

# A new practical classification of desmoplastic reaction in endoscopic forceps biopsy of colorectal cancer

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**Summary.** Background. The histopathological discrepancy between endoscopic forceps biopsy (EFB) and post-resection specimens is considered a practical clinical problem. This retrospective study aimed to determine the current diagnostic concordance between the EFB and surgical specimens of colorectal cancer (CRC) and then investigated the useful factors in EFB diagnosis.

**Methods.** We used the representative pathological data of 2188 CRCs. The comparison of histopathological discrepancy between EFB and the related surgical specimens was performed. Furthermore, 418 biopsy specimen slides in our hospital were reviewed to determine the classification of intratumor desmoplastic reaction (DR).

**Results.** Among the 2188 patients, the positive sensitivity of EFB for adenocarcinoma was 82.7%. The discrepancy rate between the EFB and surgical specimens was 10.8-40.0% corresponding to different T stages. On the basis of DR classification, 32, 131, and 255 tumors were categorized as little, moderate and extensive, respectively. The correlation between DR classification and tumor invasion based on T stage was significant (Spearman's  $\rho=0.112$ ;  $p<0.05$ ). The extensive DR provided better estimates for advanced tumors than the little and moderate DR ( $\chi^2=3.977$ ,  $p=0.046$ ). Besides DR, factors including deeper cutting the slides and histological types were significantly associated with "adenocarcinoma" diagnosis in EFB of CRCs ( $p<0.05$ ).

**Conclusion.** To the best of our knowledge, this is the first time that a DR classification specifically for EFB specimens was proposed. It might contribute to improve the accuracy of biopsy-based diagnosis of CRC.

**Key words:** Desmoplastic reaction, Colorectal cancer, Endoscopic forceps biopsy

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DOI: 10.14670/HH-18-353

## Introduction

Colorectal cancer (CRC) is a common disease and early detection can help to improve the prognosis of patients. Colonoscopy is considered to be the most effective method for detecting colorectal lesions. Moreover, diagnosis based on endoscopic forceps biopsy (EFB) specimens is not only the gold standard method for the qualitative evaluation of neoplasia but also provides essential information for clinicians to guide further treatment and surveillance plans for patients (Binefa et al., 2014; Young et al., 2014; Fotheringham et al., 2019). Unfortunately, histopathologic discrepancy exists between EFB and post-resection specimens, considered as a practical clinical problem for both clinicians and pathologists (Wei et al., 2012; Hwang et al., 2016; Park et al., 2019). Besides the limited biopsy sample, the lack of unified and specialized diagnostic system for gastrointestinal biopsy might be one pivotal reason related to the discrepancy (Kim et al., 2012; Wei et al., 2012).

It is well known that Eastern (Japanese) and Western pathologists use different criteria to establish a gastrointestinal carcinoma diagnosis, focusing on cytological dysplasia and structural invasion, respectively (Kim et al., 2011). To increase the global consistency on the diagnosis for gastrointestinal epithelial neoplasia, a series of international congresses were held aimed at establishing the terminological classifications of gastrointestinal epithelial neoplasia since 1998 (Schlemper et al., 2000c). The latest version is the "Vienna classification" with a revised version, in which the following six terms are used: (1) negative for dysplasia, (2) indefinite for dysplasia, (3) low-grade adenoma/dysplasia, (4) high-grade adenoma/dysplasia, (5) suspicious for invasive carcinoma, and (6) (invasive) carcinoma (Dixon, 2002). According to the World Health Organization (WHO), colorectal epithelial neoplasia not invading the submucosal layer is referred to as "high-grade intraepithelial neoplasia (HGIN)/dysplasia" rather than "carcinoma" because of its low risk of lymphovascular metastasis (West and



Mitsuhashi, 2005). However, the complete muscularis mucosa is only identified in two-thirds of biopsy tissues, resulting in the difficulty of recognizing the submucosal layer (Wei et al., 2012). Subsequently, some invasive CRCs are underdiagnosed as HGIN leading to undertreatment (Chen et al., 2009; MacDonald et al., 2009; Tominaga et al., 2009).

Desmoplastic reaction (DR) is the proliferation of myofibroblasts in the stroma of invasive cancer (Angeli et al., 2009; Ohno et al., 2013). The prognosis of CRC patients can be predicted by a distinctive DR categorization (Ueno et al., 2019). Recent studies revealed that detection of DR in the biopsy specimen is useful in predicting massive submucosal invasion in CRC (Kojima et al., 2010), but there are few histopathologic criteria for its evaluation. This article revealed the current diagnostic discordance between EFB and the surgically resected specimens in patients with CRC. Additionally, we firstly proposed a histological classification of DR and discussed its significance in the biopsy-based diagnosis of CRC.

