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ORIGINAL ARTICLE

HISTOPATHOLOGICAL CHARACTERISTICS OF LATERALLY SPREADING TUMOR (LST) TYPE EARLY COLORECTAL CANCER.

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Abstract Recently, laterally spreading tumor (LST) of early colorectal carcinoma has been treated by endoscopic submucosal dissection (ESD) more frequently. Many cases of early colorectal carcinomas are diagnosed as well differentiated adenocarcinomas, but histological and/or immunohistorogical heterogeneity is often seen in LSTs. In this study, we analyzed morphological characteristics of 61 lesions of LST-type early colorectal carcinoma by using histolosical and immunohistochemical procedures. Presence of high grade atypia components was significantly correlated with depth of submucosal invasion (P=0.0102), and these were predominantly located to the invasive front (deep part) of the submucosal carcinoma. Allred scores of p53 and Ki-67 labeling indices were increased in the component of high grade atypia regardless of depth of invasion. Expressions of CDX2 and CD10 were significantly upregulated in a depth-of-invasion dependent manner, and these tended to be highly expressed in the high grade atypias. In conclusion, the present study indicates that the majority of LST is histologically heterogeneic. The component of high grade atypia of LST upregulates cell proliferation, which may leed to increase the malignant potential of LST for invading the submucosa.

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Key words: Colorectal cancer; early cancer; laterally spreading tumor; endoscopic resection.

原　著

側方伸展型発育を示す早期大腸癌の組織学的特徴

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抄録　近年，側方伸展型腫瘍(laterally spreading tumor; LST)型の早期大腸癌に対する内視鏡的切除術施行が増加してきた．多くの早期大腸癌症例は高分化型腺癌と診断されているが，組織学的異型度や免疫染色の染色性は同一病変の中でも均一でないことが多い．今回我々はLST型の早期大腸癌61症例について，その組織学的・免疫組織学的特徴について検討した．早期癌の中でも高異型度成分を持つものは有意に粘膜下層浸潤を認めた(P=0.0102)．また高異型度成分は粘膜下層浸潤癌の浸潤先進部に多く認められた．p53およびKi-67の発現は，深達度にかかわらず高異型度成分に高かった．CDX2とCD10の発現は深達度が深い症例ほど高く，また高異型度成分には高い傾向があった．以上の結果より，多くのLST型早期大腸癌では組織学的多様性が認められ，高異型度の成分では細胞増殖マーカーの発現が上昇し，腫瘍悪性化，粘膜下層浸潤と関連していると考えられた．

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Introduction

Early colorectal cancer is defined as carcinoma invasion limited to the submucosa [1]. Due to recent advances in endoscopic techniques such as magnifying endoscope and narrow band imaging [2, 3] and newly developed methods for endoscopic submucosal dissection (ESD), endoscopic treatment has been performed more frequently for larger colorectal tumors that had been otherwise resected by surgery previously.

The majority of early colorectal cancer is histologically diagnosed as well differentiated adenocarcinoma because a pathological diagnosis is made based on the histological predominance of the tumor. However, many colorectal cancers consist of well differentiated adenocarcinoma as well as small components of moderately to poorly differentiated adenocarcinoma or mucinous adenocarcinoma. In addition, several researchers have proposed that colorectal adenocarcinomas should be subclassified into the two groups: low grade atypia (dysplasia) and high grade atypia (dysplasia) [4-7]. But, histopathological characterization of the low/high grade atypias has not yet clarified extensively.

Laterally spreading tumor (LST) is one of the morphologically classified superficial neoplastic lesions under endoscopic observation. LST is defined as a laterally growing lesion at least 10 mm in diameter, and is classified into the several subtypes based on the surface patterns such as granular/non-granular and homogenous/non-homogenous appearances. The endoscopic subtypes of LST are reportedly related to both histological characteristics and depth of tumor [8].

In this study, we analyzed morphological characteristics of 61 lesions of LST-type early colorectal carcinoma by using histological and immunohistochemical procedures, and discussed the process tumor growth of LST-type colorectal carcinoma.

Materials and Methods

Patient materials and tissue sampling.

We analyzed 61 lesions of LST-type early colorectal adenocarcinoma, which were endoscopically resected from 53 patients: 51 lesions resected by ESD, and 10 lesions by endoscopic mucosal resection (EMR) at Hirosaki University Hospital (Table 1). Colorectal adenocarcinoma tissues were pasted on the rubber boards, stretched on equal force, and fixed with 10% buffered formalin. These specimens were serially cut in 2 mm-width, embedded in paraffin, cut into 4 μm sections, and stained with hematoxylin and eosin (HE). After the routine histological examinations, several representative tissue sections in each case were selected, and the corresponding paraffin blocks were cut into 4 μm sections for immunohistochemistry.

