

Original Article

## Clinicopathological Study of Mucous Pooling Referred to as Mucinous Component (MUC) in Colorectal Submucosal Invasive Carcinomas

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We investigated the significance of a pool of mucin, individually termed mucinous component (MUC), at the leading invasion edge of colorectal submucosal invasive carcinoma (SIC). In particular, we studied the correlation between pathological adverse prognostic factors and stainability of the mucosubstances and tumor-associated glycoconjugates in MUC.

We demonstrated that MUCs were present in 21% of SICs, and 20% of SICs with MUC were involved in metastasis or recurrence, while only 6% of SICs without MUC were positive for it ( $p=0.005$ ).

SICs with MUC showed strong staining of carcinoembryonic antigen and Ulex europaeus I in the MUCs as well as in the cancer cells with high-grade atypia. Moreover, the mucins in MUCs predominantly stained for sialomucin.

This study was the first to investigate the importance of mucous nodules in colorectal SIC. We concluded that MUC in SIC would be an important adverse prognostic factor, and that we should at least consider employing a similar treatment strategy for it to advanced colorectal carcinoma.

**Key words:** submucosal invasive carcinoma, colorectal carcinoma, mucous pooling

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### Introduction

Colorectal mucinous carcinoma is difficult to cure by resection, and therefore has a poorer prognosis than nonmucinous colorectal carcinoma<sup>1,2,3,4,5,6,7,8</sup>. Given that most of the lesions histologically defined as mucinous carcinomas are in an advanced stage, there have been few reports describing mucinous carcinomas at an early stage<sup>9</sup>. Recently, the presence of a mucous nodule has been recognized as an adverse prognostic factor for submucosal invasive carcinoma (SIC). We have investigated the presence of a pool of mucin in SIC, and have designated it as a mucinous component (MUC), supposing that MUC may be a significant adverse prognostic factor and that SIC with MUC might represent an initial stage of advanced mucinous carcinoma<sup>10,11</sup>.

In this paper, we report the results of our investigation undertaken to correlate the presence of MUC with the other adverse prognostic factors of SIC. In this text, we will also discuss the significance of MUC in treatment of SIC.

### Patients and methods

We have studied 222 lesions of SIC with sufficient data for histopathological and statistical examination. The lesions were treated as follows: 148 lesions were surgically resected and 74 were endoscopically resected, including 41 patients who had both endoscopic and subsequent surgical resections.

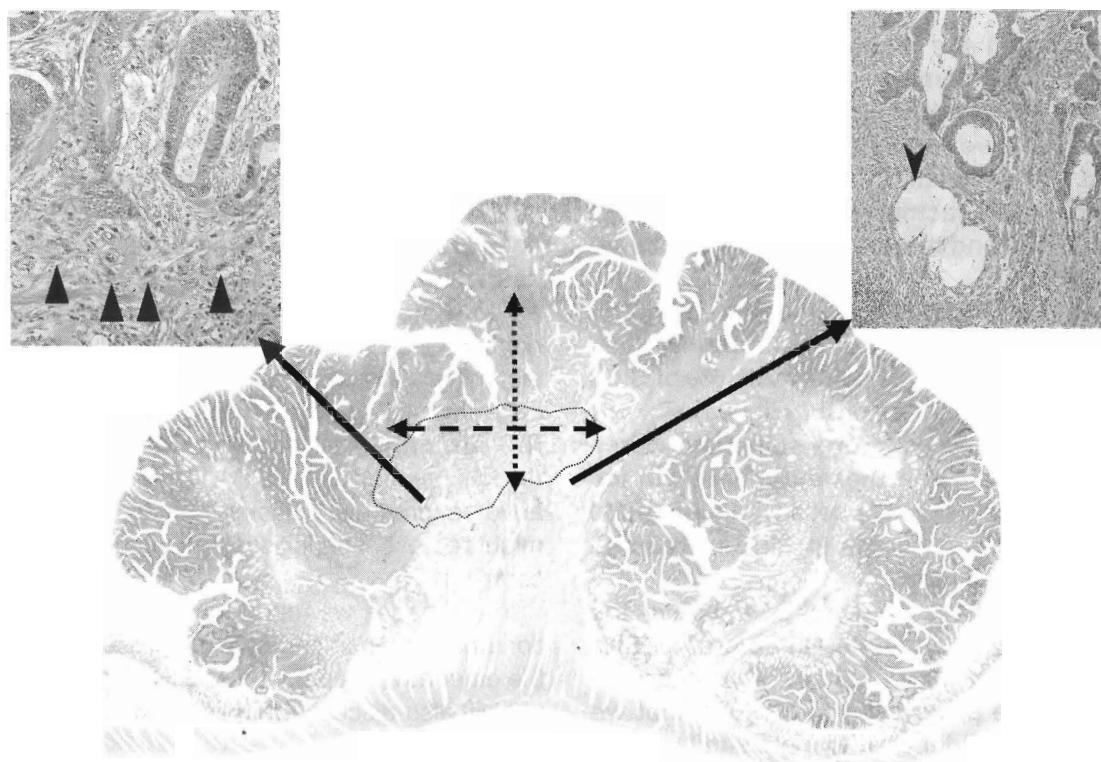
Macroscopically, early colorectal cancers were

