Title

Desmoplastic reaction in biopsy specimens of early colorectal cancer: a Japanese prospective multicenter study

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Summary

We previously reported that detection of desmoplastic reaction (DR) in pretreatment biopsy specimens was useful for the prediction of submucosal (SM) depth in nonpedunculated early colorectal cancers in a retrospective multicenter study. Here, we performed a prospective multicenter study for verification of our previous findings.

Subjects in this study were diagnosed with early colorectal cancer (Tis or T1) by endoscopy, and with adenocarcinoma from the biopsy specimens. Our target was collection of 100 cases. Fifteen institutions affiliated with the Japanese Society for Cancer of the Colon and Rectum (JSCCR) participated in this collaborative study, and the cases were provided by eleven of them. The histological findings of DR were evaluated by pathologists at each contributing institute and at the JSCCR center. SM depth was evaluated according to the General Rules for Clinical and Pathological Studies on Cancer of the Colon, Rectum and Anus of the JSCCR.

A total of 112 patients with ECRC were enrolled in this study. For nonpedunculated submucosal invasive colorectal cancers, prevalence of DR in pretreatment biopsy specimens was significantly related to histological type and SM depth. Further, presence of DR was significantly correlated with SM depth in nonpedunculated ECRCs. The sensitivity and specificity of DR detection for prediction of pSM2 (tumor invasion ≥ 1000 μm) in nonpedunculated ECRC were 94.6% and 59.0%, respectively.

Evaluation of DR in pretreatment biopsy specimens may be a useful tool for

the clinicopathological diagnosis of colorectal carcinoma with massive invasion into the submucosal layer.

Introduction

Endoscopic mucosal resection and endoscopic submucosal dissection (ESD/EMR) have become established therapies for early gastrointestinal cancer. Since deep submucosal invasive cancers increase the possibility of lymph node metastasis, it is clinically important to determine the depth of submucosal invasion (SM depth) in early colorectal cancers (ECRCs) before treatment [1-4]. The rate of lymph node metastasis is low in shallow submucosal invasive cancers, as previously reported [5]. Consequently, we revealed that the use of the pathological subclassification, which defines pSM1 as SM invasion < 1000 μm and pSM2 as SM invasion ≥ 1000 μm, in patients diagnosed with colorectal submucosal (pSM) cancer was helpful as a guide in therapeutic options.

The new endoscopic systems (narrow band imaging (NBI) and the Fuji Intelligent Chromo Endoscopy (FICE) technologies) have the capability to enhance visibility by not only magnifying the mucosal surface, but also the capillary pattern, which may prove to be increasingly useful for detection of deep submucosal invasive cancers [6-9]. Therefore, with these new technologies, it is possible to predict SM depth without performance of biopsy. However, the new endoscopic systems are not yet in general use.

Desmoplastic reaction (DR), which is characterized by the infiltration of eosinophilic myofibroblasts in the stroma of invasive cancers [10, 11], is thought to start increasing when carcinoma cells have invaded beyond the muscularis mucosae, thus, DR may be a good indicator of early invasion [12-17].

In this multicenter collaborative study led by the Japanese Society for Cancer of the Colon and Rectum (JSCCR), the usefulness of DR detection in biopsy specimens from colorectal cancer patients was evaluated as a practical alternative method to diagnose pSM2 [18].

Materials and Methods

Patients and Specimens

Eleven institutions (Teikyo University, National Defense Medical Collage, Fukuoka University Chikushi Hospital, Hyogo Collage of Medicine, Jichi Medical University, Juntendo University, Juntendo University Shizuoka Hospital, Kyorin University, Showa University Northern Yokohama Hospital, National Cancer Center, and Dokkyo University school of Medicine) affiliated with the JSCCR participated in this collaborative study. Between September 2008 and January 2010, cases diagnosed with adenocarcinoma by biopsy were enrolled in this study, and the presence of DR was evaluated in them. The features of this prospective study are as follows: 1) all cases were diagnosed with early colorectal cancer (Tis or T1) by endoscopy and with adenocarcinoma by biopsy; 2) we aimed to collect at least 100 cases; 3) information such as age, sex, tumor location, size, macroscopic type, histological type, and the presence of desmoplastic reaction was evaluated by each institution when the cases were enrolled; 4) after endoscopical or surgical therapy, information such as histological type, depth of invasion, and existence of vascular invasion and lymph node metastasis were analyzed, excluding cases that were over T1, had no surveillance, or did not undergo treatment (exclusion criteria). This study was approved by the ethics committee in the JSCCR, and all samples were collected with the patients' consent.