## Materials and methods

This was a retrospective medical record review based on the pathology database. All available specimens were formalin-fixed and paraffin-embedded. The 5- $\mu$ m-thick sections were created for hematoxylin and eosin (H&E) staining. This study was approved by the Ethics Committee of Shandong Provincial Hospital Affiliated to Shandong First Medical University and a waiver of patient consent was granted.

### Patients

A total of 3063 patients with CRCs underwent surgical resection at our hospital between January 1, 2010, and August 31, 2018. The inclusion criteria were 1) primary adenocarcinoma; 2) isolated tumor; and 3) biopsy result was available. Moreover, 875 patients were excluded because of the exclusion criteria: 1) synchronous multiple tumors; 2) insufficient biopsy results; 3) a family history of adenomatous polyposis; and 4) a medical history of related endoscopic submucosal dissection (ESD) treatment. Finally, a total of 2188 patients were enrolled in this study.

Biopsies were performed in all patients. Moreover, the pathological reports of these biopsies were divided into eight groups as follows: (1) negative for dysplasia, (2) indefinite for dysplasia, (3) low-grade adenoma/dysplasia, (4) high-grade adenoma/dysplasia, (5) suspicious for intramucosal carcinoma, (6) intramucosal carcinoma, (7) suspicious for submucosal carcinoma, and (8) submucosal or deeper invasive carcinoma.

A total of 418 patients underwent the biopsy examination in our hospital. Colonoscopy was carried out after bowel preparation (CF-Q260AI, Olympus Optical Co., Ltd., Tokyo, Japan). Patients underwent

EFB and the endoscopists decided the number of biopsies to take from each suspicious lesion of malignancy. Other patients did EFB in different referral centers. According to the Chinese Protocol of Diagnosis and Treatment of Colorectal Cancer, the official pathology report of EFB is an important basis for arranging treatment for colorectal lesions (DOI:10.3760/cma.j.cn112139-20200518-00390). Therefore, those diagnostic reports from outside our hospital were also included in the analysis.

After surgeries were performed, the pathological characteristics from all resected specimens were analyzed in accordance with the WHO classification. The pathological diagnosis of the biopsy specimen was compared with that of the postsurgical specimen.

### Classification of DR

The biopsy slides in our hospital were reviewed by the pathology department. The histological findings were determined by H&E staining alone. The intratumor DR was histologically classified as little (+), moderate (++) or extensive (+++), based on the accumulation of collagen fibers and myofibroblasts. The classifications were defined as follows: 1) little (+), stromal fibroblasts slightly increased and showed very thin bundle formation; 2) moderate (++) , fibrotic stroma moderately

**Table 1.** The basic characteristics of the enrolled cases according to the histological results from surgical specimens (n=2188).

General information	No. (%)
Sex	
Male	1326 (60.6)
Female	862 (39.4)
Age (year) (mean $\pm$ standard deviation)	16-92 (59.8 $\pm$ 11.7)
Tumor location	
Cecum	126 (5.8)
Ascending	222 (10.1)
Transverse	95 (4.3)
Descending	77 (3.5)
Sigmoid	285 (13.0)
Rectum	1383 (63.2)
Macroscopic type of lesion	
Mass	392 (17.9)
Ulcerative	1728 (79.0)
Infiltrative	68 (3.1)
Histological type	
Well-differentiated	126 (5.8)
Moderately differentiated	1770 (80.9)
por, sig, muc	292 (13.3)
Tumor size (mm)	10-170 (50.1 $\pm$ 12.0)
Depth of invasion (T stage)	
Tis	20 (0.9)
T1	81 (3.7)
T2	349 (16.0)
T3	896 (41.0)
T4	842 (38.5)

Por, poorly differentiated; sig, signet ring cell; muc, mucinous.

