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Ultrastructural Studies of Malignant Peritoneal Mesothelioma Cells in Ascitic Fluids and Tumor Tissues

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Summary. **To obtain some useful descriminants for distinguishing mesothelioma cells from adenocarcinoma cells or reactive mesothelial cells in ascitic fluids, we studied the ultrastructural features of malignant mesothelioma cells in ascitic fluids. In a total of seven cases of malignant epithelial mesothelioma, there was a characteristic form with a concentric arrangement of intracytoplasmic organelles that appeared most frequently in the fluids. Determining the ratio of length to diameter (LDR) in the microvilli was helpful in distinguishing malignant mesothelioma cells from adenocarcinoma cells or reactive mesothelial cells in ascitic fluids as well as in tumor tissues. However, the number of intermediate filaments was not considered a useful discriminant in ascitic fluids, because some gastric adenocarcinoma cells also had abundant intermediate filaments.**

INTRODUCTION

We frequently deal with cytologic specimens as well as histologic specimens from patients with malignant mesothelioma. We have demonstrated the different intensities of immunostaining between histologic and cytologic specimens of malignant mesothelioma.[1] However, the ultrastructural relationship between mesothelioma cells in fluids and those in tumor tissues has not been thoroughly elucidated. Therefore, the present study investigated the ultrastructural characteristics of epithelial type malignant peritoneal mesothelioma cells in ascitic fluids and in tumor tissues.

MATERIALS AND METHODS

Seven cases of unequivocal malignant peritoneal mesothelioma were obtained from the cytology, surgical pathology and autopsy files at Niigata University School of Medicine and surrounding community hospitals. All malignant mesothelioma examined were diffuse epithelial type mesothelioma (Table 1).

Ascitic fluids were obtained by puncture before and after treatment with anti-cancer agents. The fluid was centrifuged and the sediment was smeared on glass slides. The smears were fixed in 95% ethanol and stained by Papanicolaou's method. Tumor tissues for light microscopic and electron microscopic examinations were obtained by laparoscopic biopsy, open abdominal biopsy or autopsy.

The centrifuged sediments of ascitic fluid and small cubes of tumor tissue were fixed in 2.5% phosphate-buffered glutaraldehyde, post-fixed in 1% osmium tetroxide, and embedded in epoxy resin. Ultrathin sections were stained with uranyl acetate and lead citrate. Microvillous length was computed as the mean of the five longest surface microvilli in each case, and expressed as the ratio of length to diameter (LDR), according to Warhol et al.[2,3] Nuclear bodies were classified according to Bouteille et al.[4]

For comparative studies, we used seven malignant ascitic fluids obtained from 5 patients with poorly differentiated adenocarcinoma of the stomach and 2 patients with duct cell carcinoma of the pancreas and benign ascitic fluids which contained reactive mesothelial cells due to various non-neoplastic basic diseases, e.g., liver cirrhosis.

For statistical evaluations, the Student's t-test or

Table 1. Clinical data of patients with malignant mesothelioma used in the present study.

Case No.	Patient age/sex	Clinical manifestation	History of exposure to asbestos	Anti-cancer agents[a] used for therapy	Outcome[b]	Tissue specimen
1	44/M	Abdominal distension	No	*po*: 5-FU *ip*: ADM, MMC, OK432	DOD 1y6m	Autopsy
2	49/M	Abdominal distension	Yes	*iv*: 5-FU, CPM, MMC, CHRM *ip*: MMC, ADM, OK432	DOD 1y7m	Autopsy
3	50/M	Abdominal distension	Yes	*ip*: ADM, OK432	DOD 4m	Autopsy
4	74/M	Abdominal distension	Unknown	*iv*: CDDP *ip*: ADM	DOD 1y1m	Open biopsy
5	60/M	Abdominal distension	No	*ip*: CDDP	DOD 1y8m	Laparoscopic biopsy
6	48/F	Abdominal distension	No	*iv*: CDDP *ip*: ADM	DOD 2y4m	Open biopsy
7	61/F	Abdominal distension	No	*iv*: CDDP, ADM, CPM, 5-FU *ip*: ADM	DOD 8m	Open biopsy

[a] *po*: per os administration, *ip*: intraperitoneal administration, *iv*: intravenous administration, 5-FU: 5-Fluorouracil, ADM: Adriamycin, MMC: Mitomycin C, CPM: Cyclophosphamide, CHRM: Chromomycin A_3.
[b] DOD: died of disease.

