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# Local recurrence after successful endoscopic submucosal dissection for rectal mucinous mucosal adenocarcinoma: A case report with a molecular analysis

ESD for mucinous mucosal adenocarcinoma in the rectum

**Abstract**

BACKGROUND

Mucinous adenocarcinoma of the colorectum is a rare histological subtype and is characterized by an abundant mucinous component. Mucinous tumors are frequently observed at an advanced stage, which indicates an aggressive subtype. However, few case reports have been published, and little information is available concerning genetic alterations in mucinous adenocarcinoma.

CASE SUMMARY

A 76-year-old man underwent en-bloc endoscopic submucosal dissection (ESD) for the management of a type 0-Is+IIa lesion. A histological examination revealed an intramucosal mucinous adenocarcinoma with signet-ring cell carcinoma and well- to moderately differentiated tubular adenocarcinoma. Three years after ESD, local recurrence was detected by an endoscopic examination, and the new 0-Is+IIa lesion had a similar phenotype to the previously resected lesion. Re-ESD was chosen for the management of the recurrent tumor, and the histological examination revealed positive tumor infiltration at the vertical margin. Additional surgical resection was performed for the curative treatment. A genetic analysis showed pathogenic alterations of *RNF43* and *TP53* in the adenoma and an additional alteration of *SMAD4* in the carcinoma.

CONCLUSION

This mucinous mucosal (Tis) adenocarcinoma case was suggested to have an aggressive phenotype and a careful and close follow-up are required.

**Key Words:** case report; rectal cancer; mucinous adenocarcinoma; endoscopic submucosal dissection; TP53; colitic cancer pathway

Murakami Y, Tanabe H, Ono Y, Sugiyama Y, Kobayashi Y, Kunogi T, Sasaki T, Takahashi K, Ando K, Ueno N, Kashima S, Yuzawa S, Moriichi K, Mizukami Y, Fujiya M, Okumura T. Local recurrence after successful endoscopic submucosal dissection for rectal mucinous mucosal (Tis) adenocarcinoma: A case report with a molecular analysis. *World J Gastrointest Oncol* 2022; In press

**Core Tip**: Colorectal mucinous adenocarcinoma characterized by an abundant mucinous component is a rare histological subtype and is frequently observed at an advanced stage. Intramucosal mucinous adenocarcinoma was dissected by endoscopic submucosal dissection and local recurrence was detected three years after the treatment. A genetic analysis showed pathogenic alterations of RNF43, TP53, and SMAD4. This case of mucinous mucosal adenocarcinoma was suggested to have an aggressive phenotype based on the treatment course and advanced genotype identified by target sequencing. Careful and close follow-up should be performed, and additional surgery should be considered when managing patients with mucinous adenocarcinoma.

## INTRODUCTION

The World Health Organization classifies colorectal cancers according to the histology [1]. The most common type of colorectal cancer is adenocarcinoma, and it is frequently heterogeneous, being composed of several histological variants; mucinous adenocarcinoma, signet-ring cell carcinoma, neuroendocrine carcinoma, squamous cell carcinoma, medullary carcinoma, and undifferentiated carcinoma. The histological subtype presumably plays a role in the tumor biology and prognosis of patients. Approximately 10% of colorectal carcinomas are mucinous adenocarcinomas, which is characterized by an abundant mucinous component comprising more than 50% of the tumor volume [2]. The clinicopathological characteristics are reported as a younger age, arising from the proximal colon, and a larger tumor size [3, 4]. Signet-ring cell carcinomas are observed in about 1% of colorectal carcinoma cases and are present as single cells with abundant cytoplasmic mucin. Mucinous adenocarcinomas with signet-ring cell

carcinomas are distinctively different from adenocarcinoma in their tumor biology and aggressive phenotype [5].

