Sub-classification of type V_I pit patterns in colorectal tumors: Relation to the depth of tumor invasion

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Abstract. We analyzed pit patterns of colorectal tumors with magnifying colonoscopy, focusing on the relationship between the V_I pattern sub-classification and the depth of tumor invasion. The V_I pattern was divided into the well-demarcated and poorly demarcated subtypes. The percentage of tumors with a depth of invasion of over 1,000 μm was in the order of non-V types (type I-IV) < type V_I < type V_N. Most well-demarcated V_I lesions (80.8%) showed a depth of invasion of <1,000 μm compared to only 21.1% of poorly demarcated V_I lesions. Both the frequency of degeneration or prolapse of the epithelial lining and the frequency of desmoplastic reaction occurred in the order of non-V types < type V_I < type V_N, while the degree of histological differentiation and the frequency of residual muscularis mucosae occurred in the order of non-V types > type V_I > type V_N. Furthermore, the poorly demarcated V_I subtype showed a higher frequency of epithelial lining degeneration or prolapse and a lower frequency of residual muscularis mucosae than the well-demarcated V_I subtype. Tumors with a depth of invasion of >1,000 μm showed a higher frequency of epithelial lining degeneration or prolapse, a lower degree of histological differentiation, a higher frequency of desmoplastic reaction, and a lower frequency of residual muscularis mucosae than did tumors with <1,000 μm of invasion. These results suggest that V_I pit pattern sub-classification is a useful indicator of the depth of colorectal tumor invasion and treatment selection. A prospective study on this subject would be useful.

Introduction

When colonoscopy is performed to diagnose colorectal diseases, it is essential to determine whether a given lesion is a tumor or a non-tumor lesion, and, if the lesion is a tumor, whether it is an adenoma or a cancer. If the lesion is cancer, evaluation of the depth of invasion is also essential. Colorectal cancers limited to the mucosa are unlikely to metastasize, but those invading the submucosal layer are more likely to metastasize to the lymph nodes (1,2). Submucosal cancers with a depth of invasion of <1,000 μm rarely metastasize, whereas those with a depth of invasion of over 1,000 μm are more likely to metastasize (3-5). Therefore, distinguishing a depth of invasion of <1,000 μm (an indication for endoscopic treatment) is important for treatment selection.

Pit pattern evaluation with magnifying colonoscopy is useful for diagnosing colorectal tumors (6-10). Pits are glandular openings, about 50-100 μm in size, located on the mucosal surface. Evaluation of the pit pattern involves the size, morphology, and arrangement of the pits. Kudo *et al* observed colorectal lesions under a stereoscopic microscope and classified the pit patterns into types I through V (11): type I and II lesions are mostly non-tumor, whereas type III, IV, and V lesions are mostly epithelial tumors (6,12-14). Type I through IV lesions are primarily confined to the mucosa, and the V_N (non-structure) type refers to deeply invading tumors with a depth of invasion of over 1,000 μm (15-18). The V_I (irregular) type, however, represents tumors with varying depths of invasion, ranging from those confined to the mucosa to tumors invading the deep submucosal layers (17,19). A further sub-classification of the V_I type based on lesion depth (more or less than 1,000 μm) would assist in treatment selection.

We subdivided the V_I pit pattern into well-demarcated and poorly demarcated subtypes, and evaluate the usefulness of this sub-classification to predict the presence/absence of tumor invasion of the submucosal layer. The study was also designed to analyze the relationship of the histological architecture of tumors to the pit patterns and depth of tumor invasion.

Patients and methods

Patient selection and study design. The study involved 99 cases with colorectal tumors satisfying all of the following inclusion criteria: i) cases who had undergone endoscopic or surgical resection at the Kurume University Hospital or its affiliated facilities between April 1987 and May 2003; ii) cases in whom the preoperative pit pattern (observed under magnifying colonoscopy) could be compared with the

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pit pattern in the resected tissue observed under a stereoscopic microscope; iii) cases showing concomitant existence of the two pit patterns mentioned above; iv) cases in which post-operative histopathological evaluation was possible. All the diagnostic and treatment procedures were performed by colonoscopists trained in magnifying colonoscopy.

Two liters of a preparatory solution of electrolytes and polyethylene glycol was administered orally to each patient before colonoscopy. All colonoscopies were performed using commercially available videocolonoscopes (CF-H260Z, CF-Q240Z; Olympus Optical Co., Ltd., Tokyo, Japan) that provide both conventional and magnifying images. All lesions detected at colonoscopy were diagnosed by both magnification and chromoendoscopy using 0.075% crystal violet.

