Pure somatic pathogenic variation profiles for patients with serrated polyposis syndrome

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Abstract

Background

The serrated pathway is a distinct genetic/epigenetic mechanism of the adenoma-carcinoma sequence in colorectal carcinogenesis. Although many groups have reported the genetic-phenotypic correlation of serrated lesions (SLs), previous studies regarding the serrated pathway were conducted on patients with SLs that have heterogeneous germline genetic backgrounds. We aimed to compare pure somatic genetic profiles among SLs within identical patients with SPS as a homogenous germline background.

Methods

We analysed SLs from one patient with SPS (Case #1) and compared DNA variant profiles using targeted DNA multigene panels via NGS among the patient's hyperplastic polyp (HP), three sessile serrated lesions (SSLs), and one traditional serrated adenoma (TSA), in addition to leucocytes as a germline variant, and separately analysed three SSLs and one tubular adenoma (TA) within another patient with SPS (Case #2).

Results

In two patients, no germline pathogenic variant was observed, and a known pathogenic variant of *BRAF* (c.1799T > A, p.Val600Glu) was observed in one TSA and one SSL in Case #1, while three SSLs exhibited the *BRAF* variant in Case #2. Further, the genetic profile of TA is consistent with the adenoma-carcinoma sequence pathway profile and distinct from that of the other SLs within the same patient with SPS.

Conclusions

These findings of pure somatic genetic variant profiles among SLs with identical germline genetic background support the previous results analysed among SLs with heterogeneous germline genetic backgrounds.

Background

Colorectal cancer (CRC) is one of the most common cancers worldwide and ranks as the sixth leading cause of cancer-related deaths. [1 2] Since CRC arises from premalignant polyps, the detection and removal of these lesions decreases both CRC incidence and mortality. [3] Although conclusive evidence indicates that colorectal tumours arising from precancerous polyps are mainly categorised as traditional tubular adenomas through the adenoma-carcinoma sequence, some groups have reported that 15–30% of all CRCs are initiated from serrated lesions (SLs) rather than conventional adenomas arising through the adenoma-carcinoma sequence. [4–6] SLs are histologically heterogeneous, including benign hyperplastic polyps (HPs), precancerous sessile serrated lesions (SSLs), or traditional serrated adenomas (TSAs). [4] Among these SLs, HPs are the most frequent subtype and may be further subdivided by predominant mucin type, microvesicular, and goblet cell-rich HPs, and rarely undergo malignant progression. [7 8] SSLs are the second most common form of SLs and are estimated to account for one-third of all SLs. SSLs are recognised as important precursors of the serrated pathway showing a high CpG island methylator phenotype (CIMP). [7 8] TSAs account for a smaller proportion of SLs and are histologically defined by protuberant growth patterns with villiform projections.

The serrated pathway is a distinct genetic/epigenetic mechanism of colorectal carcinogenesis, and a distinguishing trait of the serrated pathway may be the activating variant in *BRAF* (c.1799T > A, p.Val600Glu), a component of the mitogen-activated protein kinase pathway, but this has not been fully characterised. [9–11] TSAs can be classified into the *BRAF* mutant pathway that comes from CIMP-high character followed by mutation of *TP53* and/or *RNF43* and *KRAS* mutant pathways, which exhibit CIMP-low characteristics. [12 4 10 13] Reportedly, a large proportion of microvesicular HPs contain the *BRAF* pathogenic variant, which is believed to occur early in the serrated pathway, causing constitutive activation of the mitogen-activated protein (MAP) kinase–ERK pathway and uncontrolled cell division. [9 14–19] SSLs may progress to colorectal carcinoma through *BRAF* mutation and CIMP-high/microsatellite instability (MSI)-high profiles. [13 20 21] Although many groups have reported the genetic-phenotypic correlation regarding SLs, the precise profile and mechanisms of these serrated pathways for prevention of colorectal carcinogenesis are not fully elucidated, as previous reports involved many patients with SLs with different germline backgrounds. Therefore, genetic comparison and analysis of multiple SLs within a patient should be conducted.

