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Morphogenesis of Peritoneal Metastasis in Human Gastric Cancer[1]

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ABSTRACT

A comparative light microscopic and scanning electron microscopic study of the morphogenesis of peritoneal metastasis in 34 human gastric cancers was performed. Prior to adhesion of gastric cancer cells to the peritoneum, the mesothelial cells became hemispherical and exfoliated from the peritoneum, and gastric cancer cells adhered to the naked areas of the submesothelial connective tissue. A flat metastatic tumor was formed by cancer cell proliferation in the shallow region of the peritoneum. Subsequently, after the infiltration of cancer cells into the connective and adipose tissue, the formation of a large tumor mass was observed. There was a correlation between the surface and histological structure of the metastatic tumors. In poorly differentiated cancer, the cells were isolated while in differentiated cancer, they formed nodules with indistinguishable cell boundaries.

INTRODUCTION

Peritoneal dissemination is the important factor that stands in the way of successful surgical treatment of human gastric cancer. While the mechanisms of experimental metastasis have been studied by various methods (1, 2, 4), little is known about the actual mechanism of human peritoneal metastasis.

In the present investigation, a comparative LM[2] and SEM study of the morphogenesis of peritoneal metastasis in human gastric cancer was carried out.

MATERIALS AND METHODS

The present series consists of 34 gastric cancer patients, 24 males and 10 females, ranging in age from 31 to 76 years. Nine of the patients (26.5%) had peritoneal metastasis. All patients were operated upon at the Department of Surgery of Tottori University between July 1976 and June 1977.

Eight patients with benign gastroduodenal lesions and cholelithiasis were used as the control.

The patients were laparotomized, and 1.5-sq cm specimens of the mesenteric peritoneum were removed with a scalpel and scissors. The resected specimens were rinsed in physiological 0.9% NaCl solution and fixed in 2.5% glutaraldehyde buffered with 0.1 M phosphate (pH 7.4). After postfixation in 1% osmium tetroxide, the specimens were dehydrated in graded ethanol series, immersed in isoamyl acetate, and dried by the critical-point drying method using dry ice (9). The specimens were sputter coated with platinum and observed by SEM.

Specimens containing areas of metastatic dissemination were prepared for histology. They were removed from the SEM stub with a scalpel, rehydrated, and embedded in water-miscible methacrylate by the method of Kushida *et al.* (6). Serial sections were cut with glass knives and stained with hematoxylin (Weigert) and eosin without removing the embedded matrix. LM and SEM findings were compared.

RESULTS

Peritoneum of Controls. SEM revealed the normal mesenteric peritoneum to be covered with mesothelial cells. These cells were so flat that details of the cell boundaries were indiscernible. On the cell surfaces, numerous microvilli were noted (Fig. 1).

LM revealed that the normal mesenteric peritoneum was composed of 3 layers: a single layer of flat mesothelial cells; a thin layer of submesothelial connective tissue consisting of collagen fibers oriented parallel to the peritoneal surface; and a layer of adipose tissue (Fig. 2).

Peritoneum of Patients with Peritoneal Metastasis. SEM revealed the peritoneal surface to be quite different from that of the controls. The mesothelial cells were hemispherical. The cells were separated from each other and interconnected by a few bundles of fine cytoplasmic processes.

The submesothelial connective tissue was visible between the cells, microvilli were decreased in number and length, and irregular cytoplasmic processes were noted on the cell surface (Fig. 3). Mesothelial cell exfoliation from the peritoneum was seen, and naked areas of the connective tissue were exposed to the peritoneal cavity (Fig. 4).

Since these changes were observed in most of the gastric cancer patients with peritoneal metastasis and in a few patients without peritoneal metastasis, we investigated the relationship between mesothelial cell changes and gastric cancer stage classification, using the degree of invasion to the serosa of the stomach and the extent of metastatic dissemination to the peritoneum (Table 1). The gastric cancer stage was macroscopically classified according to surgical findings (4).

No mesothelial cell changes were observed in all the patients without cancer penetration through the gastric serosa. However, the mesothelial cell changes were observed in 9 of 16 (56.0%) patients without peritoneal metastasis but with serosal cancer penetration, and the changes were observed in 8 of 9 (89.0%) patients with peritoneal metastasis. The findings suggest that the mesothelial cell changes precede peritoneal metastasis.

