Abstract. The incidence of mesothelioma is estimated to rise sharply worldwide, including Japan, in the next two decades. Molecular and proteomic studies are urgently required to elucidate the pathobiology of malignant mesothelioma. This paper describes the characterization of novel human malignant pleural mesothelioma cell lines representing the sarcomatoid, epithelioid and biphasic subtypes. Materials and Methods: Established pleural effusion fluid cell lines were observed using phase-contrast microscopy and transmission electron microscopy. The immunoreactivity of the cells was evaluated using immunohistochemistry, FACS analysis and Western blotting. The expression of SV40 large cell antigen and the EGFR mutation status were also analyzed. Results: The cell lines had different morphological and immunophenotypic characteristics. All cell lines showed immunophenotypic marker expression of vimentin, mesothelin and N-cadherin, but no expression of CEA or E-cadherin. At the electron microscopic level, a cell surface rich in microvilli confirmed mesothelial origin of the cell lines. Karyotype analyses showed complex abnormalities in all cell lines. Neither EGFR mutations relevant to tyrosine kinase inhibitor responsiveness nor the expression of SV40 large cell antigen was detected in any of the cell lines. Conclusion: FACS analysis is more sensitive for evaluating mesothelin expression than immunohistochemistry of cut specimens. Irrespective of the expression of EGFR on FACS analysis, no EGFR mutation was detected. These three cell lines may be useful for studying cellular, molecular and genetic aspects of mesothelioma.

Malignant pleural mesothelioma (MPM) is an aggressive neoplasm that arises from mesothelial cells of the serosal lining of the pleural cavity (1-4). Exposure to asbestos fibers is closely implicated in the development of MPM. Due to the long latency period after exposure and the widespread use of asbestos fibers for many years, the incidence of MPM is expected to rise sharply in the next two decades.

Several clinical issues involving the diagnosis, pathophysiology and treatment of malignant mesothelioma remain unresolved. In particular, there is no cure for MPM, and with the currently available treatment the median survival time ranges from 6 to 18 months.

MPM is classified into three major histological subtypes: epithelial, sarcomatoid and biphasic or mixed (containing both epithelial and sarcomatoid elements). Epidermal growth factor receptor (EGFR), a tyrosine kinase receptor, is overexpressed in MPM and in a wide variety of epithelial malignancies, including non-small cell lung carcinoma (NSCLC), head and neck, colon, and breast cancer (5). On immunohistochemistry, EGFR expression was observed in 11 out of 16 epithelial MPM specimens, but in only 4 out of 9 sarcomatoid and in 2 out of 9 mixed MPM specimens (5, 6).

A relatively small number of MPM cell lines have been established compared to the number of lung cancer cell lines that have been established; a limited number of cell lines are available through tissue culture banks, such as the ATCC. According to previous reports, only a few cell lines have been established from Japanese patients with MPM (7, 8). In the present study, the phenotypes of three MPM cell lines that we established were investigated using immunohistochemistry, FACS, Western blotting, and electron microscopy; their EGFR mutation status and SV40 large cell antigen expression were also determined.
Materials and Methods

Case reports

Patient 1: A 77-year-old male presented with cough and increasing dyspnea of 5 months’ duration in October 1996. Chest X-rays showed marked thickening of the right pleura. On open pleural biopsy, a diffuse mesothelioma of the sarcomatoid subtype was found. Three months later, the patient developed superior vena cava syndrome. Radiotherapy was not effective. The patient died due to respiratory failure after 1 month of treatment. The autopsy confirmed the diagnosis. The patient had been exposed to asbestos while working in a shipyard at a younger age.

Patient 2: A 75-year-old male presented with an increasing left pleural effusion in August 1999. The cytology of the effusion suggested malignant mesothelioma. Two months later, the patient developed subcutaneous extension of the pleural tumor via the thoracentesis site; on histopathological examination of the biopsy specimen, the epithelioid subtype of MPM was diagnosed. Pleurodesis was successful in controlling the pleural effusion. However, despite chemotherapy with cisplatin plus gemcitabine, the patient died of progressive respiratory failure 6 months later. The patient had no history of occupational exposure to asbestos, but, after his retirement, he had spent a lot of time repairing the suspensions and brake systems of old cars as a hobby.