Serrated polyposis syndrome (SPS) is characterised by multiple SLs located throughout the colon and is accompanied by an increased risk of CRC. The diagnosis of SPS is based on the cumulative lifetime number of HPs, TSAs, and SSPs in a patient who meets one of the two following World Health Organization (WHO) criteria, including (1) > 5SPs proximal to the rectum, all being ≥ 5 mm in size, including ≥ 2 that are ≥ 10 mm; or (2) > 20 SPs of any size distributed throughout the colon, with ≥ 5 being proximal to the rectum. [22] Therefore, it is important to compare genetic profiles among SLs within patients with SPS to understand the pure somatic genetic variant associated with the serrated pathway.

In the present study, we customised a set of targeted DNA multigene panels and used it to evaluate the variant SL profiles. Herein, we show differences in the main genetic contributors to the serrated pathway among SLs within SPS patients.

Methods

Patients

We analysed nine SLs and one non-SL (tumular adenoma) from two patients with SPS who met the WHO 2019 criteria for the diagnosis of SPS. All patients 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).

Samples, DNA extraction, and quality assessment

SL samples were obtained from the Department of Diagnostic Pathology at Hamamatsu University Hospital as formalin-fixed paraffin-embedded (FFPE) tissue from the two patients with SPS resected by EMR. We also obtained matched normal blood samples. Genomic DNA was extracted from macrodissected tumours and non-tumors using a QIAamp DNA FFPE Tissue Kit (Qiagen, Hilden, Germany), and extracted from the blood using an EZ1 DNA Blood 350 μ l Kit (Qiagen). The quality of the gDNA was analysed using the 2200 TapeStation (Agilent Technologies, Santa Clara, CA, USA) system using the TapeStation Analysis software (Agilent), which automatically determines and displays the DNA integrity number (DIN) as a measure of DNA integrity (<https://www.agilent.com/cs/library/applications/5991-5258EN.pdf>).

Next-generation sequencing (NGS)

We customised the multigene panel (72 genes; **Table 1**) by adding the QIAseq Human Colorectal Cancer Panel (71 genes, DHS-002Z; Qiagen) to the *RNF43* gene because the pathogenicity of the *RNF43* gene variant has been reported in SLs. [23] The customised multigene panel was used for library construction according to the manufacturer's instructions. The libraries were assessed using a QIAseq Library Quant Assay Kit (#QSTF-ILZ-R; Qiagen) and applied to a MiniSeq sequencer (Illumina, San Diego, CA, USA). The Qiagen web portal (<https://geneglobe.qiagen.com/jp/analyze/>) 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 (IGV; <http://software.broadinstitute.org/software/igv/home>).

IHC staining

IHC was performed as described previously. [24] Staining for the expression of MLH1 protein was performed using 10% formalin fixed for 6–48 h at room temperature, and paraffin-embedded blocks were cut into 4 µm thick serial sections. The slides were dewaxed by heating at 55°C for 30 min, followed by three 5 min washes with xylene. Subsequently, the tissues were rehydrated using a series of 5 min washes in 100%, 95%, and 80% ethanol, and distilled water. Endogenous peroxidase activity was blocked by treatment with 3% hydrogen peroxide for 10 min at room temperature. Following incubation with the protein block reagent [StartingBlock™ (TBS) Blocking Buffer; cat. no. 37542; Thermo Fisher Scientific Inc. Pittsburgh, PA, USA) for 5 min at room temperature, samples were washed twice with TBS, and incubated with the mouse monoclonal antibody Anti-MLH1 (clone G168-728; 1:50; cat. no. 554073 BD Biosciences) for 30 min at room temperature, and then incubated with dextran polymer conjugated with goat anti-mouse immunoglobulin G and horseradish peroxidase (ChemMate Envision kit; Dako; Agilent Technologies, Inc.) for 30 min at room temperature. The antigen-antibody complex was visualised using 3,3'-diaminobenzidine tetrahydrochloride and counterstained with haematoxylin for 1 min at room temperature using an autostainer (Histostainer; Nichirei Biosciences Inc.).

