Methylation of CpG island promoters at *ZNF625*, *LONRF2*, *SDC2* and *WDR17* in a patient with numerous non-granular type laterally spreading tumors and colorectal cancer: A case report

MORIYA IWAIZUMI[1], TERUMI TANIGUCHI[1], KIYOTAKA KURACHI[2], SATOSHI OSAWA[3], KEN SUGIMOTO[4], SATOSHI BABA[5], HARUHIKO SUGIMURA[6] and MASATO MAEKAWA[1]

[1]Department of Laboratory Medicine, [2]Second Department of Surgery, [3]Department of Endoscopic and Photodynamic Medicine, [4]First Department of Medicine and Departments of [5]Diagnostic Pathology and [6]Tumor Pathology, Hamamatsu University School of Medicine, Hamamatsu, Shizuoka 431-3192, Japan

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Abstract. Patients with adenomatous polyposis syndromes such as familial adenomatous polyposis are at higher risk of colorectal cancer, hence continuous management is necessary. However, little is known about the etiology of patients with numerous laterally spreading tumors (LSTs), or how genetic alterations uniquely influence LSTs in colorectal carcinogenesis. The present case report investigated a woman with >150 non-granular type LSTs (LST-NG) and one sigmoid colon cancer. After subtotal colectomy via ileorectal anastomosis, genetic and epigenetic analyses were conducted by comparing the profiles of the patient's normal colonic mucosa, four LST-NG lesions and a cancer lesion. Using customized multigene panel testing, no pathogenic germline mutations were detected, including APC regulator of WNT signaling pathway, but identified a somatic pathogenic variant of *APC* in one LST-NG lesion, and both *TP53* and F-box and WD repeat domain containing 7 somatic mutations in the cancer. Comprehensive genome-wide methylation analysis showed that CpG island promoters at zinc finger protein 625, LON peptidase N-terminal domain and ring finger 2, WD repeat domain 17 and syndecan 2 were methylated in both LST-NG and cancer, which may contribute to colorectal tumorigenesis as early as the LST-NG phase.

Correspondence to: Dr Moriya Iwaizumi, Department of Laboratory Medicine, Hamamatsu University School of Medicine, 1-20-1 Handayama, Higashi-ku, Hamamatsu, Shizuoka 431-3192, Japan
E-mail: iwaizumi@hama-med.ac.jp

Abbreviations: FAP, familial adenomatous polyposis; LST, laterally spreading tumor; LST-G, LST-granular type; LST-NG, LST-non-granular type; IRA, ileorectal anastomosis; DMS, differentially methylated site

Key words: laterally spreading tumor, laterally spreading tumor-non-granular type, colorectal cancer, zinc finger protein 625, LON peptidase N-terminal domain and ring finger 2, syndecan 2, WD repeat domain 17, methylation

Introduction

There are various types of polyposis syndrome including familial adenomatous polyposis (FAP), serrated polyposis syndrome, Peutz-Jeghers syndrome, juvenile polyposis syndrome, and *PTEN*-hamartoma tumor syndrome (1). Patients with these syndromes are at higher risk of developing colorectal cancer. Therefore, appropriate management via genetic testing and endoscopic surveillance is essential for the treatment and surgical prophylaxis of patients with colorectal polyposis. FAP is an autosomal dominant colorectal tumor syndrome caused by an *APC* pathogenic germline variant, and is characterized by the formation of numerous adenomatous polyps throughout the entire colon (2). Since 15-20% of cases are *de novo* without clinical or genetic evidence of FAP (3), it is important for clinical diagnosis to check for more than 100 colorectal adenomas via colonoscopy, regardless of family history of colorectal adenomatous polyposis (4). Each adenoma is typically polypoid in shape in patients with FAP. Therefore, it is difficult to judge whether a phenotype with vast numbers of an alternate morphological type of adenoma is associated to FAP.