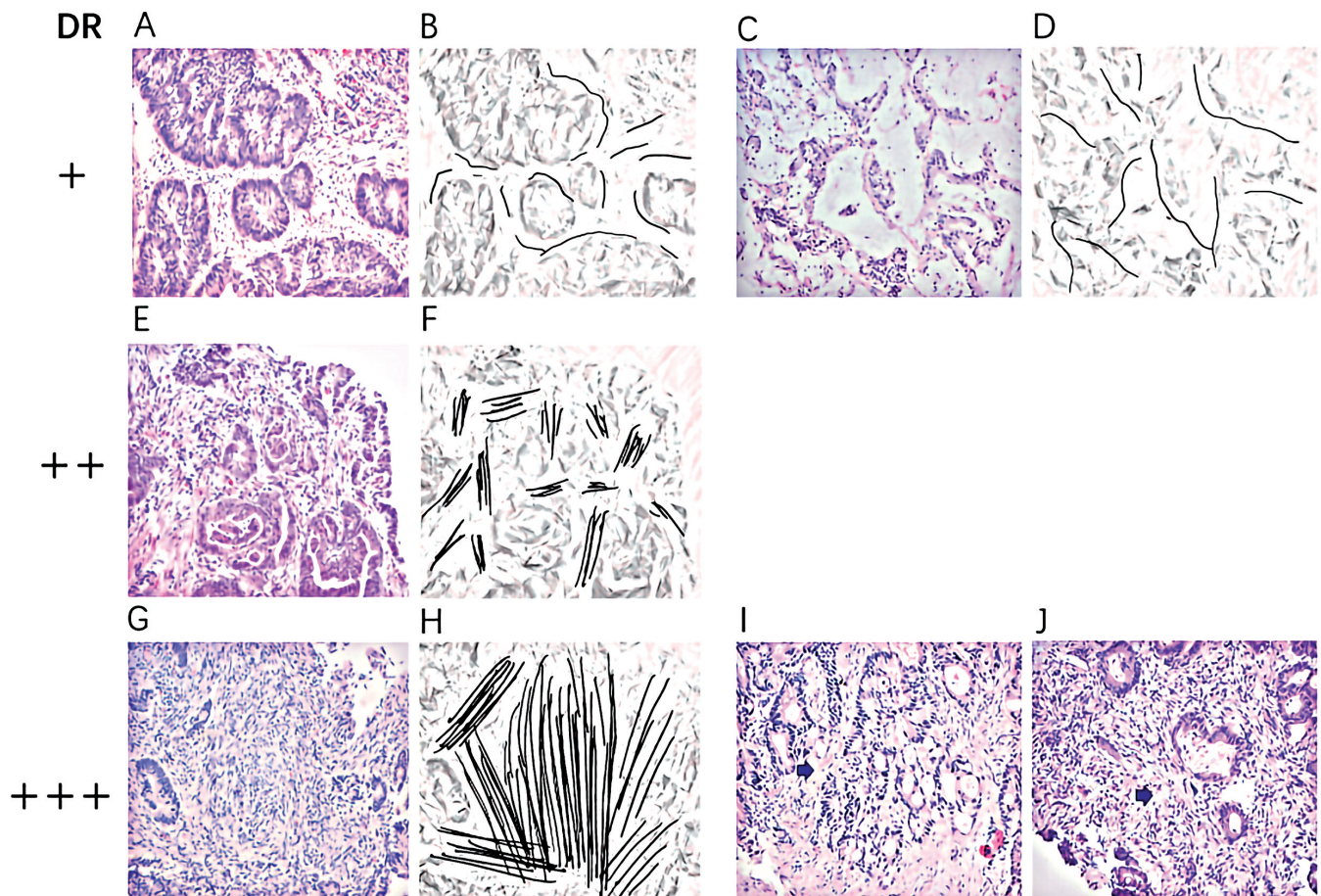
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increased and consisted of relatively thick bundle formation with randomly orientated collagen; 3) extensive (+++), cancer stroma remarkably increased and comprised of fibers stratified into multiple layers, intermingling with myxoid stroma or keloid-like collagen (Fig. 1). Pathologists should be cautious in inflammatory infiltration that does not signify the presence of DR. The slides were reevaluated by two senior pathologists with gastrointestinal expertise at low or medium magnification. If the two pathologists disagreed about the diagnosis, the findings were discussed to reach a final decision using a double-headed microscope.

*Statistical analyses*

We used the SPSS version 19.0 statistical software (International Business Machines Corp., Armonk, NY, USA) for the statistical analysis of the data. Kappa analysis was performed to test interobserver agreement.

The strength of agreement for the kappa value can be interpreted as follows: 0.01-0.20 slight agreement, 0.21-0.40 fair agreement, 0.41-0.60 moderate agreement, 0.61-0.80 substantial agreement and 0.81-0.99 excellent agreement. Spearman correlation statistics was performed for assessing the correlation between DR classification in biopsy specimens and tumor invasion in surgical specimens, with a Spearman's correlation coefficient ( $\rho$ ), which ranges from zero (no association) to 1 (perfect association). The effect of DR in predicting the progressive tumors was determined using  $\chi^2$  analysis, and Fisher's exact test was used whenever appropriate. Multivariate analyses were performed to evaluate factors contributing to "adenocarcinoma" diagnosis in EFB, using a logistic regression model. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated to estimate the association between pathological factors and histopathologic diagnosis in biopsy specimens.  $p < 0.05$  was considered significant.



