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Original

Clinicopathological and Molecular Features of Laterally Spreading Tumors

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Abstract: Colorectal flat-elevated neoplasms can be classified into small-flat adenoma and laterally spreading tumors (LSTs), which can then be sub-categorized into granular-type (LST-G) and nongranular-type (LST-NG) LSTs with possible biological differences between them. We evaluated clinicopathological features and *KRAS* / *BRAF* mutations in 24 LST-Gs and 57 LST-NGs. PCR-based pyrosequencing assays were used to determine the presence of activating mutations in codons 12 and 13 of *KRAS* and in codon 600 of *BRAF*. Significant differences between LST-Gs and LST-NGs were observed in tumor size (30 mm vs. 15 mm, $P < 0.0001$) and the frequency of *KRAS* mutations (75%, 18 / 24 vs. 5%, 3 / 57, $P < 0.0001$). For LST-NGs, the histological grade was increased with an increase in the tumor size. The frequency of submucosal cancer (SM-ca) was also higher in tumors of at least 20 mm than in tumors smaller than 20 mm ($P < 0.05$). In contrast, there was no indication of a size-dependent increase in the histological grade. No significant difference in the frequency of *KRAS* mutation in LST-Gs and LST-NGs was related to tumor size. Two subtypes of LSTs were observed to have different clinicopathological and molecular characteristics. These findings suggest that different molecular mechanisms could exist in these subtypes of colorectal flat-type neoplasms.

Key words: colorectal adenoma, colorectal carcinoma, laterally spreading tumor, *KRAS* mutation, flat adenoma

Introduction

Colorectal cancer (CRC) can develop via various molecular pathways. Most CRCs develop over a long period of time by a multistep process called the adenoma-carcinoma sequence[1)]. Approximately two-thirds of sporadic CRCs arise from conventional adenomas and usually show a protruding (polypoid) macroscopic appearance. The process of colorectal carcinogenesis often begins with inactivation of the adenomatous polyposis coli (APC) gene / β-catenin signaling pathway (the Vogelstein mode[1)], followed by *KRAS* and *TP53* mutations[2)].

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It was reported recently that colorectal neoplasias (CRNs) could be morphologically classified into polypoid and nonpolypoid, with the latter subclassified as flat-elevated or depressed tumors[3]. In addition, flat-elevated neoplasias can be classified further into small-flat adenoma or laterally spreading tumors (LSTs), which were initially reported by Kudo[3]. LSTs are characterized by lateral extension along the luminal wall with a low vertical axis, and are subcategorized into granular-type LST (LST-G) and nongranular-type LST (LST-NG). In clinical practice, LSTs are not easily found during colonoscopy, which makes it challenging for colonoscopists to distinguish them from the normal mucosa[3]. Although interval cancers may be missed during endoscopy, inadequate recognition of such tumors may result in the development of cancer.

In the present study, we examined the hypothesis that LST-Gs and LST-NGs have different clinicopathological and molecular features that could potentially help in diagnosing CRC.

Methods

Patients and samples

We examined 81 LSTs from 81 patients who underwent endoscopic resection (N＝73) or surgical resection (N＝8) at Showa University Hospital from 2004 to 2011. The samples were selected solely based on tissue availability. We excluded patients with inflammatory bowel disease or with a familial predisposition to cancers such as familial adenomatous polyposis or hereditary nonpolyposis colorectal cancer. Written informed consent was obtained from all patients. The ethics committee of Showa University School of Medicine approved the procedures for tissue collection and analysis, and informed consent was obtained from all patients.

Endoscopic evaluation and macroscopic classification

All patients were prepared for the procedure by administering 1.8 L of an oral electrolyte lavage solution. Colonoscopists with extensive experience performed all examinations using high-resolution video colonoscopes (CF-240ZI, CF-260AI, or CF-260HZI; Olympus Optical Co., Tokyo, Japan). When detected, CRNs were prospectively described based on the Paris endoscopic classification[4]. The flat-elevated lesions (type 0-IIa) were subclassified into small flat-elevated, LST-G (Fig. 1a) and LST-NG (Fig. 1b). Briefly, LSTs are defined by a large lateral diameter (＞10 mm), a low vertical axis, and lateral extension along the luminal wall. LST-Gs are composed of superficial spreading aggregates of nodules that form flat, broad-based lesions with a granulonodular and uneven surface, whereas LST-NGs have a flat smooth surface without granulonodular formation[5]. Small flat-elevated neoplasms were not included in this study. Other clinicopathological findings were determined according to the general rules of the Japanese Research Society for Cancer of the Colon and Rectum.

