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Invasive micropapillary carcinoma of the colon: an immunohistochemical study

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Abstract Invasive micropapillary carcinoma has recently been reported in various anatomic sites. In this article, we report a case of micropapillary carcinoma of the sigmoid colon. A 70-year-old Japanese woman presented with bloody stool for 2 months. Detailed examination disclosed ulcerative and localized tumor in the sigmoid colon. Histological examination of the colon tumor showed a combination of conventional adenocarcinoma (60%) and micropapillary carcinoma (40%). Immunohistochemically, micropapillary carcinoma cells were positive for cytokeratin (CK) 20, carcinoembryonic antigen, and CA125, but negative for CK7, thyroid transcription factor-1, surfactant apoprotein A, estrogen receptor, and progesterone receptor. Additionally, the immunohistochemistry of epithelial membrane antigen revealed reverse polarity of neoplastic cells. Results of conventional adenocarcinoma were basically identical to those of micropapillary carcinoma. In the stroma of both conventional adenocarcinoma and micropapillary carcinoma, many myofibroblasts were present and CD34-positive stromal cells were absent. Finally, we report the fourth case of micropapillary carcinoma arising in the colon. Immunohistochemical results of CK7(−)/CK20(+) strongly suggest the colon as a primary site of micropapillary carcinoma. Additionally, micropapillary carcinoma of the colon may cause a similar stromal reaction to conventional adenocarcinoma of the colon.

Key words Micropapillary carcinoma · Colon · CK20 · CA125

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Introduction

Invasive micropapillary carcinoma has been recently described in various anatomic sites including breast, urinary bladder, lung, ovary, salivary gland, ampullo-pancreatobiliary region, renal pelvis, and ureter.[1–18] Additionally, only three cases with invasive micropapillary carcinoma of the colon have been very recently reported.[19] In this article, we present the fourth case of colonic adenocarcinoma with micropapillary morphology arising in the sigmoid colon and discuss the usefulness of immunohistochemical study in surgical materials.

Case report

A 70-year-old Japanese woman presented with bloody stool for 2 months and consulted a general physician. Endoscopic examination of the colon disclosed ulcerative and localized tumor. Subsequently, she was transferred to the Department of Surgery, Kochi Red Cross Hospital, and underwent sigmoidectomy. Her clinical course was uneventful 1 month after the operation.

The surgically resected tumor specimen was fixed in 10% formalin and embedded in paraffin. Sections in 3 μm thick were stained with hematoxylin and eosin. Additionally, immunohistochemical stain was performed using a Histofine Simple stain-PO (multi) kit (Nichirei, Tokyo, Japan). Antibodies employed in the present study are summarized in Table 1. We considered alpha-smooth muscle actin-positive and h-caldesmon-negative stromal cells, and CD34-positive and CD31-negative stromal cells, as myofibroblasts and CD34-positive stromal cells (fibroblasts), respectively. Small sections retrieved from formaldehyde-fixed tumor tissue were fixed with 2.5% glutaraldehyde and postfixed with 0.8% osmium tetraoxide in phosphate buffer for 1 h at room temperature. After dehydration in graded ethanol, they were embedded in Epon 812. The ultrathin sections were cut with a Reichert microtome, stained with uranyl

Table 1. Antibodies employed in the present study

Antigen	Clone	Dilution	Source
Carcinoembryonic antigen	II-7	1:100	DAKO, Glostrup, Denmark
Epithelial membrane antigen	E29	Prediluted	DAKO, Glostrup, Denmark
Cytokeratin 7	OV-TL 12/30	1:100	DAKO, Glostrup, Denmark
Cytokeratin 20	Ks 20.8	1:50	DAKO, Glostrup, Denmark
Thyroid transcription factor-1	8G7G3/1	1:100	Lab Vision, CA, USA
Surfactant apoprotein A	PE10	1:50	DAKO, Kyoto, Japan
Estrogen receptor	1D5	Prediluted	DAKO, CA, USA
Progesterone receptor	PgR 636	Prediluted	DAKO, CA, USA
CA 125	M11	1:50	DAKO, Glostrup, Denmark
Alpha-smooth muscle actin	1A4	1:100	DAKO, Glostrup, Denmark
Caldesmon	h-CD	1:100	DAKO, Glostrup, Denmark
CD34	My10	1:20	Becton Dickinson, CA, USA
CD31	JC/70A	1:20	DAKO, Glostrup, Denmark

