Superficially serrated adenoma: a proposal for a novel subtype of colorectal serrated lesion

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Abstract

We describe a series of colorectal polyps characterized by mixed adenomatous and serrated features, herein referred to as superficially serrated adenomas. Twenty superficially serrated adenomas were obtained from 11 female and 9 male patients aged 62–87 years. Most lesions endoscopically appeared as small sessile polyps, but larger lesions were plaque-like (2–20 mm; median, 5 mm). Eighteen lesions (90%) were located in the sigmoid colon or rectum. They consisted primarily of straight, adenomatous glands but showed serration confined to the superficial layer. Immunohistochemistry revealed CK20 expression in the upper layer. Proliferating cells, determined by their expression of Ki-67, were localized to the middle to bottom layers. Genetic analyses identified *KRAS* mutations in 19 lesions and a *BRAF* mutation in one lesion. Furthermore, *RSPO* fusions and/or overexpression were observed in 18 lesions and truncating *APC* mutations were observed in the two remaining lesions. Consistent with the presence of WNT pathway gene alterations, all superficially serrated adenomas showed focal or diffuse nuclear β -catenin accumulation. Since concurrent *KRAS* mutations and *RSPO* fusions are reportedly common in traditional serrated adenomas, we reviewed 129 traditional serrated adenomas and found 15 lesions (12%) that were associated with superficially serrated adenoma components. Remarkably, all but one superficially serrated adenoma-associated traditional serrated adenoma exhibited concurrent *KRAS* mutations and *RSPO* fusions/overexpression. The present study suggests that superficially serrated adenoma is a morphologically and molecularly distinct type of colorectal serrated polyp that is histogenetically related to traditional serrated adenoma.

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Introduction

Epithelial colorectal polyps are classified into two major groups: conventional adenomas and serrated lesions [1–4]. Conventional adenomas consist of dysplastic glands and are further subclassified as tubular, tubulovillous, or villous, depending on their degree of villosity [2]. Serrated lesions are characterized by serrated crypt epithelium with or without dysplasia and include hyperplastic polyps, sessile serrated adenomas/polyps, and traditional serrated adenomas [3].

Conventional adenomas and serrated lesions are defined morphologically but also have different molecular backgrounds and are related to distinct tumorigenic pathways [1, 4, 5]. Conventional adenomas commonly have *APC* mutations and give rise to mismatch repair-proficient adenocarcinomas [2, 4, 6]. Serrated lesions mostly have *BRAF* or *KRAS* mutations, with dysplastic sessile serrated adenoma/polyps and traditional serrated adenomas often containing additional WNT pathway gene alterations,

predominantly *RNF43* mutations, and *RSPO* fusions [4, 5, 7–10]. Sessile serrated adenoma/polyps are recognized as precursors to *BRAF*-mutated adenocarcinomas with or without mismatch repair deficiency, whereas traditional serrated adenoma-derived adenocarcinomas are consistently mismatch repair-proficient [10–14].

Although there is still debate concerning the precise diagnostic criteria for each category, the current histological classification of colorectal polyps is generally well accepted. However, sometimes polyps with focal areas of serration are observed, which are thus difficult to classify as either a conventional adenoma or serrated lesion. A recent study analyzed a series of serrated tubulovillous adenomas and implied that they are clinicopathologically and molecularly distinct from conventional tubulovillous adenomas or traditional serrated adenomas [15]. Based on their mutation profiles and early WNT pathway activation, as indicated by nuclear β -catenin expression, serrated tubulovillous adenomas appear to be aligned more closely with the conventional pathway rather than the serrated pathway.

In the present report, we describe another previously unrecognized type of colorectal polyps showing mixed morphological features of both conventional adenomas and serrated lesions, which is herein referred to as a superficially serrated adenoma. Our study indicates that superficially serrated adenomas have a characteristic molecular background suggestive of serrated lesions and histogenetic relationship to traditional serrated adenomas.