Table 2. Cell types of neoplastic or reactive mesothelial cells.

Cell	Cell type (%)			
	A	B	C	D
Malignant mesothelioma				
Before chemotherapy	39±12	51±13	6 ± 2	4 ± 3
(n=7)	(13-52)	(34-80)	(3-10)	(1-12)
After chemotherapy	29± 9	34± 9	32± 5	5±2
(n=7)	(9-40)	(24-52)	(22-38)	(2-7)
Reactive mesothelial cells	64± 7	24± 8	8 ± 2	4 ± 1
(n=6)	(51-72)	(13-37)	(5-9)	(3-6)

Mean ± SD (range)

Table 3. LDR for microvilli of various kinds of cells.

Cell	Ascitic fluid		Tumor tissue
	Before chemotherapy	After chemotherapy	
Malignant mesothelioma	16.8±1.7	15.3±1.7	14.2±1.4
(n=7)	(14.4-19.8)	(12.4-18.1)	(12.4-16.6)
Adenocarcinoma	7.8±1.4	NT	NT
(n=7)	(6.2-10.8)		
Reactive mesothelial cells	9.8±1.6	NT	NT
(n=6)	(7.6-12.4)		

Mean ± SD (range) NT: not tested

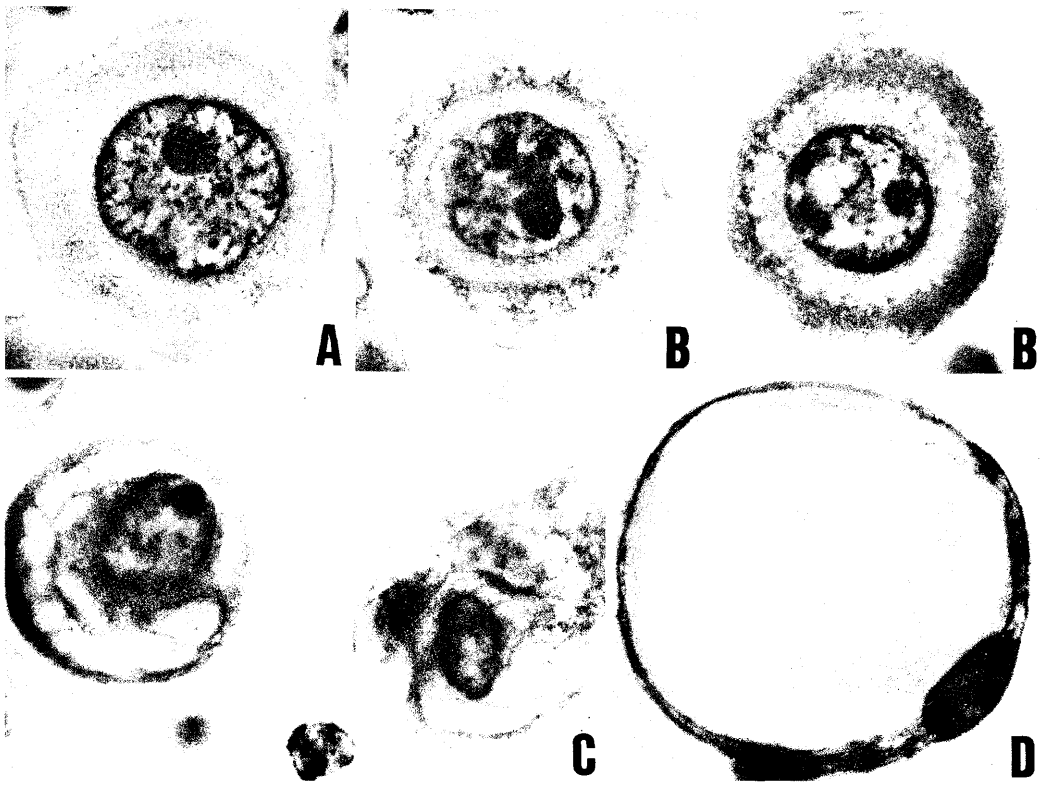


Fig. 1. Mesothelial cells in ascitic fluids. (**A**) Type A cell, (**B**) Type B cell, (**C**) Type C cell, (**D**) Type D cell. Papanicolaou's stain. ×1,700