classified according to the classification of early gastric cancers of the Japan Society for Gastroenterological Endoscopy as follows: I, protruded type; IIa, superficial elevated type; IIb, superficial flat type; IIc, superficial depressed type. Type I, the protruding type, is further divided into Ip (pedunculated), Isp (semipedunculated) and Is (sessile) subtypes. As for the superficial type, IIa aggregated (laterally spreading type) and IIa+IIc (slightly elevated type with central ulceration) are added.






For histological grade, we classified well-differentiated adenocarcinomas into two sub-types. A lesion composed mostly of well-differentiated adenocarcinoma was described as a pure type (well-1) and one combined with moderately differentiated adenocarcinoma features was described as mixed type (well-2).

Moreover, we defined poorly differentiated adenocarcinoma that was invading the interstitium at the leading edge of the lesion as single cell infiltration (SCI) and a pool of mucin ahead of the submucosal invasion of cancer as MUC (Fig.1). We used these histopathological findings as indicators of tumor invasiveness.

The extent of submucosal invasion was evaluated using the parameters of submucosal (sm) depth and width as determined by microscopic observations of the specimen with an optical micrometer. The tumors were fixed in 17% neutral buffered formalin, and then sections were cut at 2 mm to 3 mm thickness. We calculated sm depth by measuring the vertical distance of cancer invasion from the upper border of the mucosal muscular layer to the deepest portion within the submucosa. On the other hand, sm width was calculated



**Fig. 1.** Adverse prognostic factors of colorectal submucosal invasive carcinoma.

-  moderately or poorly differentiated adenocarcinoma
-  Vertical distance of submucosal invasion referred to as sm depth
-  Transverse distance of submucosal invasion termed as sm width
-  Poorly differentiated adenocarcinoma ahead of the invasion referred to as single cell infiltration (SCI)
-  A pool of mucin in the submucosal layer termed as mucinous component (MUC)

by measuring the transverse distance below the upper border of the mucosal muscular layer. As previously reported<sup>10,11</sup>, we classified the extent of invasion into three grades. Invasion less than 500  $\mu\text{m}$  in depth and 2 mm in width was classified as 'Extent I', while 'Extent II' indicated sm depth between 500  $\mu\text{m}$  and 1 mm or width between 2 mm and 4 mm. 'Extent III' designated submucosal invasion greater than 1 mm in sm depth or 4 mm in sm width (Fig. 2).

We investigated the stainability of mucin, especially that in MUC, using alcian blue (pH 2.5)-periodic acid-Schiff (AB-PAS) staining and high iron diamine-alcian blue (pH 2.5) (HID-AB) staining.

Moreover, we investigated stainability of MUC and other cancer tissues by performing carcinoembryonic antigen (CEA), carbohydrate antigen 19-9 (CA19-9) and Ulex europaeus I (UEA-I) staining using the ABC method. We attempted to make a immunohistochemical grading based on a localization pattern of these substances according to Hamada's method<sup>12</sup>.

Grade I: Apical type. Substances are restricted predominantly to the apical border of the malignant glands.

Grade II: Cytoplasmic type. Substances are demonstrated not only apical surfaces but also on the basolateral surfaces and in the cytoplasm as fine granules.

Grade III: Stromal type. Substances are found over the entire surfaces and cytoplasm on the malignant cells, and is diffusely distributed in the surrounding stroma adjacent to the basal membrane of the malignant glands.