Although the main colorectal tumorigenesis pathway is via protruded adenomas through the adenoma-carcinoma sequence, some colorectal cancers (CRCs) develop from these flat lesions via a *de novo* pathway (5,6). Kudo *et al* first called these flat, early lesions laterally spreading tumors (LSTs), and classified the horizontal growth lesions into two subtypes: the LST-granular type (LST-G), with granules and nodules on the tumor surface; the LST-nongranular type (LST-NG), with flat, smooth surfaces (7,8). In terms of histopathology, most LST-G and LST-NG lesions comprise tubular or tubulovillous adenomas, although the molecular characteristics of the two subtypes differ. Hiraoka *et al* demonstrated that LST-G lesions have *KRAS* mutations and are intermediate with regard to hypermethylation, whereas LST-NG lesions exhibit little pathogenic variation (9). Sakai *et al* reported that a *TP53* mutation

occurred during the development of cancer in both LST-NG and LST-G lesions (10,11). Although the authors reported the molecular characteristics of tumor progression from LST to CRC, the collected tumor samples were obtained from patients subjected to various environmental factors, including different lifestyles and microbiomes, as well as various genetic germline backgrounds. Therefore, ideal molecular analysis should be performed using normal colonic mucosa, LSTs, and CRCs from patients with identical backgrounds.

Herein, we investigate a patient with >150 LST-NG lesions in the entire large intestine and one adenocarcinoma in the sigmoid colon, which has not been previously reported. The lesions appeared to have the FAP phenotype, but otherwise atypical in that all the adenomatous lesions were LST-NG. The cancer lesion of this case might have been developed by somatic mutation of *FBXW7* and/or *TP53*, differently with the tumorigenesis by germline *APC* mutation followed by acquired *APC* disfunction in patients with FAP. We further demonstrate the genomic/epigenomic difference among the normal colonic mucosa, LST-NGs, and cancer lesions of the patient as tumor progression occurs via accumulation of epigenetic alterations as well as pathogenic mutations of tumor suppressor genes. In this study, for the first time, we report that the hypermethylation of *ZNF625*, *LONRF2*, *SDC2*, and *WDR17* as well as somatic mutation of *FBXW7* and/or *TP53* contribute to tumorigenesis from LST-NG.

Case report

A 72-year-old female presented at our hospital after a positive fecal occult blood test without any change to her bowel habits. She had no family history of colorectal polyposis or colorectal cancer. A complete colonoscopy revealed more than 150 amelanotic, flat, elevated lesions in the entire large intestine with melanotic background mucosa owing to prolonged self-administration of Sennoside A+B calcium (Fig. 1A). All the flat lesions were LST-NG, and the biopsy samples obtained from the lesions were pathologically diagnosed as tubular adenomas. Esophagogastroduodenoscopy was also performed and that no fundic gland polyposis was observed. After 4 years of annual endoscopic surveillance, one of the LST-NG lesions had developed into an adenocarcinoma in the sigmoid colon (Fig. 1A). Since LST-NG is a pre-cancerous condition, a subtotal colectomy by ileorectal anastomosis (IRA) was performed. The postoperative histopathology results demonstrated the presence of a tubular adenocarcinoma and multiple tubular adenomas (Fig. 1B and C). No cases of both multiple LST-NG lesions and CRC have been previously reported; we hypothesize that the tumorigenesis might have had a genetic/epigenetic cause. The patient provided written informed consent, and the study was approved by the Institutional Review Board of the Hamamatsu University School of Medicine (approval no. 17-222).

For genetic analysis, we first performed multigene panel testing using MiSeq sequencer (Illumina) and collected one normal colonic mucosa (N), four LST-NG lesions (L1-L4), and one cancer sample (C) from the patient immediately following IRA, extracted the DNA, and froze the fresh samples for storage. The extracted DNA was quantified using a Qubit dsDNA BR Assay Kit (Q32850; Thermo Fisher Scientific) on a Qubit 2.0 Fluorometer (Q33216, Thermo Fisher Scientific), and was prepared for shearing according to the SureSelect XT HS Target Enrichment System Manual (Agilent Technologies). Custom capture probes were designed using SureDesign (Agilent Technologies) covering the exons and boundary regions of 96 genes, including *APC*. For library preparation, a SureSelect XT HS Reagent Kit (G9702A, Agilent Technologies) was used according to the manufacturer's instructions. In brief, pre-enriched adapter-ligated libraries were prepared. Quality and quantity of libraries were determined by 4150 TapeStation System (G2992AA, Agilent Technologies) using D1000 ScreenTape (5067-5582, Agilent Technologies). 3.8 pmol of each library was used for hybridization. Subsequently, custom capture probes were hybridized to target sequences to enable sequence enrichment using streptavidin beads. Post-enrichment, libraries were quantified, pooled, and sequenced using MiSeq Reagent Kit v3 (MS-102-3001, Illumina) on a MiSeq sequencer. SureCall v4.0.1.46 (Agilent Technologies) and VariantStudio software (Illumina) were used for data analysis and alignment. GRCh37 was used as the reference genome.