Statistical analyses

Statistical analyses were performed using IBM SPSS Statistics for Windows (version 25; IBM Corp., Armonk, NY, USA), and a value of $P < 0.05$ was considered statistically significant.

Results

Clinicopathological features

We analysed SLs from two patients with SPS (Cases #1 and #2). Case #1 was a 41-year-old man who initially exhibited a positive faecal occult blood test (FOBT). He underwent colonoscopy and six protruded lesions were detected throughout the colon, and all lesions were located proximal to the rectum (Fig. 1, Table 2). We performed endoscopic mucosal resection (EMR) on all lesions, and SLs (one HP, three SSLs, and two TSAs) were diagnosed histopathologically in each resected specimen. SPS was also diagnosed in the patient because he met the WHO 2019 criteria for the diagnosis of SPS. The patient did not have a first-degree relative with SPS. Case #2 is a 68-year-old woman who underwent colonoscopy as part of a routine medical examination, and six protruded lesions were detected throughout the colon. All lesions were located proximal to the rectum (Fig. 1, Table 2) and were endoscopically resected. Among the lesions, five were histologically SSLs, and one was non-SL (tubular adenoma). Case #2 was diagnosed with SPS because she met the WHO 2019 criteria.

DNA quality for analysis

DNA quality data are presented in Table 2. It is particularly important to determine whether the DNA extracted from FFPE has not degraded prior to NGS analysis. Therefore, we observed the quality of the gDNA using the 2200 TapeStation system and determined the DNA integrity number (DIN) as a measure of DNA integrity. A favourable DIN (≥ 4.0) was observed in five of nine (55.6%) samples, and these samples were ≤ 2 years old, whereas three of four samples exhibited low DIN numbers (< 4.0) at 2 years of age ($P = 0.018$). However, the depths of the molecular-tagged sites and all examined sites were favourable.

Somatic variant profile of analysed lesions from patients with SPS

The patient in Case #1 exhibited all pathological SL types (HP, SSL, and TSA) and we analysed the DNA variant profile of one HP, three SSLs, and one TSA, in addition to leucocytes as a germline variant profile (Table 3, **Supplemental Table 1**). We detected three germline variants (*DCC*; c.2277T > G, p.Ile759Met, *MSH3*; c.1718G > A, p.Arg573Lys, and *BAX*; c.32G > A, p.Gly11Glu), which were observed in all five patients. When focusing on gene variants known to be associated with SLs, a known pathogenic variant of *BRAF* (c.1799T > A, p.Val600Glu) was detected in one SSL located in the ascending colon (#1–4) and one TSA in the transverse colon (#1–5) among the six SLs. One SSL in the ascending colon displayed a splice site variant at *RNF43* (c.687G > A) without any *BRAF* variant. No *KRAS* pathogenic variants were detected among the six lesions in the patients. Five DNA mismatch repair (MMR) genes are known to cause MSI-H when these functions are disrupted (*MLH1*, *MSH2*, *MSH6*, *PMS2*, *EPCAM*). The SSL with the *BRAF* c.1799T > A pathogenic variant located in the ascending colon also displayed the *MLH1* variant (c.687G > A, p.Val213Glu).

The patient in Case #2 had only one type of SL (three SSLs), but it is unique that we could compare the DNA profile of three SSLs with that of one non-SL lesion (tubular adenoma) in addition to leucocytes as a germline variant profile (Table 4, **Supplemental Table 2**). We detected five germline variants (*BRCA1*; c.670 + 1G > T, splice site variant, *BRCA2*; c.2350A > G, p.Met784Val, *BRCA2*; c.3420T > A, p.Met784Val, *AXIN2*; c.2140C > T, p.Arg714Trp, *MET*; c.4141G > A, p.Ala1381Thr), which were observed in all four patient lesions. A known pathogenic variant of *BRAF* (c.1799T > A, p.Val600Glu) was detected in all SSLs analysed (#2–1, #2–3, and #2–4), whereas we detected another two *BRAF* variants, not known to be pathogenic in the previous database, in tubular adenomas of patients. No *KRAS* or *RNF43* variants were detected among the four lesions, including tubular adenomas. Interestingly, a tubular adenoma displayed two pathogenic variants that are highly associated with the adenoma-carcinoma sequence (*APC*; c.4249_4265delATTATAAGCCCCAGTGA, p.Ile1417SerfsTer4, *TP53*; c.818G > A, p.Arg273His), but not the other three SSLs.