Statistically, we expressed values as means  $\pm$  SE, and determined that below 5% was significantly different using the Student's t test and chi square test.

## Results

### *Adverse prognostic factors for metastasis or recurrence of SIC*

We compared background and prognostic factors between SICs with metastasis or recurrence (metastatic group) and SICs without metastasis or recurrence (non-metastatic group). Our study showed no differences between the two groups with regard to age at onset or gender. As for the location and diameter of the tumor, although there were no significant differences of other prognostic factors between the rectum and colon SICs, SICs in the rectum and tumors of greater diameter were found more frequently in the metastatic group than in the non-metastatic group ( $p = 0.003$ ). In terms of gross type, all metastatic group SICs were classified as types Is, Isp or IIa + IIc. The metastatic or recurrence rates of each histopathological type were as follows: 4% in well-1 lesions, 9% in well-2 lesions and 17% in moderately differentiated adenocarcinomas ( $p = 0.008$ ). Moreover, SICs positive for lymph vessel invasion ( $p < 0.0001$ ), venous vessel invasion ( $p = 0.01$ ), SCI ( $p < 0.0001$ ) and MUCs ( $p = 0.005$ ) were more apt to have the metastases or recurrences than SICs negative for those adverse prognostic factors (Table 1).

### *Stainability of mucous in SIC*

Most of the mucin in goblet cells of the background mucosa was acid mucin, which became blue upon AB (pH 2.5)-PAS staining. Sialomucin became blue with HID-AB (pH 2.5) staining and sulfomucin became partially black in the deep crypts.

As for cancer glands, brush borders and goblet cells became blue upon with AB - PAS staining with acid mucin. In the lumens of cancer glands, neutral and acid mucins were mixed. As for acid mucins, sialomucin

sm depth (mm)	sm width (mm)		
	$\leq 2$	2~4	$4 \leq$
$\leq 500$	Extent-I		
500~1000		Extent-II	
$1000 \leq$			Extent-III

Fig. 2. Classification of the extent of invasion into three grades using sm depth and sm width.

**Table 1.** Correlation between metastases or recurrences and adverse prognostic factors in submucosal invasive carcinoma

Prognostic Factors	Metastasis or Recurrence		Total No.	p-value
	Present (%)	Absent (%)		
<b>Location</b>				
Colon	6(5)	126(95)	132	0.003
Rectum	14(16)	71(84)	85	
<b>Gross type</b>				
Ip	0(0)	45(100)	45	NS
Isp	1(11)	8(89)	9	
Is	16(15)	90(85)	106	
Ila	0(0)	14(100)	14	
Ila-aggretd	0(0)	10(100)	10	
Ila+Ilc	3(12.5)	21(87.5)	24	
Ilc	0(0)	3(100)	3	
<b>Histological grade</b>				
Well-1	2(4)	45(96)	47	NS
Well-2	12(9)	128(91)	140	
Moderately differentiated	5(17)	24(83)	29	
Poorly differentiated	0(0)	2(100)	2	
Signet ring cell	1(100)	0(0)	1	
<b>Lymphatic vessel invasion</b>				
Present	18(20.7)	69(79.3)	87	<0.0001
Absent	2(2)	130(98)	132	
<b>Venous vessel invasion</b>				
Present	12(16)	63(84)	75	0.01
Absent	8(6)	137(94)	145	
<b>SCI</b>				
Present	19(18)	89(82)	108	<0.0001
Absent	1(1)	110(99)	111	
<b>MUC</b>				
Present	9(20)	37(80)	46	0.005
Absent	11(6)	165(94)	176	

was more frequently found than sulfomucin. The mucins in MUCs predominantly stained for sialomucin.

#### *Stainability of tumor-associated carbohydrate antigen*

Mucin, which stained either moderately or extremely positive, was present in 73% of SICs upon CEA staining, 63% of SICs upon CA 19-9 staining and 80% of SICs upon UEA-I staining. In the metastatic SICs, all lesions were stained more than moderately positive upon CEA and UEA-I staining, and 83% were stained similarly with CA 19-9 staining (Table 2).

Cancer cells showed the same intensity of staining for both CEA and UEA-I, but stained more diffusely for CEA than UEA-I. MUC was markedly stained with UEA-I compared with other antigens. UEA-I was stained more intensely on poorly differentiated adenocarcinoma than well-differentiated adenocarcinoma, and mucin circumscribing cancer cells in the lymphatic vessels was stained strongly in several lesions.

