous and occasional prominent nucleoli, and mostly non-stratified. In some cases, cytoarchitectural atypia is seen predominantly in deep/basal crypts (also known as crypt cell dysplasia).
Eosinophilic (goblet cell-deficient/depleted dysplasia)	Tubular architecture. Goblet cells are completely or near completely absent. Enterocyte-type cells with eosinophilic cytoplasm and nuclei being slightly enlarged or elongated and hyperchromatic, with no or mild stratification. Eosinophilic secretion within cryptal lumen may present.
Serrated (serrated dysplasia, NOS)	Serrated architecture in most of the lesion, may be mixed with villous architecture, involving upper portion or full thickness. Serrated crypts are distorted and complex, with no or occasional basal crypt dilatation or inversion, and no ectopic crypts (*i.e.*, no characteristic features of SSA or TSA). Enterocyte-type cells may show slightly eosinophilic and/or slightly mucin-rich cytoplasm, and with nuclei being small or mildly enlarged, oval-to-round, with inconspicuous or prominent nucleoli.
SSA-like (sessile serrated adenoma/lesion-like)	Serrated lesion with characteristic features of SSA, including dilatation of crypt base in boot- or L-shape, or in inverted T-shape, with enterocyte-type cells showing no or slightly eosinophilic and/or slightly mucin-rich cytoplasm, and with nuclei being small or mildly enlarged and mostly non-stratified, with inconspicuous nucleoli.
TSA-like (traditional serrated adenoma-like)	Mixed tubulovillous and serrated architecture, with ectopic crypts, and enterocyte-type epithelial cells showing eosinophilic cytoplasm and mildly elongated nuclei.

*References 12-14

Table 3. Comparison of Histocytologic Features between Carcinoma-related, Endoscopically Polypoid and Nonpolypoid Lesions

Histomorphologic features	Carcinoma-related lesions (N = 52) % (n)	Non-polypoid lesions (N = 34) % (n)	Combined (N = 86) % (n)	Polypoid lesions (N = 57) % (n)	Sporadic adenomas in IBD (N = 32) % (n)	Sporadic adenomas in non-IBD (N = 60) % (n)
Glandular architecture						
Tubular	54 (28)	26 (9)	43 (37)	84 (48)	84 (27)	68 (41)
Tubulovillous	27 (14)	35 (12)	34 (29)	9 (5)*	13 (4)	21 (13)
Villous	8 (4)	6 (2)	7 (6)	0	3 (1)	11 (7)
Serrated	11 (6)	33 (11)	26 (20)	7 (4)*	0	0
Novel IBD dysplasia classification						
Adenomatous	62 (32)	59 (20)	61 (52)	93 (54)	97 (31)	100 (60)
Mucinous/Hypermucinous ("mucinous")	10 (5)	8 (3)	9 (8)	0	0	0
Sessile serrated adenoma-like ("SSA-like")	0	6 (2)	2 (2)	7 (4)	0	0
Traditional serrated adenoma-like ("TSA-like")	2 (1)	0	1 (1)	0	0	0
Dysplasia with terminal differentiation ("Differentiated")	9 (5)	3 (1)	7 (6)	0	0	0
Goblet cell deficient/depleted ("Eosinophilic")	9 (5)	3 (1)	7 (6)	0	0	0
Serrated NOS	8 (4)	21 (7)	13 (11)	0	0	0
Mixed adenomatous and non-adenomatous lesions/epithelial changes						
Mucinous (hypermucinous epithelium)	17 (9)	6 (2)	13 (11)	4 (2)	6 (2)	5 (3)
TSA-like	2 (1)	3(1)	2 (2)	0	0	0
Differentiated	6 (3)	6 (2)	6 (5)	0	0	0
Eosinophilic (goblet cell depletion/eosinophilic cytoplasm)	13 (7)	9 (3)	12 (10)	2 (1)*	3 (1)*	3 (2)*
Serrated (sawtoothed epithelial configuration)	23 (12)	6 (2)	16 (14)	0	0	5 (3)
Mixed with more than 2 types	6 (3)	3 (1)	5 (4)	0	0	0
Adenomatous lesions with some peculiar features	(N = 36)	(N = 23)	(N = 59)	(N = 53)	(N = 32)	(N = 60)
Combined inflammatory pseudopolyp	20 (7)	35 (8)	25 (15)	0	0	0
Dysplastic island(s) amidst granulation tissue	17 (6)	22 (5)	19 (11)	0	0	2 (1)
Pleomorphic and disarrayed nuclei	36 (13)	39 (9)	37 (22)	4 (2)**	3 (1)**	3 (2)**
Micropapillary or hobnailing surface epithelium	17 (6)	22 (5)	19 (11)	0	0	2 (1)**