**Fig. 1.** Desmoplastic reaction (DR) classification in colorectal cancer (CRC). **A, C.** Little (+) DR in the well and poorly-differentiated CRCs, respectively; **B, D.** Schematics for **(A)** and **(C)**, thin black lines representing DR; **E.** Moderate (++) DR in the well-differentiated CRC; **F.** Schematic for **(E)**, fasciculate black lines representing for DR; **G, I, J.** Extensive (+++) DR in the well-differentiated CRC; **H.** Schematic for **(G)**, laminar black lines representing DR; **I.** Arrow, myxoid stroma; **J.** Arrow, keloid-like collagen. x 100.

**Results**

*Clinicopathological features*

The baseline characteristics of the enrolled 2188 patients (1326 men and 862 women; age, 16-92 years) with CRCs are summarized in Table 1. Regarding tumor location, 126 lesions (5.8%) were observed in the cecum, 222 (10.1%) in the ascending colon, 95 (4.3%) in the transverse colon, 77 (3.5%) in the descending colon, 285 (13.0%) in the sigmoid colon, and 1383 (63.2%) in the rectum. The tumor size was 50.1±12.0 mm. According to the pathological analysis, approximately four-fifths of the patients had moderately-differentiated adenocarcinoma with muscularis or deeper invasion.

*Discrepancy between EFB and tumor invasion in patients with colorectal cancer*

The numbers of the pathological reports of EFB in

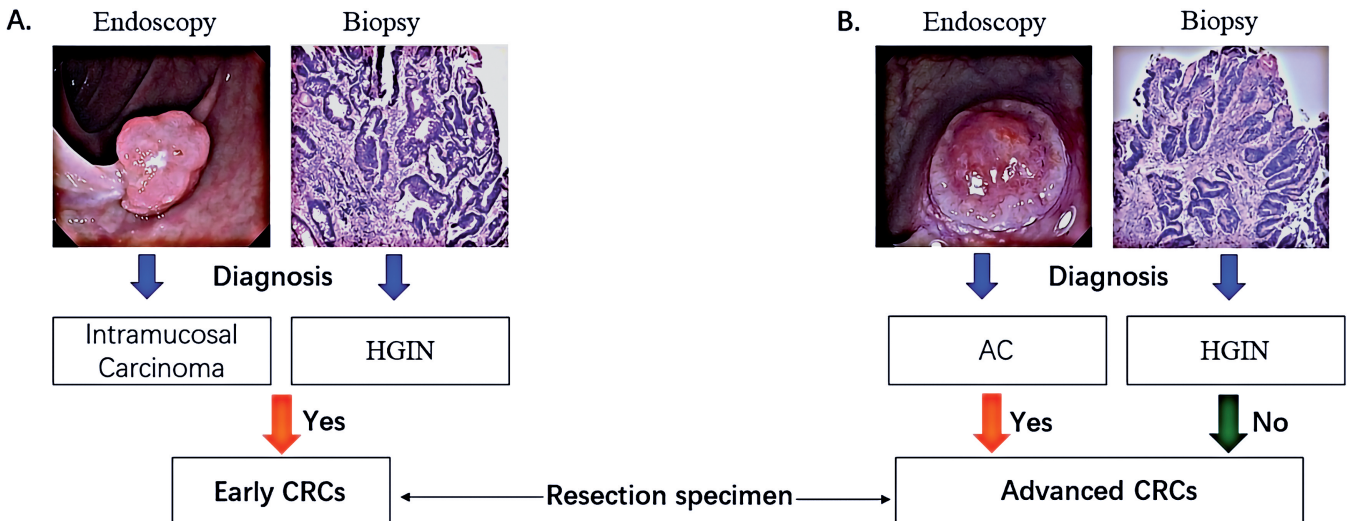
all patients were 16 (0.7%) in “negative for dysplasia”, 8 (0.4%) in “indefinite for dysplasia”, 31 (1.4%) in “low-grade adenoma/dysplasia”, 111 (5.1%) in “high-grade adenoma/dysplasia”, 29 (1.3%) in “suspicious for intramucosal carcinoma”, 67 (3.1%) in “intramucosal carcinoma”, 134 (6.1%) in “suspicious for submucosal carcinoma”, and 1792 (81.9%) in “submucosal or deeper invasive carcinoma”, respectively.

