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BRIEF REPORT

Identification of a novel *PRR15L-RSPO2* fusion transcript in a sigmoid colon cancer derived from superficially serrated adenoma

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Abstract

Superficially serrated adenoma (SuSA) is a recently proposed subtype of colorectal serrated lesion. We here report a sigmoid colon cancer derived from SuSA, which exhibited aggressive clinical behavior. Endoscopically, the tumor appeared as a superficial elevated lesion with a large nodule. Histological examination of the surgically resected material showed tubular adenocarcinoma associated with SuSA. Although tumor invasion was limited to the submucosal layer, lymph node and extranodal metastases were detected. The patient subsequently developed peritoneal metastases and died 15 months after surgery. Molecular analyses identified a *KRAS* mutation and a novel *PRR15L-RSPO2* fusion, which retains the entire coding region of *RSPO2*, in both SuSA and adenocarcinoma components. The present study demonstrates the malignant potential of SuSA and expands the spectrum of *RSPO* fusions in colorectal neoplasms.

Keywords Colorectal cancer · R-spondin · Superficially serrated adenoma · *KRAS*

Introduction

Although conventional adenomas are the major precursors of colorectal cancers, recent studies suggested that up to 30% of colorectal cancers are derived from serrated lesions [1]. Serrated lesions include hyperplastic polyp (HP), sessile serrated adenoma/polyp (SSA/P), and traditional serrated adenoma (TSA) [2]. Among these, SSA/P and TSA are precursors of adenocarcinomas, whereas HP is thought to have negligible malignant potential [3]. In addition to these established histological subtypes of serrated lesions, we recently suggested superficially serrated adenoma (SuSA) as a novel subtype of serrated lesion [4]. SuSAs are mostly located in the rectosigmoid colon and endoscopically appear as sessile polyps or plaque-like lesions. They are histologically characterized by mixed adenomatous and serrated features and primarily consist of straight, adenomatous glands but exhibit serration confined to the superficial layer.

Mutations leading to mitogen-activated kinase pathway activation, either *BRAF* or *KRAS* mutations, are thought to be initial and virtually ubiquitous molecular alterations in the serrated pathway of tumorigenesis [1]. In addition, dysplastic serrated lesions frequently have alterations in WNT pathway genes [5–7]. However, in contrast to conventional adenomas, which mostly have *APC* mutations, serrated lesions preferentially harbor *RNF43* mutations and *RSPO* fusions [5–7]. Importantly, most SuSAs have *KRAS* mutations and *RSPO* fusions/overexpression, indicating that they represent a genetically homogenous group of lesions [4]. Furthermore, these genetic features support their classification as a serrated lesion.

Interestingly, SuSAs are often associated with TSAs, implying their histogenetic relationship [4]. Considering the

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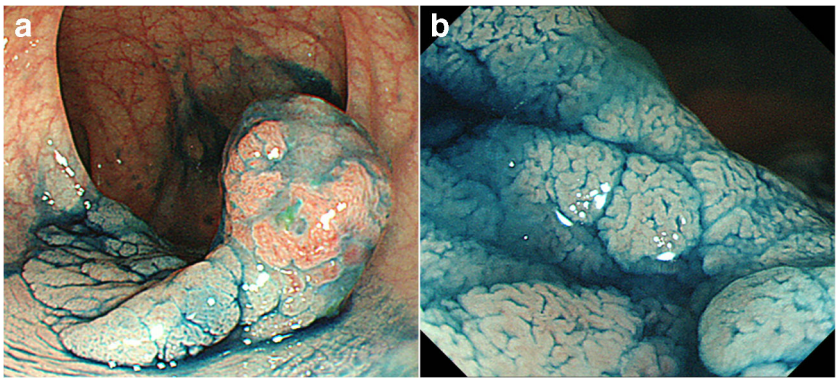

Fig. 1 Endoscopic features of the lesion. **a** Chromoendoscopy with indigo carmine dye shows a superficial elevated lesion with a nodule. There is a depressed area on the top of the nodule. **b** A magnified chromoendoscopy image with indigo carmine dye of the superficial elevated portion reveals lobulated surface and fern-like appearance

malignant potential of TSAs, it is expected that SuSA is also a precursor to colorectal adenocarcinomas. However, there have been no cases directly supporting this notion. We here present an adenocarcinoma derived from SuSA, which resulted in a fatal outcome. Furthermore, our molecular analysis identified a previously unreported *RSPO2* fusion transcript in this lesion.