*$p < 0.05$, ** $p < 0.001$, as compared to carcinoma-related lesions and nonpolypoid lesions

Table 4. Recommendations for approaching endoscopic surveillance biopsy of suspicious lesion and diagnosis of IBD-associated neoplastic precursors

First and most important - **To have knowledge of the endoscopic appearance of the lesion** (ideally by SCENIC terminology and definition): ***Polypoid*** versus ***Nonpolypoid***

- Polypoid: pedunculated or sessile
- Nonpolypoid: superficial elevated (if it is multinodular), flat, or depressed
- Additional descriptions: ulceration within the lesion, distinct or indistinct border, smooth surface

To determine microscopically if the lesion shows conventional adenomatous dysplasia

For adenomatous lesion: to distinguish between sporadic adenoma and true CAD

- If polypoid, dome shaped, with distinct border and smooth surface: most likely sporadic adenoma, less likely CAD
- If nonpolypoid, unlikely sporadic adenoma
- Add comment/note in pathology report to provide clinician with the grade of dysplasia and opinion favoring one or the other and suggesting endoscopic correlation; do not use the term "DALM" anymore

- *Features of exclusion of CAD (i.e., favoring sporadic adenoma)*: "top-down" pattern of dysplasia; symmetric contour

- *Features favoring CAD*: (1) architecturally haphazard and cytologically irregular; (2) significant nuclear pleomorphism and disarray; (3) unusual and peculiar features, including hobnailing or micropapillary surface, microvesicular or bubbling mucin-rich cytoplasm; (4) mixed/intermingled with inflammatory polyp and/or granulation tissue; (5) isolated dysplastic island(s) in the midst of colitic mucosa; (6) deep crypts-predominant cytoarchitectural atypia; (7) mixed non-adenomatous epithelial changes, including serration, hypermucinous change, or goblet cell depletion with or without eosinophilic cytoplasm

For lesion without frank adenomatous dysplasia: To identify the primary cytoarchitectural features

- If prominent epithelial changes (serrated, mucinous, and eosinophilic, etc.), likely a non-adenomatous / non-conventional lesion (variant of CAD)
- If only focal epithelial changes, rule out inflammatory polyp, and be descriptive only in report
- Be alert to mild cytologic atypia and atypia in basal crypts; keep the differentiated dysplasia in mind
- Add comment/note in pathology report to provide clinician with brief explanation of the diagnosis and terminology, and include a clear message about the likely neoplastic nature of the lesion

DISCUSSION

The distinctive histomorphologic features of the true IBD/colitis-associated dysplastic (CAD) lesions have never been fully identified, therefore the hardcore morphologic criteria for reliably distinguishing CAD from sporadic adenomatous polyps in diagnostic pathology practice have been lacking and problematic.

Many attempts have been made, mostly around two decades ago [5-11], to distinguish CAD (commonly termed DALM then), particularly the presumably adenoma-like dysplastic lesions (*a.k.a.*, adenoma-like polyp, adenoma-like mass, and adenoma-like DALM), from conventional/sporadic adenomatous polyps. In summary, those studies have proposed that the colitis-associated lesions generally tend to (1) be architecturally and cytologically irregular, including varying size and shape of glands, variation in nuclear features (configuration, size, chromatin, and orientation), and dystrophic goblet cells; (2) be more likely villous; (3) be more likely in full-thickness or 'bottom-up', rather than 'top-down' pattern of dysplasia and proliferation zone; (4) be associated with flat dysplasia in the surrounding or adjacent mucosa (so that "judging the lesion by the company of the epithelium it keeps" used to be a recommended approach); (5) show mixed dysplastic and non-dysplastic epithelium/crypts in the top of lesion; (6) have marked inflammatory cell infiltrate in lamina propria; and (7) show large variation in appearance and width of stroma. Some investigators also tried to explore immunohistochemical markers such as p53 and β-catenin [17,18]. However, none of these features is sufficiently objective and specific to be regarded as rigid criteria of CAD, especially when one deals with representative endoscopic biopsies of lesions. Ultimately, a better judgement can only be made by the endoscopic appearance, while the histopathological evaluation can only determine if there is cytologic dysplasia.