Immunohistochemical examinations.

Immunohistochemical examination was performed on deparaffinized sections using the standard avidin-biotin-peroxidase complex (ABC) method with an automated immunostainer (Benchmark XT; Ventana Medical System, Tucson, AZ, USA) [9]. The antibodies used were p53 (DO-7, 1:50 dilution; DakoCytomation, Glostrup, Denmark), Ki-67 (MIB-1, 1:100 dilution; DakoCytomation, Glostrup, Denmark), CDX2 (CDX2-88, 1:100 dilution; BioGenex, San Ramon, CA, USA), CD10 (56C6, 1:1 dilution; Nichirei, Tokyo, Japan), MUC2 (Ccp58, 1:50 dilution; Novocastra, Newcastle, UK) [10, 11].

Evaluation and classification.

We measured the maximum size and the maximum height of the tumors as well as the height of surrounding non-neoplastic mucosa. Histological grade was classified

Table 1 Clinicopathological features of LST-type early colorectal carcinomas

Patients	n=53	
Age (mean, SD)	69.1	±10.2
Sex		
Male	34	
Female	19	
	n=61	
Location		
Right sided colon	32	
Left sided colon	29	
Histological grade (dominant) *		
tub1	60	
tub2	1	
Histological grade (subclassification) *		
tub1	26	
tub1>pap	5	
tub1>pap, tub2	7	
tub1>tub2	20	
tub1>tub2, muc	2	
tub2>tub1	1	
Depth of invasion **		
M	25	
MM	18	
SM	18	
Size of tumor (mm)	30	±2.2
Height of tumor (μm)	2823.8	±3007.0
Height of non-neoplastic mucosa (μm)	313.1	±104.8

* Histological grades are classified according to the Japanese Classification of Colorectal Carcinoma (1): tub1, well differentiated adenocarcinoma; tub2, moderately differentiated adenocarcinoma; pap, papillary adenocarcinoma; and muc, mucinous adenocarcinoma.
** M, mucosal carcinoma without muscularis mucosae invasion; MM, mucosal carcinoma with muscularis mucosae invasion; and SM, submucosal invasive carcinoma.

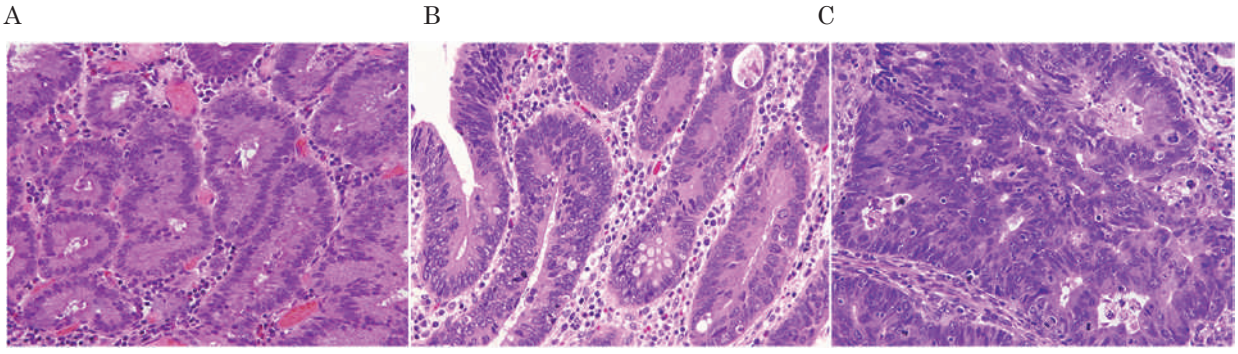

Figure 1 Histological grade of carcinoma.
Low grade atypia of well differentiated tubular/papillary adenocarcinoma (L-well) (A). High grade atypia of well differentiated tubular/papillary adenocarcinoma (H-well) (B). High grade atypia of moderately differentiated tubular adenocarcinoma (H-mod) (C).

into the three groups as follows: (1) low grade atypia of well differentiated tubular/papillary adenocarcinoma (L-well), (2) high grade atypia of well differentiated tubular/papillary adenocarcinoma (H-well), and (3) high grade atypia of moderately differentiated tubular adenocarcinoma (H-mod) (Figure 1). There were no lesions of low grade atypia of moderately differentiated tubular adenocarcinoma. Distribution of histological