Case report

A 57-year-old woman with no medical history presented with hematochezia. Total colonoscopy revealed a superficial elevated lesion with a large nodule, measuring 20 mm in size, in the sigmoid colon (Fig. 1a). The superficial elevated portion showed lobulated surface in white light images. Chromoendoscopy revealed tubular pits having areas with fern-like appearance, suggestive of serrated lesion (Fig. 1b). The nodular portion appeared reddish and had a demarcated depressed area. Based on these findings, we suspected an adenocarcinoma with submucosal invasion whereas the superficial elevated component appeared as a non-invasive precursor component.

The patient underwent laparoscopic sigmoidectomy. Histological examination of the lesion showed tubular adenocarcinoma invading the deep submucosal layer in the area recognized as a nodule in the endoscopic observation (Fig. 2a, b). The superficial elevated portion of the tumor was SuSA, which consisted of straight adenomatous glands and showed serration in the superficial layer (Fig. 2c–e). Immunohistochemistry for MIB1 showed localization of proliferative cells in the middle to bottom layers. Although SuSAs are often associated with a TSA, this lesion did not have a TSA component. While no vascular invasion was detected, a lymph node metastasis and an extranodal metastasis were detected.

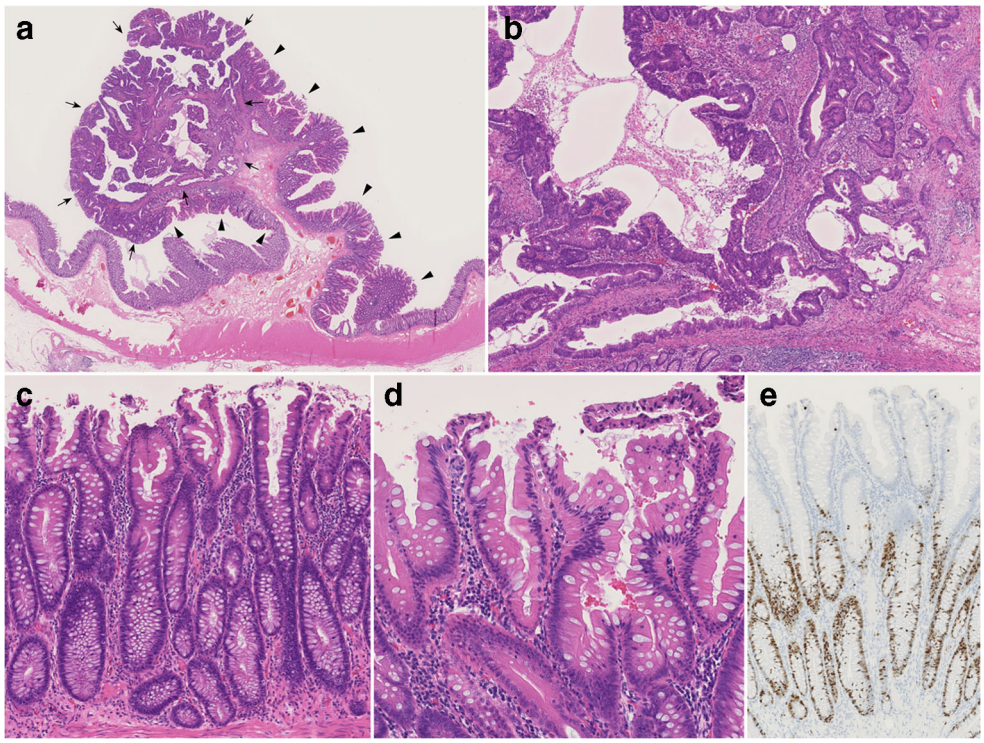

Fig. 2 Histological features of the lesion. **a** Panoramic view of the lesion. The tubular adenocarcinoma component (arrows) is located within SuSA (arrowheads). **b** Tubular adenocarcinoma forming irregular glands invades the submucosal layer. **c**, **d** The superficial elevated component is SuSA composed of adenomatous glands with superficial serration. **e** MIB1 staining of the SuSA component. MIB1-positive proliferative cells are confined to the middle to lower layers

The patient received adjuvant chemotherapy with capecitabine for 6 months, but ovarian and peritoneal metastases were detected 11 months after surgery. She was treated with fluorouracil, leucovorin, and oxaliplatin (mFOLFOX6) plus bevacizumab but died of tumor progression after 4 months.