Tissue sample preparation

To extract genomic DNA, 20 formalin-fixed paraffin-embedded samples and 61 frozen

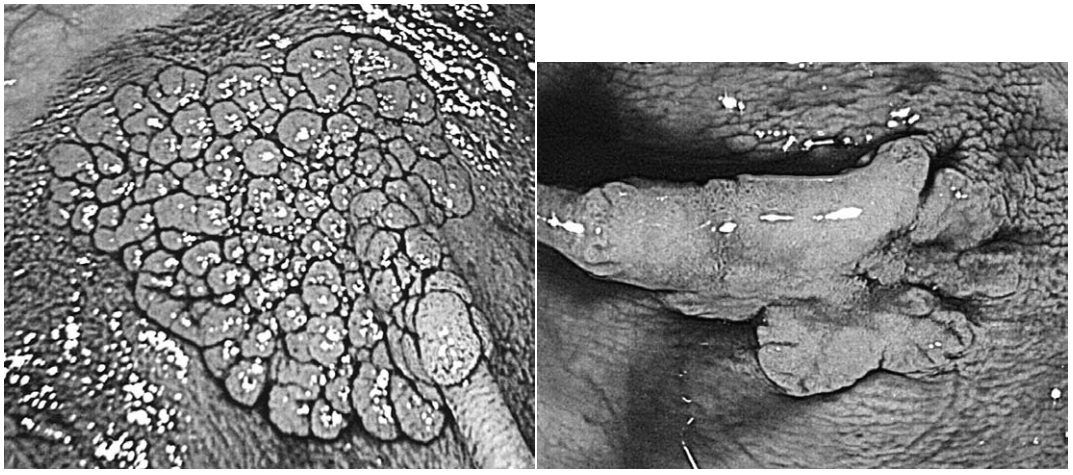

Fig. 1. Endoscopic appearance of a) granular-type laterally spreading tumor (LST-G) and b) nongranular-type (LST-NG) LST.

tissue samples were used. The frozen tissue samples were obtained from colonoscopic biopsy specimens and stored at −80°C. We distinguished between neoplastic and nonneoplastic areas of the biopsied tissues based on the pit patterns visible on chromoendoscopic examination. DNA was extracted from frozen tissue samples by using standard proteinase K / phenol / chloroform methods. In addition, 10 μm thick sections were obtained from archival formalin-fixed, paraffin-embedded tumor tissues. After microdissection for these histological sections, DNA was extracted using the QIAamp DNA mini kit (QIAGEN Inc., Valencia, CA).

KRAS and BRAF gene mutations

Samples were analyzed using PCR-based pyrosequencing to determine the presence of activating mutations in codons 12 and 13 of *KRAS* and in codon 600 of *BRAF*, as described previously[6, 7].

Data analysis and statistics

We calculated the mean, median, ranges, and 95% confidence interval (95% CI). The difference in continuous variables (age, tumor size, methylation density) between two groups was analyzed using the Mann-Whitney U test. Categorical variables were compared between clusters using the χ^2 test, or Fisher's exact test when testing small samples. All tests were two sided. $P < 0.05$ was considered statistically significant.

Results

Table 1 summarizes the clinicopathological and molecular features of 81 LSTs. LST-Gs were significantly larger than the LST-NGs ($P < 0.0001$); however, there were no significant differences in patient gender or age, tumor location, histology, or treatment between LST-Gs and LST-NGs. Our testing of *KRAS* and *BRAF* mutation status revealed a significant difference in the frequency of *KRAS* mutations between LST-Gs and LST-NGs (75% vs. 5%,

Table 1. Clinicopathological and molecular characteristics of patients with laterally spreading tumor

		LST		
		Granular Type (%) (N = 24)	Nongranular Type (%) (N = 57)	*P* value
Gender	Male	11 (46)	39 (68)	0.056
	Female	13 (54)	18 (32)	
Age	(median, yrs)	73.5	68.0	0.138
	(range, yrs)	(55–89)	(53–82)	
Location	Proximal	13 (54)	29 (51)	0.787
	Distal	11 (46)	28 (49)	
Size	(median, mm)	30	15	< 0.0001
	(range, mm)	(12–73)	(10–39)	
Histology	Adenoma			
	LGD	15 (63)	33 (58)	0.833
	HGD	6 (25)	18 (32)	
	Submucosal cancer	3 (13)	6 (11)	
Treatment	Endoscopic	21 (88)	52 (91)	0.607
	Surgical	3 (13)	5 (9)	
KRAS mutation	Presence	18 (75)	3 (5)	< 0.0001
	Absence	6 (25)	54 (95)	
BRAF mutation	Presence	0	0	NA
	Absence	24 (100)	57 (100)	

HGD, high-grade dysplasia; LGD, low-grade dysplasia; LST, laterally spreading tumor; NA, not applicable.

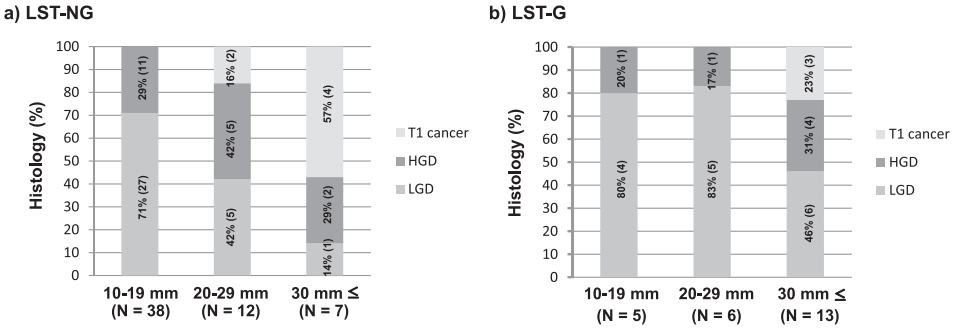

Fig. 2. Correlation between the tumor size and histological grade; a) nongranular-type laterally spreading tumor (LST-NG), b) granular-type LST (LST-G). T1 cancer was histologically defined as submucosal cancer.