Patient 3: A 55-year-old female presented with a right pleural effusion and a large tumor abutting on the right upper mediastinum in April 2004. Histopathological examination of a CT-guided biopsy specimen revealed a malignant pleural mesothelioma. She had 4 cycles of chemotherapy consisting of cisplatin and gemcitabine and achieved partial response. However, the patient developed superior vena cava syndrome and brain metastasis. The patient received brain irradiation followed by chemotherapy. Fourteen months after her initial chemotherapy, the patient died due to progressive tumor spread to both lungs. Autopsy confirmed the presence of the biphasic subtype of MPM. Neither the patient nor her husband had a history of asbestos exposure.

Reagents and antibodies. RPMI 1640 medium was purchased from Sigma-Aldrich (St. Louis, MO, USA) and fetal calf serum (FCS) was purchased from GIBCO (Invitrogen Life Technologies, Carlsbad, CA, USA). Anti-mesothelin monoclonal antibody, referred to as MB, was a kind gift from Dr. Ira Pastan (NIH, Bethesda, MD, USA) (9). Anti-calretinin and anti-WT-1 polyclonal rabbit antibodies were purchased from NovoCastra (Newcastle-upon-Tyne, UK) and Santa Cruz Biotechnology (Santa Cruz, CA, USA), respectively. Monoclonal antibodies against HBME1, CK5/6, vimentin, p53, thrombomodulin, EMA (DAKO Japan, Kyoto), AE1/AE3 (Boehringer-Mannheim, Mannheim, Germany), CAM5.2 (Becton-Dickinson, Franklin Lakes, NJ, USA), EGFR (Calbiochem, Cambridge, MA, USA), and TTF-1 (NeoMarkers, Fremont, CA, USA) were obtained from commercial sources (Table I).

In the present study, calretinin, WT-1, HBME1, CK5/6, vimentin, thrombomodulin and mesothelin were used as mesothelial markers. In contrast, CEA, AE1/AE3, CAM5.2 and EMA were used as epithelial markers. Calretinin is a 29 kDa calcium-binding protein that is abundantly expressed in neural tissue. WT-1 is the product of the Wilms’ tumor gene, a tumor suppressor located at the locus 11p3. HBME-1 is a monoclonal antibody generated against the suspension of a malignant mesothelioma cell line. CK5/6 represents a type of basic intermediate filament cytoskeletal protein. Thrombomodulin (CD141) is a 75 kDa glycoprotein in endothelial cells and in a variety of other cell types, including mesothelial cells. Mesothelin is a 40 kDa cell surface protein that is strongly expressed in normal mesothelial cells and malignant mesothelioma. On the other hand, TTF-1 (thyroid transcription factor-1) is highly expressed in type II pneumocytes, as well as in the majority of pulmonary adenocarcinoma and small cell carcinoma, but not in malignant mesothelioma. p53 protein is aberrantly expressed in some of malignancies as a consequence of the mutation of the p53 tumor suppressor gene located at 17p13.

Culture and isolation of clones used for cell proliferation. Pleural effusion fluid was obtained from each patient under sterile conditions after obtaining written informed consent. One sample was obtained from the patient with the sarcomatoid subtype of MPM, one sample was obtained from the patient with the epithelial subtype of MPM, and samples were obtained from the patient with the biphasic subtype of MPM. The pleural effusion fluid samples were overlaid on Ficoll-Hypaque gradient and centrifuged at 1500

Table I. Summary of antibodies used for immunohistochemistry.