Endoscopically or surgically resected tissue specimens were mounted on a plate and fixed in 10% formalin for 24 h. They were washed with water and stained with Carrazzi's hematoxylin, followed by observation under a stereoscopic microscope for pit patterns on the surface of each lesion immersed in water, and then photographed.

Pit pattern diagnosis. We classified pit patterns based on the classification of Kudo (11,19). The type I pit pattern represents regular round crypts, the type II pattern represents stellar or papillary crypts, the type III pattern represents small tubular or roundish crypts (III_S) or large tubular or roundish crypts (III_L), the type IV pattern consists of branch- or gyrus-like crypts, and the type V pattern consists of irregular crypts (V_I) or non-structural crypts (V_N) (Fig. 1). V_I was further divided into the well-demarcated subtype (showing clear pit contours) or the poorly demarcated subtype (without clear pit contours) (Fig. 2). For lesions exhibiting multiple pit patterns, the pit pattern of the most atypical area was adopted. We collectively counted types I through IV as non-V types.

Histopathological analysis. After observation, a cut line was made at the center of lesions measuring <5 mm, and multiple cut lines were placed at intervals of 2 mm in lesions measuring >6 mm in size. Sections were prepared and subjected to hematoxylin and eosin staining. The sections were assessed by two pathologists as follows:

i) Evaluation of the depth of tumor invasion: according to the method of Kitajima *et al* (3), the vertical depth of invasion of each lesion invading the submucosal layer was measured, and the lesions were divided into two groups: lesions with a depth of invasion of <1,000 μm (including lesions confined to the mucosa) and lesions with a depth of invasion of over 1,000 μm.

ii) Evaluation of the histological architecture: four histological indicators were assessed. The degeneration/prolapse of the epithelial lining was rated as present or absent (Fig. 3A). The degree of histological differentiation of cancer was rated as well differentiated, moderately differentiated, or poorly differentiated (Fig. 3B). The desmoplastic reaction was rated as absent (-), present but without reduced gland density (+), or present with reduced gland density (++) (Fig. 3C). Residual muscularis mucosae were rated as present (++), partially present (+), or absent (-) (Fig. 3D).

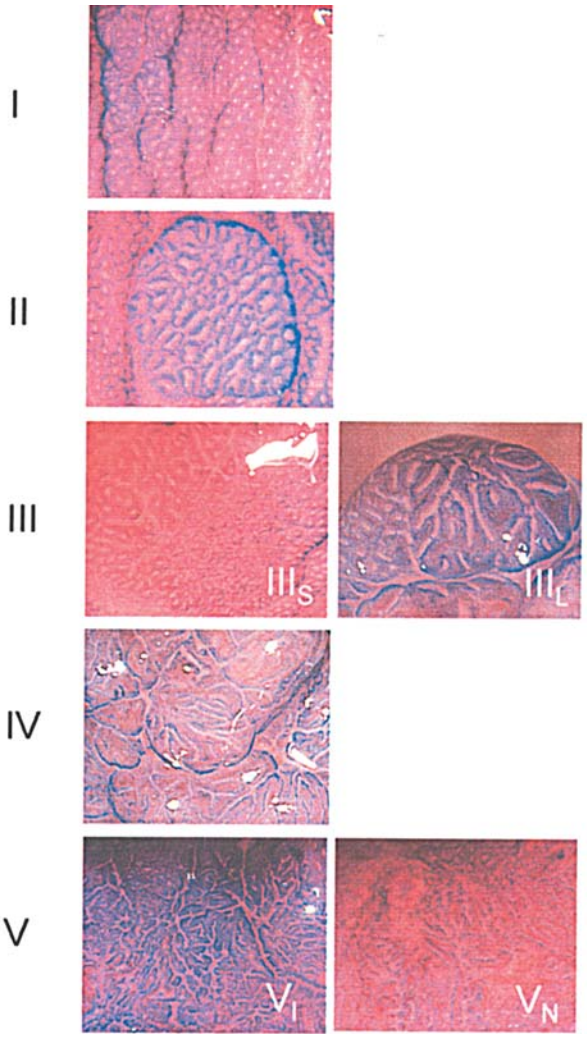

Figure 1. Classification of the magnified features of the pit patterns in colorectal lesions according to the classification of Kudo: type I, round pits; type II, stellar or papillary pits; type III_S, small tubular or roundish pits; type III_L, large tubular or roundish pits; type IV, branch-like or gyrus-like pits; type V_I, irregular pits; type V_N, non-structural pits.