Since most SuSAs harbor *KRAS* mutations and *RSPO* fusions/overexpression [4], we analyzed the presence of these genetic alterations in SuSA, adenocarcinoma, and the metastatic lesion. DNA and RNA samples were prepared from microdissected specimens of the respective components [7]. Genetic alterations in frequently mutated regions of *APC*, *BRAF*, *CTNNB1*, and *KRAS* were analyzed by Sanger sequencing as described previously [5]. The results showed the common presence of *KRAS* c.35G>A (G12D) mutation in all the three components whereas *APC*, *BRAF*, and *CTNNB1* mutations were absent in all components. *RSPO2* and *RSPO3* expression levels were determined using quantitative reverse transcription (RT)-PCR, as described previously [7]. Exons 3–4, but not exons 1–2, of *RSPO2* were found to be overexpressed in SuSA, carcinoma, and metastasis (Fig. 3a). *RSPO3* expression levels were marginal. Although the unbalanced overexpression of exons 3–4 of *RSPO2* implies the presence of a fusion transcript [7], we failed to detect previously reported *RSPO2* fusions by RT-PCR (data not shown). Therefore, we conducted the 5′ rapid

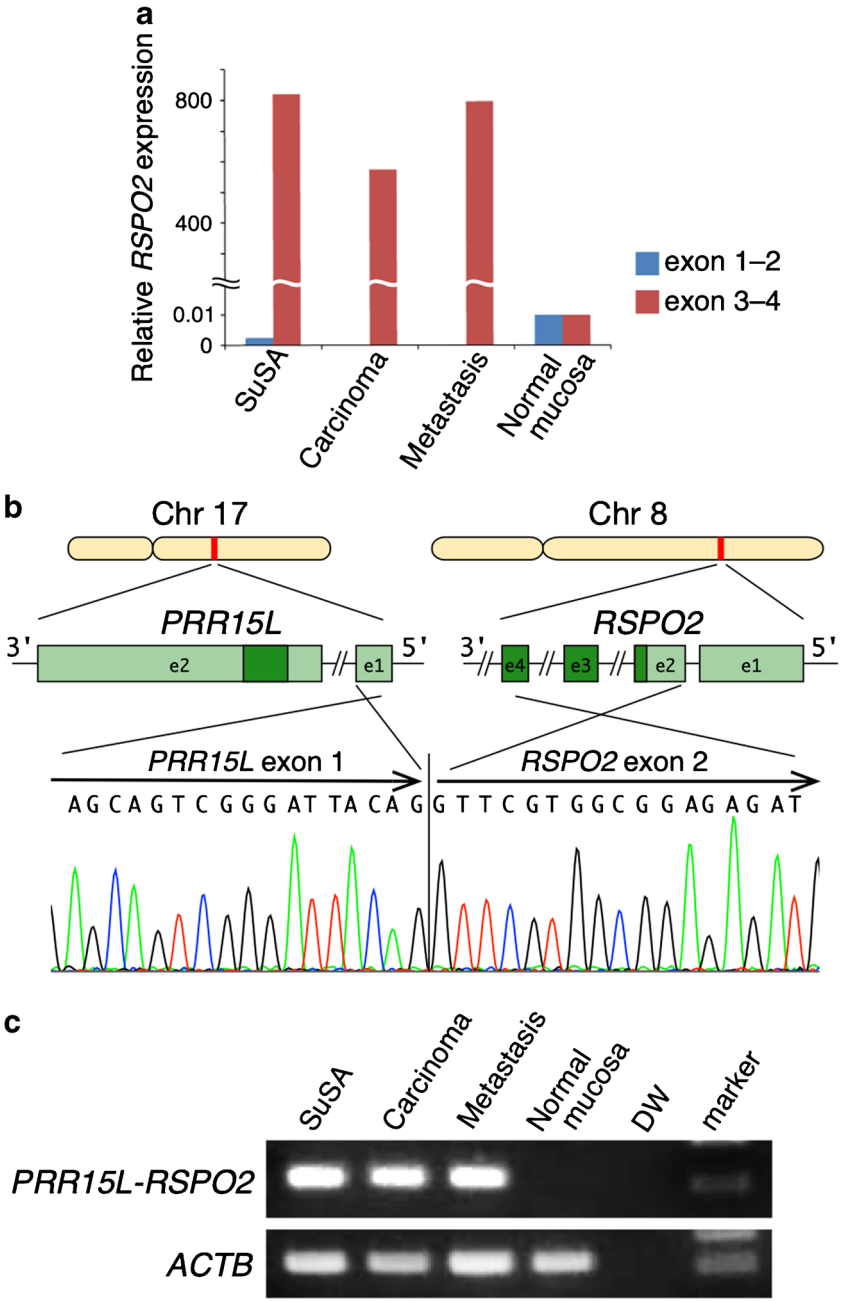

Fig. 3 Identification of a *PRR15L-RSPO2* fusion. **a** Quantitative RT-PCR analysis of *RSPO2*. Exons 3–4 of *RSPO2* are overexpressed whereas exons 1–2 expression is marginal in SuSA, carcinoma, and metastasis. **b** Schematic view of the putative *PRR15L-RSPO2* fusion structure. Exon 1 of *PRR15L*, encoding the 5′ untranslated region, is fused to exon 2 of *RSPO2*, which contains the translation initiation site. Dark green indicates protein-coding regions. Sanger sequencing of the *PRR15L-RSPO2* fusion junction is indicated below. **c** RT-PCR amplification of *PRR15L-RSPO2* fusion. *ACTB* served as a positive control