<table>
<thead>
<tr>
<th>Markers</th>
<th>Primary antibody</th>
<th>Source</th>
<th>Dilution</th>
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<td>NovoCastra x100</td>
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<td>WT-1</td>
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<td></td>
<td>Vimentin</td>
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<td></td>
<td>Thrombomodulin</td>
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<tr>
<td></td>
<td>Mesothelin</td>
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</tr>
<tr>
<td></td>
<td>EMA</td>
<td>monoclonal</td>
<td>DAKO x10</td>
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rpm for 25 minutes to remove erythrocytes. Isolated mononuclear cell fractions containing tumor cells were washed and distributed in 75-cm² flasks. Cells were cultured in RPMI 1640 medium supplemented with 10% heat-inactivated FCS and maintained at 37°C in a humidified atmosphere of 5% CO₂ and 95% air. The medium was changed every 3 or 4 days. The established cell lines that were isolated from the pleural effusion fluid were subcultured for over 50 passages; they have continued to grow well to the present time, more than one year from initial culturing.

Cell growth curve and tumorigenicity in nude mice. The cells were plated at 1x10⁶ cells in a 35-mm Petri dish in triplicate and the cell number in each well was counted daily for 7 days. The doubling time of each cell line was calculated based on the exponential growth phase.

The cultured cells (4x10⁴) of each MPM cell line were injected subcutaneously into the dorsal side of two nude mice. The animals were examined every week for the development of tumors. All animal care was in accordance with institutional guidelines. The tumor tissue that developed was excised and fixed in 10% formalin and processed for routine histopathological examination.

Immunohistochemistry. The cells, detached using trypsin-EDTA, were washed and centrifuged. The cell pellets were fixed in formalin and embedded with paraffin; cut specimen slides were then prepared. Cut cell samples in paraffin-embedded specimens rather than cytospin slides of fixed cells were chosen for three reasons. The first is that we wanted to compare the immunophenotypes of the MPM cells derived from the established cell lines with those of the pathology specimens. The second is that cytospin slides are difficult to stain with an intra-nuclear antigen, such as WT-1. The third is that all components of cell membranes, cytoplasm, and cell nuclei can be clearly stained in cut specimens rather than in cytospin slides.

Immunohistochemical studies were carried out for mesothelial markers (calretinin, WT1, HBME, CK5/6, vimentin, thrombomodulin and mesothelin), epithelial markers (CEA, AE1/AE3, CAM5.2 and EMA), p53 and TTF-1. All immunostaining of the slides was carried out using the automated Ventana system (Ventana Medical Systems, Inc., Tucson, AZ, USA) that uses an indirect avidin-biotin-peroxidase technique with diaminobenzidine as the chromagen for antigen localization (10). For controls, isotype-matched antibodies were used, or the primary antibodies were omitted. Primary antibodies were appropriately diluted and used, as shown in Table I. Reacted antigen with antibodies was evaluated semiquantitatively scored as negative (less than 5%), + positive (5-50%), and 2+ positive (50-100%).

FACS analyses. Cultured cells (2x10⁶) were detached with trypsin-EDTA (Sigma-Aldrich). Each sample was washed twice in FACS buffer (PBS plus 0.05% fetal bovine serum and 0.1% sodium azide). One hundred microliters of either anti-human epithelial antigen (Ber-EP4; DakoCytomation, Glostrup, Denmark), anti-EGFR (DakoCytomation), or anti-mesothelin antibody (MB) at a concentration of 1 μg/10⁶ cells (9) were added to the cells; subsequently, the cells were incubated for 30 minutes at 4°C. The cells were then washed twice with FACS buffer, resuspended in 100 μl of FITC-conjugated goat anti-mouse IgG (Beckman Coulter, Fullerton, CA, USA) and incubated for 30 minutes at 4°C. Finally, cells were washed twice and analyzed using FACS Calibur (Becton-Dickinson Japan, Tokyo) and CellQuest software. As a negative control, an irrelevant, isotype-matched, monoclonal antibody was used instead of the primary antibody. The three MPM cell lines and A549 (a lung adenocarcinoma cell line) cells were examined in parallel.