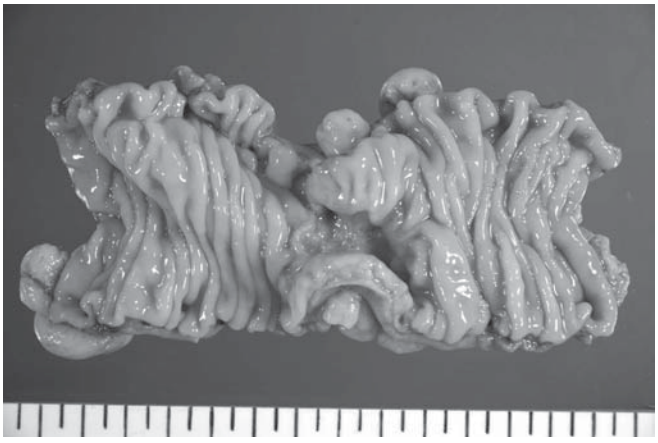

Fig. 1. Macroscopic findings of the colon tumor. An ulcerative and localized tumor is observed in the sigmoid colon

acetate and lead citrate, and examined with an electron microscope (JEM-100S; JEOL, Tokyo, Japan).

Pathological findings

Macroscopically, the ulcerative and localized tumor, which measured 3.5 × 2.7 cm, was observed in the sigmoid colon (Fig. 1). Microscopically, proliferation of atypical glandular cells was observed and the cribriform proliferating pattern was also identified. Additionally, tumor consisting of 10–20 neoplastic cells without fibrovascular core resulting in the micropapillary configuration was observed. Spaces reminiscent of vascular lumens were seen between micropapillary carcinoma cells and the stroma (Fig. 2A,B). There was a natural transition between conventional tubular carcinoma (60% of total tumor area) and micropapillary carcinoma (40% of the total tumor area). In depth, the tumor invaded into the subserosal layer, and the histological type at the deepest area showed micropapillary carcinoma. Marked infiltration into lymphatic vessels and mild infiltration into veins of neoplastic cells was observed. The metastasis of one regional lymph node was present.

Immunohistochemical results are summarized in Table 2. Both conventional adenocarcinoma cells and micropapillary carcinoma cells were positive for carcinoembryonic antigen (CEA) and cytokeratin (CK) 20 (Fig. 3A). However, both components were negative for CK 7, thyroid transcription factor (TTF)-1, surfactant apoprotein A (SPA), estrogen receptor (ER), and progesterone receptor (PgR).

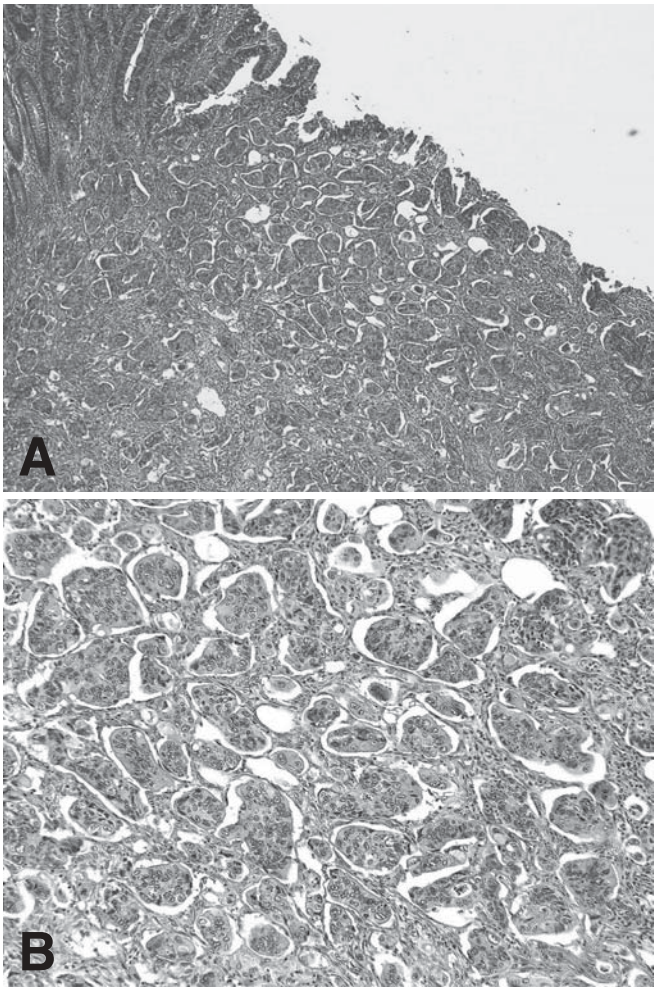

Fig. 2. Microscopic findings of the colon tumor. A tumor consisting of 10–20 neoplastic cells without fibrovascular core proliferates in the stroma. Tumor clusters are surrounded by clear empty spaces. **A** Low magnification. The component of conventional tubular adenocarcinoma is observed at the *left upper side*. **B** High magnification