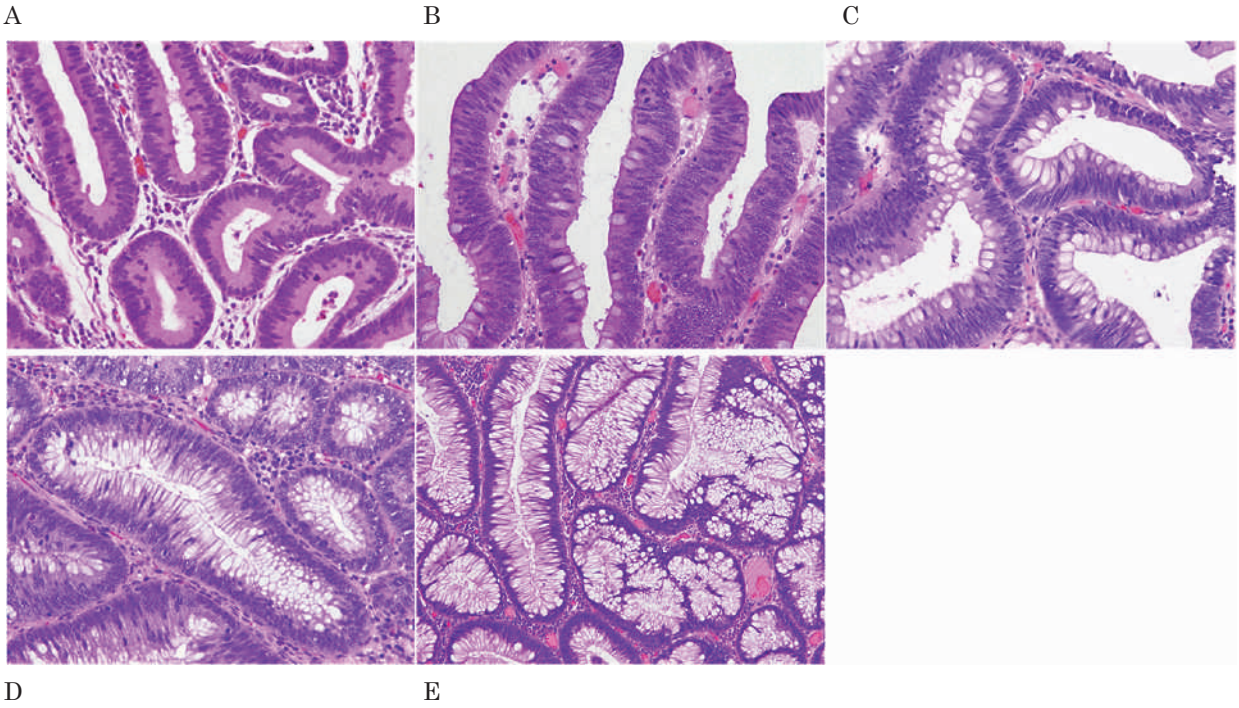

Figure 2 Mucus amount.
Mucus amount of tumor cells was evaluated as follows: score 1, poor (A); score 2, relatively poor (B); score 3, moderate (C); score 4, rich (D): score 5, very rich (E).

grades was mapped in each representative section. Mucus amount of the tumor cells was evaluated as follows; score 1, poor mucus and few goblet cells; score 2, relatively poor; score 3, moderate as non-neoplastic mucosa; score 4, rich; and score 5, very rich in mucus amount (Figure 2). Assessments of immunohistochemical staining for p53 and CDX2 were followed to the scores advocated by Allred, et al (Allred score, Figure 3) [12]. This method was originally used to assess immunostaining signals of estrogen receptor (ER) and progesterone receptor (PgR) of breast cancer. A proportion score (PS) is assigned that represents the estimated proportion of positive tumor cells on the entire slide. An intensity score (IS) is assigned that estimates the average staining intensity of positive tumor cells. The PS and IS are added to obtain a total score (TS). Positive immunoreactivities for Ki-67, CD10 and MUC2 were evaluated by percentage in the tumor tissues.

Statistical analysis.

The data of the size and the height of tumors, as well as the height of non-neoplastic mucosa were analyzed by one-way ANOVA, using Tukey-Kramer test with set α priori to 0.05. The components of histological grades seen in one lesion, and its depth of invasion were analyzed by Spearman rank correlation. Histopathological scores among the groups of histological grade and depth of tumor invasion were analyzed by Steel-Dwass test.

Results

Morphological characteristics of LST

Clinicopathological and morphological features of LSTs are summarized in Tables 1 and 2. There was an inverse correlation between the depth of tumor and the size of