In general, nonpolypoid (non-adenoma-like) dysplastic lesions showed a higher probability to be associated with carcinoma, whereas polypoid (adenoma-like) lesions were not significantly associated [19,20]. In addition, non-adenoma-like lesions were found to have different genetic alterations from adenoma-like lesions [21,22], whereas the adenoma-like lesions in IBD and the sporadic adenomas in non-IBD individuals were genetically very similar and therefore are most likely the same in biologic nature [22,23].

In recent years, our knowledge about IBD-associated neoplastic precursors is further complicated by the recognition of several forms of non-adenomatous (non-conventional) neoplastic or putatively neoplastic epithelial changes, including serrated, (hyper)mucinous, eosinophilic (or goblet cell depleted), and differentiated (with terminal epithelial differentiation, also known as crypt cell dysplasia) lesions [12-16]. Some investigators also proposed additional types of dysplasia with Paneth cell differentiation, SSA-like, and TSA-like [14]. These less common non-conventional lesions usually have only minimal or no frank cytologic atypia/dysplasia, so they are also known as non-adenomatous lesions [15,16], although they are still considered as variants of dysplasia and termed in general as *non-conventional dysplasia* by some investigators [14]. Of these variants, the serrated lesions are relatively more common, and importantly these serrated lesions often show no characteristic features of SSA/SSL or TSA and are therefore recommended to be classified as serrated lesion not otherwise specified (serrated lesion NOS) [12,14]. The other terminology coexisting in recent literature, '*serrated epithelial change* (SEC)' [24,25], is still poorly define. On the other hand, the typical SSAs/SSLs are also seen in IBD patients, with a similar incidence and distribution pattern as seen in general population [26-28]. Of note, some SSAs/SSLs in early stage may appear only mildly elevated but not sufficiently polypoid on endoscopy.

Taken all of these together into the context, the pathology of IBD-associated neoplastic precursors is actually in a wide spectrum and more complex than what we have defined in the past. It is not possible to develop a simple criterion for the differential diagnosis. Instead, to collectively identified certain distinctive morphologic features would be more practical and useful.

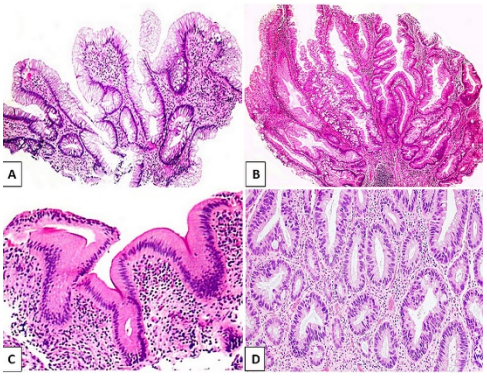
In the present study, we employed a different approach in attempt to further identify the true colitis-associated precancerous lesions and thus to address the lingering question. First, we retrospectively searched for the background lesions in patients who have already developed invasive carcinoma, hence the association of these lesions and carcinoma was doubtless. Second, we paid our attention specifically to the endoscopically nonpolypoid lesions that presumably do not belong to conventional adenomas. Third, we comparatively analyzed the difference between nonpolypoid and polypoid lesions and the similarities between the polypoid lesions in IBD and the sporadic adenomatous polyps in non-IBD individuals. In summary, our study results have demonstrated that (1) the precursor lesions related to IBD-associated enteric carcinomas are almost always nonpolypoid in macroscopic/endoscopic appearance; (2) nearly half of the carcinoma-related lesions are non-adenomatous (non-conventional) lesions with only mild or focal conventional adenomatous cytologic dysplasia, and commonly have mixed adenomatous and non-adenomatous features; (3) the carcinoma-related adenomatous lesions commonly show some peculiar histocytologic features, particularly with mixed features of inflammatory pseudopolyp characterized by significant necroinflammatory component and crypt dropout. Most of these features we report here have not been described before. These seemingly distinctive morphologic characteristics, although still not absolutely specific or unique, can be used as features of inclusion for identifying the colitis-associated dysplasia on endoscopic biopsies when