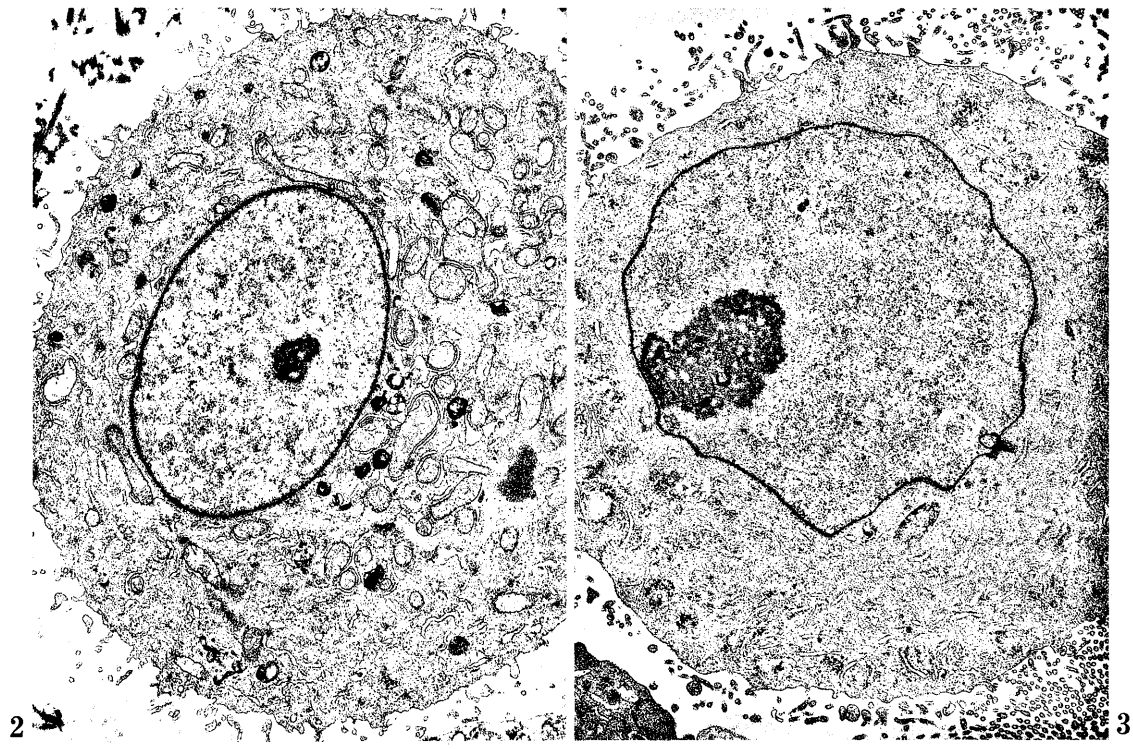


Fig. 2. Mitochondria-rich Type A cell. Uranyl acetate and lead citrate. ×4,700

Fig. 3. Type A cell of simple form. Uranyl acetate and lead citrate. ×4,700

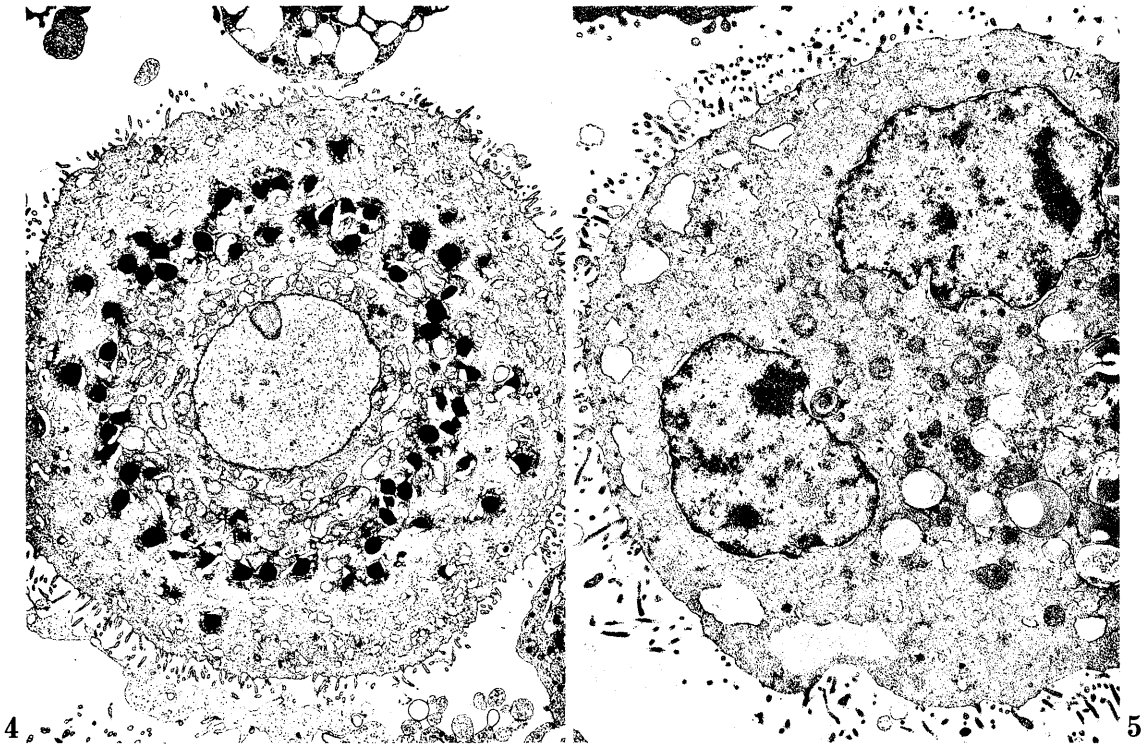


Fig. 4. Type B cell characterized by numerous slender microvilli on the entire cell surface and the concentric arrangement of intracytoplasmic organelles. Uranyl acetate and lead citrate. ×4,700

Fig. 5. Type C cell. Vacuolization is beginning at the mitochondria and endoplasmic reticulum. Uranyl acetate and lead citrate. ×4,700