The multistep model of carcinogenesis has been proposed in colorectal cancer. Biallelic *APC* mutations are known to be an early event leading to adenomas, followed by activating mutations in *KRAS* in the advancing adenoma, and *TP53* mutations during the transition to malignancy in common colorectal cancers [6]. Gene expression-based subtyping in colorectal cancers has been widely accepted as a relevant source of disease stratification. The genetic pathway of mucinous adenocarcinoma has also been investigated, suggesting a separate genetic pathway with high mutations in *K-RAS* and *TP53* [7]. However, these mutation frequencies have differed among reports, indicating that colorectal mucinous adenocarcinomas are still heterogeneous phenotypes [3, 7, 8]. Detailed examinations are required for the genetic analysis of mucinous adenocarcinoma in the colorectum.

Although the overall survival of mucinous carcinoma patients was shown to be poorer than that of adenocarcinoma patients in some reviews, the prognosis and the treatment options for mucinous adenocarcinoma remain controversial [3, 9, 10]. Mucinous adenocarcinomas are frequently found in the advanced stages, so patients with mucinous carcinoma therefore show a poor prognosis. In contrast, only a few cases of intramucosal mucinous colorectal carcinoma have been reported thus far, so the prognosis of the patients with early mucinous adenocarcinoma treated by endoscopic resection is unclear [11, 12].

We herein report a case of early mucinous rectal adenocarcinoma was removed by endoscopic submucosal dissection (ESD) with recurrence observed after three years.

## CASE PRESENTATION

### *Chief complaints*

A 76-year-old man with a history of hematochezia underwent colonoscopy at a hospital, and an elevated tumor was observed in the lower rectum. He was referred to our department.

### *History of present illness*

The patient had a history of hematochezia, with no abdominal symptoms.

### *History of past illness*

He had a history of appendectomy due to appendicitis and prostate cancer surgery.

### *Personal and family history*

The patient had no family history of malignant tumors.

### *Physical examination*

Physical examination was unremarkable, and his abdomen was soft, nontender, and nondistended with no palpable mass.

### *Laboratory examinations*

A routine laboratory examination showed normal blood counts with a white blood cell count of 5930/μl, red blood cell count of 4920/μl, and platelet count of 24.2/μl. Tumor markers were within the normal range; carcinoembryonic antigen (CEA) was 3.2 ng/mL, and carbohydrate antigen 19-9 (CA19-9) was <2 U/mL.

### *Imaging examinations*

Colonoscopy revealed a 0-Is+IIa lesion with slight bleeding (Figure 1A), chromoendoscopy emphasized a laterally spreading flat lesion (IIa) at the base of the protrusion (Is) (Figure 1B), and magnified endoscopy with narrow-band imaging (NBI) revealed an intense irregular micro-vascular pattern in the Is lesion and regular caliber vascular pattern in the IIa lesion (Figure 1C, D). Computed tomography (CT) did not show any lymph node metastasis or distant metastasis.

## FINAL DIAGNOSIS

The tumor was diagnosed as a mucosal rectal carcinoma.

## TREATMENT

The tumor was removed by ESD. In the procedure, diluted hyaluronic acid solution was injected into the submucosa at the anal side of the tumor. Repeated local injections were needed while the submucosa was dissected just above the muscular layer toward the proximal side of the tumor. The en-bloc resected tissue with the lateral normal epithelia measured 30 mm (Figure 2A).

A microscopic examination revealed an intramucosal mucinous adenocarcinoma with signet-ring cell carcinoma and well- to moderately differentiated adenocarcinoma, with a negative margin in both the vertical and horizontal aspects (Figure 2B). A marked mucous lake was found in the center of the tumor, with signet-ring cell spreading in mucin (Figure 2C). A basal spreading tumor 22 mm in diameter was composed of adenoma with mild to moderate dysplastic cells. The pathological diagnosis was Tis, ly0, v0, Stage 0.