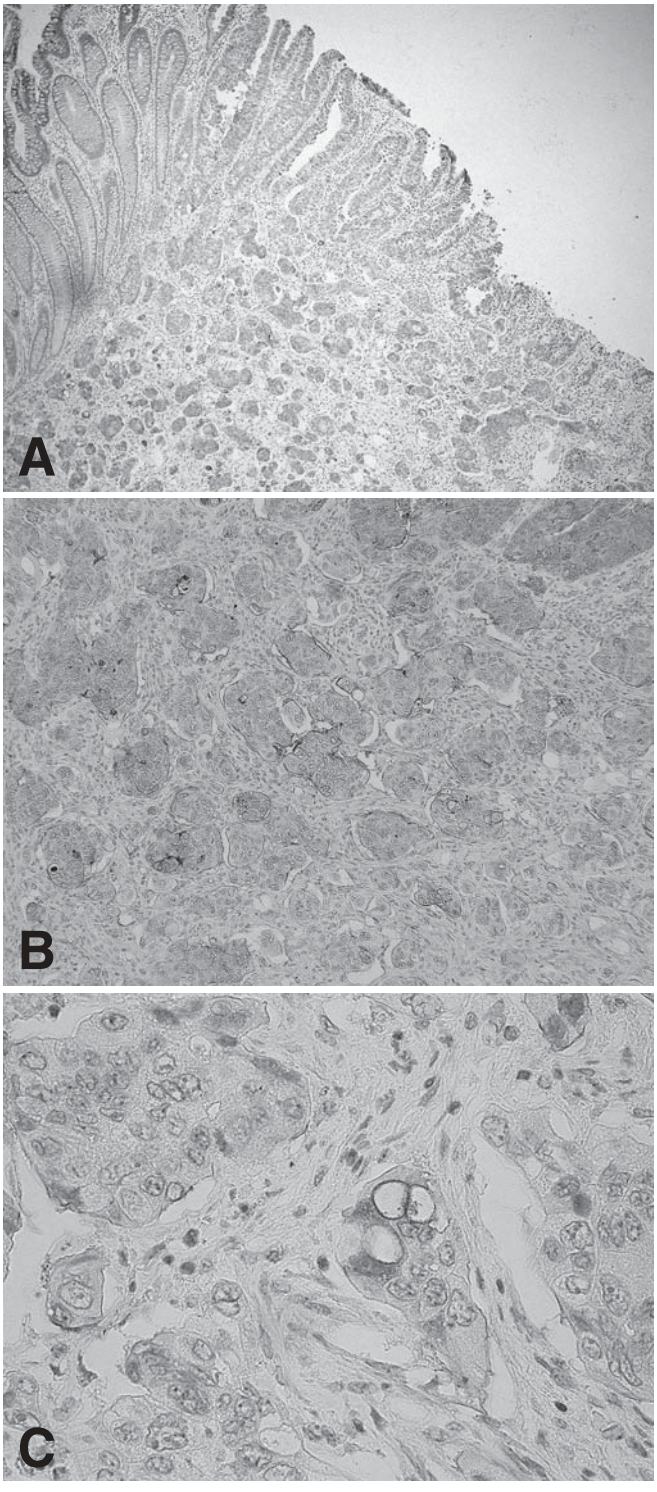

Fig. 3. Immunohistochemical results of micropapillary carcinoma of the colon. **A** Cytokeratin 20. Neoplastic cells of micropapillary carcinoma as well as conventional adenocarcinoma were positive for cytokeratin 20. **B** Epithelial membrane antigen (EMA). Reverse polarity, namely, an "inside-out" pattern, is confirmed. **C** CA125. Micropapillary neoplastic cells are focally positive for CA125