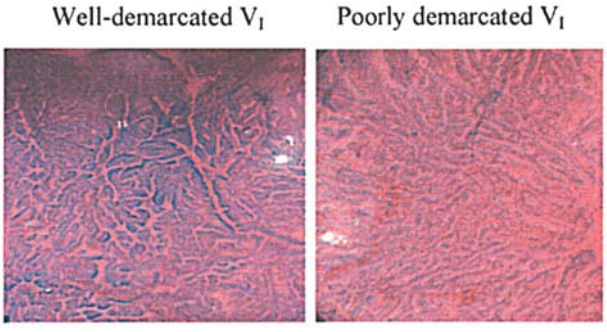

Figure 2. Sub-classification of the Type V_I pit pattern in colorectal tumors. V_I pits were divided into two subtypes: the well-demarcated subtype (showing clear pit contour) and the poorly demarcated subtype (without clear pit contour).

Statistical analysis. The χ^2 test, two-way analysis of variance, and Mann-Whitney U test with Bonferroni's correction were used for testing differences among the three groups. $P<0.05$ was regarded as statistically significant.

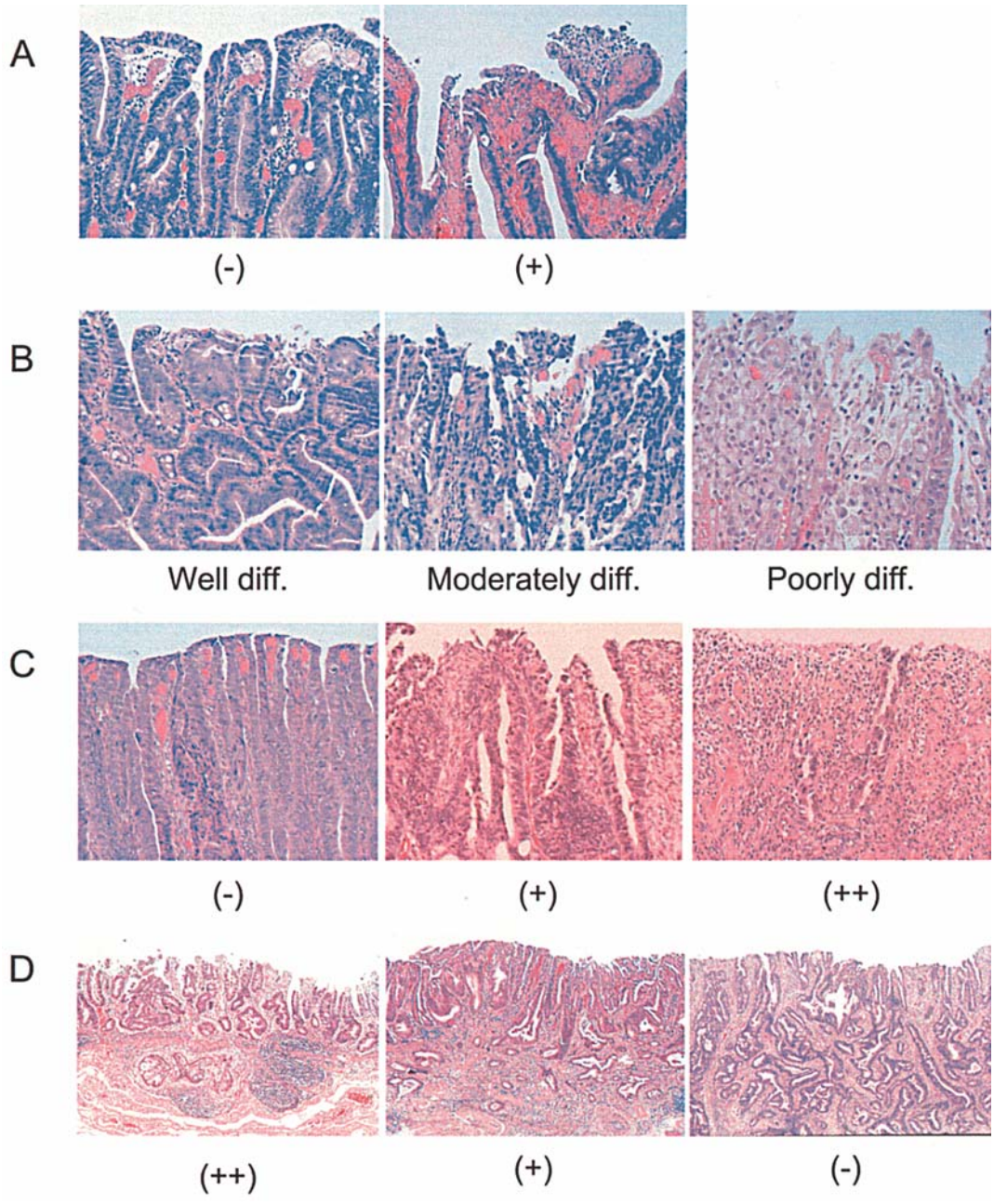

Figure 3. Classification of colorectal tumors according to histological architecture. Four histological indicators were assessed: (A) Degeneration/prolapse of the lining epithelium was rated on a two-category scale: present, absent. (B) The degree of histological differentiation of the cancer was rated on a three-grade scale: well differentiated, moderately differentiated, poorly differentiated. (C) Desmoplastic reaction was rated on a three-grade scale: absent (-), present but without a reduction of the gland density (+), and present and accompanied by a reduction of the gland density (++). (D) Residual muscularis mucosae was rated on a three-grade scale: present (++), partially present (+), absent (-).

Results

Relationship between the pit pattern and depth of tumor invasion. The depth of tumor invasion was <1,000 μm for all types other than type V (Table I). Most well-demarcated V_I lesions, 80.8%, showed a depth of invasion of <1,000 μm compared to only 21.1% of poorly demarcated V_I lesions ($P<0.0001$). All V_N lesions showed a depth of tumor invasion of >1,000 μm. These results indicate that the depth of tumor invasion increases in the order of non-V types < well-demarcated V_I subtype < poorly demarcated V_I subtype < V_N type ($P<0.0001$).

Using these data, we tested the validity of classifying all non-V type and well-demarcated V_I subtypes as having lesion depths of <1,000 μm, and of classifying poorly demarcated V_I subtypes and V_N type as having lesion depths >1,000 μm (Table II). In this manner an overall correct diagnosis rate of 90.9% was achieved. For the group with a lesion depth >1000 μm, sensitivity was 80.8%, specificity was 94.5%, and the true positive rate was 84.0%.