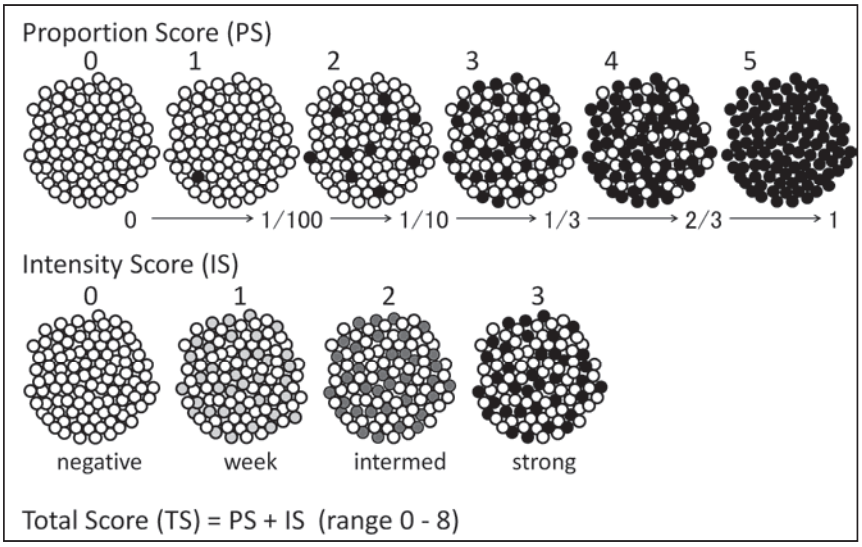

Figure 3 Allred score.

A method for scoring immunostaining signals by Allred, et al. −12). This score is originally used to assess expressions of estrogen receptor −ER) and progesterone receptor −PgR) of breast cancer. A proportion score −PS) is assigned that represents the estimated proportion of positive tumor cells on the entire slide. An intensity score −IS) is assigned that estimates the average staining intensity of positive tumor cells. The PS and IS are added to obtain a total score −TS). TS of 3 or more are reported as positive.

Table 2 Morphological characteristics of LST-type early colorectal carcinoma

	Depth	mean ±SD	*P*
Size of tumor (mm)	M	34.8 ±20.2	0.046
	MM	31.3 ±12.7	
	SM	21.9 ±14.2	
Height of tumor (μm)	M	2400.0 ±2680.6	0.168
	MM	3947.2 ±3460.7	
	SM	2288.9 ±2808.6	
Height of non-neoplastic mucosa (μm)	M	282.0 ±95.6	0.151
	MM	338.9 ±115.8	
	SM	330.6 ±100.2	

M, mucosal carcinoma without muscularis mucosae invasion; MM, mucosal carcinoma with muscularis mucosae invasion; and SM, submucosal invasive carcinoma.

tumor; i.e. submucosal carcinomas were smaller than mucosal carcinomas (P=0.046) (Figure 4). Histological grades of LSTs are shown in Table 3. Presence of the component of high grade atypia (H-well, H-mod) was significantly correlated with depth of submucosal invasion (P=0.0102). Various patterns of distribution regarding histological grades were observed (Figure 5 and Table 4). In the cases of mucosal carcinoma, the component of high grade atypia was mainly found at the superficial part of LST, while that was predominantly located to the invasive front (deep part) of the submucosal carcinoma.

Histological phenotypes of LST

Amount of the mucus and results of immunohistochemistry are summarized in Table 5 and Figure 6. A representative case of LST is shown in Figure 7. Amount of the

Table 3 Histolosical grades of LST-type early colorectal cancer

	total	M	MM	*SM*
n	61	25	18	18
L-well	4	2	1	1
L-well + H-well	20	11	8	1
H-well	7	3	1	3
L-well + H-well +H-mod	1	0	1	0
L-well + H-mod	8	4	1	3
H-well + H-mod	21	5	6	10
			$P = 0.01022$	
			rs = 0.33157	

L-well, low grade atypia of well differentiated tubular/papillary adenocarcinoma; H-well, high grade atypia of well differentiated tubular/papillary adenocarcinoma; H-mod high grade atypia of moderately differentiated tubular adenocarcinoma; M, mucosal carcinoma without muscularis mucosae invasion; MM, mucosal carcinoma with muscularis mucosae invasion; and SM, submucosal invasive carcinoma.