All detected variants were validated using Integrative Genomics Viewer 2.9.2 (Broad Institute, Cambridge, MA, USA). We detected no pathogenic variants, including *APC*, in the normal mucosa. Of the four LST-NG lesions, only one had a pathogenic variant of *APC* (NM_000038.5: c.2396_2397delAT, NP_000029.2: p.Tyr799CysfsTer3), which was a somatic change because there was no mutation of *APC* in the normal colonic mucosa. In contrast, pathogenic mutations in both *TP53* (NM_000546.5: c.499_500delCA, NP_000537.3: p.Gln167AlafsTer13) and *FBXW7* (NM_033632.3: c.1513C>T, NP_361014.1: p.Arg505Cys) were detected in the cancer (Table I). These results suggest that the patient had no possibility of developing FAP because there was no *APC* mutation in the normal mucosa, and the somatic mutation of *FBXW7* and/or *TP53* contributed to tumorigenesis.

As tumor progression generally occurs by accumulation of epigenetic alterations as well as pathogenic mutations of tumor suppressor genes, it is important to understand the role of DNA methylation in tumorigenesis. Therefore, we next conducted a comprehensive genome-wide analysis using an Infinium MethylationEPIC BeadChip Kit (Illumina) according to the manufacturer's recommendations. Briefly, bisulfite-treated DNA was subjected to whole-genome amplification before fragmentation and precipitation. The resuspended DNA was subsequently hybridized to probes attached to the BeadChips (Illumina), which contained >850,000 CpG sites, and the nonhybridized DNA was removed. The attached probes were then subjected to single-base extension and stained. The BeadChips were scanned using the iScan™ system (Illumina) according to the manufacturer's recommendations. The red and green signals from the iScan™ system were converted into unmethylated and methylated signals. For each CpG site in the CpG island gene region, a DiffScore value of >100 between the normal mucosa and the cancer was defined as the absolute DMS value of the sample and calculated using GenomeStudio FrameWork v2011.1 software (Illumina) and the R statistical environment (version 3.1.3). Among the 766 detected DMSs, we selected one methylated CpG site from each of the cancer