The time interval between EFB and related operation was 7.8±6.0 days. After the evaluation of surgical specimens, the comparison of histological results with those of biopsy specimens was performed. The biopsy was positive for “adenocarcinoma” in 1792 of the 2168 patients, resulting in a sensitivity of 82.7%. The rate of muscularis mucosae presence in EFB was 32.8% (137/418), and only 90 of the 137 patients showed that the muscularis mucosae was invaded. Five patients with intramucosal tumors were overdiagnosed with advanced carcinomas. Furthermore, 16 patients were underdiagnosed with benign lesions on biopsy. Details

**Table 2.** Discrepancy between the pathological reports of biopsy and the tumor invasion from surgical specimens (n=2188).

Surgical specimen	Biopsy specimen								Discrepancy
	Diagnosis subtype, n (%)								
T stage	1	2	3	4	5	6	7	8	n (%)
Tis	0	0	2 (10.0)	9* (45.0)	2* (10.0)	1* (5.0)	1 (5.0)	5 (25.0)	8 (40.0)
T1	0	0	3 (3.7)	7 (8.5)	1 (1.2)	5 (6.1)	2* (2.4)	64* (78.0)	16 (19.5)
T2	1 (0.3)	3 (0.9)	3 (0.9)	18 (5.1)	5 (1.4)	11 (3.1)	26* (7.4)	283* (80.9)	41 (11.7)
T3	4 (0.4)	3 (0.3)	8 (0.9)	47 (5.2)	9 (1.0)	26 (2.9)	57* (6.4)	743* (82.8)	97 (10.8)
T4	11 (1.3)	2 (0.2)	15 (1.8)	30 (3.6)	12 (1.4)	24 (2.9)	48* (5.7)	697* (83.1)	94 (11.2)

Diagnosis of biopsy 1, negative for dysplasia; 2, indefinite for dysplasia; 3, low-grade adenoma/dysplasia; 4, high-grade adenoma/dysplasia; 5, suspicious for intramucosal carcinoma; 6, intramucosal carcinoma; 7, suspicious for submucosal carcinoma; 8, submucosal or deeper invasive carcinoma; \*Histological assessment is consistent between the biopsy and surgical specimens.



**Fig. 2.** Comparison of the diagnosis of endoscopy and biopsy in early (A) and advanced (B) colorectal cancer. HGIN, high-grade intraepithelial neoplasia; AC, adenocarcinoma; CRC, colorectal cancer.

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regarding the histological discrepancy rate between EFB and surgical specimens are shown in Table 2. Although the highest discrepancy rate was 40.0% in the Tis stage group, there was no significant difference among the five T stage groups ( $p>0.05$ ). In addition, we separately analyzed the discrepancy in 418 cases, in which both EFB and surgical specimens were from our hospital. As a result, it was similar to the data from all patients (sensitivity, 81.4%; discrepancy rate, 8.5%-50.0%).

### Diagnostic concordance between the endoscopy and EFB

418 patients underwent endoscopic examination before surgery in our hospital. We compared the diagnostic concordance of endoscopy and biopsy with surgical specimens. The consistent rate of diagnosis for early CRCs was similar in both endoscopy and biopsy, and that for advanced CRCs was lower in biopsy (Table 3, Fig. 2).

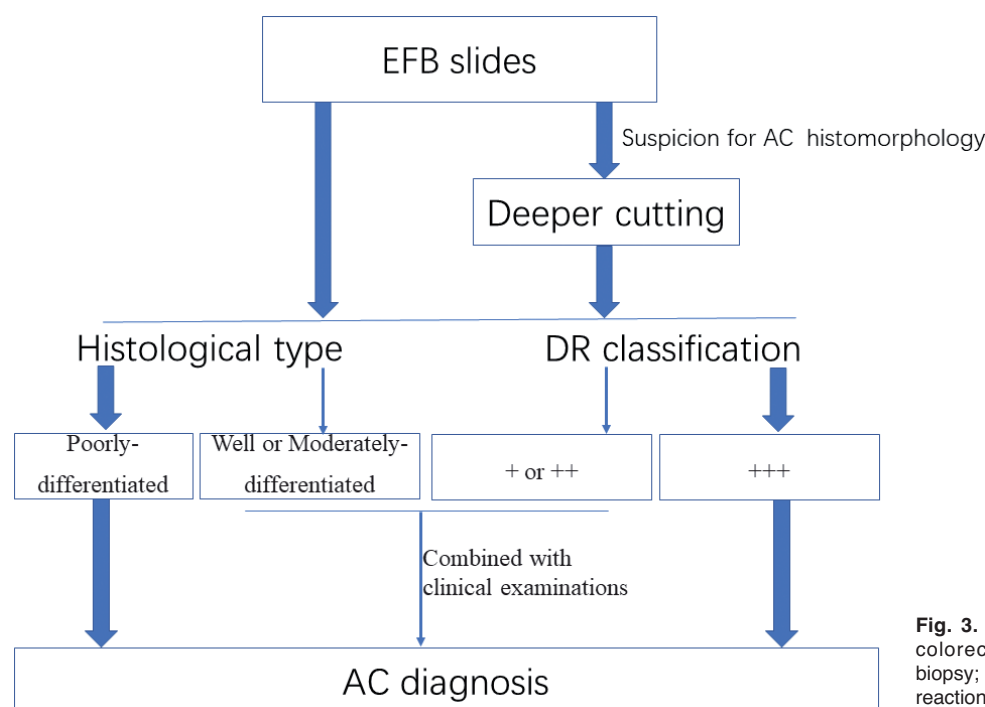
### Correlation between pathological features in EFB and tumor invasion