the endoscopy images are not readily available to the pathologists. Of note, on the other hand, the 'top-down' pattern of dysplasia and symmetric contour are reliable features of exclusion for colitis-associated dysplasia and strongly favor sporadic adenoma; (4) nonpolypoid lesions are almost identical to those proven carcinoma-related lesions, therefore further suggestive of their true colitis-associated neoplastic lesions in nature; and (5) polypoid lesions in IBD are basically identical to the sporadic adenomas in non-IBD individuals, including those occurred in colitic mucosa, therefore the polypoid lesions very unlikely represent CAD, although they may occasionally progress to carcinoma in IBD patients as they often do in general population. To make these points more clinically relevant and practical, we suggest that if the lesion is known to be nonpolypoid and/or if any of the above distinct histocytologic features are found as they have higher association of carcinoma-related lesions, one should correlate with clinical risk with regards to follow-up surveillance colonoscopy, as opposed to polypoid lesion and/or lesions without any of the features. To include a similar text in the pathology report to deliver alert and explanatory message to the caring clinician would be helpful. In the end, based on the present study and many other descriptions in literature and recent updates, the authors summarize all of the favorable and unfavorable features (in Table 4), in attempt to propose a practical recommendation for how to approach the endoscopically visible neoplastic precursor lesions in IBD patients, particularly on the surveillance endoscopic biopsies.

Parenthetically, the invisible lesions (usually found on ransom biopsies) were not specifically addressed in our study because it was difficult for us to retrieve sufficient cases. Although some of our carcinoma-related lesions seemed to be in that category, but it was not possible for us to retrospectively confirm or restore the original macroscopic view of the original mucosa. It is our impression that most of the invisible lesions were the conventional adenomatous dysplasia, and

some may also be any of the non-conventional dysplasia. However, the distribution rate of each pattern in the invisible lesions remains to be addressed by future studies.