MLH1 status of SLs

Since SLs, especially SSLs and a partial group of TSAs, display MSH-H characteristics due to hypermethylation of *MLH1*, [4] we attempted IHC staining for MLH1 protein for all nine lesions with detected DNA variant profiles. Among the nine lesions, we detected no lesions with defective MLH1 proteins.

Mutational Signature patterns

We examined types of combination of a substituted base plus the flanking 5' and 3' bases and to see the tendency of mutation signature according to the classification of mutational signature (MS). As shown in Table 5, the majority type of the single base substitution (SBS) was C > T, followed by T > C among nine SSLs. Especially, C > T SBS tends to be commonly observed in HP (#1–2) and TSA (#1–5) of case1 and TA (#2–2) of case 2, and T > C substitution was occurred in one SSL (#2–4) of case2, speculating that both non-SSL serrated lesions such as HP, TSA and non-SLs as TA may be characterized as MS pattern seen by aging. We further checked the MS patterns by combination set of a substituted base plus the flanking 5' and 3' bases (**Supplemental Table 3**). Among nine lesions, we observed

nine substitutional sets of CCG > CTG, six set of TCG > TTG and GTG > GAG, and five set of ACC > AGC and GCT > GGT, but these were not satisfied the typical MS.

Discussion

Several groups have reported the genetic-phenotypic correlation with SLs via analysis of many SL patients, but the patients' germline genetic background is not homogenous, and these reports do not conclusively indicate whether SLs arise from "pure" somatic genetic and/or epigenetic alterations or not. Therefore, this study aimed to analyse and compare the somatic genetic profiles among multiple SLs within a patient with SPS. Our study demonstrates that (a) favourable DNA samples (≥ 4.0 DIN) can be obtained from FFPE tissues ≤ 2 years old to detect appropriate somatic DNA profiles using NGS, (b) pure somatic SL DNA profiles within a SPS patient were compatible with previous SL reports using patients with heterogeneous germline genetic backgrounds, and (c) pure DNA profiles of TA are quite different from that of other SLs within a patient with SPS. To our knowledge, this is the first study to demonstrate a pure somatic genetic profile compared among SLs within a patient.

Although FFPE tissue is generally prepared for histopathological diagnosis, it can also be used for molecular diagnosis, such as genomic analysis using NGS, as it can be clearly observed from the histopathological picture that the lesion is a tumour within the FFPE sample. This applies to our study in that SLs are histopathologically varied, and it is important to determine the location of SL. Therefore, we used FFPE samples, detected the SL location, and extracted DNA only at this location. Further, it is important to analyse NGS with high-quality DNA samples, and evaluate the quality of pre-analytical and analytical processes, especially when using DNA from FFPE due to the tendency of the sample to degrade. In our study, we evaluated the DNA derived from FFPE by DIN and found that DNA was degraded in FFPE tissues stored for more than two years, which is consistent with recent reports by other groups showing that the high degradation of DNA extracted from FFPE is unsuitable for sequence analysis. However, NGS analysis in our study was successful even with aged FFPE samples, probably because the amplicon for analysis was designed to be as small as 200 bp, even if the nucleic acid was degraded. [25–27] Thus, it is important to consider the best sample from which to obtain DNA to match the progress of genomic analysis techniques, such as long-read sequencing. [28]