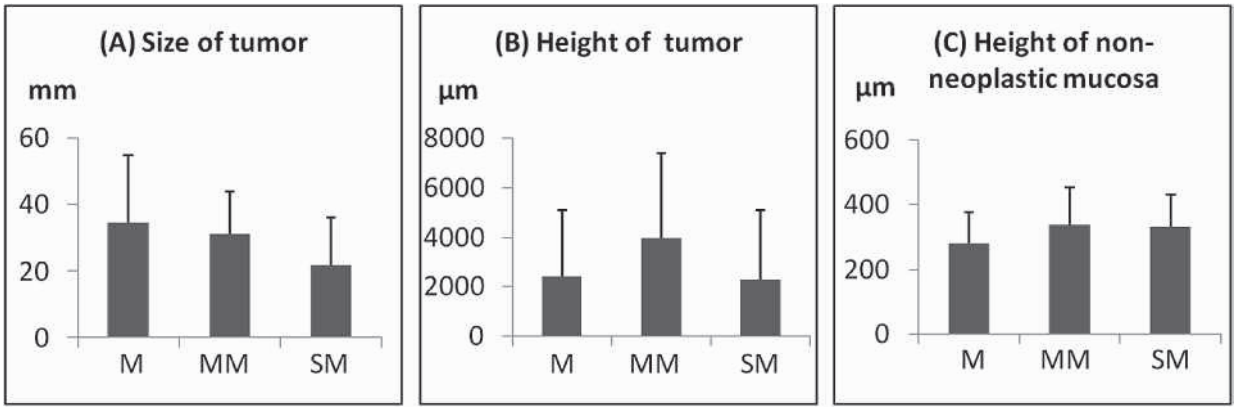

Figure 4 Morphological characteristics of LST-type early colorectal carcinoma.
M, mucosal carcinoma without muscularis mucosae invasion; MM, mucosal carcinoma with muscularis mucosae invasion; and SM, submucosal invasive carcinoma.

mucus of LSTs was significantly decreased in the component of high grade atypia regardless of depth of invasion. Allred scores of p53 and Ki-67 labeling indices were increased in the component of high grade atypia regardless of depth of invasion. Expressions of CDX2 and CD10 were significantly upregulated in a depth-of-invasion dependent manner, and revealed a tendency of higher expression in the high grade atypia. Degree of MUC2 expression showed a similar tendency to that of mucus amount, but was decreased dependently to depth of invasion.

Discussion

LST is one of the morphologically classified superficial neoplastic lesions at least 10 mm in diameter. The majority of LSTs belongs to mucosal carcinoma, but some LSTs invade the submucosa [8]. Therefore, accurate indication of endoscopic treatment for LST is an important issue for gastrointestinal endoscopists. However, clinicopathological characteristics of LST have not been well understood yet. In this study, we analyzed morphological characteristics of LST and demonstrated increased expression of Ki-67/p53/CD10 in the high grade atypia of