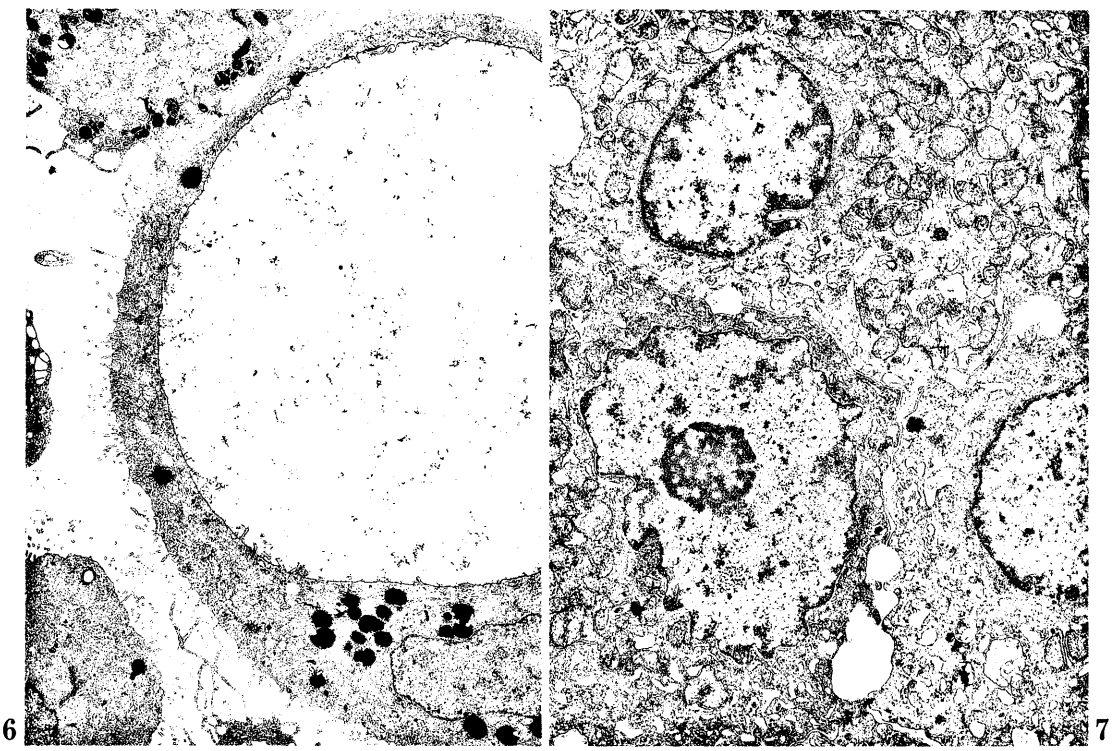


Fig. 6. Type D cell. The signet-ring like feature is due to an intracytoplasmic neolumen. Uranyl acetate and lead citrate. ×1,500

Fig. 7. Malignant mesothelioma cells