Many groups have reported the influence of pathological genetic variations of *BRAF*, such as c.1799T > A and p.Val600Glu, on the progression of HPs, SSLs, TSAs, and *KRAS* pathogenic variants for HPs and TSAs, but these analyses were performed among patients with heterogeneous germline backgrounds. [10–13] To detect a pure somatic genetic variation profile, we compared the genetic profiles of dome-serrated lesions within identical patients. In Case #1, a known pathogenic variant of *BRAF* (c.1799T > A, p.Val600Glu) was detected in one SSL (#1–4) and one TSA (#1–5). Previous reports have demonstrated that the *BRAF* variant was found in almost all SSLs, where there are two distinct pathways for progression to TSAs, that is, the *KRAS*-driven pathway and the *BRAF*-driven pathway, and it is compatible that we detected the *BRAF* variant commonly in two different SLs in patient #1. [12 13 20 21] When we focus on the two lesions, it is interesting that genetic profiles, other than that of the *BRAF* variant, appear quite different (*BLM*, *AXIN2*, *CDC27*, and *MLH1* in #1–4, and *RET*, *ERBB2*, *STK11*, and *TCERG1* in #1–5, as seen in Table 4). Therefore, the two SLs must be initiated by the common *BRAF* pathogenic variant, followed by progression via the accumulation of different genetic profiles, but further accumulated findings should be considered. In Case #2, all SSLs displayed pathogenic variants of *BRAF* (c.1799T > A, p.Val600Glu), as expected from previous reports. [14] In addition, it is interesting that we confirmed the adenoma-carcinoma pathway by detecting somatic *APC* deletion (c.4249_4265delATTATAAGCCCCAGTGA) as a driver variant (VAF: 55.4%) in TA (#2–2). Notably, the somatic genetic profile of the TA is quite different from other SSLs within Case #2 (#2–1, #2–3, #2–

4), which indicates that the serrated pathway in the SSL adenoma-carcinoma sequence in TAs does not have common driver variants at the initiation stage, and that the accumulated genetic variant profile is distinct between the two pathways.

RNF43 has been reported as one of the key genes when pathogenic germline or somatic variants are detected in SLs. [23 29 30] Giannakis *et al.* demonstrated that somatic mutations in *RNF43* occur in 18.9–17.6% of CRC cases, and the majority of *RNF43* somatic mutations were truncating events. Despite this, it is still unclear from their reports whether CRC arises from SLs. [29] Taken together, it is possible that the somatic *RNF43* splice-site variant detected in our study in SSLs of Case #1 (#1–3) is pathogenic in the serrated polyposis-cancer sequence, although additional questions remain as limitations, such as the existence of two hits for the lesion by genetic or epigenetic alteration.

As for epigenetic features in SLs, it has been reported that silencing of *MLH1* plays an important role in the progression of SLs, especially with the *BRAF* pathogenic variant, [4 16] but in our IHC study, no deficiency of MLH1 protein could be seen among SLs in two patients with SPS. Apparently, this result does not agree with a previous report, but it is not clear whether *MLH1* was silenced to completely suppress the expression of MLH1 protein. Moreover, it must be noted that previous clinical reports have demonstrated that deficient-MMR has not been identified in HPs, TSAs, or SSLs, but has been reported in SSL with dysplasia (SSLD) only. [21 31] Additionally, SSLD is the only pre-cancerous colorectal lesion in which *MLH1* is methylated. [32] Regarding the occurrence of deficient MMR, patients with pre-cancerous lesions, especially with SSLD, require careful surveillance after resection.

Among the SLs and non-SLs (TA), we examined SBS type and checked which substitutional types would be correspond to the MS pattern previously reported [33]. Interestingly, we found that HP, TSA, and TA have similar substitutional pattern in that these three lesions have mainly observed C > T characterized as aging, and SBS of SSL is distinct from these three lesions. To confirm our results from multigene panel testing, more huge number of SBS should be analyzed using whole exome sequencing or whole genome sequencing.