Histology

The tumors were initially observed by conventional endoscopy, and subsequently diagnosed as adenocarcinoma by biopsy. They were classified macroscopically into pedunculated or nonpedunculated type, as previously reported [5]. Then, the invasion depth of the lesions was examined in the specimens obtained by surgical or endoscopic resection. The resected colon tissues were cut for evaluation of SM depth, which was done according to the General Rules for Clinical and Pathological Studies on Cancer of the Colon, Rectum and Anus of the JSCCR [19]. Briefly, EMR/ESD specimens were cut at 2 mm intervals, and surgically-resected colonic tissues were cut along the long axis at 2 to 4 mm intervals for sections stained with hematoxylin and eosin. The histological type of the deepest invasive portion was classified as well-, moderately-, or poorly-differentiated adenocarcinoma in accordance with the World Health Organization classification. SM depth was measured according to the General Rules for Clinical and Pathological Studies on Cancer of the Colon, Rectum and Anus of the JSCCR. The presence of DR in biopsy specimens was evaluated as previously described, and criteria for assessment of DR is shown in Table 1 [20, 21].

Statistical analysis

We performed χ^2analyses to determine the correlation between the various pathological parameters, and Fisher's exact test was also used as necessary. For continuous variables, two-group comparisons were performed with the

parametric two-sample Student's t-test and nonparametric Mann-Whitney U-test. All tests were two-tailed, with differences reported as significant if $P < 0.05$.

Results

One hundred and twelve cases were enrolled in this study, including 78 males and 34 females. DR was detectable in pretreatment biopsy specimens from 53 patients (47. 3%) (Table 2). We analyzed 81 cases upon omission of those that fell into the exclusion criteria.

Clinicopathological features

The clinicopathological features of the 81 patients (60 males and 21 females, mean age 67.5 ± 10.1 years) with early colorectal cancer are summarized in Table 3. With regard to histological type, 64 lesions (79%) were well-differentiated adenocarcinomas, and 14 lesions (17.3%) were moderately-differentiated adenocarcinomas. Macroscopically, 5 lesions (6.2%) appeared pedunculated, and 76 lesions (93.8%) were nonpedunculated. DR was detectable in pretreatment biopsy specimens from 40 patients (49.4%) (Table 3).

Relationship between clinicopathological features and presence of DR in biopsy specimens from patients with ECRC

There were significant differences between the DR-positive and DR-negative groups regarding distribution of the 3 histological types, well-, moderately-, and poorly-differentiated. There were also significant differences regarding distribution of the two groups of invasion depth, m, sm1; and sm2. However, none of the other factors, including age, sex, tumor size, and tumor location,

were significantly related to DR positivity in the pretreatment biopsy specimens of ECRC cases (Table 3).

Relationship between depth of submucosal invasion and presence of DR in biopsy specimens from patients with ECRC

According to the SM depth, we divided the patients into two groups, the pM and pSM1 group where SM invasion < 1000 μm and the pSM2 group where SM invasion ≥ 1000 μm, and investigated the relationship between SM depth and DR positivity. DR was detectable in pretreatment biopsy specimens from 3 of the 27 patients in the pM and pSM1 group, and in 37 of the 54 patients in the pSM2 group (Table 4). DR positivity had a sensitivity of 92.5% (37 out of 40) and specificity of 58.6% (24 of 41) for determination of pSM2 cases.

In addition, DR was detectable in 2 of the 25 patients in the pM and pSM1 group with nonpedunculated ECRC while it was demonstrated in 35 of 51 patients in the pSM2 group with nonpedunculated ECRC. DR positivity in nonpedunculated ECRCs had a sensitivity of 94.6% (35 of 37) and specificity of 59.0% (23 of 39) for determination of pSM2 cases (Table 5).