Adhesion of Cancer Cells to the Peritoneum. Fig. 5 showed that cancer cells did not adhere to the mesothelial cells but rather to the naked areas of the submesothelial connective tissue. The cancer cell surface appeared rough and revealed irregular cytoplasmic processes and short microvilli that were different from regenerating mesothelial cells, which revealed stubby microvilli and delicate folds (10).

Cancer Cell Proliferation. SEM revealed cancer cell proliferation in the naked area of the connective tissue and formation of a small, flat tumor. The surface of this metastatic tumor was

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[2] The abbreviations used are: LM, light microscopy; SEM, scanning electron microscopy.

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Table 1

Mesothelial cell changes and presence or absence of peritoneal metastasis and serosal invasion

Classification is based on the general rules for the gastric cancer study in surgery (4).

	P_0[a]	P_1	P_2	P_3	Total
S_0	NNNNN NN				7
S_1	NN				2
S_2	NNNN CCC	N	CC		10
S_3	NNN CCCCC C		CCC	CCC	15
Total	25	1	5	3	34

[a] P's: P_0, no disseminating metastasis; P_1, disseminating metastasis to the adjacent peritoneum; P_2, few metastases to the distant peritoneum; P_3, numerous metastases to the distant peritoneum. S's: S_0, no serosal cancer invasion; S_1, serosal cancer invasion suspected; S_2, definite serosal cancer invasion; S_3, cancer infiltration to other organ(s). N, patients with normal mesothelial cells; C, patients with mesothelial cell changes.

partially covered with fibrin-like substances (Fig. 6).

LM of a histological section of the specimen shown in Fig. 6 revealed cancer cell proliferation in the shallow connective-tissue layer (Fig. 7).

Cancer Cell Infiltration into the Connective and Adipose Tissue. SEM revealed that the cancer cells proliferated and formed a tumor mass. The metastatic tumors were of different appearance; their surface structures can be roughly classified into 2 types. One is the nodular type, consisting of a dense mass of different-sized cancer nodules with indistinguishable cell boundaries (Fig. 8). The other is the diffuse type, consisting of a mass of isolated cancer cells (Fig. 10).

LM revealed cancer cell infiltration into the deep connective and adipose tissue with strong proliferation of the connective tissue (Figs. 9 and 11). There was a correlation between the histological and the surface structure. Histologically, the nodular type is a differentiated cancer (Fig. 9), and the diffuse type is a poorly differentiated cancer (Fig. 11).

DISCUSSION

The present findings suggest that, prior to gastric cancer cell adhesion to the peritoneum, mesothelial cells become hemispherical, exfoliation occurs, and the naked areas of the submesothelial connective tissue are exposed to the peritoneal cavity. These findings coincide with early experimental studies (1, 2, 4). However, while Birbeck and Wheatley (1) described mesothelial cell exfoliation as a rare phenomenon, in our study, it was not so rare. This may be because SEM facilitates the closer observation of a larger area of the peritoneal surface. Therefore, we feel that SEM is very useful for the study of peritoneal metastasis.

The observation that the cancer cells did not adhere to the mesothelial cells but rather to the naked connective tissue areas is in agreement with those of other workers (1, 2, 4) and supports the hypothesis that the tumor cells have a strong affinity for injured areas of the peritoneum (2). However, human cancer cell adhesion to mesothelial cells cannot be ruled out because serial observations which are possible in experimental animals are not feasible in humans. Kudo *et al.* (5) found that, in rats, AH100B ascites hepatoma cells adhere to mesothelial cells. This suggests that adhesion to the peritoneum may be dependent on the tumor cell strain.

Preceding the infiltration into the deep submesothelial connective tissue, shallow cancer cell proliferation was noted. It is conceivable that the submesothelial connective tissue fibers, which are oriented parallel to the peritoneal surface, prevent the immediate cancer cell infiltration into the deep connective tissue (7).