The present study has several limitations: i) IHC for DNA MMR was performed only for pre-cancerous lesions and not cancerous lesions, because cancer was not found in the two analysed SPS patients; ii) the analysed number of patients with SPS was small; and iii) the methylation profile was not evaluated. These findings require further investigation in future studies. However, to the best of our knowledge, the present study is the first to focus on pure somatic genetic variant profiles of multiple SLs compared within a SPS patient, and it may represent the first step in understanding the mechanism behind the differentiation into different histological types of serrated lesions.

In conclusion, we compared pure somatic genetic profiles among SLs within patients with SPS as a homogenous germline background using NGS. A known common pathogenic *BRAF* variant could be seen in SSLs and TSAs, but another genetic profile of each lesion appeared quite different. In addition, the genetic profile of TA is consistent with the profile of the adenoma-carcinoma sequence pathway and distinct from that of other SLs within the same patient with SPS. More research is required to understand the molecular mechanisms underlying the progression to SL for patient care and colorectal cancer prevention.

Abbreviations

Colorectal cancer (CRC); Serrated lesion (SL); hyperplastic polyp (HP); sessile serrated lesion (SSL); traditional serrated adenoma (TSA); mitogen-activated protein (MAP); CpG island methylator phenotype (CIMP); microsatellite instability (MSI); MSI-high (MSI-H); Serrated polyposis syndrome (SPS); World Health Organization (WHO); endoscopic mucosal resection (EMR); DNA mismatch repair (MMR); SSL with dysplasia (SSLD)

Declarations

Contributors: MI conceived the study. MH, TT, and MM analysed and interpreted the data. SO, KS and SB interpreted clinicopathological feature. MI, and MM drafted the manuscript and critically revised it for important intellectual content. All authors read and approved the final manuscript.

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Competing interests None declared.

Ethics approval The present study was approved by the Ethics Committee of Hamamatsu University School of Medicine (approval no. 17-222). All methods were performed in accordance with the Declaration of Helsinki.

Availability of data and materials statement The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

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Tables

Table 1 List of genes analysed in the study

<i>BRAF</i>	<i>SMAD4</i>	<i>AXIN2</i>	<i>CDC27</i>	<i>ENG</i>	<i>GPC6</i>	<i>MLH3</i>	<i>PMS1</i>	<i>SLC9A9</i>
<i>FBXW7</i>	<i>STK11</i>	<i>BAX</i>	<i>CDH1</i>	<i>EP300</i>	<i>GREM1</i>	<i>MSH2</i>	<i>PMS2</i>	<i>SMAD2</i>
<i>KRAS</i>	<i>TCF7L2</i>	<i>BLM</i>	<i>CDK4</i>	<i>EPCAM</i>	<i>KIT</i>	<i>MSH3</i>	<i>POLD1</i>	<i>SRC</i>
<i>CTNNB1</i>	<i>TP53</i>	<i>BMPR1A</i>	<i>CDKN2A</i>	<i>ERBB2</i>	<i>MAP2K4</i>	<i>MSH6</i>	<i>POLE</i>	<i>TCERG1</i>
<i>NRAS</i>	<i>ACVR1B</i>	<i>BRCA1</i>	<i>CHEK2</i>	<i>FGFR3</i>	<i>MAP7</i>	<i>MUTYH</i>	<i>PTEN</i>	<i>TGFBR2</i>
<i>PIK3CA</i>	<i>AKT1</i>	<i>BRCA2</i>	<i>CTNNA1</i>	<i>FLCN</i>	<i>MET</i>	<i>MYO1B</i>	<i>PTPN12</i>	<i>WBSCR17</i>
<i>APC</i>	<i>ATM</i>	<i>BUB1B</i>	<i>DCC</i>	<i>FZD3</i>	<i>MIER3</i>	<i>PALB2</i>	<i>RET</i>	<i>SCG5</i>
<i>DMD</i>	<i>ATP6V0D2</i>	<i>CASP8</i>	<i>EGFR</i>	<i>GALNT12</i>	<i>MLH1</i>	<i>PIK3R1</i>	<i>RPS20</i>	<i>RNF43</i>

Table 2 Clinicopathological features, DNA quality, quantity, and NGS depth of FFPE samples and blood samples