As far as the molecular features of CAD, it is beyond the scope of this study and discussion here, although we mentioned some interesting molecular findings in a few representative lesions. In brief, based on considerable studies so far, no any single or panel of specific or unique molecular marker(s) have been found in CAD. It appears that the molecular changes in colitis-associated neoplasia are essentially those identified in sporadic colorectal neoplasms, including many driver gene mutations, DNA methylation, and mismatch repair deficiency; however, the frequency and timing of the occurrence and spectrum of the molecular events are different and variable [29-33]. Epigenetic changes are also believed to contribute significantly to the development of CAD. We have recently found that *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, MLH1/PMS2 loss in a subset of adenomatous, mucinous and eosinophilic but not in differentiated and serrated lesions, and SAT-B2 or PTEN loss and IMP3 overexpression were seen in a small subset of lesions [15].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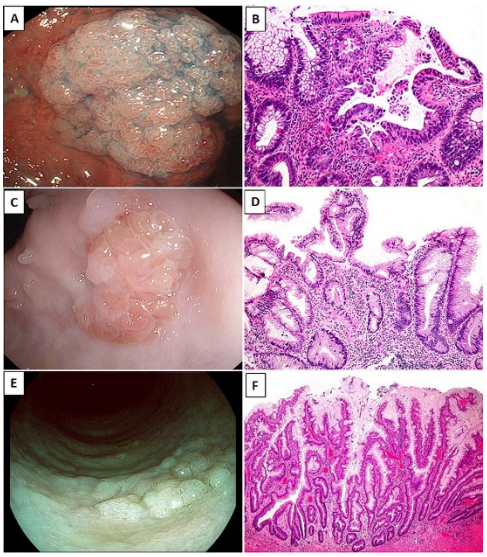
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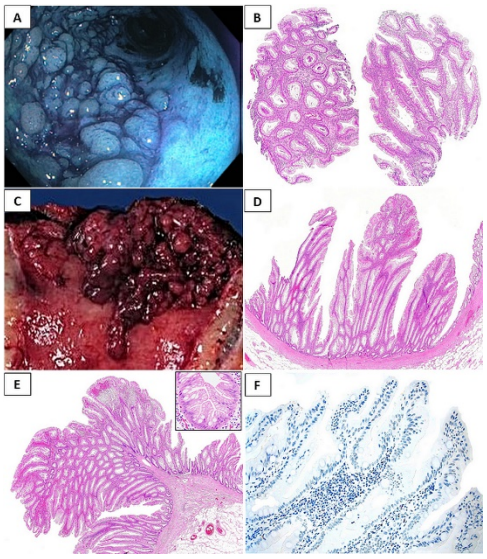
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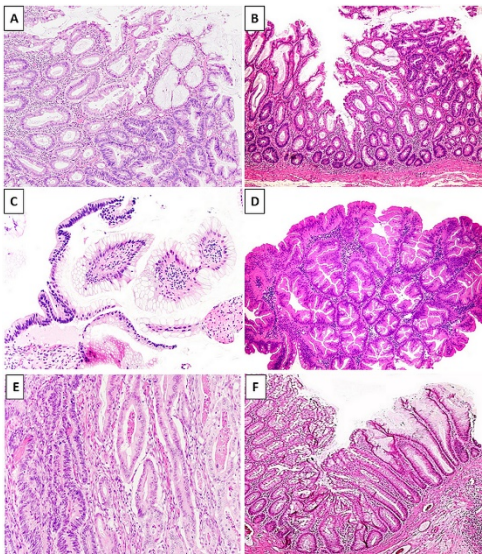
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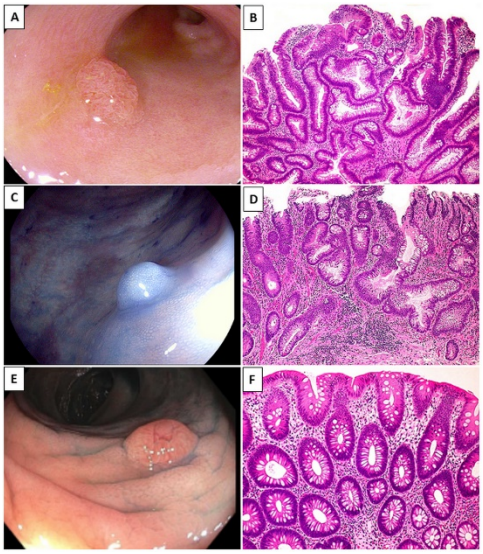
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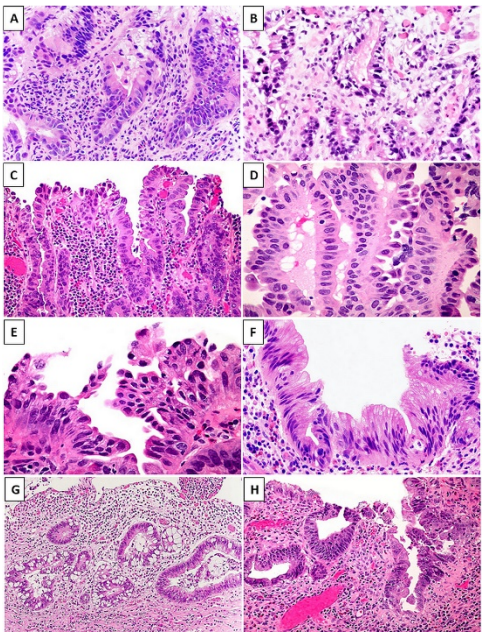
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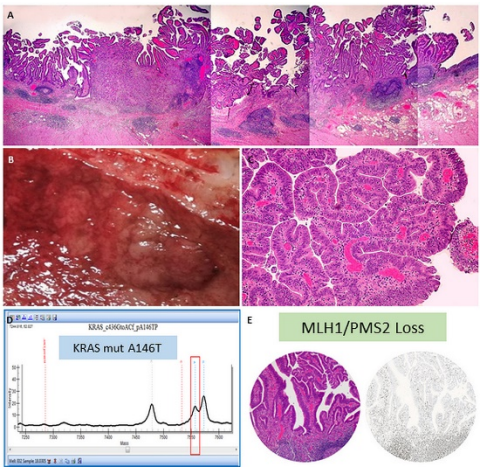
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Figures Legends

Figure 1. **Representative micrographs of non-adenomatous neoplastic precursor lesions**. (A) Mucinous (hyperpmucinous) lesion. (B) Serrated NOS lesion. (C) Eosinophilic (goblet cell depleted) lesion. (D) Differentiated lesion (dysplasia with terminal epithelial differentiation) (all ×100).