SIC showed weak staining with CA19-9, but the cancer tissues except for MUC sometimes became stained.

### Correlation of MUC with other prognostic factors

There was no relationship between MUCs and age of onset, gender, gross type, diameter, location and histopathological type of SIC.

As for vessel invasion, we did find that SIC positive for venous invasion were apt to have MUC ( $p = 0.13$ ) and SIC positive for lymphatic invasion had MUC with a significant difference ( $p = 0.039$ ) (Table 3).

With respect to sm depth, SICs with MUC had significantly greater sm depths than those without MUC ( $p = 0.0003$ ) (Fig. 3), but there was no difference in sm width between the two groups. In addition, the greater the extent of invasion, the higher was the positive rate of MUC in SICs ( $p = 0.048$ ) (Table 4).

**Table 2.** Stainability of carbohydrate antigens in submucosal invasive carcinoma

	Stainability			
	None	Grade I	Grade II	Grade III
CEA	1 (3.3%)	7 (23.3%)	9(3) (30%)	13(3) (43.3%)
CA19-9	9(1) (30%)	2 (6.7%)	12(3) (40%)	7(2) (23.3%)
UEA-1	3 (10%)	3 (10%)	10(3) (33.3%)	14(3) (48.6%)

( ): No. of lesions in metastatic group

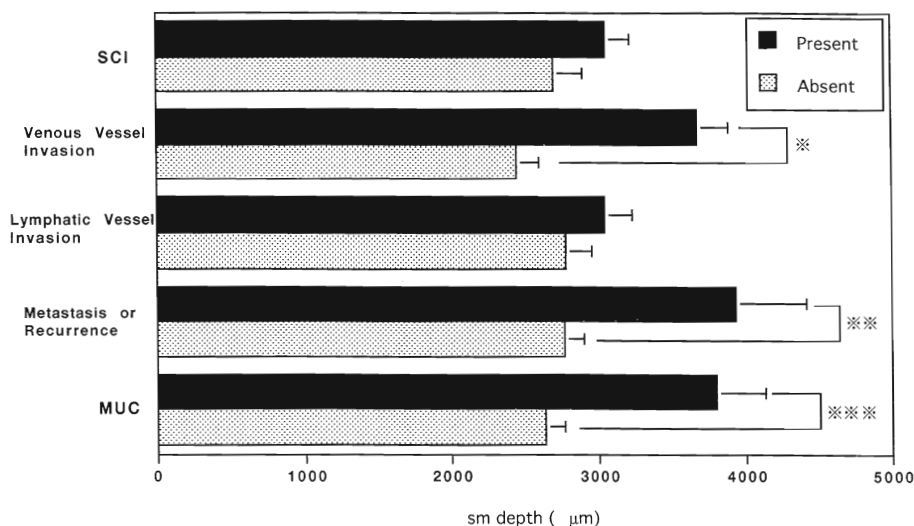
### Discussion

There have been several reports concerning the adverse prognostic factors in SIC lesions: histological grade, level of invasion, vessel invasion, and poorly differentiated adenocarcinoma ahead of the invasion<sup>9,13</sup>. We have reported that a pool of mucin ahead of the invasion "front," referred to as MUC, is an adverse prognostic factor of SIC<sup>10,11</sup>.

In our present study, cancer glands neighboring MUCs showed high-grade atypia, similar to the type noted in advanced mucinous carcinoma. Colorectal mucinous carcinoma had almost reached an advanced stage in these cases, and therefore data on early stage disease has rarely been reported<sup>1,2,4,5,7,8,14</sup>.

Nivantvongs<sup>9</sup> reported that mucinous carcinoma was discovered in 8% of SIC cases, and was more common if the SIC was defined as having a considerable amount of mucous in the interstitium of the polyp. Tsuchiya et al.<sup>15</sup> reported that an ascending colon SIC measuring 11 mm in diameter had the histological appearance of a mucinous carcinoma in the submucosal layer, involving a remote lymph node.

We have defined MUC as a pool of mucin present at the leading edge of the invasion adjacent to cancer glands with high-grade atypia regardless of the amount of mucous. Researchers have demonstrated that the main substance of epithelial mucin is glycoprotein, and that goblet cells are filled with secretory type glycoprotein in the large secretory granules of api-



**Fig. 3.** Correlation between sm depth and adverse prognostic factors. Three factors were significantly associated with vertical distance of invasion termed as sm depth. Means  $\pm$  s.e.m. \* $p < 0.0001$ , \*\* $p = 0.0101$ , \*\*\* $p = 0.0003$ .