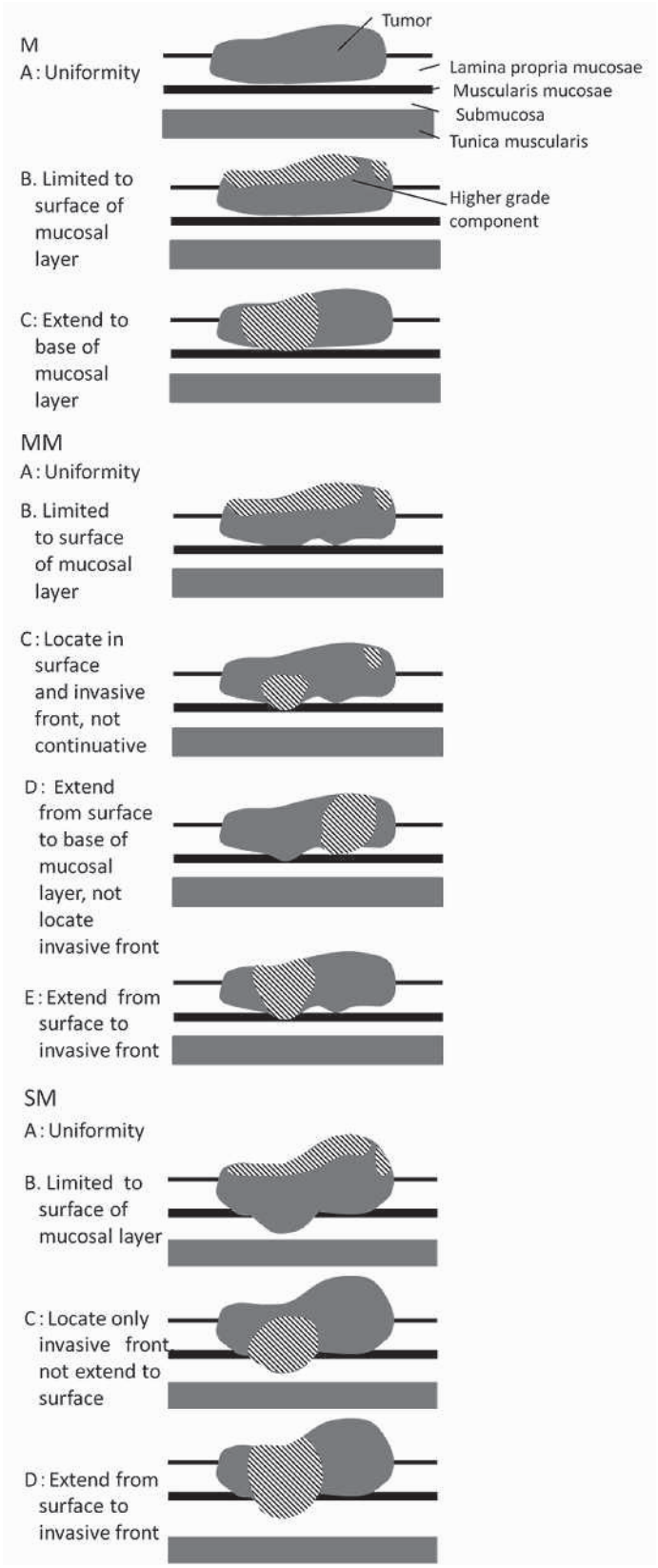

Figure 5 Distribution patterns of histolosical grades of LST-type early colorectal cancer.
M, mucosal carcinoma without muscularis mucosae invasion; MM, mucosal carcinoma with muscularis mucosae invasion; and SM, submucosal invasive carcinoma.

Table 4 Distribution patterns of histolosical grades of LST-type early colorectal cancer.

Mucosal carcinoma without muscularis mucosae invasion (M)			
A	5	L-well	2
		H-well	3
B	10	L-well + H-well	7
		L-well + H-mod	3
C	10	L-well + H-well	4
		L-well + H-mod	1
		H-well + H-mod	5
Mucosal carcinoma with muscularis mucosae invasion (MM)			
A	2	L-well	1
		H-well	1
B	5	L-well + H-well	4
		H- well + H-mod	1
C	1	H-well + H-mod	1
D	2	L-well + H-well	1
		H-well + H-mod	1
E	8	L-well + H-well + H-mod	1
		L-well + H-well	3
		L-well + H-mod	1
		H-well + H-mod	3
Submucosal invasive carcinoma (SM)			
A	4	L-well	1
		H-well	3
B	1	L-well+ H-mod	1
C	1	H-well + H-mod	1
D	12	L-well + H-well	1
		L-well + H-mod	2
		H-well + H-mod	9

adenocarcinoma, suggesting the potential for invasion to the submucosa.

Early colorectal cancer is defined as carcinoma invasion limited to the submucosa. The majority of the early carcinomas are diagnosed as well differentiated adenocarcinoma because of the histological predominance. However, many cases of well differentiated adenocarcinoma have small components of moderately or poorly differentiated adenocarcinoma or mucinous adenocarcinoma inside. Such histological heterogeneity has been well described in invasive carcinomas [13-15]. Presence of poorly differentiated carcinoma and higher grade atypia (dysplasia) at the deepest level of tumor invasion (invasive front) means the higher malignant potential even if they are not predominant. In this study, we demonstrated clearly the histological heterogeneity of LST-type early colorectal carcinoma. Some of the mucosal carcinomas had a component of high grade atypia while the others showed histological homogeneity. The component of high grade atypia was located mainly at the superficial part of LST, which might to be caused by chronic irritation from the intestinal contents, like foods and feces. On the other hand, the majority of carcinomas invasive to the submucosa showed the component of high grade atypia at the invasive front (deep part) and the superficial part of LSTs. The component of high grade atypia in turn showed a high malignancy potential, resulting in more cases with submucosal invasion. More, it was also associated with a decreased amount of mucus, suggesting a decrease in neoplastic differentiation. MUC2 is one of important mucin proteins, and is a marker of goblet cells of

Table 5 Mucus amount and immunohistochemical results.

depth	M			MM			SM		
histological grade	L-well	H-well	H-mod	L-well	H-well	H-mod	L-well	H-well	H-mod
n	17	19	9	11	16	8	5	14	13
mucus amount *#	3.2 ± 1.3	2.7 ± 1.1	2.9 ± 1.2	3.5 ± 1.4	2.5 ± 1.4	1.9 ± 1.2	3.2 ± 1.5	2.4 ± 1.2	1.5 ± 0.5
p53 *#	3.3 ± 1.7	4.7 ± 1.7	4.7 ± 1.5	5.0 ± 1.8	6.3 ± 1.5	6.0 ± 1.6	3.6 ± 1.1	6.0 ± 1.4	5.7 ± 2.4
CDX2 *#	5.1 ± 3.2	5.4 ± 2.8	5.8 ± 2.7	6.2 ± 2.6	6.2 ± 1.7	6.0 ± 1.7	6.4 ± 1.7	6.5 ± 0.9	6.6 ± 1.2
Ki-67 *#	36.5 ± 16.5	52.1 ± 14.0	63.3 ± 13.2	33.6 ± 14.3	45.0 ± 15.9	51.1 ± 145	38.0 ± 4.5	57.1 ± 11.4	70.0 ± 10.8
CD10 *#	0.6 ± 2.4	10.5 ± 31.5	0.0 ± 0	15.5 ± 35.0	6.9 ± 22.4	31.4 ± 43.0	26.0 ± 39.7	29.2 ± 36.4	52.3 ± 36.1
MUC2 *	51.8 ± 31.7	72.1 ± 19.9	61.1 ± 34.8	42.0 ± 28.6	47.3 ± 32.6	42.5 ± 34.5	42.0 ± 29.5	43.1 ± 29.5	20.0 ± 24.1

* Difference among histological grades.
# Difference among depth of invasion.