Western blot analysis. Cells were harvested with 0.2% EDTA and solubilized in a lysis buffer supplemented with a protease inhibitor cocktail and PMSF. Lysates were clarified by centrifugation at 4°C, and an equivalent amount of protein from each sample was used to analyze the profile of N- and E-cadherin expression. Lysate samples were mixed with an equal volume of SDS buffer and boiled at 95°C for 5 min. The samples were subjected to SDS-polyacrylamide gel electrophoresis using a denaturing 10-20% gradient. Proteins were transferred to the PVDF membrane, then blocked for 1 h in TBS containing 0.1% v/v Tween 20 and 5% non-fat dry milk. The membranes were incubated overnight at 4°C with primary mouse monoclonal antibody, including anti-N-cadherin (C20820; Pharmingen/Transduction Laboratories, San Diego, CA, USA), or anti-E-cadherin (C70320; Pharmingen/Transduction Laboratories), respectively. Anti-b-actin mouse monoclonal antibody (AC-15; Sigma-Aldrich) was used as an internal control. Antibody reactive bands were visualized by anti-mouse horseradish peroxidase-conjugated antibody diluted 10,000-fold which was applied for 2 h at room temperature. The chemiluminescent signal was captured using ECL plus detection reagents (Amersham Biosciences, Buckinghamshire, UK) according to the manufacturer's instructions and then exposed on Kodak BioMax Light film.

Transmission electron microscopy (TEM). Detached cells were washed and centrifuged at 400 g. The pellets were prefixed for 10 minutes at 4°C in 0.2% glutaraldehyde in cacodylate buffer, recentrifuged and then fixed for 1 h in 2% glutaraldehyde in cacodylate buffer at 4°C. The cells were then washed in the same buffer and post-fixed in 1% osmium tetroxide at 4°C for 1 h. The samples were dehydrated in a graded alcohol series, followed by propylene oxide, then embedded in Epon (Luveak 812; Nacalai Tesque Inc., Kyoto, Japan). After double staining with uranyl acetate and lead citrate, ultrathin sections were examined using a Hitachi H-7100 electron microscope (Hitachi High-Technology, Tokyo, Japan).

Chromosomal analysis. Cytogenetics and karyotyping were conducted using cells in tissue culture following in situ treatment with colcemide for 3 h at a final concentration of 0.1 μg/ml. The cells were then treated with hypotonic solution and fixed in a methanol-acetic acid solution. Trypsin-Giemsa stain banding was performed to assign all chromosomes of the karyotype. Chromosome counts were made on well-spread metaphases.

EGFR mutation analysis. Genomic DNA was extracted from each of the detached culture cell pellets after centrifugation according to conventional methods. EGFR mutations on exons 18 through 21 were examined according to previously described methods (11, 12). Primers for EGFR (exons 18-21) analysis were the same as used in previously published papers. PCR was conducted using PlatinumTaq DNA polymerase purchased from Invitrogen Life Technology (Carlsbad, CA, USA). All sequencing reactions were performed in both forward and reverse directions. Direct DNA sequencing of PCR products was performed using the ABI genetic...
analyzer and the Big-Dye terminator cycle sequencing kit (Version 1.1; Applied Biosystems, Foster City, CA, USA). All sequencing was confirmed at least twice from independent PCR products.

**PCR analysis for SV40 Tag.** The possibility of SV40 infection of the cell lines was determined by PCR using the following primers: SV3.for 5’-TGA GGC TAC TGC TGA CTC TCA ACA-3’ and SV.rev 5’-GCA TGA CTC AAA AAA CTT AGC AAT TCT G-3’, which amplify a 105-bp fragment of Tag (13). The amplification products were resolved in an ethidium bromide-stained 2% agarose gel. All three cell lines, as well as positive and negative controls, were analyzed for SV40 Tag. Plasmid containing SV-40 large cell antigen, pEF 321-T, which was kindly provided by Dr. Yusuke Imai of the Department of Microbiology at Kochi University Medical School, was used as the positive control.