**Table 3.** Correlation between MUCs and adverse prognostic factors

Prognostic factors	MUC		Total No.	p-value
	Present(%)	Absent(%)		
<b>Location</b>				
Colon	18(21)	67(79)	85	NS
Rectum	27(20)	105(80)	132	
<b>Gross type</b>				
Ip	10(23)	34(77)	44	NS
Isp	1(11)	8(89)	9	
Is	25(22)	81(78)	106	
Ila	0	14(100)	14	
Ila aggregated	3(30)	7(70)	10	
Ila+Ilc	5(21)	19(79)	24	
Ilc	0	3(100)	3	
<b>Histological grade</b>				
well-1	7(15)	40(85)	47	NS
well-2	30(21)	110(79)	140	
Moderately differentiated	6(21)	23(79)	29	
Poorly differentiated	0	2(100)	2	
Signet ring cell	1(100)	0(0)	1	
<b>Lymphatic vessel invasion</b>				
Present	24(28)	63(72)	87	0.039
Absent	21(16)	110(84)	131	
<b>Venous vessel invasion</b>				
Present	21(28)	54(72)	75	NS
Absent	24(17)	120(83)	144	
<b>SCI</b>				
Present	25(23)	83(77)	108	NS
Absent	20(18)	91(82)	111	

**Table 4.** Correlation between the extent of invasion and adverse prognostic factors

Extent of Invasion	Number of Lesions	Lymphatic Invasion (%)	Venous Invasion (%)	SCI (%)	MUC (%)	Metastasis or recurrence(%)
Extent-I	10	0	0	0	0	0
Extent-II	19	26	0	32	5	0
Extent-III	185	45	40	54	25	12

\*p &lt; 0.05, \*\*p &lt; 0.005, \*\*\*p &lt; 0.001.

cal cytoplasm. According to the mechanism of carcinogenesis of colorectal epithelial cells, terminal sugar sequences of mucin type glycoprotein, i.e., sial-

ic acid, N-acetylgalactosamine and fucose, would be changed<sup>16,17,18</sup>. This was appreciated by the increased diastase-PAS staining of cancer mucin, probably

because cancer-associated sialomucin has a relative deficiency of O-acetyl substituents.

Shamsuddin<sup>19</sup> reported that histochemical studies for the detection of epithelial acidic mucosubstances showed that sialomucin predominated in the colon mucosa harboring a carcinoma irrespective of the location of the tumor, whereas colon mucosa from otherwise normal individuals and patients with non-cancerous diseases showed a predominance of sulfomucin. In our study, mucin that was secreted from cancer cells, especially, MUC, also showed an extreme predominance of sialomucin.

The well-known binding affinities of lectins for sugars had been exploited in studies of cancer-associated changes in glycoconjugate structures. Glycoproteins binding to UEA-I lectin, which recognized the terminal  $\alpha$ -L-fucose residue, have been noted as having a specific binding site on carcinoma tissue. The emergence of fetal antigens presumably occurred as a consequence of the disappearance of gene expression, which was switched off during normal development. CEA, which is one of the typical oncofetal antigens in colorectal carcinoma tissue, is very similar to the mucin-type glycoprotein given that it possesses the redundant terminal carbohydrate residue. Matsushita et al.<sup>20</sup> reported that CEA from carcinoma tissue was found to have the same electrophoretic mobility as UEA-I binding glycoproteins. As for our cases, the greater the atypia of the cancer cells, the more diffuse was the CEA and UEA-I staining. Mucin in MUC showed greater stainability with UEA-I than CEA.

It has been proposed that the sialylation of carbohydrates and cancer metastasis were intimately related. Nakayama et al.<sup>21</sup> reported that sialyl Lewis (SLA) antigen expression in primary colorectal carcinoma correlated significantly with hematogeneous recurrences in the liver and the lung. CA19-9 was detected by the monoclonal antibody NS19-9 obtained from mice immunized with the colon cancer cell line SW 1116. The antigenic determinant of CA19-9 was SLA. We demonstrated that 63% of all SICs and 83% of metastatic group SICs were moderately or extremely positive for CA19-9 immunohistological staining. Although there was CA19-9 staining in either the interstitium and vessels at the leading edge of invasion, the MUC was weakly stained with CA19-9.