Materials and methods

Samples

This study was approved by the Ethics Committee of the National Cancer Center, Tokyo, Japan. All tissue samples were obtained by endoscopic resection at the National Cancer Center Hospital, Tokyo, Japan, or at the National Cancer Center Hospital East, Chiba, Japan. The present study analyzed 20 colorectal superficially serrated adenomas, which consisted of adenomatous glands but showed superficial serration. Seven lesions were retrieved from the case files of S.S. The remaining 13 cases were identified through a histological review of approximately 9500 colorectal polyps, that were originally diagnosed as tubular adenoma, from the National Cancer Center Hospital, Tokyo, Japan. In addition, we histologically re-evaluated 129 traditional serrated adenomas, which had been previously characterized for their clinicopathological features and mutation profiles [8, 16], in order to determine whether any were associated with superficially serrated adenoma components.

Immunohistochemistry

Immunohistochemical analyses were performed on formalin-fixed paraffin-embedded specimens. Ten goblet cell-rich hyperplastic polyps and 10 low-grade tubular adenomas were also examined for comparison. All goblet cell-rich hyperplastic polyps had *KRAS* mutations, whereas the tubular adenomas had *APC* mutations [8], indicating that these lesions have prototypical genetic features. Antigen retrieval was performed by autoclaving the samples in 10 mM citrate buffer (pH 6.0) for 10 min. The following primary antibodies were used: anti-CK20 (Ks 20.8; 1:50; Dako, Glostrup, Denmark), anti-Ki-67 (MIB-1; 1:100; Dako), anti-MYC (Y69; 1:200; Abcam, Cambridge, UK), anti- β -catenin (14; 1:200; BD Biosciences, Franklin Lakes, NJ, USA), and anti-MLH1 (ES05; 1:200; Dako). An automated stainer (Dako) was used in accordance with the manufacturer's protocol. ChemMate EnVision (Dako) methods were used for detection. For β -catenin staining, nuclear expression in <10%, 10–50%, or >50% of cells was classified as –, +, or ++, respectively [8]. For MLH1 staining, lesions devoid of nuclear staining were considered MLH1-expression-deficient, using lymphocytes and endothelial cells as internal positive controls.

DNA and RNA extraction

Deparaffinized, 10- μ m-thick sections from each paraffin block were microdissected under a microscope using sterilized toothpicks in order to enrich for tumor content. Five goblet cell-rich hyperplastic polyps and five tubular adenomas were also analyzed for comparison. In superficially serrated adenoma-associated traditional serrated adenomas, the superficially serrated adenoma and traditional serrated adenoma components were separately dissected. The microdissected samples were subjected to DNA and RNA extraction using the QIAamp DNA FFPE Tissue Kit (Qiagen, Hilden, Germany) and RNeasy FFPE Kit (Qiagen), respectively.

Next-generation and Sanger sequencing

Next-generation sequencing targeting frequently mutated regions of *KRAS*, *NRAS*, *BRAF*, *CTNNB1*, *APC*, *RNF43*, and *GNAS* was performed as described previously [17]. All of the detected mutations were verified by Sanger sequencing. The mutation analysis of superficially serrated adenoma-associated traditional serrated adenomas was performed by Sanger sequencing as described previously [16].

Conventional and quantitative RT-PCR

Following reverse-transcription (RT) reactions, complementary DNA sample quality was assessed by PCR

amplification of a 220-bp fragment of *ACTB* [8]. RT-PCR targeting known *RSPO* fusions, including *EIF3E-RSPO2* and *PTPRK-RSPO3*, was performed as described previously [8]. Quantitative PCR for *RSPO2* and *RSPO3* was performed using KOD SYBR qPCR Mix (TOYOBO, Tokyo, Japan). *RSPO2* and *RSPO3* expression levels were determined using *GUSB* as a standard and compared with four samples from normal colon mucosa. Expression levels threefold higher than those seen in the normal colon mucosa were regarded as overexpression.

Statistical analysis

Fisher's exact test and Welch's *t* test were used to analyze categorical variables and continuous variable, respectively. *P* values <0.05 were considered to indicate statistical significance.