We detected DR and accompanied inflammation in 418 EFB specimens. On the basis of DR classification, 32, 131, and 255 tumors were categorized as little (+), moderate (++) and extensive (+++), respectively. Table 4 shows that the interobserver agreement was excellent between two pathologists ( $\kappa=0.830$ ,  $p<0.001$ ). The association between DR classification and tumor invasion based on T stage was analyzed (Table 5). The DR classification was shown to have a significant correlation with tumor invasion, which was divided into early stage (Tis-T1) and advanced stage (T2-4) (Spearman's  $\rho=0.112$ ;  $p=0.022$ ). Subsequently, we assessed the effect of DR in EFB specimens in predicting the progressive tumors. The extensive DR provided better estimates for advanced tumors than the little and moderate DR (+ and ++ vs. +++:  $\chi^2=3.977$ ,

**Table 3.** Diagnostic concordance between the endoscopy and biopsy with surgical specimens (n=418).

T stage	Total number, n	Endoscopy, n (%)				Biopsy, n (%)			
		NSC	SSC	DSC	Concordance	NSC	SSC	DSC	Concordance
Tis	3	0	1 (33.3)	2 (66.7)	0	1 (33.3)	1 (33.3)	1 (33.3)	1 (33.3)
1	9	0	2 (22.2)	7 (77.8)	2 (77.8)	2 (22.2)	0	7 (77.8)	7 (77.8)
2	64	1 (1.6)	1 (1.6)	62 (96.9)	62 (96.9)	6 (9.4)	6 (9.4)	52 (81.3)	52 (81.3)
3	176	3 (1.7)	6 (3.4)	167 (94.9)	167 (94.9)	15 (8.5)	22 (12.5)	139 (79.0)	139 (79.0)
4	166	1 (0.6)	1 (0.6)	164 (98.8)	164 (98.8)	17 (10.2)	11 (6.6)	138 (83.1)	138 (83.1)

NSC, no submucosal carcinoma; SSC, suspicious for submucosal carcinoma; DSC, definite for submucosal or deeper invasive carcinoma.



**Fig. 3.** Diagram for biopsy-based diagnosis of colorectal cancer. EFB, endoscopic forceps biopsy; AC, adenocarcinoma; DR, desmoplastic reaction.

p=0.046). However, this effect was not found in the little and moderate DR groups (+ vs. ++:  $\chi^2=1.702$ , p=0.192).

The positivity of mucosal ulcer was 25.0% (1/4) in the Tis group, 37.5% (3/8) in the T1 group, 40.6% (26/64) in the T2 group, 55.7% (98/176) in the T3 group, and 46.4% (77/166) in the T4 group. In contrast with DR, the inflammation positivity between the intra- and submucosal adenocarcinoma was insignificantly different (p>0.05).

*Pathological factors associated with “adenocarcinoma” diagnosis in EFB*

These factors, including deeper cutting the slides, histological types, and DR classification, were significantly associated with “adenocarcinoma” diagnosis in the pretreatment biopsy specimens of CRC cases. However, the quantity of biopsy tissues did not have statistical significance (Table 6). Based on the result, we suggested a diagnosis process for CRC with the biopsy specimen (Fig. 3).