Relationship of histological architecture to the pit pattern and depth of tumor invasion. We next analyzed the relationship of histological architecture to the pit pattern (Table III). The

Table I. Relationship between the V_I sub-classification and depth of tumor invasion.

	Pit pattern				
	Non-V types	Well-demarcated V_I	Poorly demarcated V_I	V_N	Total
<1000 μm	48 (100%)	21 (80.8%)	4 (21.1%)	0	73
>1000 μm	0	5 (19.2%)	15 (78.9%)	6 (100%)	26
	48	26	19	6	99

Table II. Diagnostic value of the V_I sub-classification for assessment of the tumor depth.

	>1000 μm		
Overall correct diagnosis rate	Sensitivity	Specificity	True positive rate
84.0% (90/99)	90.9% (21/26)	80.8% (69/73)	94.5% (21/25)

frequency of degeneration or prolapse of the epithelial lining (P<0.0001) and the frequency of desmoplastic reaction (P<0.0001) occurred in the order of non-V types < type V_I < type V_N, while the degree of histological differentiation (P=0.0105) and the frequency of residual muscularis mucosae (P<0.0001) occurred in the order of non-V types > type V_I > type V_N. The poorly demarcated V_I subtype showed a higher frequency of epithelial lining degeneration or prolapse (P=0.0032) and a lower frequency of residual muscularis mucosae (P=0.0037) than the well-demarcated V_I subtype, but there was no difference in the degree of histological differentiation (P=0.0904) or the frequency of desmoplastic reaction (P=0.3206).

Tumors with a depth of invasion of >1,000 μm showed a higher frequency of epithelial lining degeneration or prolapse (P<0.0001), a lower degree of histological differentiation (P<0.0001), a higher frequency of desmoplastic reaction (P<0.0001), and a lower frequency of residual muscularis mucosae (P<0.00019) than tumors with a depth of invasion of <1,000 μm (Table IV).

Table III. Relationship between the pit pattern and the histological architecture.