generally have equally distributed intracytoplasmic organelles in the tumor tissue. Uranyl acetate and lead citrate. ×1,400

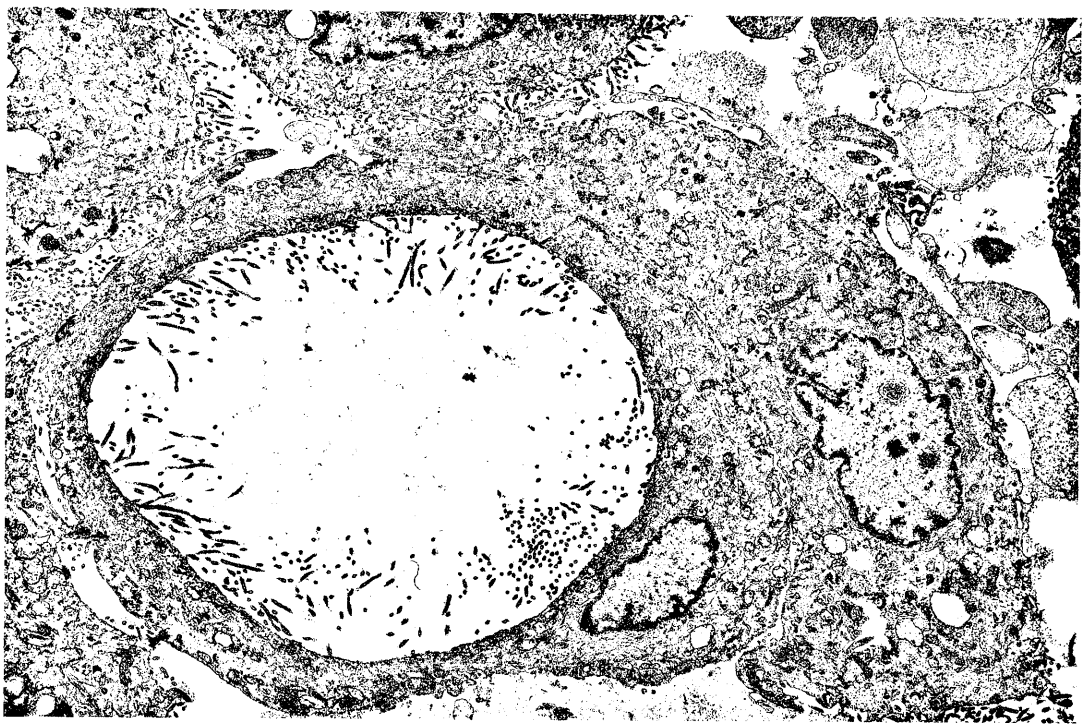
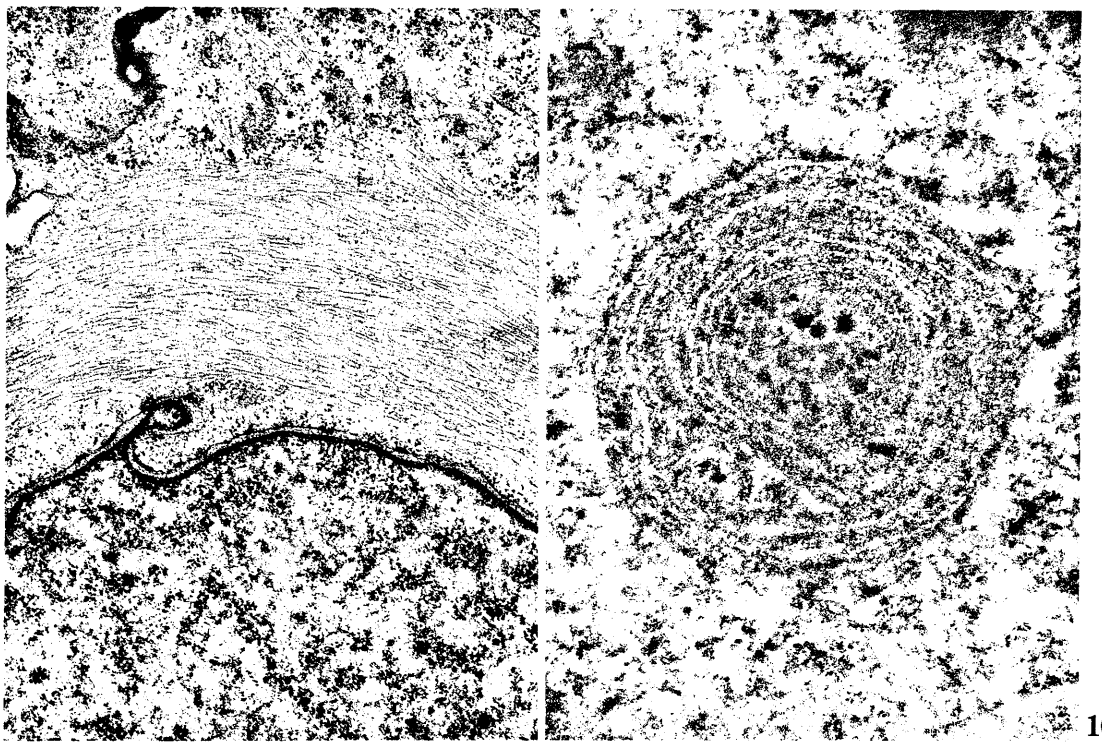