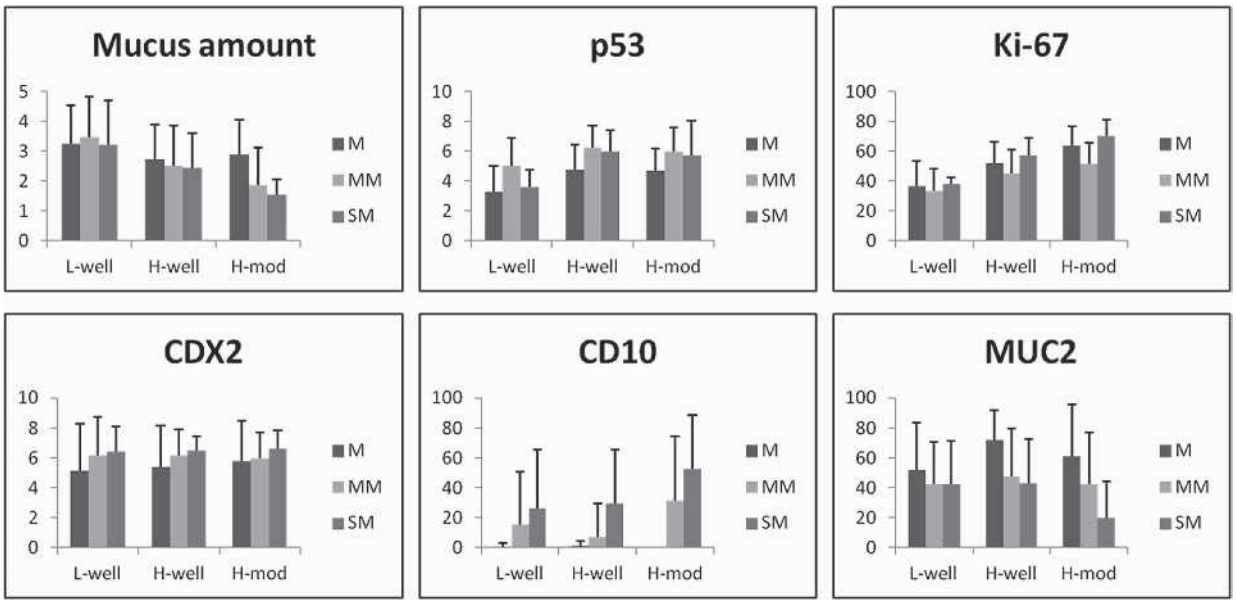

Figure 6 Mucus amount and immunohistochemical results.

the intestinal epithelium. Therefore, degree of MUC2 expression was parallel to the amount of mucin, which was decreased in the high grade atypia.

Ki-67 is a nuclear protein that is associated with cellular proliferation, therefore, it is present during all active phases of a cell cycle (G1, S, G2 and mitosis). The p53 protein regulates the cell cycle. As it also functions as a tumor suppressor it is involved in preventing cancer. Imunoreactivities of both Ki-67 and p53 have been recognized as significant markers of cell proliferation [16, 17]. In our study, expressions of Ki-67 and p53 were increased in the component of high grade atypia, and these results were deemed to be compatible with the functions of both proteins. Expression of CDX2 has been reported to decrease in the high grade and advanced stage colorectal carcinoma [18-20]. However, the expression of CDX2 was increased in the LST-type colorectal carcinoma that invaded the submucosa in our study. The discrepancy of these results is thought to come from the defferent background stages of colorectal cancer, i.e. advanced cancers in the previous studies and early cancers in our study. CD10 is expressed at the brush border of normal small intestinal epithelium. Recently