The number of cases with pedunculated ECRCs was small (n=5), thus, we were unable to investigate the relationship between SM depth and DR positivity in these cases.

Although DR wasn't detectable in pretreatment biopsy specimens from 17 patients of the pSM2 group, nevertheless, DR positivity was shown in the resected specimens from all of them (Table 6). This discrepancy may have

arisen due to some technical issues, including: 1) the size or site of the biopsy samples was unsuitable (8 lesions), and 2) the diagnosis of the biopsy specimens was incorrect (9 lesions). Taking account of these factors, the sensitivity and specificity of DR positivity was readjusted to 93.9% (46 of 49) and 75.0% (24 of 32), respectively, for determination of pSM2 cases (Table 7). Correspondingly, sensitivity and specificity of DR positivity in nonpedunculated ECRCs was 95.7% (44 of 46) and 76.7% (23 of 30), respectively, for determination of pSM2 cases (Table 8).

Discussion

Early colorectal cancer is highly curable by early detection and with appropriate therapy, however, the prevalence of lymph node metastasis is 10%-15% in patients with ECRC, especially in cases of deep submucosal invasive cancer in whom the risk is further increased, thus, the development of better diagnostic tools is necessary [2, 5, 22, 23]. The recent evolution of endoscopic instruments and techniques has promoted the use of endoscopic therapy, such as endoscopic mucosal resection and endoscopic submucosal dissection (EMR/ESD), for ECRC [24-26]. Guidelines for the treatment of colorectal cancer have also been standardized [27].

We have previously reported that the risk of lymph node metastasis in pSM2 cases is higher than that in pM and pSM1 patients [5]. Accordingly, determination of SM depth before endoscopic therapy is important and may avert needless treatment in those who may not benefit from this procedure. Recent progress in new endoscopic systems has made it possible to determine SM depth without performance of biopsy. However, few studies about this new system have been reported, and its usability in the clinic is uncertain, thus, the use of biopsy specimens for diagnosis of colorectal cancer is still the standard method in Japan. The aim of this study was to establish a better tool to determine SM depth from biopsy specimens based on DR detection.

The macroscopic classification of ECRCs falls into two categories,

pedunculated and nonpedunculated, based on the presence or absence of a stalk, respectively. The risk of lymph node metastasis in pedunculated ECRCs is significantly lower than that of nonpedunculated ECRCs [28, 29], thus, it is clinically beneficial to extract pSM2 lesions from patients with nonpedunculated ECRCs.

We had previously assessed the presence of DR in pretreatment biopsy specimens of ECRCs to predict the SM depth in a retrospective multicenter study, and we showed that there was a correlation between presence of DR and increased SM depth [18]. Here, in this prospective study, we verified the previous findings by demonstrating that the DR positivity rate in pretreatment biopsy specimens was significantly higher in ECRCs with an SM depth of ≥1000 μm (pSM2 group) than in cases where the SM depth was < 1000 μm (pM or pSM1 group). From our previous study, we found that the positive predictive value of prevalence of DR in predicting pSM2 was 91.9%, and the negative predictive value of absence of DR in predicting pM or pSM1 was 23.3%. This result suggested that DR positivity can predict pSM2.

Based on the results of the previous retrospective study, we performed power analysis (using power= 80% and $\alpha = 5\%$) to calculate the required sample size for detecting a relationship between SM depth and DR positivity. The required sample size was 34 cases per group, for a total of 68 cases, thus, our objective was to collect at least 100 cases. We were able to obtain only 27 cases belonging to the pM and pSM1 group, which was slightly less than the

ideal number, but we were able to enroll 54 cases in the pSM2 group, and a total of 81 cases was sufficiently powered to detect a relationship between SM depth and DR positivity.