Researchers have postulated that there is a close correlation between the expression of high molecular weight sialoglycoproteins and the progression of colorectal carcinoma to the metastatic phenotype. Irimura<sup>22</sup> has reported that an induction of high molec-

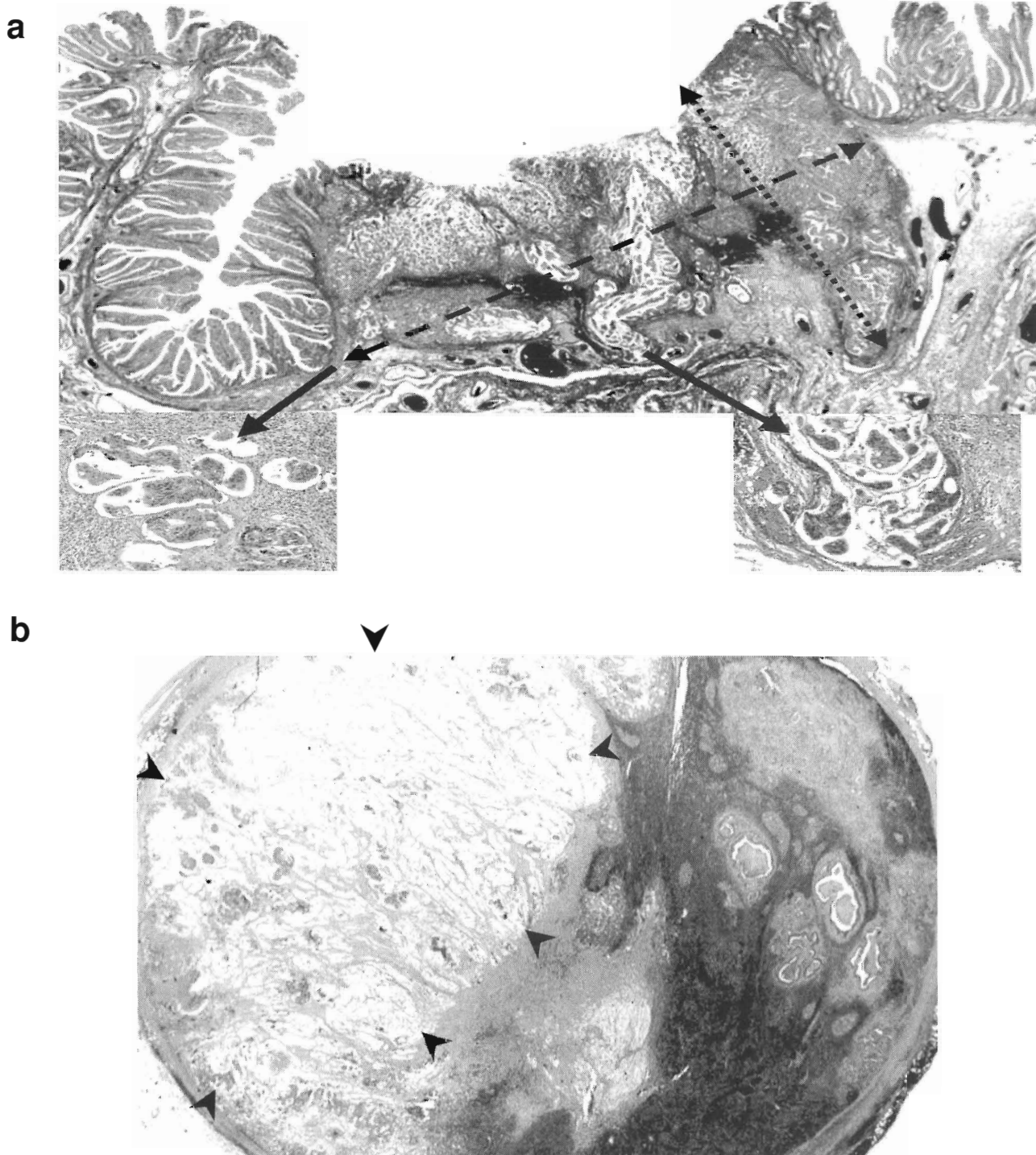
ular-weight sialoglycoprotein production probably provides colorectal carcinoma cells with an enhanced capacity to metastasize by means of their ability to evade immune defense mechanisms and to form emboli with platelets<sup>25</sup>. Several recent studies demonstrated that mucinous carcinoma may represent a distinct genetic and molecular entity<sup>23,24,25,26,27,28,29,30,31,32</sup>.