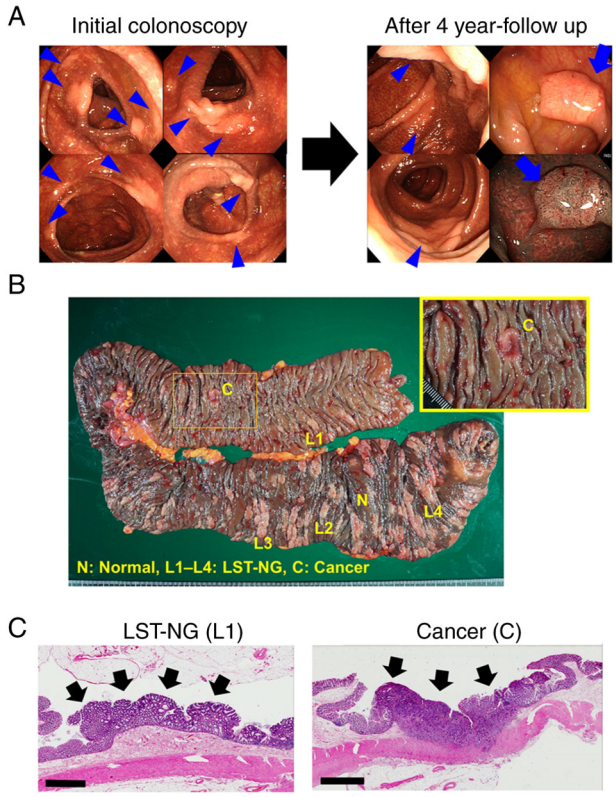


Figure 1. Clinicopathological features of the analyzed patient. (A) The left panel shows the initial endoscopic findings. Multiple LST-NG lesions (arrowheads) were identified in the entire large intestine. The right panel shows that one of the LST-NG lesions developed into an adenocarcinoma in the sigmoid colon (arrows) during the 4-year annual endoscopic surveillance. (B) A surgical specimen of the large intestine had >150 LST-NG lesions and a cancer lesion. One normal mucosa, four LST-NG lesions (L1-L4) and one cancer lesion were collected and analyzed. (C) Histopathology of an LST-NG lesion and a sigmoid adenocarcinoma (arrows) (scale bar, 3-mm). LST-NG, laterally spreading tumor-nongranular type lesions.

and normal mucosa samples, and performed clustering analysis of the normal mucosa sample, four LST-NG lesions, and cancer sample, as shown in Fig. 2A. In most DMSs, the methylation levels of the LST-NG lesions were the same as those in the normal mucosa (Group a), as previously demonstrated using sets of patients with LST (9-11). We next focused on the minority group (Group b) in which the DMS levels of the LST-NG lesions were as high as those in the cancer sample (Fig. 2B). DMS occurred at the CpG islands of the following nine genes: *ZNF625*, *LONRF2*, *MSC*, *OPLAH*, *PCDHGA4*, *GSG1L*, *BEND5*, *SDC2*, and *WDR17*. We further checked the methylation values at the CpG islands including the DMS site detected in Group b (Fig. 3). Among the regions, the CpG sites of *ZNF625*, *LONRF2*, *MSC*, and *OPLAH* were methylated in all four LST-NG lesions as in the cancer sample, and *SDC2* and *WDR17* were methylated in three of the LST-NG lesions. In the CpG islands in *GSG1L*, *BEND5*, and *PCDHGA4*, the CpG sites were not methylated as in the cancer lesions. When we observed on the gene regions of these CpG sites, we noticed that *ZNF625*, *LONRF2*, *SDC2*, and *WDR17* might have been methylated at the promoter regions in both the LST-NG lesions and the cancer sample, because these methylated CpG sites were located 200 bases upstream of the transcriptional start site, or 5' untranslated region. This suggests that methylation-silenced *ZNF625*, *LONRF2*, *SDC2*, and *WDR17* play roles in tumorigenesis as early as the LST-NG phase.

Discussion

The diagnosis and management of patients with polyposis syndromes is constantly evolving, owing to new scientific and technological advances with respect to the identification of causative genes, and the increased sophistication of endoscopic treatments for polyps. However, we were uncertain as to how to categorize the patient in the present study who had numerous LST-NG lesions, among the various colorectal polyposis syndromes, as the present work is the first report of a patient with >150 LST-NG lesions that developed a CRC during endoscopic surveillance. Our genomic and epigenomic analyses showed that: (1) no germline *APC* pathogenic variant was detectable via the multigene panel testing; (2) there was only one somatic *APC* frameshift mutant site in one of the four LST-NG lesions; (3) the somatic mutations of *TP53* and *FBXW7* were only present in the cancer sample; (4) there was methylation of the promoter CpG islands in *ZNF625*, *LONRF2*, *SDC2*, and *WDR17* in most of the LST-NG lesions as well as the cancer lesion.

FAP is clinically diagnosed when approximately ≥100 adenomatous polyposis are detected in the large intestine, irrespective of the presence or absence of a family history of FAP, as 15-20% of FAP cases are *de novo* (3,4,12). Therefore, we first suspected the indexed patient had sporadic FAP since there were >100 adenomatous lesions throughout her large intestine and she had no family history of FAP-associated lesions.