amplification of cDNA ends as described previously [7] and identified a novel *PRR15L-RSPO2* fusion (Fig. 3b). We then confirmed the expression of the *PRR15L-RSPO2* fusion using RT-PCR using a primer pair encompassing the fusion junction (5′-TTGCTTCCCGAGTCTCACCC-3′ and 5′-TTGCTTCCCGAGTCTCACCC-3′). Expression of the *PRR15L-RSPO2* fusion transcript was observed in SuSA, adenocarcinoma, and metastasis, but not in the normal mucosa (Fig. 3c). Immunohistochemistry showed the retained expression of mismatch repair proteins, MLH1, MSH2, PMS2, and MSH6.

Discussion

We recently proposed SuSA as a new subtype of colorectal polyps [4]. SuSA exhibits distinct clinicopathological features, including preferential localization in the rectosigmoid colon, mixed adenomatous and serrated morphology, and frequent association with TSAs. However, the clinical significance of SuSA has remained unclear. The present case clearly shows that SuSA is a premalignant lesion that can directly progress to adenocarcinoma without transition to TSA.

Consistent with the results of our previous study that most SuSAs have *KRAS* mutations and *RSPO* fusions/overexpression, in the present study, we identified a novel *PRR15L-RSPO2* fusion concurrent with a *KRAS* mutation. Since the *PRR15L* exon 1 encodes a 5′ untranslated region, the *PRR15L-RSPO2* fusion transcript is expected to retain the entire *RSPO2* coding region. *RSPO* fusions are recently identified genetic alterations that potentiate ligand-dependent WNT pathway signaling. In colorectal neoplasms, *RSPO* fusions are mutually exclusive with other WNT pathway gene alterations, including *APC* and *CTNNB1*, supporting their critical role in WNT pathway activation [6, 8]. *PTPRK-RSPO3* and *EIF3E-RSPO2* fusions are recurrent *RSPO* fusions; however, several other *RSPO* fusions have been described in single cases [7–10]. The identification of the *PRR15L-RSPO2* fusion expands the variations of *RSPO* fusions in colorectal neoplasms. Since *RSPO* fusions are regarded as a potential therapeutic target, our observations have potential clinical significance [11].

Previous studies have reported variable frequency of *RSPO* fusions in colorectal cancer, ranging from 0.35 to 10% [8, 12, 13]. However, considering that *RSPO* fusions are exclusive to SuSAs and TSAs among the precursor lesions, a recognizable subset of colorectal cancers are thought to be derived from SuSA or TSA. Thus far, no studies have been conducted on the clinicopathological characteristics of colorectal cancers with *RSPO* fusions. Although the outcome of the present case was fatal, the prognostic and biological relevance of *RSPO* fusions in colorectal cancer remains to be clarified.

Recently, Bettington et al. reported a series of small colorectal polyps characterized by typical TSA cytology covering the luminal surface [14]. They showed that a histologically similar component can be identified in the periphery of TSAs and suggested that these small polyps represent early forms of TSA. Furthermore, the authors suggested that SuSA is better regarded as a *KRAS*-mutated early form of TSA. However, although SuSAs may be found in association with TSAs, they can also present as large plaque-like lesions up to 20 mm in size, without transition to TSAs [4]. Additionally, the present case indicates that SuSA can directly progress to invasive carcinoma. Based on these findings, we believe that SuSA is a precursor to, but not an immature form of, TSA and its uniform histological and genetic features warrant its recognition as a distinct entity.

The present case demonstrates the malignant potential of SuSA. The identification of a novel *RSPO* fusion broadens the spectrum of *RSPO* fusions in colorectal neoplasms and supports the critical role of *RSPO* fusions in the tumorigenesis of SuSA. Although the risk of malignant progression needs to be determined, SuSA, in addition to SSA/P and TSA, may also need to be recognized as a premalignant lesion related to the serrated pathway.

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Compliance with ethical standards

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

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