**Results**

**Characterization of tumor cell lines.** This study describes the establishment of three major subtypes of human malignant pleural mesothelioma cell line. Up to the time of writing this paper, the primary cells isolated from the pleural exudates have been subcultured for over 50 passages. Phase-contrast microscopy demonstrated sarcomatoid (patient 1), epithelioid (patient 2), and biphasic subtypes (patient 3), as shown in Figure 1; they were designated as MPM1, MPM2 and MPM3, respectively. All cell lines grew as adherent layers with no floating cells at confluence in the culture media.

Based on each cell line’s cell growth curve, the doubling time was calculated as 26 hours for the sarcomatoid subtype, 46 hours for the epithelioid subtype and 43 hours for the biphasic subtype (Figure 1). The three cell lines were morphologically different on phase-contrast microscopy. The MPM1 subtype showed elongated spindle cells growing in multiple layers (Figure 2A). The MPM2 subtype grew polygonal cells (Figure 2B). The MPM3 subtype consisted of a mixture of epithelioid polygonal cells and characteristic elongated spindle cells (Figure 2C).

To evaluate the tumorigenicity of these three MPM cell lines, each cell line was inoculated into two athymic nude mice. Within one month after the subcutaneous injection of MPM cells, a visible subcutaneous tumor developed in both of the nude mice that were inoculated with the MPM1 and the MPM2 cell lines; however, no visible tumors developed in the two nude mice inoculated with the MPM3 cell line. On histopathological examination, hematoxylin-eosin staining demonstrated that inoculated MPM1 cells developed tumor tissues compatible with the sarcomatoid subtype (Figure 3A), while the MPM2 cells developed tumor tissues compatible with the epithelioid subtype of MPM (Figure 3B).

**Immunophenotypic analysis.** The cell lines’ immunoreactivity was evaluated using various mesothelial and epithelial markers. A summary of the antibody reactivity of the original tissues and the MPM cell lines is shown in Table II.
MPM1 was positive for vimentin, AE1/AE3 and CAM5.2. MPM2 was positive for calretinin, WT1, vimentin, AE1/AE3 and CAM5.2. MPM3 was positive for WT1, vimentin, AE1/AE3 and CAM5.2. All cell lines were positive for vimentin, AE1/AE3 and CAM5.2. MPM2 and MPM3 showed nuclear positive staining for p53. All cell lines were negative for mesothelin, CEA and EGFR. Representative immunohistochemistry results are shown in Figure 4.

Based on the immunohistochemistry results, the immunoreactivity of the MPM cell lines did not increase compared to the original tumors. In contrast, phenotype loss was frequently observed in the MPM2 and MPM3 cell lines. Specifically, during the process of immortalization, MPM2 lost immunoreactivity for CK5/6 and HBME1, while MPM3 lost immunoreactivity for calretinin and HBME1 compared to the original tumor immunoreactivity status, as shown in Table II.
FACS analysis. FACS analysis using an indirect immunofluorescence procedure was performed. MPM1, MPM2 and MPM3 cells expressed mesothelin. MPM2 and A549 cells expressed Ber-EP4 antigen. All cell lines expressed EGFR. Taken together, this indicates that each MPM cell line is composed of a single population of mesothelin-expressing cells (Figure 5).

Western blot analyses. The expression of the cell adhesion molecules, N-cadherin and E-cadherin, was determined using Western blot analysis, since these molecules have been reported to be expressed aberrantly in MPM and their presence is useful for differentiating the cells from lung adenocarcinoma. N-cadherin was expressed in all three cell lines. On the other hand, E-cadherin was exclusively detected in a lung adenocarcinoma cell line (PC9), as shown in Figure 6.