$P < 0.0001$); however, no *BRAF* mutation was found in either tumor type.

We then performed more detailed analyses of the relationship between tumor size and histological grade or *KRAS* mutation. For LST-NGs, the histological grade was increased with an increase in tumor size (Fig. 2a), with the frequency of submucosal cancer (SM-ca) higher

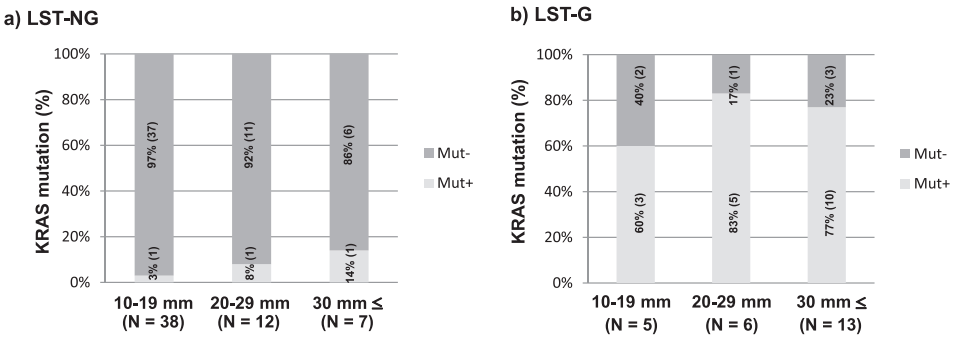

Fig. 3. Correlation between the tumor size and *KRAS* mutation; a) nongranular-type laterally spreading tumor (LST-NG), b) granular-type LST (LST-G).

in tumors larger than or equal to 20 mm compared to tumors less than 20 mm in size ($P < 0.05$). In contrast, although the frequency of SM-ca was also significantly higher in LST-Gs larger than or equal to 30 mm than in those smaller than 30 mm, there was no indication of a size-dependent increase in the histological grade (Fig. 2b). With regard to *KRAS* mutation and tumor size, the frequency of mutation was relatively higher in LST-NGs 30 mm or larger and in LST-Gs 20 mm or larger, compared to smaller tumors (Fig. 3); however, the difference was not significant.

Discussion

We evaluated the clinicopathological features and *KRAS*/*BRAF* mutational status in LST-Gs and LST-NGs collected from patients in our hospital, based on previous reports of distinct biological differences between these two tumor subtypes[8, 9]. Our study revealed several distinguishing clinicopathological and molecular features between LST-NGs and LST-Gs.

There was a significant difference in tumor size between LST-Gs and LST-NGs (median size, 30 mm vs. 15 mm). However, although LST-Gs showed no significant correlation between tumor size and histological grade, we observed a significantly higher frequency of SM-ca in LST-NGs 20 mm or larger than in those less than 20 mm in size. Although it did not reach statistical significance, the frequency of SM-ca was also higher in LST-NGs 30 mm or larger than in those 20～29 mm (57% vs. 16%). These results indicated that histological grade of LST-NGs increases with size. In contrast, only 23% (3/24) of large LST-Gs, at least 30 mm in size, showed SM-ca. Also, of three LST-Gs with SM-ca, one showed massively submucosal invasion (data not shown), and macroscopically, it had a large nodule. Uraoka *et al*[10] reported that the presence of a large nodule in LST-Gs is associated with submucosal invasion, suggesting that two types of LSTs have different manners of tumor progression from adenoma to cancer, and are implicated in the strategy of endoscopic treatment.

In this study, *KRAS* mutations were significantly more frequent in LST-Gs than LST-NGs. Some previous studies reported a lower frequency of *KRAS* mutation in flat-type neoplasms, compared to the protruded type[11, 12]; however, other studies showed the opposite result in flat-type neoplasms[13]. Based on the present findings and these other studies, the clinicopathological

and molecular features of colorectal neoplasms indicated that we should separately examine small (less than 10 mm) and large (10 mm or larger) flat-type neoplasms.

Mutations in *KRAS* or *BRAF*, which activate the mitogen-activated protein kinase cascade and malignant transformation, are a key event in colorectal carcinogenesis[14]. We showed a significant difference in the frequency of *KRAS* mutations between LST-Gs and LST-NGs, while the macroscopic appearance of these neoplasms revealed both to be flat-type. This might be related to the biological difference in LSTs; however, additional molecular changes should be investigated in LST-NGs to clarify the clinical significance of findings such as ours.

In summary, two subtypes of LST were observed to have different clinicopathological and molecular characteristics, suggesting that different molecular mechanisms may exist in these subtypes of colorectal flat-type neoplasms.

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Conflict of interest

The authors declare that they have no competing interests with regard to this manuscript.

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