Cancer cell infiltration into the connective and adipose tissue resulted in the formation of a large tumor mass with simultaneous connective tissue proliferation. Histologically, the nodular-type metastatic tumors were identified as differentiated cancer and the diffuse type as poorly differentiated cancer. These findings are in accord with the SEM study of Mishima *et al.* (8) on the human gastric carcinomatous mucosa.

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REFERENCES

1. Birbeck, M. S. C., and Wheatley, D. N. An electron microscopic study of the invasion of ascites tumor cells into the abdominal wall. Cancer Res., *25:* 490–497, 1965.
2. Buck, R. C. Walker 256 tumor implantation in normal and injured peritoneum studied by electron microscopy, scanning electron microscopy, and autoradiography. Cancer Res., *33:* 3181–3188, 1973.
3. Japanese Research Society for Gastric Cancer. The general rules for the gastric cancer study in surgery. Jpn. J. Surg., *3:* 61–71, 1973.
4. Kaneshima, S., Kudo, H., Kosaka, H., Iitsuka, Y., Kimachi, H., Takeuchi, T., and Koga, S. A scanning electron microscopic study on implantation of Ehrlich ascites tumor cells in the peritoneal layer. Yonago Acta Med., *20:* 101–107, 1976.
5. Kudo, H., Kiyasu, Y., Kimachi, H., Kaneshima, S., and Koga, S. Scanning electron microscopic observations on the peritoneal dissemination of the rats implanted with the ascites hepatoma cells AH100B. J. Clin. Electron Microsc., *10:* 649–650, 1977.
6. Kushida, T., Nagato, Y., and Kushida, H. A new embedding method employing water-miscible methacrylates for light microscopy. Proc. 10th Int. Cong. Anat. (Tokyo), 503, 1975.
7. Leighton, J., Kalla, R. L., Kline, I., and Belkin, M. Pathogenesis of tumor invasion. 1. Interaction between normal tissues and "transformed" cells in tissue culture. Cancer Res., *19:* 23–27, 1959.
8. Mishima, T., Ishida, T., Baba, N., Kawai, T., Hojo, Y., Izuo, M., and Kawabata, I. Scanning electron microscopic observations on the surface of human gastric cancer. J. Clin. Electron Microsc., *10:* 352–353, 1977.
9. Tanaka, K., and Iino, A. Critical point drying method using dry ice. Stain Technol., *49:* 203–206, 1974.
10. Watters, W. B., and Buck, R. C. Scanning electron microscopy of mesothelial regeneration in the rat. Lab. Invest., *26:* 604–609, 1972.