Ultrastructural analysis. Two cell lines (MPM1 and MPM2) were observed on TEM. The cultured MPM1 and MPM2 strains were similar and had common findings, as shown in Figure 7. The majority of cells from each cell line exhibited typical long thin, occasionally branching surface microvilli. Both cell lines had large nuclei with focal indentations in association with clumped chromatin and large nucleoli (Figure 7A and C). In the cytoplasm, well-developed Golgi apparatuses and many polysomes were found (Figure 7A). Some of the cultured cells contained glycogen granules and intermediate filamentous bundles (Figure 7C). In MPM2 cells, annulate lamellae and intranuclear filamentous bundles were also detected. Interestingly, many fine and long microvilli, sometimes showing branching, were observed on the surface of the cultured cells (Figure 7B). Moreover, in the intracellular space, microvillous projections were seen between adjoining cultured cells (Figure 7D).

Karyotypic examination. All cell lines exhibited abnormal karyotypes with aneuploidy of hypotetraploid (MPM1), hypodiploid (MPM2) and hyperdiploid (MPM3) varieties. Karyotype analyses showed more numerically and less structurally complex abnormalities in the MPM1 cell line than in the MPM2 and MPM3 cell lines (Figure 8). The data did not show a particular translocation or abnormality that was specific for MPM.

EGFR mutation status. Each cell line’s EGFR mutations in exons 18, 19, 20, and 21 were examined. No cell lines had any mutations from exon 18 through to exon 21.
Figure 4. Representative immunohistochemical staining results of the MPM cell pellets that were formalin-fixed and paraffin-embedded. MPM1 cells stained positively for vimentin (A) and AE1/AE3 (B). MPM2 cells stained intensely positively for calretinin (C) and positively for WT-1 (D). MPM3 cells stained positively for vimentin (E) and CAM5.2 (F). Original magnification, x400.
PCR results on SV40 large cell antigen expression. Based on PCR analysis, no SV40-positive amplification was detected in any of the three cell lines despite constant SV40 amplification in the positive control (pEF 321-T).

Discussion

Establishing stable and immortal cell lines could provide an essential tool for studying MPM at the cellular and molecular levels and for testing therapeutic approaches. Especially in Japan, the establishment of human mesothelioma cell lines has been infrequently reported (7, 8, 14-16). In the present study, cells from the pleural effusion fluid of MPM patients were used to establish homogenous cultures of three different MPM subtypes. These cell lines were studied to identify marker expression differences, which might be useful in the further histological characterization of the respective primary tumors.

Compared to the original immunoreactivity status of the tumor, the loss of phenotype was frequently observed in the MPM cell lines. Specifically, MPM1 conserved immunoreactivity solely for vimentin; MPM2 conserved immunoreactivity for calretinin, WT-1 and vimentin, but lost immunoreactivity for CK5/6 and HBME1; while MPM3 conserved immunoreactivity for WT-1 and
vimentin, but lost immunoreactivity for calretinin and HBME1. In the present study, immunohistochemical staining of MPM cell pellets demonstrated that vimentin is the sole common and well-conserved mesothelial marker in all MPM cell lines; this is in agreement with previous reports (14, 16). However, vimentin is currently a marker for all three MPM cell subtypes, as well as fibroblastic cell types. It should be noted that fibroblasts are negative for AE1/AE3 and CAM 5.2.

Quite recently, mesothelin has been reported as a new clinical marker for MPM (17). The FACS analysis was performed to test for the expression of mesothelin, EGFR and Ber-EP4 in fresh cells. Mesothelin and EGFR were expressed in all three MPM cell lines. In contrast, Ber-EP4 was detected only on MPM2 cells. FACS analysis using fresh cells could evaluate mesothelin and EGFR expression with greater sensitivity than immunohistochemistry using cut specimens. FACS analysis also showed that the three MPM cell lines were each composed of a single population of MPM cells without fibroblast contamination.

On Western blot analysis, N-cadherin was intensely expressed on MPM cells (Figure 5); this result is compatible with previous reports (18, 19). E-cadherin was strongly expressed in PC9, a lung adenocarcinoma cell line, but not in any of the three MPM cell lines. Consequently, the triple combined use of immunohistochemistry on cut specimens, FACS analysis using fresh cells and Western blot analysis is of great benefit for analyzing immunophenotypic markers of MPM cells.