Results

Twenty superficially serrated adenomas were obtained from 11 female and 9 male patients who were aged 62–87 years

(Table 1). Eighteen lesions (90%) were located in the sigmoid colon or rectum. They were 2–20 mm in size (median, 5 mm). Endoscopically, they were mostly small sessile polyps, but larger lesions appeared flat/plaque-like (Fig. 1). Histologically, superficially serrated adenomas primarily consisted of straight adenomatous glands but also showed serration in the superficial layer (Fig. 2a–e). The tumor cells were columnar and had uniform basal elongated nuclei (Fig. 2f). The overlying epithelium showed a tufted appearance but did not have the abundant eosinophilic cytoplasm seen in traditional serrated adenomas. None of the lesions showed a villous growth pattern. The bottom of the crypts was generally straight and lacked sessile serrated adenoma/polyp-like features, but larger lesions had some dilated glands. None of the lesions were associated with other types of polyps, such as hyperplastic polyps or sessile serrated adenoma/polyps.

We performed comparative immunohistochemical analyses of superficially serrated adenomas to normal colon mucosa, goblet cell-rich hyperplastic polyps, and tubular adenomas (Fig. 3). Since lesions of each type showed similar staining results, representative findings have been presented. CK20 was expressed in the upper layer of goblet

Table 1 Clinicopathological and molecular features of superficially serrated adenomas

Sample	Age/sex	Location	Size (mm)	β-catenin ^a	MLH1 ^b	WNT pathway gene alterations	MAPK pathway gene mutations	<i>GNAS</i> mutation
SuSA01	62/F	Rectum	5	++	+	<i>PTPRK</i> (e1)- <i>RSPO3</i> fusion	<i>KRAS</i> c.34G>T	—
SuSA02	68/F	Rectum	15	+	+	<i>PTPRK</i> (e1)- <i>RSPO3</i> fusion	<i>KRAS</i> c.35G>T	—
SuSA03	77/F	Sigmoid	20	+	+	<i>RSPO3</i> overexpression	<i>KRAS</i> c.34G>T	—
SuSA04	63/F	Rectum	10	++	+	<i>RSPO3</i> overexpression	<i>KRAS</i> c.35G>A	—
SuSA05	72/F	Sigmoid	5	+	+	<i>PTPRK</i> (e1)- <i>RSPO3</i> fusion	<i>KRAS</i> c.35G>T	—
SuSA06	62/M	Sigmoid	18	+	+	<i>RSPO2</i> overexpression	<i>KRAS</i> c.35G>T	—
SuSA07	66/M	Rectum	5	++	+	<i>PTPRK</i> (e7)- <i>RSPO3</i> fusion	<i>BRAF</i> c.1799T>A	—
SuSA08	78/F	Transverse	3	+	+	<i>PTPRK</i> (e1)- <i>RSPO3</i> fusion	<i>KRAS</i> c.35G>A	—
SuSA09	79/M	Sigmoid	4	+	+	<i>RSPO3</i> overexpression	<i>KRAS</i> c.35G>A	—
SuSA10	69/M	Rectum	9	++	+	<i>APC</i> c.3961_3964delAGCGinsCC ^c	<i>KRAS</i> c.38G>A	c.602G>A
SuSA11	62/F	Ascending	2	+	+	<i>RSPO3</i> overexpression	<i>KRAS</i> c.35G>A	—
SuSA12	71/M	Sigmoid	5	++	+	<i>PTPRK</i> (e7)- <i>RSPO3</i> fusion	<i>KRAS</i> c.35G>A	—
SuSA13	65/F	Rectum	9	++	+	<i>PTPRK</i> (e1)- <i>RSPO3</i> fusion	<i>KRAS</i> c.35G>A	—
SuSA14	87/M	Rectum	5	+	+	<i>PTPRK</i> (e7)- <i>RSPO3</i> fusion	<i>KRAS</i> c.35G>A	—
SuSA15	83/M	Sigmoid	2	++	+	<i>PTPRK</i> (e7)- <i>RSPO3</i> fusion	<i>KRAS</i> c.35G>A	—
SuSA16	76/M	Rectum	5	+	+	<i>RSPO3</i> overexpression	<i>KRAS</i> c.35G>C	—
SuSA17	71/F	Rectum	4	++	+	<i>PTPRK</i> (e7)- <i>RSPO3</i> fusion	<i>KRAS</i> c.183A>C	—
SuSA18	64/F	Rectum	4	++	+	<i>RSPO2</i> overexpression	<i>KRAS</i> c.35G>T	—
SuSA19	72/M	Rectum	3	++	+	<i>PTPRK</i> (e1)- <i>RSPO3</i> fusion	<i>KRAS</i> c.34G>T	—
SuSA20	70/F	Sigmoid	3	++	+	<i>APC</i> c.4438C>T ^c	<i>KRAS</i> c.38G>A	—