Fig. 8. Type D-like cell in the tumor tissue. The cell has large intracytoplasmic neolumen. Uranyl acetate and lead citrate. ×1,900



9 10

Fig. 9. Gastric adenocarcinoma cell containing abundant intermediate filaments. Uranyl acetate and lead citrate. ×21,250

Fig. 10. Type III nuclear body observed in a mesothelioma cell. Uranyl acetate and lead citrate. ×42,500

Wilcoxon's test was used.

RESULTS

Light microscopic findings

Mesothelial cells in ascitic fluid were first classified into four types. Type A, Type B and Type C cells were characterized by homogenously stained, concentric lamellar and vacuolated cytoplasm, respectively. Type D cells showed a signet-ring like appearance (Fig. 1). Treatment with anti-cancer agents increased the number of Type C cells irrespective of the kind of agent. Type B cells appeared more frequently in the ascitic fluids of malignant mesothelioma than in those of reactive mesothelial cells ($p<0.01$) (Table 2).

Electron microscopic findings

The Type A group observed under light microscopy was ultrastructurally composed of three kinds of cells with diffuse distribution of intracytoplasmic organelles, namely, an intracytoplasmic organelle-rich (especially, mitochondria and/or filament-rich) form (Fig. 2), simple (intracytoplasmic organelle-poor) form (Fig. 3) or intermediate form. The characteristic concentric lamellar features of the Type B group were due to the numerous slender microvilli on the entire cell surface and the concentric arrangement of intracytoplasmic organelles, lysosomes, lipid droplets or glycogen particles (Fig. 4). Cells of Type C had variously sized intracytoplasmic vacuoles. The initiation of vacuolization was observed at the mitochondria and endoplasmic reticulum (Fig. 5). The signet-ring like appearance of the Type D cell was due to an intracytoplasmic neolumen (Fig. 6).

Tumor tissues from all cases examined were mainly composed of Type A-like cells. Both the intracytoplasmic organelle-rich form and the simple form were observed within the same tumor (Fig. 7). Type D-like cells were also occasionally found (Fig. 8). The postmortem tumor tissues frequently contained vacuolated tumor cells corresponding to Type C. However, cells which corresponded to typical Type B were seldom found.