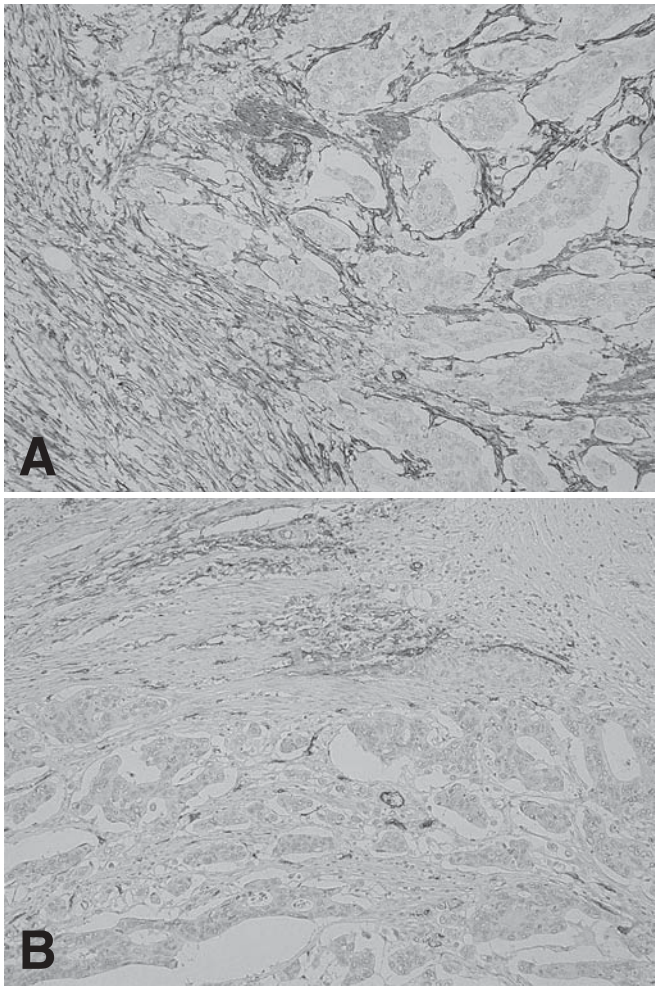

Fig. 4. Immunohistochemical results of the stroma of micropapillary carcinoma. **A** Alpha-smooth muscle actin. Many myofibroblasts are seen. **B** CD34. No CD34-positive stromal cells are observed in the stroma. In contrast, the surrounding intact stroma (*top*) contains CD34-positive stromal cells

Immunohistochemistry of epithelial membrane antigen (EMA) disclosed reverse polarity of the micropapillary carcinoma component, namely, the "inside-out" pattern (Fig. 3B). Additionally, micropapillary carcinoma cells were focally reactive for CA125 (Fig. 3C). In the stroma of both conventional adenocarcinoma and micropapillary carcinoma, many myofibroblasts were observed (Fig. 4A). In contrast, CD34-positive stromal cells were completely absent in the stroma of both carcinoma (Fig. 4B).

Ultrastructurally, vacant spaces were observed between neoplastic cells and stromal cells (Fig. 5A), and neoplastic cells contained rough abundant endoplasmic reticulum and some mitochondria in the cytoplasm (Fig. 5B).

Discussion

Recently, micropapillary carcinoma has been reported in various anatomic sites.[1–19] Except for the ovary, micropapillary carcinoma arising in many organs seems to pursue an

Table 2. Immunohistochemical results of the present tumor and comparison with micropapillary carcinoma in other anatomic sites

Antigen	Adenocarcinoma	Micropapillary carcinoma	Breast	Urinary bladder	Lung	O vary	Salivary duct
CEA	d, +	d, +					
EMA	f, +	f, +					
CK 7	–	–	+	+	+	+	+
CK 20	d, +	d, +	–	+	–	–	–
TTF-1	–	–	–	–	+	–	?
SPA	–	–	–	–	+	–	?
ER	–	–	–~+	–	–	–~+	–
PgR	–	–	–~+	–	–	–~+	–
CA125	–	f, +	–	+	?	+	?

f, focal; d, diffuse, –, negative; +, positive
Source: Nassar H. Adv Anat Pathol 2004;11:297–303[10]