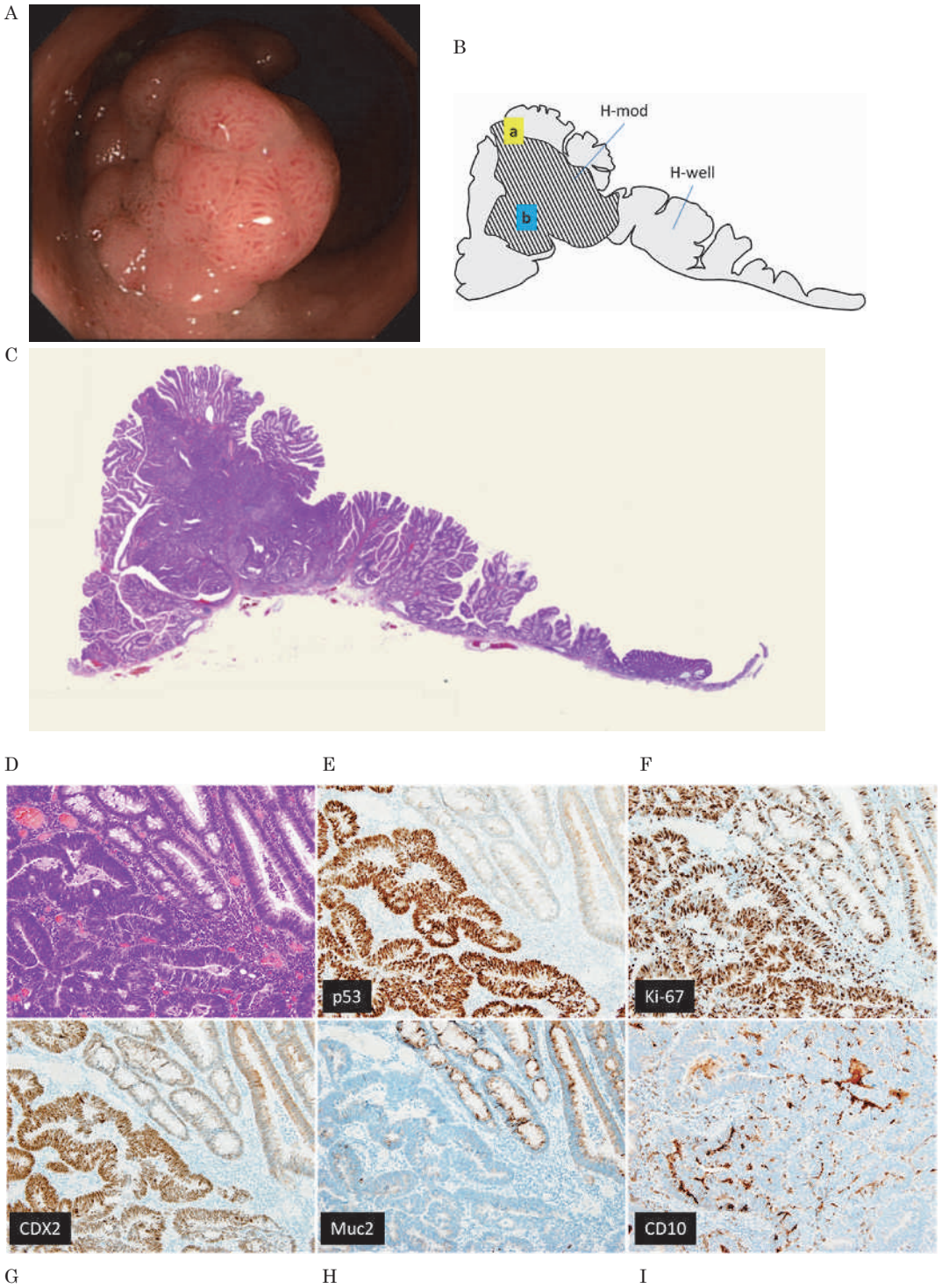

Figure 7 A representative case of LST-type early colorectal carcinoma.

A LST-type tumor of the rectum was resected by ESD. Histopathological diagnosis was well differentiated adenocarcinoma (tub1>tub2), the depth was submucosa up to 700 μm, the size was 30 x 23 mm in maximum diameter, the tumor height was 10 mm, and there is no invasion to veins or lymph ducts.
(A) Endoscopic view. (B) This lesion has two components of histological grade, H-well and H-mod. Location pattern is SM-D type in correspondence to Figure 5. (C) Loupe image of representative sections. (D-H) Highly magnified microscopic images of area 'a' in (B). (D) In HE stain, the tumor is clearly divided into H-well and H-mod components. Scores of mucus amount are 5 / 1 in H-well / H-mod. (E) p53 = 3 / 8.(F) Ki-67 = 30 / 80. (G) CDX2 = 6 / 7. (H) MUC2 = 90 / 10. (I) CD10 staining, a highly magnified image of area 'b' in (B). CD10 is negative in H-well component and focally positive in H-mod component, scores are 0 / 50.

it has been disclosed as a marker of higher malignant potential of colorectal cancer [21-23], and its expression is reportdely related to both submucosal invasion and grade of atypia of colorectal cancer [7]. Our result was compatible with that of the previous study [7]. CD10 expression and the above mentioned Ki-67/p53 immunoreactivities were thought to be associated with malignant potentials of the component of the high grade atypia of LST.

In conclusion, the present study indicated that the majority of LSTs was histologically heterogeneic. In addition, the component of high grade atypia of LST showed upregulated cell proliferation, which was suggested to leed to an increased the malignant potential of LST to invade the submucosa.

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