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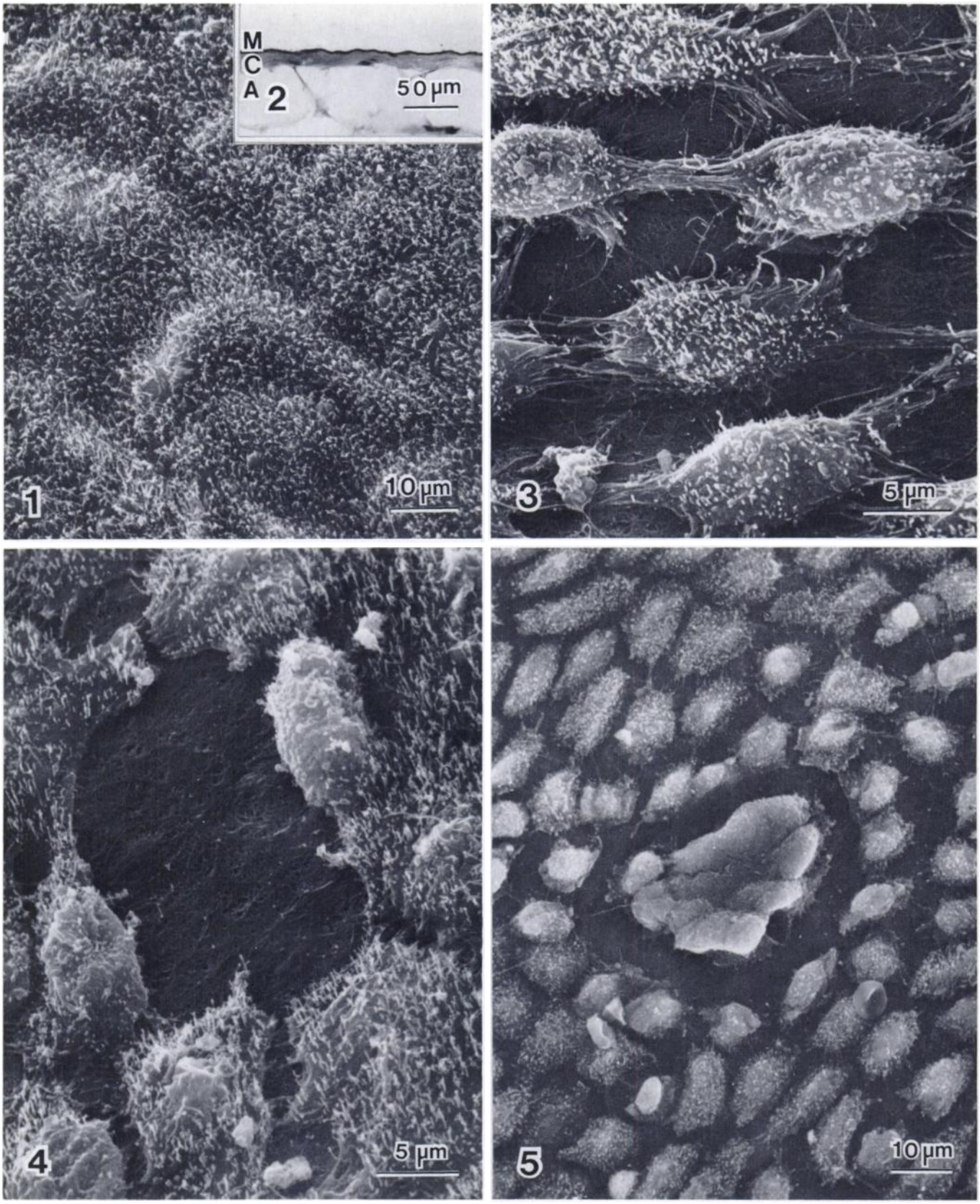

Fig. 1. SEM of the normal mesenteric peritoneum. The peritoneal surface is covered with numerous microvilli. Mesothelial cell boundaries cannot be discerned easily. × 1250.

Fig. 2. LM of the normal mesenteric peritoneum revealing a single layer of mesothelial cells (*M*), a thin layer of connective tissue (*C*), and a layer of adipose tissue (*A*). H & E, × 200.

Fig. 3. SEM of the mesenteric peritoneum of a gastric cancer patient with peritoneal metastasis. The mesothelial cells are hemispherical and separated from each other. The submesothelial connective tissue is visible between the cells. Irregular cytoplasmic processes and the decreasing of microvilli in length and number are noted on the cell surface. × 3000.

Fig. 4. Exfoliation from the peritoneum is noted, and the naked area of the submesothelial connective tissue is exposed to the peritoneal cavity. × 3000.

Fig. 5. SEM showing cancer cell adhesion to the naked area of the submesothelial connective tissue. The surface of cancer cells is covered with short microvilli and irregular cytoplasmic processes. × 1250.