The patient's adenomatous lesions were LST-NG, which are histologically the same as in protruding adenomatous polyposes (tubular adenomas) but differ morphologically to the naked eye. Moreover, no cases have been reported of FAP with multiple LST-NG lesions. Therefore, we explored the germline pathogenic variants using the normal colonic mucosa and customized multigene panel test and found no pathogenic variants, including *APC*, suggesting that the patient had no genetic evidence of FAP. The limitation of our analysis was that we could not completely exclude hereditary polyposis syndrome since the multigene panel did not include polyposis-related genes such as *MUTYH* and *BMPR1A*. Therefore, it is necessary to conduct whole exome/genome sequencing to detect any unknown germline genetic alterations to confirm the presence of new types of hereditary polyposis syndrome.

The pathogenicity of somatic variants of cancer including CRC is assessed by examining general population data, functional data, predictive data, cancer hotspots, and computational evidence (13). Therefore, numerous patients with CRC and healthy controls have been registered to establish reliable evidence. However, the registered CRC patients' own environmental factors and gut microbial compositions considerably differ. The potential role of epigenetic alterations has been reported in links between obesity, gut microbiota, and colorectal cancer. For colorectal cancer progression, high-fat diet-induced obesity leads to epigenetic remodeling of the acetylation landscape based on the gut microbiota, promotes changes in DNA methylation, and enhances production of

Table I. Genetic alterations in normal colonic mucosa, four LST-NG lesions (L1-L4) and one colonic cancer lesion.

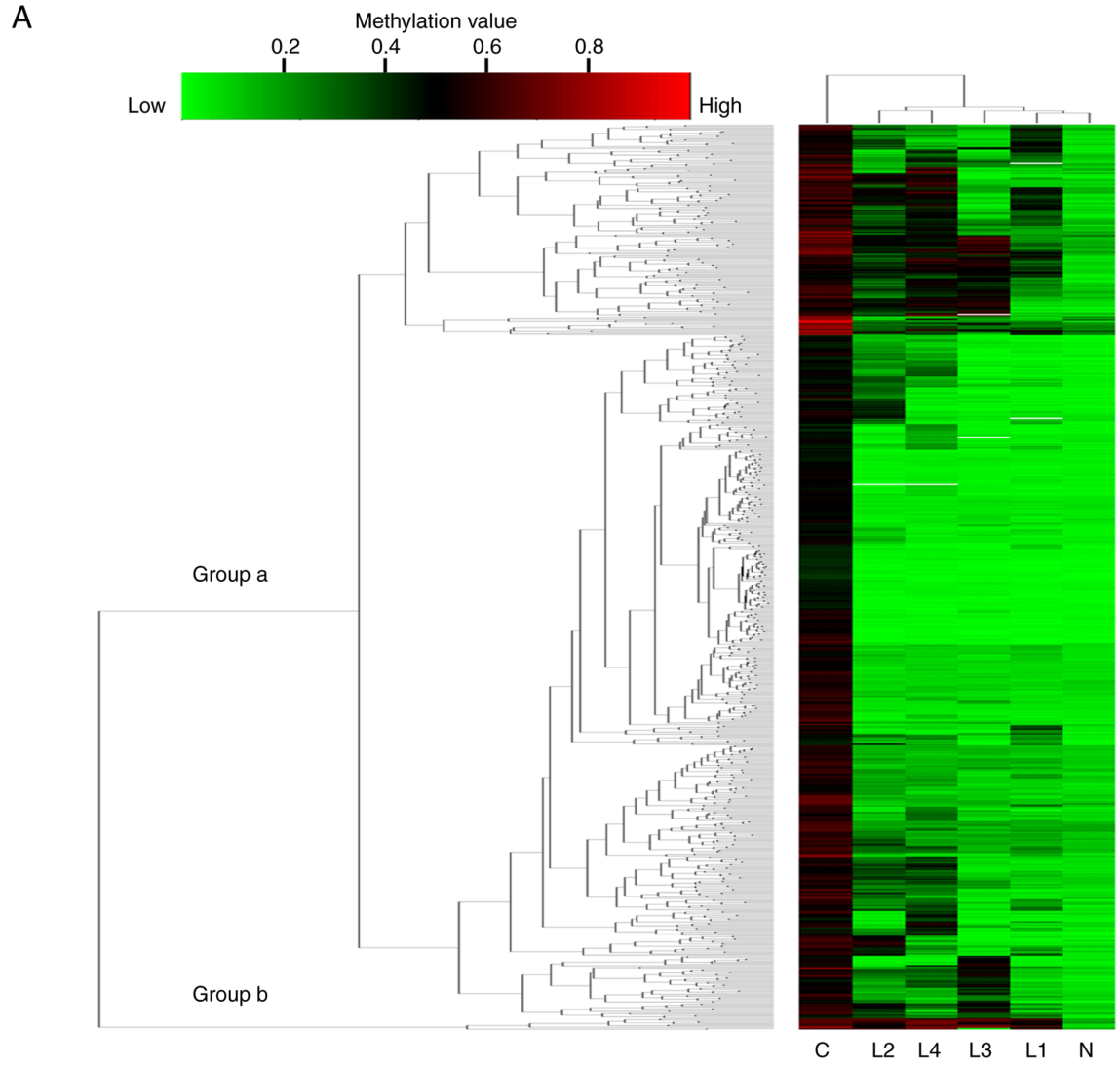
Gene	Clinically relevant variants	Type of mutation	COSMIC legacy identifier	COSMIC significance	Variant allele frequency (%)					
					N	L1	L2	L3	L4	C
TP53	NM_000546.5 c.499_500delCA p.Gln167Alafs Ter13	Frameshift	44275	N/A	0	0	0	0	0	19
FBXW7	NM_033632.3 c.1513C>T p.Arg505Cys	Missense	22975	Pathogenic	0	0	0	0	0	35
APC	NM_000038.5 c.2396_2397delAT p.Tyr799CysfsTer3	Frameshift	4167217	N/A	0	0	0	0	20	0