M male, F female

^a Nuclear β-catenin expression: +, 10–50%; ++, >50%

^b MLH1 expression: +, retained

^c Protein-truncating mutations

Fig. 1 Endoscopic findings of superficially serrated adenomas. **a** Small sessile polyp with mildly lobulated surface in the rectum (SuSA01). **b, c** Small sessile polyps in the rectum (**b**, SuSA07; **c**, SuSA19). **d** Large slightly elevated lesion in the sigmoid colon highlighted by chromoendoscopy (SuSA03)

Fig. 2 Histological features of superficially serrated adenomas. **a, b** Low-power images of polyps showing proliferation of adenomatous glands and superficial serration. **c** Abrupt transition of the neoplastic glands and normal mucosa at the periphery of the lesion. **d** Tufting of the overlying epithelium. **e** Straight tumor glands lacking serration in the middle to bottom layer. **f** Glands lined by columnar epithelium with uniform elongated basal nuclei

Fig. 3 Comparative immunohistochemical analysis. **a–d** Normal mucosa. **e–h** Goblet cell-rich hyperplastic polyp. **i–l** Superficially serrated adenoma. **m–p** Tubular adenoma. CK20 is expressed in the upper layer of normal mucosa, goblet cell-rich hyperplastic polyp, and superficially serrated adenoma (**b, f, j**). Tubular adenoma showing diffuse weak CK20 expression (**n**). Ki-67 and MYC expression is localized to the bottom layer in normal mucosa and goblet cell-rich hyperplastic polyp (**c, d, g, h**), whereas distributed in the middle to lower layers in superficially serrated adenoma (**k, l**) and in the upper layer in tubular adenoma (**o, p**)

cell-rich hyperplastic polyps and superficially serrated adenomas, similar to normal mucosa, indicating preservation of surface differentiation (Fig. 3b, f, j) [18, 19]. In contrast, tubular adenomas showed minimal to weak CK20 staining throughout the crypts (Fig. 3n). Ki-67 expression was localized to the bottom of the crypts in both normal mucosa and goblet cell-rich hyperplastic polyps (Fig. 3c, g). In superficially serrated adenomas, the Ki-67-positive cell compartment extended to the upper half of the crypts (Fig. 3k), while tubular adenomas showed expression predominantly in the upper layer (Fig. 3o). The distribution pattern of MYC-positive cells appeared similar to that of the proliferating cells in each type of lesion (Fig. 3d, h, l, p). Nuclear β -catenin expression was absent in both normal

mucosa and goblet cell-rich hyperplastic polyps (Fig. 4a, b) while appearing either focal or diffuse in superficially serrated adenomas and diffuse in tubular adenomas (Fig. 4c, d).

Next-generation sequencing identified activating *KRAS* mutations in 19 of 20 superficially serrated adenomas and a *BRAF* mutation in the one remaining (Table 1). Two lesions had truncating *APC* mutations and one lesion had a *GNAS* mutation. These results were confirmed by Sanger sequencing. *NRAS*, *CTNNB1*, and *RNF43* mutations were absent. RT-PCR identified *PTPRK-RSPO3* fusions in 11 lesions (Fig. 5a, b). Quantitative PCR revealed overexpression of *RSPO3* exons 3 and 4 in 16 superficially serrated adenomas (Fig. 5c), including all the 11 lesions containing *PTPRK*-

Fig. 4 β -Catenin expression in normal mucosa, a goblet cell-rich hyperplastic polyp, a superficially serrated adenoma, and a tubular adenoma. **a, b** Epithelial cells of normal mucosa (**a**) and a goblet cell-rich hyperplastic polyp (**b**) show exclusively membranous β -catenin expression. **c** A superficially serrated adenoma shows moderate nuclear β -catenin staining in addition to membranous staining. **d** A tubular adenoma shows diffuse strong nuclear β -catenin accumulation