Case	Sample #	Location	Pathology	Size (mm)	Storage year	Qubit dsDNA conc. (ng/ μ L)	DIN*	Mean depth for molecular tagged sites	Mean depth for all examined sites
Case #1 41 years old Male	#1-1	Ascending Colon	SSL	15	4	42.4	2.4	41.5	152.6
	#1-2	Transverse Colon	HP	7	5	13.7	2.8	35.6	170.8
	#1-3	Ascending Colon	SSL	15	2	22.9	4.3	168.3	388.6
	#1-4	Ascending Colon	SSL	15	2	29.8	4.5	232.9	499.8
	#1-5	Sigmoid Colon	TSA	25	4	24.8	2.4	134.9	332.1
	NT	Transverse Colon	TSA	10	NT	NT	NT	NT	NT
	#1-0	Peripheral Blood	Normal leukocyte	-	-	57.6	NT	157.4	355.95
Case #2 68 years old Female	#2-1	Transverse Colon	SSL	20	1	13.3	5.1	159.7	374.8
	#2-2	Cecum	TA	10	2	24.3	3.1	148.1	421.8
	#2-3	Descending Colon	SSL	15	1	21.6	5.6	281.5	619.9
	#2-4	Transverse Colon	SSL	15	1	29.8	4.1	301.4	653.3
	NT	Transverse Colon	SSL	7	NT	NT	NT	NT	NT
	NT	Descending Colon	SSL	10	NT	NT	NT	NT	NT
	#2-0	Peripheral Blood	Normal leukocyte		-	86.7	NT	158.4	389.2

DIN: DNA Integrity Number, NT: not tested, *: Median wasn't available on the Qiagen web portal.

HP hyperplastic polyp, SSL sessile serrated lesion, TSA traditional serrated adenoma, TA tubular adenoma

Table 5. Mutational signature of the single base substitutions (SBS)

Sample ID		C>A	C>G	C>T	T>A	T>C	T>G	SBS No.
		G>T	G>C	G>A	A>T	A>G	A>C	
#1-1	SSL (A)	0	3	3	0	3	1	10
#1-2	HP (T)	0	0	7	0	3	1	11
#1-3	SSL (A)	1	2	2	0	0	0	5
#1-4	SSL (A)	0	0	0	3	1	2	6
#1-5	TSA (S)	0	2	5	1	1	1	10
#2-1	SSL (T)	1	2	1	1	2	0	7
#2-2	TA (C)	0	1	4	2	1	1	9
#2-3	SSL (D)	0	0	0	1	0	0	1
#2-4	SSL (T)	1	2	1	1	5	1	11
		3	12	23	9	16	7	70

HP hyperplastic polyp, SSL sessile serrated lesion, TSA traditional serrated adenoma, TA tubular adenoma, C>G Moderate mutational process activated at different times, C>A Strong exogenous mutational process, C>T Endogenous mutational process.

Due to technical limitations, Table 3 and 4 are only available as a download in the Supplemental Files section.

Figures

Figure 1

Endoscopic/histological features of analyzed lesions. (A) Case #1. A 41-year-old man who initially exhibited a positive fecal occult blood test (FOBT). He underwent colonoscopy and six protruded lesions were detected throughout the colon, and SLs (one HP, three SSLs, and two TSAs) were diagnosed histopathologically in each resected specimen. (B) Case #2. A 68-year-old woman who underwent colonoscopy as part of a routine medical examination, and six protruded lesions were detected throughout the colon. Among the lesions, five were histologically SSLs, and one was non-SL (tubular adenoma). SSL sessile serrated lesion, HP hyperplastic polyp, TSA traditional serrated adenoma, TA tubular adenoma, A Ascending Colon, T Transverse Colon, S Sigmoid Colon, C Cecum, D Descending Colon, NT not tested

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Table3and4.docx](#)
- [BMCGHidakaSuppltable1.xlsx](#)
- [BMCGHidakaSuppltable2.xlsx](#)
- [BMCGHidakaSuppltable3.xlsx](#)