LST-NG, laterally spreading tumor-nongranular type; N, normal colonic mucosa; L1-L4, four LST-NG lesions; C, colonic cancer lesion; *FBXW7*, F-box and WD repeat domain containing 7; *APC*, APC regulator of WNT signaling pathway.

deoxycholic acid, a secondary bile acid that is produced solely by Gram-positive gut bacteria and known to cause DNA damage through reactive oxygen species production (14). Therefore, pure genetic/epigenetic factors for colorectal tumorigenesis should be detected under the same environmental and microbiological conditions if possible. One way of accomplishing that is to compare genetic/epigenetic profiles among the normal mucosa, pre-cancerous lesions, and cancer lesions obtained from patients with identical backgrounds at the same time. In the present study, we analyzed the normal colonic mucosa, LST-NG lesions, and sigmoid cancer lesion of the same patient obtained immediately following colorectal resection.

In the present study, genetic analysis using customized multigene panels revealed only one *APC* frameshift variant in one LST-NG lesion, while the remaining three LST-NG lesions had no pathogenic variants. Metz *et al* reported that more than 90% of the LST lesions examined exhibited an *APC* mutation, but it did not exhibit the mutation frequency of an LST-NG lesion (15). Sugimoto *et al* detected loss of heterozygosity (LOH) at the *APC* locus in 60% of the LST-NG lesions they examined, whereas only 28% LST-G lesions harbored the mutation (16). In contrast, precise analysis by Sugai *et al* showed that null *APC* variants (i.e., nonsense and frameshift type pathogenic variants) were numerous in LST-G lesions compared with LST-NG lesions (17). These previous reports suggest that somatic pathogenic *APC* variants play a role in the occurrence of LST-NG lesions but are not the main contributors to tumorigenesis. In the present study, we detected somatic pathogenic alterations in *TP53* and *FBXW7*, both of which variants were only present in the cancer lesion. Previous studies have demonstrated that the synergistic contributions of wild type FBXW7 and TP53 proteins contribute to the suppression of gastrointestinal cancer (18,19), and most *FBXW7* mutations in cancers, including CRC, exhibit a *TP53* mutation (20-22). Therefore, we speculated that our study patient had a cancer lesion that simultaneously lost the two tumor-suppressors that usually cooperate in the inhibition of tumorigenesis. In addition, Sakai *et al* have suggested that the *TP53* mutation is more closely involved at an earlier stage in LST-NG lesions than in LST-G lesions during cancer development (11). In the same manner, the *TP53* mutation might occur in an earlier phase of the patient's cancer lesions than the phase in which LST-NG lesions appear, and the mutation may continuously influence sigmoid tumor progression to the advanced level.