Thus far, no absolutely specific or exclusive antibodies have been identified that could be used to diagnose malignant mesothelioma. Therefore, these tumors are usually evaluated using a panel of antibodies (20, 21). The representative positive markers include cytokeratin (CK) 5/6, calretinin, WT-1, thrombomodulin, HBME-1, and mesothelin. Currently, the negative markers include CEA, MOC31, TTF-1, and Ber-EP4.

On the other hand, morphological studies using electron microscopy remains an alternative important method for the diagnosis of MPM. On TEM, thin, elongated, sometimes branching, microvilli were seen on the surface of the established MPM1 and MPM2 cell lines; this finding is the most remarkable and reliable morphological feature of MPM cell lines and is consistent with previous studies (22, 23).

Karyotyping showed that the sarcomatoid subtype (MPM1) had more numerous and less structurally complex abnormalities than the epithelioid subtype (MPM2). In addition, all three cell lines were negative for SV40 on PCR analysis, although SV40 has been implicated as the cause of human MPM (13, 24). SV40 may not be involved in the development of MPM.

Recent reports (11, 25, 26) have indicated that EGFR mutation is frequently present in female, non-smoker, east-Asian lung cancer patients who have an adenocarcinoma histology associated with gefitinib responsiveness. However, the cause of these somatic EGFR mutations remains unknown. MPM cells, as well as lung cancer cells, have a high rate of EGFR expression in immunohistochemical studies (5, 6). All three MPM cell lines had enhanced EGFR protein expression on FACS analysis but not on immunohistochemistry. They had no EGFR mutation ranging from exon 18 through 21. Thus, the absence of any EGFR mutation is one of the favorable data supporting the belief that these cell lines are unlikely to be from lung adenocarcinoma in origin.

In conclusion, we have established and characterized three novel human malignant mesothelioma cell lines; these cell lines are designated as MPM1 (sarcomatoid subtype), MPM2 (epithelioid subtype) and MPM3 (biphasic subtype). Based on the results of the present study, the common characteristic phenotype of these three MPM cell lines regardless of their histological subtype, is positive expression of mesothelin, vimentin and N-cadherin, concomitant with a lack of expression of CEA and E-cadherin. Our MPM cell lines are useful for the analysis of different MPM subtypes. They might also be useful for molecular and therapeutic studies, given

Figure 6. Western blot analysis of N-cadherin and E-cadherin. Each lane was loaded with 10 μg of total cell lysate from MPM1 (lane 1), MPM2 (lane 2), MPM3 (lane 3), and PC9, a lung adenocarcinoma cell line (lane 4). N-cadherin was detected in all three MPM cell lines, but not in PC9. In contrast, E-cadherin was detected only in PC9. β-Actin is shown as an internal control at the bottom.
Figure 7. Electron micrographs of MPM1 cells (A and B) and MPM2 cells (C and D) exhibiting long surface microvilli that are characteristic of mesothelioma cells (original magnification x 3,000). Golgi apparatus (G) and intercellular spaces (arrows) are shown. Scale bar = 2 μm.
that, in the next two decades, the incidence of mesothelioma is expected to rise sharply in Japan and the world in general.

References


Figure 8. Representative karyotypic analyses of the sarcomatoid subtype MPM1 (A), the epithelioid subtype MPM2 (B) and the biphasic subtype MPM3 (C). Many structural anomalies including monosomies, trisomies and unassignable chromosomes can be seen. The sarcomatoid cell line (MPM1) was hypotetraploid with a modal chromosome number of 79, the epithelioid cell line (MPM2) was hypodiploid with a modal chromosome number of 40, while the biphasic cell line (MPM3) was hyperdiploid with a modal chromosome number of 44.


Received August 17, 2007
Revised November 14, 2007
Accepted December 11, 2007