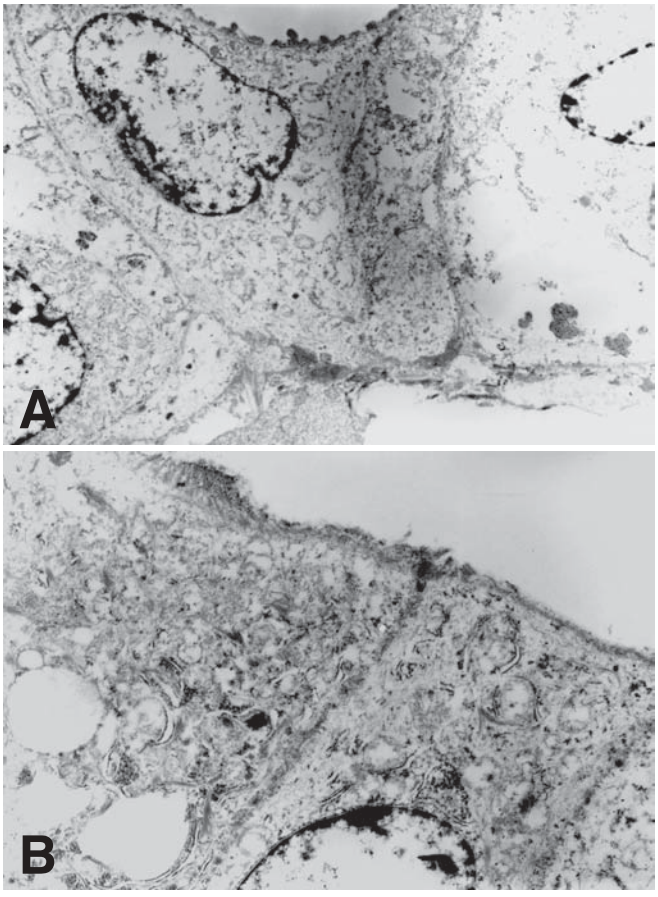

Fig. 5. Ultrastructural findings of micropapillary carcinoma. **A** Vacant space (*bottom*) was observed around the neoplastic cells. **B** Neoplastic cells of micropapillary carcinoma contain abundant rough endoplasmic reticulum and some mitochondria

aggressive clinical course because of frequent invasion into lymphatic spaces, metastasis into lymph nodes, or systemic metastasis. Micropapillary carcinoma of the colon macroscopically seems to be indistinguishable from conventional adenocarcinoma. Microscopically, micropapillary carcinoma in the present case was associated with conventional adenocarcinoma. According to the previous report, the tumor was nearly completely composed of micropapillary carcinoma in one of three cases, and the tumor was associated with conventional adenocarcinoma and concurrent mucinous carcinoma in two remaining cases.[19]

Immunohistochemical study seems to be supportive in deciding the primary site of micropapillary carcinoma of unknown origin;[10] namely, results of TTF-1(+)/SPA(+)/CK7(+)/CK20(–) suggest a pulmonary origin.[7,10] The combination of ER(+ or –)/PgR(+ or –)/CK7(+)/CK20(–) suggests that the tumor may arise from the breast or ovary.[2,10] The combination of CK7(+)/CK20(+)/CA125(+) suggests that the tumor may originate from the urinary bladder.[5,10] In the present case, neoplastic cells showed the CK7(–)/CK20(+) pattern. These results seem to reflect the immunohistochemical phenotype of conventional adenocarcinoma of the colon.[19] Interestingly, neoplastic cells were also positive for CA125. Therefore, pathologists should be careful about the evaluation of immunohistochemical results because micropapillary carcinoma arising in ovary and urinary bladder is also positive for CA125.[5,10] However, we consider that the immunohistochemical results of CK7(–)/CK20(+) exclusively suggests the colon origin as a primary site of micropapillary carcinoma. On the other hand, we have previously elucidated the increased number of myofibroblasts and decreased number of CD34-positive stromal cells in the stroma of colorectal adenocarcinoma and carcinoid tumor, and breast cancers.[2,3,20] In the present study, we have found that micropapillary carcinoma of the colon may cause the similar stromal reaction to conventional adenocarcinoma. This result suggests the invasive nature of micropapillary carcinoma of the colon.

Additionally, we ultrastructurally studied micropapillary carcinoma of the colon for the first time. In the present study, we confirmed the present of vacant spaces between neoplastic cells and stroma as micropapillary carcinoma of the breast.

Finally, we reported here a case of micropapillary carcinoma arising in the sigmoid colon. Immunohistochemical panel including CK7, CK20, TTF-1, SPA, ER, PgR, and CA125 may be helpful in deciding the primary sites of micropapillary carcinoma of unknown origin. Pathologists should recognize that invasive micropapillary carcinoma of the colon as well as ovary and urinary bladder may show a positive reaction for CA125. Our preliminary report suggests that micropapillary carcinoma of the colon may possess an invasive nature because of the increased number of myofibroblasts in the stroma.

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