We further performed epigenome-wide analysis to determine whether there were any pathogenic epigenetic alterations that cause tumorigenesis after the LST-NG phase. Of the 766 DMSs identified, 756 were hypermethylated only in the cancer lesion, and the methylation levels in the LST-NG lesions were as low as in the normal colonic mucosa (Group a). This result was expected since Sakai *et al* were able to cluster LSTs into two epigenotypes in 108 LST samples (51 LST-G and 57 LST-NG lesions), i.e., intermediate- and low methylation-groups. The authors found that the intermediate methylation epigenotype was associated with LST-G lesion morphology, while the low methylation LSTs mostly reflected LST-NG lesion morphology (10,11). When we assessed the remaining 10 DMSs, all of which were categorized in the same cluster group (Group b), we noticed that the methylation levels of all 10 DMSs were as high as those in the cancer lesions. Moreover, all 10 DMSs were located in the CpG island region, indicating that the genes where the 10 DMS were located play roles in tumorigenesis by silencing the pre-cancerous phase of the LST-NG lesions. We further determined whether the CpG island regions, including the 10 DMSs, were methylated to the same extent in the LST-NG lesions as in the cancer lesion and found that *ZNF625*, *LONRF2*, *SDC2*, and *WDR17* may have been methylated at the promoter region in both the LST-NG lesions and the cancer lesion. Among those four genes, *SDC2* has been investigated most extensively regarding methylation in colorectal neoplasms. *SDC2* has the chromosomal locus 8q22.1 and encodes syndecan-2 protein.



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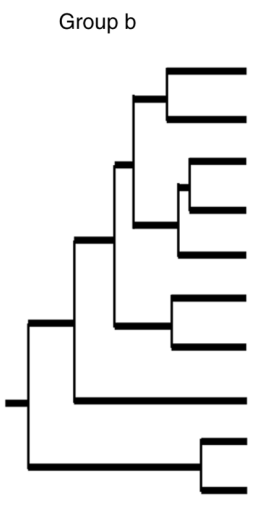

Chromosome containing the CpG (GRCh 37)	Chromosomal coordinates of the CpG (GRCh 37)	Gene
19	12267464	*ZNF625*
2	100938799	*LONRF2*
8	72756153	*MSC*
8	145106438	*OPLAH*
5	140856330	*PCDHGA4*
16	28074384	*GSG1L*
1	49242513	*BEND5*
8	97506675	*SDC2*
4	176987020	*WDR17*
4	176987009	*WDR17*

Figure 2. Methylation profile focused on DMSs in the analyzed patient. (A) Clustered heatmap composed of 766 DMSs. For each CpG site in the CpG islands of the gene region, a DiffScore value of >100 between the normal mucosa and the cancer was defined as the absolute DMS value of the sample. In the majority of DMSs, the methylation levels of the LST-NG lesions were the same as those of the normal mucosa (Group a). However, there were small clusters with methylation levels as high as those of the cancer lesion (Group b). (B) Heatmap focused on Group b with information about each DMS. DMSs, differentially methylated sites; *ZNF625*, zinc finger protein 625; *LONRF2*, LON peptidase N-terminal domain and ring finger 2; *MSC*, musculin; *OPLAH*, 5-oxoprolinase, ATP-hydrolysing; *PCDHGA4*, protocadherin gamma subfamily A, 4; *GSG1L*, GSG1 like; *BEND5*, BEN domain containing 5; *SDC2*, syndecan 2; *WDR17*, WD repeat domain 17.