In the present study, we clearly demonstrated that the presence of DR was significantly correlated with SM depth in nonpedunculated ECRCs. The sensitivity and specificity that the presence of DR predicts pSM2 in nonpedunculated ECRCs was 94.6% and 59.0%, respectively. These findings suggest that detection of DR in pretreatment biopsy specimens may be useful for the prediction of SM depth, especially in nonpedunculated ECRCs with slight submucosal invasion for which EMR/ESD treatment may be applicable.

However, a potential problem with this method is low specificity, since 17 of 54 cases in the pSM2 group were DR-negative. These biopsy specimens were reviewed and re-evaluated by our project research members, which revealed that DR was in fact present in the resected sections, but not the biopsies, from all 17 cases. This discrepancy may have arisen due to some technical issues, including: 1) the size or site of the biopsy samples from 8 lesions was unsuitable to correctly identify the presence of DR, and 2) 9 lesions were misinterpreted as DR-negative when in fact they were positive. Upon combination of the DR findings from both the biopsies and the resected tissues, sensitivity and specificity were improved to 95.7% and 76.7%, respectively. Additionally, a receiver operating characteristics (ROC) curve was generated, and the area under the curve (including 95% CI) was

calculated to determine the best discriminating level of SM depth for DR detection. ROC analysis confirmed 950 μm as the best diagnostic cut-off value of SM depth for DR detection, and 50 μm, which is the difference between the value of 950 μm as determined by COV and 1000 μm, the defining value of pSM2, is an acceptable measurement error range. This result provides a basis for determining that DR is good indicator of pSM2. Following a consensus meeting among JSCCR members, we established the following criteria for pathologic detection of DR: 1) the existence of a carcinoma is necessary; 2) histological findings of infiltrating carcinoma do not signify the presence of DR; 3) DR contains areas of collagen fiber accumulation and myofibroblast proliferation, but inflammatory infiltration does not signify the presence of DR; and 4) special staining procedure is not required for detection of DR.

Desmoplastic reaction (DR), which is characterized by the infiltration of eosinophilic myofibroblasts in the stroma of invasive cancer [10, 11], is thought to start increasing when carcinoma cells have invaded beyond the muscularis mucosae [12-17]. Nakada et al. have preliminarily reported that the presence of DR in the surface of resected ECRC predicts deep invasion into the submucosa [30]. However, DR wasn't compared between pSM1 and pSM2 groups in their study. This is the first study to analyze the correlation between SM depth and the presence of DR prospectively.

In conclusion, the detection of DR in pretreatment biopsy specimens may be useful for the prediction of SM depth in nonpedunculated ECRCs, thus,

notation of DR status in a pathology report of ECRCs allows the direction of patients towards appropriate therapy, such as EMR/ESD treatment.

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Disclosure Statement

The authors have no conflict of interest.

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Table 1

Criteria for detection of DR.

1) Existence of carcinoma is required for detection of DR.
2) The histological findings of infiltrating carcinoma do not signify the presence of DR.
3) DR contains areas of collagen fiber accumulation and myofibroblasts proliferation, but inflammatory infiltration does not signify the presence of DR.
4) Special staining procedure isn't required for detection of DR.

DR, desmoplastic reaction.

Table 2

Clinicopathological features of all patients.

Clinicopathological features	Number of patients (%)
Sex	
Male	78 (69.6)
Female	34 (30.4)
Age (year)	66.6±10.4
Tumor location	
Rectum	32 (28.6)
Sigmoid	32 (28.6)
Descending	7 (6.3)
Transverse	13 (11.6)
Ascending	18 (16.1)
Cecum	10 (8.8)
Histological type	
Well-differentiated	78 (69.6)
Moderately-differentiated	25 (22.3)
por, sig, muc	9 (8.0)

Tumor size (mm)	22.9 ±13.3
Desmoplastic reaction in biopsy specimens	
Positive	53 (47.3)
Negative	59 (52.7)

por, poorly-differentiated; sig, signet ring cell; muc, mucinous.

Table 3

Clinicopathological features of the 81 patients who were further analyzed.