There was a tendency for LDR to be greater in mesothelioma cells from asicitic fluid rather than those in the tumor tissue from the same host. The average LDR in malignant mesothelioma before anti-cancer chemotherapy was 16.8, while those in gastric and pancreatic adenocarcinoma cells and reactive mesothelial cells were 7.8 and 9.8, respectively (Table 3). The LDR in malignant mesothelioma cells in ascitic fluids was significantly greater than those in gastric and pancreatic adenocarcinoma cells and reactive mesothelial cells ($p<0.01$).

Although perinuclear intermediate filament bundles were frequently observed in the malignant mesothelioma cells, some gastric adenocarcinoma cells also contained abundant perinuclear intermediate filament bundles (Fig. 9).

In Cases 2 and 7, nuclear bodies were found in ascitic fluids both before and after anti-cancer chemotherapy as well as in tumor tissues. Tumor cells from Case 2 contained Type I, II and III nuclear bodies, while Case 7 tumor cell contained mainly Type III nuclear bodies (Fig. 10). There was no significant difference between ascitic fluids and tumor tissues in the type and number of nuclear bodies. We failed to detect any nuclear bodies in all reactive mesothelial cells examined.

DISCUSSION

The difficulty in distinguishing malignant mesothelioma from metastatic adenocarcinoma or hyperplastic mesothelium is a well recognized problem in both histopathology and cytopathology. A number of investigators have emphasized that immunohistochemical methods are helpful for a differential diagnosis.[5-8] However, we have clearly demonstrated different intensities of immunostaining in histologic and cytologic specimens of malignant mesothelioma.[1]

Previous studies also demonstrated the usefulness of electron microscopy in distinguishing mesotheliomas from adenocarcinomas, mainly in tissue.[9-12] Although some investigators demonstrated that both mesotheliomas and adenocarcinomas retain their ultrastrctural characteristics in fluids,[13-15] the correlation between cell features in fluids and in tissues has not yet been throughly elucidated.

Our findings indicate that there is a fundamental difference between the mesothelial cells in fluids and in tissues. Type B cells are frequently found in fluids but seldom in tissue samples. This phenomenon may be explained by the following: Malignant mesothelioma cells and reactive mesothelial cells probably form a polyhedron in tissues. Free and solitary mesothelial cells, both neoplastic and reactive, become globular in fluids. Therefore, intracytomic organelles are rearranged concentrically and the new free cell surface produces numerous new microvilli. In malignant mesotheliomas, the tumor cells can proliferate in fluids. This is the reason Type B cells were more abundant in ascitic fluids. However,

reactive mesothelial cells in ascitic fluids are mainly supplied by exfoliation from the serosal surface.

In tissue specimens of mesothelioma, some investigators observed that mesotheliomas had a significantly greater microvillous LDR than adenocarcinoma.[2,3,10] Kobzil et al.[13] suggested the usefulness of determining LDR of cells in serous fluids. The present study indicates that LDR determination is helpful for effusion samples. Neoplastic mesothelial cells in ascitic fluids could be distinguished from reactive mesothelial cells as well as adenocarcinoma cells by determining LDR.

Differences in intermediate filament content have also been stressed as a useful discriminant in tissue sections.[3] The materials in that study were adenocarcinomas of the lung or breast. Indeed, well developed, long microvilli, intermediate filaments, junctional structures, an absence of eletron-dense secretory granules, an electron-lucent appearance of the tubular lumen and intracellular vacuoles equipped with microvilli, and the presence of a basal lamina are all common features of typical diffuse malignant epithelial mesothelioma.[16] However, some gastric poorly differentiated adenocarcinoma cells have fairly abundant intermediate filaments in their cytoplasm. Therefore, we do not consider intermediate filament content to be a useful discriminant in ascitic fluid samples, unlike malignant pleural effusion samples which frequently contain pulmonary adenocarcinoma cells.

Although nuclear bodies which are intranuclear inclusions existing in various kinds of cells are often observed in tumors, including malignant mesothelioma,[4,17] they may be more frequently observed in tumor tissues treated with anti-cancer agents or irradiation.[18] In two of seven malignant mesotheliomas, we successfully detected nuclear bodies in tumor cells in ascitic fluids obtained before anti-cancer chemotherapy, while we failed to find any nuclear bodies in reactive mesothelial cells in ascitic fluids. The presence of a nuclear body may suggest neoplastic mesothelial cells rather than reactive mesothelial cells.

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