## OUTCOME AND FOLLOW-UP

Colonoscopy and CT were conducted every six months within one year and the annual surveillance was conducted thereafter. An ESD scar was observed by the colonoscopy for two years (Supplementary Figure 1), and a 0-Is+IIa lesion was revealed at the ESD scar three years later (Figure 3A). Chromoendoscopy emphasized a laterally spreading flat lesion (IIa) at the base of the protrusion (Is) (Figure 3B, C), and magnified endoscopy with crystal violet staining revealed an intense irregular micro-vascular pattern in the Is lesion and an invisible vascular pattern in the IIa lesion (Figure 3D, E). Three years after ESD, CT showed no sign of lymph node metastasis. Re-ESD was chosen for the recurred lesion, and a microscopic examination of the ESD specimens revealed a submucosal invasive mucinous adenocarcinoma with well- to moderately differentiated adenocarcinoma, and positive vertical margin (Figure 3F). The mucinous adenocarcinoma had infiltrated the submucosal lesion under the IIa lesion.

Based on these results, additional surgical resection was recommended, and Mile's operation was performed (Supplementary Figure 2). The mucinous adenocarcinoma had infiltrated the muscle layer with lymphatic invasion, but there were no lymph node metastases. The eventual histological diagnosis was T2, N0, M0, Stage I. The postoperative course was normal, and the patient has not had any recurrence for one year.

*Next generation sequencing*

We analyzed the genetic alterations of the lesions in a previous report [13]. In brief, genomic DNA was isolated from formalin-fixed paraffin embedded (FFPE) specimens using a GeneRead DNA FFPE kit (Qiagen, Hikden, Germany). The mutation profile was determined by target amplicon sequencing using a GeneStudio S5 system (Thermo Fisher Scientific, Carlsbad, CA). A colorectal cancer associated gene panel including *ARID1A, MUTYH, NRAS, CTNNB1, PIK3CA, FBXW7, APC, BRAF,* and *KRAS*[14, 15] and a pancreatic ductal adenocarcinoma (PDA)-medium panel including *KRAS, TP53, SMAD4, CDKN2A, GNAS, BRAF, PIK3CA, RNF43, STK11,* and *HRAS* were designed using the Ampliseq Designer website (https://www.ampliseq.com). Variants were identified using the Variant Caller plugin (version 5.0.4.0). Genetic mutations of colorectal cancers reported in the Catalogue Of Somatic Mutations In Cancer (COSMIC) database (**https://cancer.sanger.ac.uk/cosmic/**) was identified.

A total of four pathogenic mutations were observed with the PDA-medium panel in the genetic analysis (Table 1). *TP53*p.R273H and *RNF43* p.W159Afs*8 were found in the adenoma and *SMAD4* p.D351G, and *PIK3CA* p.T74N were additionally found in the adenocarcinoma (Figure 4). No mutations were detected in any other genes, including *KRAS*, *GRAS*, *BRAF*, *STK11*, and *HRAS*. The *TP53* mutation in the tumor cells was confirmed with p53 immunohistochemistry. Mutations were not found in the colorectal cancer-associated gene panel, indicating no somatic mutations in *APC*. The protocol of the genetic analysis was approved by Asahikawa Medical University

Research Ethics Committee, and a written informed consent was obtained from the patient.

## DISCUSSION

A mucosal mucinous adenocarcinoma with signet-ring cell carcinoma in the rectum was en-bloc resected by ESD, but recurrence was observed at the scar three years later. A heterogenous histology wherein mucinous and tubular adenocarcinoma were developed from adenoma was observed, and a genetic analysis revealed that a single mutated pathway was associated with these tumors. Thus, the mucinous adenocarcinoma was deemed to have originated from a laterally spreading adenoma with initial mutations in the *RNF43* and *TP53* cancer suppresser genes. The aggressive genotype corresponded to the clinical aggressive phenotype.

Mucinous colorectal adenocarcinomas account for approximately 10% of colorectal carcinomas, while signet-ring cell carcinoma is rare (about 1%) in the incidence [16]. Signet-ring cell carcinoma has not been well evaluated due to its low incidence and the fact of the most cases are found at an advanced stage [17]. Interestingly, since some signet-ring cell carcinomas were found in combination with adenoma, the origin of the carcinoma was speculated to be originated from the adenoma [18, 19]. Our case supports this theory, as the elevated lesion (Is) with an irregular vascular surface pattern developed from the IIa lesion with a regular vascular pattern. The endoscopic findings indicated the malignant histology of the tumors, with Is and IIa lesions constructed from the carcinoma and adenoma, respectively. A histological feature of this tumor was its heterogenicity, comprising mucinous, tubular adenocarcinoma, and adenoma components.