**Discussion**

CRC is one of the most common malignancies of the digestive system (Zheng et al., 2016). Hence, improvement in the accuracy of biopsy diagnosis from patients with CRCs is urgently and significantly required, establishing the proper treatment and then gradually reducing the significant economic health

burden of this disease in the long term. In this study, we revealed the current diagnostic discordance regarding the histopathologic diagnosis between EFB and surgically resected specimens from CRC patients and then firstly suggested a histological classification of DR, which could be helpful in the diagnosis of advanced tumor based on EFB specimens.

Although significant progress has been made in the diagnostic procedure of gastrointestinal cancer, EFB is still the standard and obligatory pretreatment for further surgical or oncologic therapy (Liu and Cheung, 2019). However, the accuracy of pathological diagnosis from biopsy specimens is not optimal (Lee et al., 2010). More advanced or aggressive CRC is associated with a higher incidence of “HGIN” on biopsy-based pathological examination. This has been considered a practical clinical problem for Chinese clinicians until recently (Wei et al., 2012). In the current study, we investigated this issue by comparing the histopathological discrepancy between EFB and the corresponding resection specimens. A total of 17.2% (376/2168) of patients with CRC would be underdiagnosed according to their biopsy diagnosis. Generally, the histopathologic discrepancies between EFB and the resection specimens would be reduced as the invasive depth of the tumor increases (Park et al., 2019). The tendency in this study was similar to previous ones, showing the highest discrepancy rate in the Tis group. However, the difference was not statistically significant.

The inaccuracy of the biopsy diagnosis is possibly attributed to two reasons. One is the presence of different diagnostic criteria. It is well known that there are two main diagnostic systems for gastrointestinal epithelial neoplasia including the Eastern and Western criteria (Nishimura et al., 2019). The “Eastern” concept

**Table 4.** Result of DR classification in EFB evaluated by two pathologists (n=418).

Doctor 1	Doctor 2	+	++	+++	Total
+		25	5	0	30
++		10	110	9	129
+++		0	13	246	259
Total		35	128	255	418

Desmoplastic reaction classification +, little; ++, moderate; +++, extensive; DR: desmoplastic reaction; EFB: endoscopic forceps biopsy.

**Table 5.** Association between desmoplastic reaction classification in biopsy specimens and tumor invasion in surgical specimens (n=418).

T stage	Total number, n	Desmoplastic reaction, n (%)		
		+	++	+++
Tis	3	1 (33.3)	1 (33.3)	1 (33.3)
1	9	2 (22.2)	4 (44.4)	3 (33.3)
2	64	3 (4.7)	21 (32.8)	40 (62.5)
3	176	6 (3.4)	54 (30.7)	116 (65.9)
4	166	20 (12.0)	51 (30.7)	95 (57.2)

Desmoplastic reaction classification +, little; ++, moderate; +++, extensive.

**Table 6.** Multivariate analyses of factors predicting “adenocarcinoma” diagnosis in biopsy specimens (n=418).

Factors	Multivariate analysis		
	Odds ratio	95% confidence interval	P-value
Number of tissue blocks			0.653
≤3 (n=113)	1	Reference	
>3 (n=305)	1.143	0.638-2.048	
Deep cutting of slides			<0.001
Yes (n=48)	1	Reference	
No (n=370)	0.261	0.132-0.516	
Histological type			0.005
Well or Moderately- differentiated (n=377)	1	Reference	
Poorly-differentiated (n=41)	19.782	2.467-158.661	
DR classification			<0.05
+ (n=32)	1	Reference	
++ (n=131)	2.641	1.105-6.314	
+++ (n=255)	7.784	3.281-18.466	

DR, desmoplastic reaction.

is actually derived from Japanese pathologists, who diagnose carcinoma based on the cytological findings (Schlemper et al., 2001). On the contrary, a structurally invasive focus is required for Western pathologists, who emphasize the possibility of metastases only after tumor invasion (Schlemper et al., 1997). Considering the persistent efforts of the international experts from digestive pathology, “Vienna classification” and its revised version were generated, effectively narrowing the gap between the “Eastern” and “Western” criteria (Schlemper et al., 2000c; Guindi and Riddell, 2001; Vindigni et al., 2010). However, the “Vienna classification” is not specific for the pathological diagnosis of biopsy specimens. Recent studies have revealed that although the Vienna classification is used, the problem regarding frequent diagnostic discrepancies between EFB and resection specimens would still remain (Kasuga et al., 2012). Additionally, the “Vienna classification” is significantly subdivided into four categories, resulting in inter-pathologist inconsistency and confusing clinicians in daily clinical practice (Ponz de Leon and Di Gregorio, 2001). The other reason is the difficulty of establishing the diagnosis of submucosal invasion. Several authorities support the hypothesis that “intraepithelial neoplasia” is more proper than “carcinoma” in describing the colorectal neoplastic lesions unless submucosal invasion is observed (Schlemper et al., 2000c). Nevertheless, a definite sign of tumor cell invading the muscularis mucosa is not easily recognized in biopsy specimens, specifically in advanced CRC (Wei et al., 2012). In our study, the rate of muscularis mucosae presence was only 32.8%. Compared with endoscopy, the diagnostic concordance with resection specimen for advanced CRCs was lower in biopsy. Some advanced CRCs were underestimated from the biopsy specimens in routine work, especially by the pathologists without much related diagnosis experience. As mentioned above, the application and comprehensive understanding regarding the definition and diagnostic criteria for colorectal intraepithelial neoplasia are significantly heterogeneous in China (Huang, 2005). Using the broader, rather than finer classifications to assess the degree of dysplasia substantially could improve the reliability in reality (Terry et al., 2002).