Clinicopathological features	DR- positive(%) (n = 40)	DR-negative (%) (n = 41)	P value
Sex			
Male	29 (72.5)	31 (75.6)	0.804
Female	11 (27.5)	10 (24.4)	
Age (year)	67.0 ± 11.6	68.1 ± 8.49	0.620
Appearance			
Pedunculated	3 (7.5)	2 (4.9)	0.675
Nonpedunculated	37 (92.5)	39 (95.1)	
Histological type			
Well-differentiated	26 (65)	38 (92.7)	0.017
Moderately-differentiated	12 (30)	2 (4.9)	
por, sig, muc	2 (5)	1 (2.4)	
Depth of invasion			
pM+pSM1	3 (7.5)	24 (58.5)	<0.001
pSM2	37(92.5)	17 (41.5)	

por, poorly-differentiated; sig, signet ring cell; muc, mucinous.

pM+pSM1, SM invasion < 1000 μm; pSM2, SM invasion ≥ 1000 μm

Table 4

Relationship between depth of submucosal invasion and DR in biopsy specimens of patients.

Depth of submucosal invasion	Number of patients	DR	
		DR - negative	DR - positive
pSM2	54	17	37
pM + pSM1	27	24	3

DR, desmoplastic reaction.

pSM2, SM invasion ≥ 1000 μm; pM + pSM1, SM invasion < 1000 μm

Sensitivity : (37/37+3) x 100 = 92.5 %

Specificity : (24/24＋17) x 100= 58.6 %

Table 5

Relationship between depth of submucosal invasion and DR in biopsy specimens of patients with nonpedunculated type.

Depth of submucosal invasion	Number of patients	DR	
		DR - negative	DR - positive
pSM2	51	16	35
pM + pSM1	25	23	2

DR, desmoplastic reaction.

pSM2, SM invasion ≥ 1000 μm; pM + pSM1, SM invasion < 1000 μm

Sensitivity : (35/35+2) x 100 = 94.6 %

Specificity : (23/23＋16) x 100= 59.0 %

Table 6

Re-evaluation of 17 specimens, DR – negative and pSM2.

	Biopsy specimen	Resected lesion	Cause
1	DR (-)	DR (+)	Error in biopsy site (lesion was too big to obtain suitable biopsy sample)
2	DR (-)	DR (+)	Same as above
3	DR (-)	DR (+)	Same as above
4	DR (-)	DR (+)	Same as above
5	DR (-)	DR (+)	Same as above
6	DR (-)	DR (+)	Same as above
7	DR (-)	DR (+)	Technical error (biopsy specimen ~~is~~ was too small to evaluate DR)
8	DR (-)	DR (+)	Same as above
9	DR (+)	DR (+)	DR-positive upon re-evaluation by the Central Review
10	DR (+)	DR (+)	Same as above
11	DR (+)	DR (+)	Same as above
12	DR (+)	DR (+)	Same as above

13	DR (+)	DR (+)	Same as above
14	DR (+)	DR (+)	Same as above
15	DR (+)	DR (+)	Same as above
16	DR (+)	DR (+)	DR-positive upon re-evaluation at affiliated research center
17	DR (+)	DR (+)	Same as above

DR, desmoplastic reaction.

Table 7

Summary of re-evaluation of relationship between depth of submucosal invasion and DR in biopsy specimens of patients.

Depth of submucosal invasion	Number of patients	DR	
		DR - negative	DR - positive
pSM2	54	8	46
pM + pSM1	27	24	3

DR, desmoplastic reaction.

pSM2, SM invasion ≥ 1000 μm; pM + pSM1, SM invasion < 1000 μm

Sensitivity : (46/46+3) x 100 = 93.9 %

Specificity : (24/24＋8) x 100= 75.0 %

Table 8

Re-evaluation of relationship between depth of submucosal invasion and DR in biopsy specimens of patients with nonpedunculated type.

Depth of submucosal invasion	Number of patients	DR	
		DR - negative	DR - positive
pSM2	51	7	44
pM + pSM1	25	23	2

DR, desmoplastic reaction.

pSM2, SM invasion ≥ 1000 μm; pM + pSM1, SM invasion < 1000 μm

Sensitivity : (44/44+2) x 100 = 95.7 %

Specificity : (23/23＋7) x 100= 76.7 %