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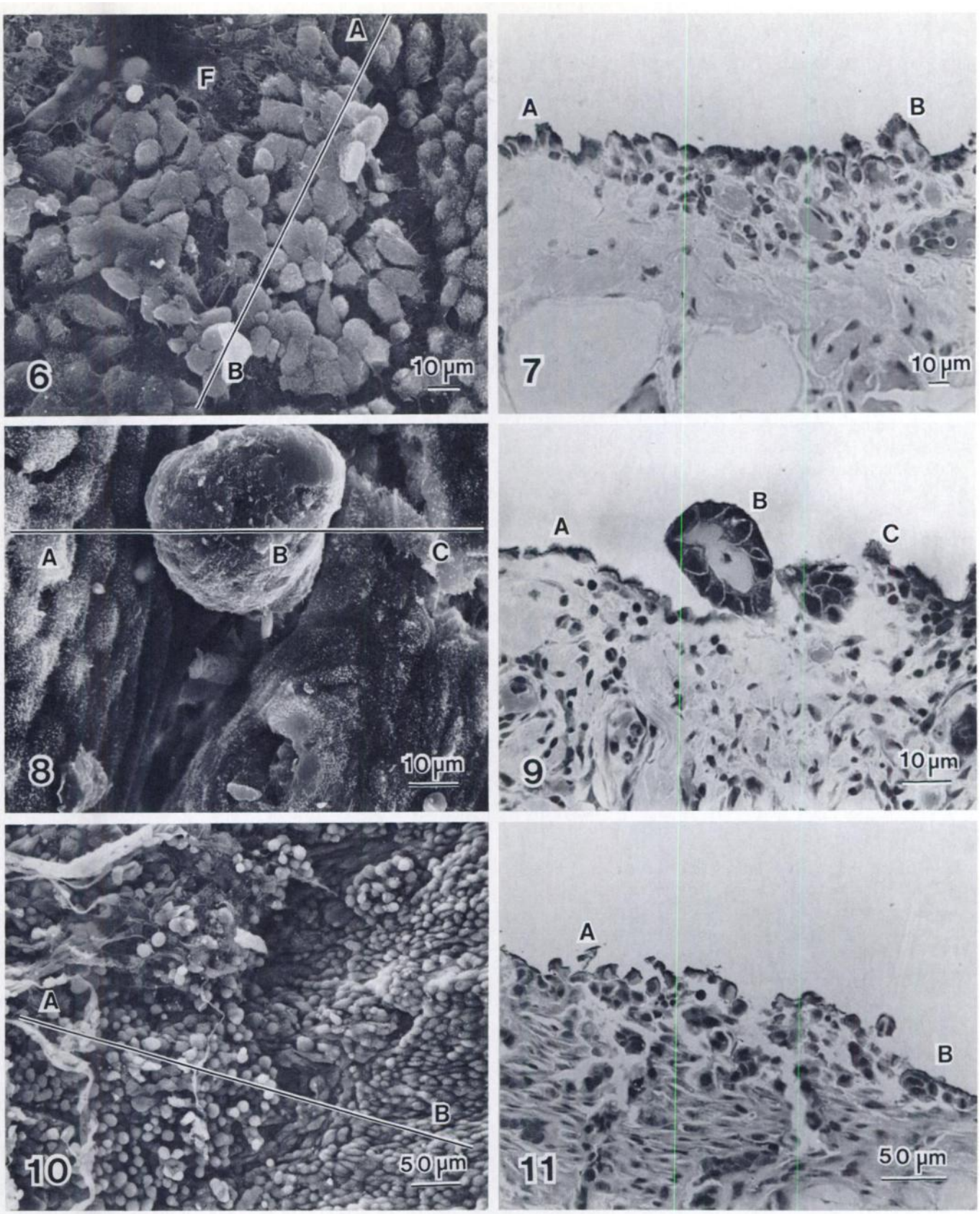

Fig. 6. SEM showing cancer cell proliferation in the shallow region of the peritoneum. The tumor surface is partially covered with a fibrin-like substance (*F*). *A* and *B*, the plane of histological section that is shown in Fig. 7 × 700.

Fig. 7. LM of a histological section of the specimen shown in Fig. 6. The cancer cells proliferated in the shallow region of the peritoneum. H & E, × 500.

Fig. 8. Fig. 8 is a SEM of a nodular-type peritoneal metastatic tumor. Proliferation of the connective tissue is seen. *A* to *C*, the planes of histological section shown in Fig. 9. Fig. 9 is a LM of Fig. 8. These figures show the nodular-type tumor to be a differentiated cancer. H & E, × 1000.

Fig. 9. Legend as in Fig. 8.

Fig. 10. Fig. 10 is a SEM of diffuse-type peritoneal metastatic tumor. Proliferation of the connective tissue is seen. *A* and *B*, the plane of histological section shown in Fig. 11. Fig. 11 is a LM of Fig. 10. These figures show the diffuse-type tumor to be a poorly differentiated cancer. H & E, × 200.

Fig. 11. Legend as in Fig. 10.

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