DR accompanied with tumor invasion is being increasingly observed. Kimura et al. investigated the presence of DR in pretreatment biopsy specimens from early CRC patients. They found that the presence of DR was significantly associated with submucosal invasion depth. The positive predictive value of DR in predicting submucosal invasion depth ( $\geq 1000 \mu\text{m}$ ) was 82.4-91.9% (Hirose et al., 2010; Kimura et al., 2012; Ohno et al., 2013). CRC with submucosal invasion depth  $\geq 1000 \mu\text{m}$  is usually treated surgically (Tanaka et al., 2015). As a result, DR in EFB specimens was considered to be a good indicator for the clinicopathological diagnosis of massive submucosal invasion. However, a recent report showed that DR was also present in the intramucosal

invasive carcinoma (Fukami et al., 2018). This contradictory finding might be related to the heterogeneity of DR. Using the existing DR categorization (Ueno et al., 2004), which assesses DR on the front of tumors, in the EFB specimens is obviously unreasonable. Therefore, we divided the intratumor DR in EFB into three groups, including little, moderate and extensive, which had the distinctive histomorphology on the basis of the combination of collagen fibers and myofibroblasts. Our data implied that a statistically significant correlation between the DR classification and tumor invasion was observed. Moreover, the extensive but not little or moderate DR had the remarkable effect of predicting the advanced tumors.

Inflammatory background can make the recognition of DR difficult. The erosion or ulcer is also characterized by the accumulation of collagen fiber and myofibroblasts which accompany the infiltrating inflammatory cells (Okamoto et al., 2013). According to our study and other Chinese studies, the macroscopic type of CRC in Chinese individuals is generally ulcerative (Xu et al., 2009; Dai et al., 2017). It may not be necessary to distinguish the stromal reaction caused by the tumor from the ulcer surrounded with malignant cells since the deep ulcer also has a disrupted muscularis mucosal layer.

Besides DR, we also investigated the association between other pathological features and biopsy diagnosis. Our results showed that the tumor differentiation was associated with “adenocarcinoma” diagnosis in the pretreatment biopsy specimens of CRC cases. This result is comprehensible because these pathological features may have a positive association with tumor stromal production, which would lead to a good concordance of “adenocarcinoma” diagnosis among pathologists (Kimura et al., 2012; Kojima et al., 2017). In contrast to deeper cutting the slides, increasing the quantity of biopsy tissue blocks did not contribute to the “adenocarcinoma” diagnoses.

In clinical practice, the possibility of sampling error in endoscopic biopsies is always observed, not taking into account the presence of nearby invasion (Schlemper et al., 2000a,b). In our study, the diagnoses of 16 cases were tumor-negative based on biopsy specimens from CRC patients. Therefore, to assess the depth of invasion, some useful supplementary examinations should be used, including ultrasonographic (Ell et al., 2000) and radiographic examinations.

This study had the following limitations. This was a retrospective study, and the number of biopsy samples was not associated with the resection specimens. Moreover, our study design included only surgically resected CRCs, excluding cases with endoscopically resected early CRCs. Hence, the accuracy of colonoscopic biopsy in establishing the diagnosis of CRC could result in bias. Thus, a prospective, multicenter study is required to confirm the results.

In conclusion, we suggest a semiquantitative DR classification and related diagnosis process, which might

contribute to improve the accuracy of biopsy-based diagnosis of CRC.

*Conflict of interests.* All authors declare that they have no conflicts of interests.

*Funding.* This work was supported by National Science Foundation of China [81500413] and Shandong Province Natural Science Foundation [ZR2014HQ064].

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Accepted June 15, 2021