Figure 2. **Endoscopically nonpolypoid (non-adenoma-like) lesions and corresponding histomorphologies** (×100). (A/B) A lesion in ascending colon in a 45 y/o female with longstanding CD. A- Dye chromoendoscopy with methylene blue. B- Adenomatous low-grade dysplasia.; (C/D) A lesion in sigmoid colon in a 76 y/o male with UC. C- White light endoscopy, D- Serrated lesion on the biopsy of the corresponding lesion. (E/F) A lesion in descending colon in a 49 y/o male with UC: E- Virtual chromoendoscopy. F- Mucinous lesion on the biopsy of the corresponding lesion.

Figure 3. A case of serrated NOS lesion. A large multinodular and villous lesion in sigmoid in a 50-year-old man with longstanding UC: (A/B) Lesion seen endoscopically (dye chromoendoscopy with methylene blue) and microscopically (×100) on the first colonoscopy. The lesion extended up to 4 cm in length and occupied two thirds of the bowel circumference. The index biopsy was diagnosed as “hyperplastic polyp”, and DNA flow cytometry detected no abnormal DNA content. No immediate resection was performed. Multiple subsequent biopsies were diagnosed as “inflammatory polyps”. The lesion was surgically resected 4 years later. (C to F) Lesion seen on the resected specimen (C) and microscopically (D/E, ×40 and ×100). The lesion increased in size, up to 6 cm in length and involving the entire circumference of bowel. Microscopically, the lesion was composed of numerous prominent projections in which the

lesional epithelium is largely arranged in pronounced serrated architecture and mixed with villous architecture. However, it showed no or occasional basal crypt dilatation and no ectopic crypts. The epithelial cells are lack of conventional cytologic dysplasia, although focally show slight enlarged nuclei (insect in E). F. p53 immunohistochemical stain showed loss of p53 expression in lesional epithelial cells (×200).

Figure 4. Mixed morphologic non-adenomatous features seen in some adenomatous lesions. (A/B) Mixed adenomatous and serrated changes (×100). (C) Mixed adenomatous and mucinous changes (×200). (D) A lesion with serration, eosinophilic cytoplasm, and mild cytologic atypia, representative of a TSA-like lesion (×100). (E) Mixed adenomatous and eosinophilic lesion (on the right half of micrograph) in which there is lack of goblet cells and mild cytologic atypia (×100). (F) Mixed mucinous and serrated changes (×100).

Figure 5. **Endoscopically polypoid (adenoma-like) lesions and corresponding histomorphologies** (×100). (A/B) A lesion in ascending colon in a 52 y/o female with CD; (C/D) A lesion in rectum in a 49 y/o male with UC (C- Dye chromoendoscopy with methylene blue). (E/F) A lesion in descending colon in a 58 y/o male with UC.

Figure 6. Unusual peculiar morphologic features of colitis-associated adenomatous dysplasia. (A/B) Adenomatous dysplasia with unusually greater degree of nuclear pleomorphism and irregular arrangement (×200). (C/D) Hobnailing surface of dysplastic epithelium (E ×100, F ×200). (E) Micropapillary surface of dysplastic epithelium (×200). (F) Dysplastic epithelium with microvesicular cytoplasm (×200). (G/H) Dysplastic epithelium in a background of granulation tissue/inflammatory pseudopolyp.

Figure 7. A representative case of colitis-associated dysplasia (conventional/adenomatous lesion). A sessile lesion in rectum stump in 43-year-old female with longstanding UC and status post total colectomy 3 years ago: (A) A panoramic view of the major portion of the lesion (×20), with superficially invasive carcinoma (in the yellow-circled area). (B) Macroscopic view of the lesion on gross image of the fresh surgical specimen. (C) Adenomatous lesion on the surface, in tubulovillous architecture, with prominent intratumoral lymphocytes (intraepithelial lymphocytosis) (×200). (D) *KRAS* gene mutation (A146T) detected. (E) The same area of the lesion on H&E stain (left) and on immunostain for MLH1/PMS2 (loss) (right).

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