The genetic approach is expected to clarify the tumor progression pathway. Adenoma-carcinoma sequences are proposed in common adenocarcinoma, in which sequential mutations of *APC*, *KRAS*, *TP53*, and *SMAD4* take place. However, a recent genetic examination of colorectal adenocarcinoma with a mucinous component indicated fewer *TP53* mutations than in classical adenocarcinoma [20]. In contrast, our

genetic analyses of this mucinous adenocarcinomas with its adenoma component showed *TP53* p.R273H (c.818G>A, COSM10660) which is a frequent variant in common colorectal cancers. Mutations of *APC* and *KRAS*, which are frequently observed in colorectal cancers, were not observed in either adenocarcinoma or adenoma. *RNF43*W159Afs*8 (c.474_476delCTGinsA, not in COSMIC database) was found in all areas of the tumor, and *SMAD4* D351G (c.1052A>G, COSM373800) was found in areas of the carcinoma. The mutation sequence in progression from adenoma to adenocarcinoma was initiated from *RNF43* and *TP53,* followed by *SMAD4.* The process is similar to that observed in colitis-associated cancers, wherein somatic mutations of *RNF43* are the driver genetic alteration linking chronic inflammation and cancer development in colitic cancers [21]. A lack of *APC* mutations in mucinous cancer supports similarity to the colitic cancer pathway. Coincidentally, one of the characteristics of colitis-associated cancer is a high proportion of mucinous or signet-ring cell carcinomas. The histological nature was corresponding to the genetic characteristics.

The mucinous colorectal adenocarcinoma-mucosal type, which was endoscopically resected in this case, was first reported in 2010 [22]. Until now, no other mucinous adenocarcinoma treated endoscopically has been reported in the English literature. Our tumor was extremely rare, since it was a limited intramucosal lesion and removed endoscopically. The presence of mucosal carcinoma with adenoma and the genetic analyses of the lesions indicated the initiation of mucinous carcinogenesis. In this case presentation, the genetic mutational analysis confirmed that the mucinous adenocarcinomas were derived from adenoma, and the involvement of the RNF43 pathway in mucinous carcinogenesis was proposed. In addition, the mucinous adenocarcinoma in this initial lesion was limited to the mucosal layer without a negative margin in the vertical or horizontal aspects at the primary treatment. However, the mucinous adenocarcinoma recurred three years later. These findings suggested that mucinous adenocarcinoma possessed an aggressive genotype, so even mucosal tumors should receive close follow-up. The Japan Gastroenterological Endoscopy Society

guidelines for ESD/EMR note that Tis carcinomas generally do not metastasize to lymph node or other organs [23]. Additional surgical resection should be considered for T1 carcinoma with malignant indictors, including poorly differentiated phenotypes (e.g., mucinous adenocarcinoma). The European Society of Gastrointestinal Endoscopy Guidelines recommend additional surgical intervention "in the cases with massive submucosal invasion, undifferentiated adenocarcinoma, positive or nonevaluable vertical margins, and/or lymphovascular infiltration by cancer cells" [24]. Mucosal (Tis) carcinoma with mucinous component is included in these criteria. The treatment strategy for mucosal (Tis) mucinous adenocarcinoma remains to be addressed.

One limitation associated with the present study warrants mention. The patient ultimately underwent Mile's operation after undergoing ESD twice through the clinical course. Endoscopic ultrasound (EUS) is useful for evaluating the depth of submucosal invasive lesions in the rectal tumors before endoscopic resection [25]. The second ESD procedure might have been avoided if a more accurate pretreatment diagnosis concerning the depth of the local recurrence had been available.

## CONCLUSION

This case of mucinous mucosal adenocarcinoma case was suggested to have an aggressive phenotype based on the treatment course and the advanced genotype detected by target sequencing. Careful and close follow-up should be performed when managing patients with mucinous adenocarcinoma.

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