Figure 3. Methylation profile focused on CpG island regions including DMSs categorized in group b. The methylation values of the CpG island regions around the DMS site in Group b were determined. In the cancer lesions, all the CpG island regions, including each DMS, were methylated in all nine genes, and the CpG sites of *ZNF625*, *LONRF2*, *MSC* and *OPLAH* were methylated in all four LST-NG lesions. *SDC2* and *WDR17* were methylated in three LST-NG lesions. In the CpG islands in *GSG1L*, *BEND5* and *PCDHGA4*, the CpG sites were not methylated as cancer lesions. TSS200, 200 bases upstream of the TSS; TSS500, 500 bases upstream of the TSS; TSS1500, 1,500 bases upstream of the TSS; 5'UTR, within the 5' untranslated region, between the TSS and the ATG start site; body, between the ATG and stop codon. TSS, transcriptional start site; DMSs, differentially methylated sites; LST-NG, laterally spreading tumor-nongranular type lesions; *ZNF625*, zinc finger protein 625; *LONRF2*, LON peptidase N-terminal domain and ring finger 2; *MSC*, musculin; *OPLAH*, 5-oxoprolinase, ATP-hydrolysing; *PCDHGA4*, protocadherin gamma subfamily A, 4; *GSG1L*, GSG1 like; *BEND5*, BEN domain containing 5; SDC2, syndecan 2; *WDR17*, WD repeat domain 17.

The syndecan-2 protein participates in cell proliferation, cell migration, and cell-matrix interactions via its extracellular matrix proteins receptor (23-27), and altered syndecan-2 expression has been detected in several different tumor types (28-30). As reported, overexpressed *SDC2* has tumor activity in CRC (31-33), and it apparently exerts its oncogenic character when activated. Oh *et al* first reported the hypermethylation of *SDC2* in colorectal adenoma as well as in CRC, indicating its contribution to tumorigenesis (34). Their results have been corroborated by other groups, and various useful evaluation methods using stool samples, blood, and urine have been demonstrated (35-40). In contrast to many previous reports concerning *SDC2* in colorectal adenomas and CRC, little is known about alterations in *ZNF625*, *LONRF2*, and *WDR17* concerning CRC tumorigenesis. *ZNF625* has the chromosomal locus 19p13.2 and encodes zinc finger protein 625, which is predicted to enable DNA-binding transcription factor activity, but has not yet been completely analyzed. Lin *et al* reported that among 228 hypermethylated promoter-associated CpG islands, *ZNF625* is one of the most frequently hypermethylated genes in colorectal cancer (41). *LONRF2* has the chromosomal locus 2q11.2 and encodes LON peptidase N-terminal domain and ring finger 2. The gene is conserved in various species including chimpanzees, mice, dogs, chickens, zebrafish, and frogs. Hua *et al* reported *LONRF2* hypermethylation in the rectal adenocarcinomas from 171 patients (42). *WDR17* has the chromosomal locus 4q34.2, encodes WD repeat-containing protein 17, and is abundantly expressed in the retina and testes. It has been suggested as a candidate gene for retinal disease (43). In anal cancer, *WDR17* is hypermethylated, regardless of HIV infection status (44), but to date there has been no report on *WDR17* methylation in CRC. Considering the findings of this study along with those of previous studies, we suggest that *SDC2* hypermethylation contributes to colorectal tumorigenesis at the adenoma stage, and is not limited to LST-NG lesions. Although little is known about the methylation of *ZNF625*, *LONRF2*, and *WDR17* concerning CRC tumorigenesis, it is possible that methylation of the CpG island promoters at *ZNF625*, *LONRF2*, and *WDR17* plays a unique key role in the tumorigenesis of LST-NG lesions.

However, the present study has some limitations: i) the genetic/epigenetic analysis was performed using a different number of samples in each group, that is, only one cancer lesion, four LST-NG, and one normal mucosa; ii) only one patient was analyzed.

In conclusion, we successfully demonstrated the acquired genomic/epigenomic status of pre-cancerous and cancerous phases under identical germline and environmental conditions by analyzing a patient with multiple LST-NG lesions and sigmoid colon cancer. We detected four genes methylated at the CpG island promoters during the LST-NG lesion phase. Although rare, patients with both pre-cancer and cancer lesions should be further investigated to elucidate the contribution made by pure somatic genomic/epigenomic alterations to tumorigenesis.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

MI conceived the study. MI, TT, and MM analyzed and interpreted the data. KK, SO, KS, HS and SB interpreted clinicopathological features. MI and MM drafted the manuscript and critically revised it for important intellectual content. MI and MM confirm the authenticity of all the raw data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of Hamamatsu University School of Medicine (approval no. 17-222).

Patient consent for publication

The patient provided written informed consent for the participation and publication of her data and images.

Competing interests

The authors declare that they have no competing interests.

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