MUCs were recognized in 21% of all SICs. On the other hand, MUCs were present in 45% of metastatic group SICs. When 50% or more of the tumor was mucinous, the tumor could be classified as mucinous carcinoma. Mucinous carcinomas had been classified into several subtypes as a result of previous investigations. Studies on the prognosis of advanced mucinous carcinoma had been based mainly on pathological features, such as the grade of differentiation of the cancer cells in the mucous lake and the ratio of the area of the mucous component to other carcinoma tissue. Saigusa<sup>33</sup> reported classification of mucinous carcinomas into two subtypes, i.e., fixed and floating types, according to the distribution of cancer cells in the mucous nodule. They determined that the fixed type mucinous carcinoma was predominant for sulfomucin and the floating type was predominant for sialomucin, which was found most commonly in the rectum and had a poor prognosis.

Wolfmann<sup>34</sup> reported that mucinous carcinomas could be grouped into two subtypes, i.e., intracellular and extracellular types. In that study, he demonstrated that the former type was composed of poorly differentiated adenocarcinoma and was apt to have a poor prognosis involving remote lymph nodes or the peritoneum. On the contrary, the latter type was usually consisted of a well-differentiated adenocarcinoma, for which there was no difference in prognosis as compared to non-mucinous adenocarcinoma.

Umpleby<sup>1</sup> defined colorectal carcinoma as mucinous carcinoma only when the mucous component comprised over 60% of the entire cancer tissue. He reported that both the curative resection rate and cumulative five-year survival rate were lower than in non-mucinous carcinomas provided that the mucous lake comprised more than 80% of the colorectal carcinoma tissue, which was designated as "high mucin content". Minsky<sup>2</sup> reported that colorectal carcinomas with a colloid pattern in more than two thirds of the tumor volume could be considered colloid carcinomas, and when this pattern was less than two thirds, it could be referred to as adenocarcinoma with colloid features. There was no difference in prognosis between those expressed by Dukes classification, but colloid carcino-





**Fig. 4.** SIC with MUC showing remote lymph node and synchronous multiple liver metastasis

Fig. 4-a: A 75 mm sized ascending colon SIC was resected by right hemicolectomy. Moderately differentiated adenocarcinoma showing Extent-III of invasion (H&E: x 2.5).

◄.....► sm depth : 2575 mm

◄--► sm width : 7.3 mm

There were lymphatic invasion (*lower left*) and venous invasion (*lower right*) with MUC in the submucosal layer (H&E: x 25).

Fig. 4-b: Remote lymph node was involved by mucinous carcinoma (▼) (H&E: x 5).



ma was prevalent in the rectum with a higher rate of local recurrence and peritoneal dissemination.

In general, according to the rules for clinical and pathological studies on cancer which were established by the Japanese Research Society for Cancer of the Colon and Rectum, mucinous carcinoma was defined as that having mucous nodules comprising over 50% of the carcinoma tissue. Sadahiro<sup>35</sup> reported that there was a mucous component in more than 30.2% of colorectal carcinomas invading deeper than the submucosal layer, and 49% of those carcinomas with mucous nodules comprising less than 10% of the lesion. He concluded that colorectal carcinomas with a mucous lake comprising more than 10% of the lesion had a poorer prognosis.

Previous reports demonstrated that it was difficult to perform curative resections for mucinous carcinomas that had invaded deeper than the proper muscular layer. They reported that advanced mucinous carcinoma tended to show lymph node metastasis and peritoneal dissemination despite comparatively low-grade atypia of the cancer cells.

In our study of SICs, only one of 46 lesions with MUC showed a mucous nodule formation comprising over 50% of the whole carcinoma tissue, with most having less than 10%. However, SICs with MUC had correlated significantly with the extent of invasion, especially sm depth, and showed higher metastatic and recurrence rates than those without MUC (Fig. 4).

We conclude that since SICs with MUC are associated with many adverse prognostic factors similar to those of advanced mucinous carcinoma, we should design a therapeutic plan after meticulous pathological investigation of resected specimens, particularly in cases of resected polyps using endoscopic polypectomy technique.

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