RSPO3 fusions. *RSPO3* exons 1 and 2 overexpression, suggestive of overexpression of full-length *RSPO3*, was observed in five lesions. Two lesions that lacked *RSPO3* overexpression showed overexpression of *RSPO2* exons 1 and 2, and 3 and 4, indicating overexpression of full-length *RSPO2*. Collectively, 18 superficially serrated adenomas showed *RSPO* fusions and/or overexpression (Table 1). *RSPO* fusions/overexpression was mutually exclusive with *APC* mutations. Although we histologically reexamined the *APC*-mutated lesions, these were basically indistinguishable from the other superficially serrated adenomas, whereas one lesion (SuSA20) showed slightly lesser superficial serration. Overexpression of *RSPO2* and *RSPO3* was absent in all goblet cell-rich hyperplastic polyps and tubular adenomas.

Since concurrent *RSPO* fusions/overexpression and *KRAS* mutations are common genetic features of traditional serrated adenomas [8, 16], we histologically reviewed 129 traditional serrated adenomas and identified 15 (12%) that were associated with a superficially serrated adenoma component (Table 2 and Supplementary Table 1). The patients with superficially serrated adenoma-associated traditional serrated adenomas were 44–80 years of age and predominantly female in contrast to those without superficially serrated adenoma components, which occur more frequently in male patients. All of the superficially serrated adenoma-associated traditional serrated adenomas were located in the sigmoid colon or rectum. The traditional serrated adenoma and superficially serrated adenoma components were usually recognizable as polypoid and sessile areas, respectively, in both endoscopic and histological examination (Fig. 6) and were histologically well-

Table 2 Comparison of colorectal traditional serrated adenomas with and without superficially serrated adenoma components

	Superficially serrated adenoma component		<i>p</i> value
	Present (<i>n</i> = 15)	Absent (<i>n</i> = 114)	
Age (years), median (range)	66 (44–80)	68 (34–85)	0.61
Female/male	11/4	37/77	0.0035
Proximal/distal	0/15	23/91	0.071
Polyp size (mm), median (range)	13 (6–49)	7 (2–58)	0.060
Typical cytology ^a	0/2/13	0/7/107	0.28
Slit-like serration ^a	5/6/4	12/33/69	0.015
Ectopic crypt foci ^a	2/3/10	43/32/39	0.012
High-grade component	7 (47%)	14 (9%)	0.0032
Nuclear β -catenin ^b	13 (87%)	96 (84%)	1.0
<i>BRAF</i> mutation	0 (0%)	80 (70%)	1.1×10^{-7}
<i>KRAS</i> mutation	15 (100%)	32 (28%)	5.0×10^{-8}
<i>RSPO</i> fusion or overexpression	14 (93%)	33 (29%)	2.0×10^{-7}

^a <10%/10–50%/>50%

^b Nuclear β -catenin expression in $\geq 10\%$ of cells

demarcated. Superficially serrated adenoma-associated traditional serrated adenomas showed more prominent ectopic crypt foci and less slit-like serration and were more likely to have high-grade components compared with those without superficially serrated adenoma components. The superficially serrated adenomas associated with traditional

Fig. 5 Gene expression analyses of *RSPO* in superficially serrated adenomas. **a** RT-PCR amplifications of *RSPO* fusions. *PTPRK* exon 1-*RSPO3* and *PTPRK* exon 7-*RSPO3* fusions were detected in two (SuSA13 and 19) and four (SuSA12, 14, 15, and 17) superficially serrated adenomas, respectively. *EIF3E*-*RSPO2* fusions were not detected in any samples. *ACTB* served as a positive control. **b** Sanger sequencing of *RSPO* fusion junctions and *KRAS*. The *PTPRK* exon 7-

RSPO3 fusion product was sequenced using a reverse primer. **c** *RSPO* expression analysis in superficially serrated adenomas. Quantitative RT-PCR analysis of *RSPO2* exons 1 and 2, and exon 3 and 4 (left), and *RSPO3* exons 1 and 2, and exons 3 and 4 (right). Expression levels are indicated relative to normal colon mucosa. Five goblet cell-rich hyperplastic polyps (HP) and five tubular adenomas (TA) were also analyzed for comparison

serrated adenomas showed immunohistochemical features similar to solitary superficially serrated adenomas, including CK20, Ki-67, and MYC expression patterns (Supplementary Figure 1). Focal or diffuse nuclear β -catenin expression was observed in 11 of 15 lesions (73%). The extent of β -catenin expression was similar between the two components in most lesions but more extensive in the traditional serrated adenoma component of some. MLH1 expression was consistently retained in both components.