	Pit pattern			
	Non-V types (%)	Well-demarcated V_I (%)	Poorly demarcated V_I (%)	V_N (%)
Degeneration/prolapse of the lining epithelium				
(-)	42 (87.5)	17 (65.4)	4 (21.1)	0
(+)	6 (12.5)	9 (34.6)	15 (78.9)	6 (100)
Degree of superficial differentiation				
Well differentiated	12 (85.7)	20 (90.9)	12 (63.2)	1 (16.6)
Moderately differentiated	2 (14.3)	2 (9.1)	6 (31.6)	4 (66.8)
Poorly differentiated	0	0	1 (5.2)	1 (16.6)
Superficial desmoplastic reaction				
(-)	48 (100)	25 (96.2)	16 (84.2)	3 (50)
(+)	0	0	1 (5.3)	0
(++)	0	1 (3.8)	2 (10.5)	3 (50)
Residual muscularis mucosae				
(++)	48 (100)	22 (84.6)	7 (36.8)	1 (16.7)
(+)	0	3 (11.5)	7 (36.8)	3 (50)
(-)	0	1 (3.9)	5 (26.4)	2 (33.3)
Total	48	26	19	6

Table IV. Relationship between depth of tumor invasion and histological architecture.

	Depth of tumor invasion	
	<1000 μm (%)	>1000 μm (%)
Degeneration/prolapse of lining epithelium		
(-)	59 (76.6)	4 (18.2)
(+)	18 (23.4)	18 (81.8)
Degree of superficial differentiation		
Well differentiated	33 (84.6)	12 (54.5)
Moderately differentiated	6 (15.4)	8 (36.4)
Poorly differentiated	0	2 (9.1)
Superficial desmoplastic reaction		
(-)	76 (98.7)	16 (72.7)
(+)	0	1 (4.5)
(++)	1 (1.3)	5 (22.8)
Residual muscularis mucosae		
(++)	74 (96.1)	4 (18.2)
(+)	2 (2.6)	11 (50)
(-)	1 (1.3)	7 (31.8)
	77	22

Discussion

The first reports on the pit patterns of the colorectal mucosa were made from studies of normal colorectal mucosa by Bank *et al* (20) and Tada *et al* (21). Subsequently, reports of the pit patterns in colorectal tumors began to be published (11). The recent development of magnifying endoscopes allows observation of pit patterns with a higher resolution (6-10). We performed the present study primarily to evaluate the usefulness of V_I pit pattern sub-classification for diagnosing the depth of colorectal tumor invasion. The study was also designed to analyze the relationship of the histological architecture to the pit pattern and the depth of tumor invasion.

All tumors of types I to IV, but not type V, showed a depth of tumor invasion of <1,000 μm. All V_N type lesions showed a depth of invasion of over 1,000 μm, consistent with previous reports (18,22). The depth of tumor invasion was <1,000 μm in 80.0% of the well-demarcated V_I lesions, but only in 21.1% of the poorly demarcated V_I lesions. Thus, the percentage of deeply invading lesions follows the order of non-V types < well-demarcated V_I subtype < poorly-demarcated V_I subtype < V_N type.

We then added the V_I pit pattern sub-classification to the classification of Kudo (11,19) as a paradigm for diagnosing the depth of tumor invasion. Using this system, we identified lesions with a depth of invasion of >1,000 μm with a satisfactorily high probability (sensitivity 80.8%, specificity 94.5%, and true positive rate 84.0%). The V_I type represents lesions with depths of invasion ranging from those confined to the mucosa to lesions invading the deep layers of the submucosa, requiring establishment of a sub-classification to distinguish them. Our V_I pit pattern sub-classification is useful for determining whether or not the depth of tumor invasion is <1,000 μm.

We also found that the frequency of degeneration or prolapse of the epithelial lining and the frequency of desmoplastic reaction occurred in the order of non-V types < well-demarcated V_I subtype < poorly demarcated V_I subtype < type V_N, while the degree of histological differentiation and the frequency of residual muscularis mucosae occurred in the order of non-V types > well-demarcated V_I subtype > poorly demarcated V_I subtype > type V_N. These results suggest that degeneration or prolapse of the epithelial lining is induced by weakening of the superficial layer of the lesion due to the disappearance of the muscularis mucosae, an increased desmoplastic reaction, and/or reduction of tumor differentiation during the course of tumor invasion.

Poorly demarcated V_I pits are identified by a reduction in the chromatic response to crystal violet due to the destruction of epithelial cells. When combined with the finding that degeneration or prolapse of the epithelial lining occurs more frequently with increasing tumor depth, the formation of poorly demarcated V_I pits may be at least partially attributable to deeper invasion of the tumor.

In conclusion, our pit pattern classification (including the V_I type sub-classification) is useful for diagnosing the depth of colorectal tumor invasion and therefore for treatment selection. A prospective study to validate the usefulness of this sub-classification would be useful.

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