Referring to the results of our previous mutation and expression analyses [8, 16], we noted that all superficially serrated adenoma-associated traditional serrated adenomas had *KRAS* mutations and all but one lesion (93%) showed *RSPO* fusions and/or overexpression. One lesion (7%) had a *GNAS* mutation. We then analyzed the presence of these mutations and *RSPO* fusions/overexpression in each component. The results showed that all the mutation and *RSPO*

expression statuses were concordant between the two components of each lesion (Fig. 7 and Supplementary Table 1).

Discussion

Our histological and immunohistochemical analyses demonstrated that superficially serrated adenomas share several common features with low-grade tubular adenomas. Superficially serrated adenomas primarily comprise straight adenomatous glands and exhibit nuclear accumulation of β -catenin and overexpression of MYC, suggestive of WNT pathway activation [20]. Unlike tubular adenomas, however, proliferative cells localize to the middle and lower layers of the mucosa while the superficial epithelium exhibits serration and CK20 expression; these findings more

Fig. 6 A traditional serrated adenoma associated with a superficially serrated adenoma. **a** Endoscopic image showing a protruding polyp within a slightly elevated sessile lesion (arrowheads). **b** Panoramic view showing a polypoid component with lobulation and a sessile component (arrowheads). **c** Traditional serrated adenoma component exhibiting classical histological features such as villous architecture and ectopic crypt formation. **d, e** Superficially serrated adenoma component. Non-serrated adenomatous glands occupy the middle to lower layer, whereas the upper layer shows serration

closely resemble those of goblet cell-rich hyperplastic polyps. We may need to consider sessile serrated adenoma/polyps with dysplasia while formulating the differential diagnosis. However, superficially serrated adenomas lack non-dysplastic sessile serrated adenoma/polyp components and are mostly localized to the distal colon [3, 14]. Collectively, superficially serrated adenomas exhibit mixed adenomatous and serrated features and are distinct from currently recognized categories of colorectal polyps.

Mutation and gene expression analyses indicated that superficially serrated adenomas are fairly genetically homogeneous, as most harbor *KRAS* mutations together with *RSPO* fusions and/or overexpression. Most serrated lesions exhibit either *KRAS* or *BRAF* mutations that lead to mitogen-activated protein kinase (MAPK) pathway activation, although the frequencies vary. For example, *KRAS* mutations are reportedly common in goblet cell-rich hyperplastic polyps and traditional serrated adenomas but rare in microvesicular hyperplastic polyps and sessile serrated adenoma/polyps [4, 8]. The WNT pathway gene

mutation profiles are also distinct among the different polyp types. *RSPO* fusions, which activate WNT signaling by inducing the overexpression of R-spondin and down-regulation of RNF43 [21], are present in one-third of traditional serrated adenomas but absent in conventional adenomas [8, 16]. A previous study by our group also identified the overexpression of full-length *RSPO* as an alternative mechanism of WNT activation in a subset of traditional serrated adenomas [16]. *APC* mutations, which were detected in two superficially serrated adenomas, are present in the majority of conventional adenomas but infrequently detected in serrated lesions. Therefore, the concurrent presence of *KRAS* mutations and *RSPO* fusions/overexpression in most superficially serrated adenomas supports the classification of superficially serrated adenoma as a serrated lesion.

We also noted an association with a superficially serrated adenoma component in a subset of traditional serrated adenomas. Strikingly, all but one superficially serrated adenoma-associated traditional serrated adenoma exhibited

Fig. 7 *RSPO* expression in superficially serrated adenoma-associated traditional serrated adenomas. **a–c** RT-PCRs targeting the *PTPRK* exon 1-*RSPO3* (**a**), *PTPRK* exon 6-*RSPO3* (**b**), and *PTPRK* exon 7-*RSPO3* (**c**) fusions of superficially serrated adenoma and traditional serrated adenoma components of superficially serrated adenoma-associated traditional serrated adenomas. *ACTB* served as a positive control. **d** Sanger sequencing results for the *RSPO* fusion junctions and *KRAS*. The *PTPRK* exon 7-*RSPO3* fusion product was sequenced using a reverse primer. **e, f** Quantitative RT-PCR analysis of the

RSPO2 exons 1 and 2 and exons 3 and 4 (**e**) and *RSPO3* exons 3 and 4 (**f**) junctions of the superficially serrated adenoma and traditional serrated adenoma components of superficially serrated adenoma-associated traditional serrated adenomas. Expression levels are indicated relative to normal colon mucosa. Notice that *RSPO3* overexpression was detected in both components in all the lesions with *RSPO* fusions and/or overexpression. T traditional serrated adenoma, S superficially serrated adenoma, N normal mucosa

KRAS mutations and *RSPO* fusions and/or overexpression. Furthermore, the superficially serrated adenoma and traditional serrated adenoma components of these lesions consistently exhibited concordant *KRAS* mutation and *RSPO* expression statuses. Whereas previous studies demonstrated that *KRAS*-mutated traditional serrated adenomas are rarely associated with precursor polyps, superficially serrated adenomas may be an important precursor of *KRAS*-mutated traditional serrated adenomas. Simultaneously, our observations imply that the combination of a *KRAS* mutation and an *RSPO* fusion is not sufficient to induce the characteristic protuberant morphology of a traditional serrated adenoma

and that additional unidentified factors are required for the development of traditional serrated adenomas.

Traditional serrated adenomas, particularly those with *BRAF* mutations, are often associated with hyperplastic polyps or sessile serrated adenoma/polyps [13, 22–27]; however, only a few studies have recognized the association of a discrete adenomatous component with traditional serrated adenomas [22, 25, 27]. Notably, one study described the association of a “tubular adenoma-like lesion,” which may be consistent with superficially serrated adenoma, in 10 of 107 traditional serrated adenomas [25]. These tubular adenoma-like lesions reportedly comprised straight

adenomatous glands, with Ki-67-positive proliferating cells localized to the lower two-thirds of the crypts. Furthermore, a magnified endoscopic examination of these lesions revealed a hyperplastic polyp-like surface pattern. Although this study did not provide the detailed clinicopathological or molecular features of traditional serrated adenomas associated with a tubular adenoma-like lesion, it appears to suggest the common presence of superficially serrated adenoma-associated traditional serrated adenomas in different case series.

It may be tempting to regard superficially serrated adenoma as a variant of traditional serrated adenoma, based on their frequent coexistence and overlapping mutation profiles. However, superficially serrated adenomas were mostly small sessile polyps that lacked the classical morphological features of traditional serrated adenomas, including the typical cytology, ectopic crypt formation, and slit-like serration [13]. Thus, it is difficult to identify a morphological link between solitary superficially serrated adenomas and traditional serrated adenomas. Indeed, most superficially serrated adenomas examined in the present study were originally diagnosed as low-grade tubular adenomas. Additionally, when superficially serrated adenomas were observed in association with traditional serrated adenomas, these two components were sharply demarcated. Moreover, superficially serrated adenomas exhibit a rather uniform genetic profile, unlike traditional serrated adenomas, which harbor diverse sets of WNT and MAPK pathway gene mutations [8].

In conclusion, the present study has characterized the morphological and molecular features of colorectal superficially serrated adenoma. The concurrent presence of *KRAS* mutations and *RSPO* fusions/overexpression is a common molecular feature of superficially serrated adenomas and suggests that these can be classified as serrated lesions, despite their predominantly adenomatous morphology. Although superficially serrated adenomas appear as solitary polyps, they may be associated with traditional serrated adenomas. Given the frequent associations and overlapping mutation profiles of superficially serrated adenomas and traditional serrated adenomas, superficially serrated adenomas might be an important precursor to *KRAS*-mutated traditional serrated adenomas. Further studies are needed to establish superficially serrated adenoma as a distinct histological subtype of serrated lesions; however, we believe that the recognition of this entity contributes to the appropriate histological classification of colorectal polyps and a better understanding of colorectal tumorigenesis.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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