Characterization of Three Human Malignant Mesothelioma Cell Lines

Marjan A. Versnel, Henk C. Hoogsteden, Anne Hagemeijer, Monique J. Bouts, Theo H. van der Kwast, Mick Delahaye, Gert Schaart, and Frans C. S. Ramaekers

ABSTRACT: Three human malignant mesothelioma cell lines, designated Mero-14, Mero-25, and Mero-41, have been isolated from effusions and from autopsy material of confirmed cases of malignant mesothelioma. Light and electron microscopy, cytogenetics, growth requirements, and intermediate filament expression of these cell lines were studied and, where possible, compared with the original tumor material of the patient. Cytologic and ultrastructural morphology was consistent with the mesothelial nature of the cells. All cell lines displayed a hyperdiploid karyotype similar to that of the tumor cells obtained directly from the patient. All three malignant mesothelioma cell lines had marker chromosomes 1, 3, 9, and 22, as well as other markers that were occasionally present in these cell lines and in other malignant mesotheliomas studied. Growth kinetic studies in medium supplemented with epidermal growth factor (EGF) showed increased proliferation and a decreased proliferation in medium supplemented with hydrocortisone (HC) or EGF plus HC. The three malignant mesothelioma cell lines were positive for the cytokeratins 7, 8, 18, and 19 based on immunofluorescence and immunoblotting tests with chain-specific monoclonal antibodies. The characteristics of these cell lines support the assumption that Mero-14, Mero-25 and Mero-41 are derived from malignant mesotheliomas and have retained their original character.

INTRODUCTION

Human malignant mesotheliomas are rare tumors that occur most frequently in the pleura and less frequently in the pericardium and peritoneum. A strong relationship has been found between asbestos exposure and the development of a mesothelioma [1]. Despite better controlled use of asbestos, the incidence of mesothelioma is still increasing because of the long lag time between asbestos exposure and the appearance of the tumor.

The first symptom of malignant mesothelioma is usually a pleural effusion. Cytologic diagnosis is difficult as there are no consistent morphologic criteria for dif-
ferentiation between benign and malignant mesothelial cells on the one hand and adenocarcinoma cells on the other [2]. Additional techniques, such as immunocytochemistry, electron microscopy, morphometry, and chromosomal analysis have proved useful but are still not sufficient to establish the diagnosis of malignant mesothelioma with certainty in all cases [3–6].

Biochemical analyses of the cytoskeletal composition of mesothelioma cells have suggested the usefulness of intermediate filament typing. Cytokeratin #5 seems to be a valuable marker for discriminating between malignant mesotheliomas and adenocarcinomas [7]. Coexpression of vimentin and cytokeratins often occurs in malignant mesotheliomas and has also been proposed to be discriminative between mesotheliomas and adenocarcinomas [8–10]. However, some of the latter cancers may occasionally show simultaneous expression of both cytokeratins and vimentin [11–14]. Specific tumor cell lines are invaluable tools to gather more insight into the cellular and molecular biologic characteristics of malignant mesotheliomas. We report here on three continuously growing malignant mesothelioma cell lines that were established from patient material. Light and electron microscopy, cytogentic, growth requirements, and intermediate filament expression patterns of these cell lines have been studied and, where possible, compared to the original patient material.

MATERIALS AND METHODS

Patient material

Malignant mesothelioma cell lines were derived from pleural effusion of patients Me-14 and Me-41 and from autopsy tumor material of patient Me-25. All three patients were men over the age of 60, with a history of asbestos exposure. All patients presented with dyspnea, weight loss, and a large pleural effusion on chest x-ray. There were no signs of other malignancies. All patients died within 1 year after the first symptoms.

Isolation of Cell Lines and Growth Conditions

Mero-14 and Mero-41 were isolated from the pleural effusion of patients Me-14 and Me-41, respectively. Mero-25 was isolated from autopsy material of patient Me-25. The effusion cells were collected in 50-ml tubes with 5000 IU heparin (Organon, Oss, Holland) and 5 ml Ham's F10 medium (GIBCO, Paisley, United Kingdom) supplemented with 15% fetal calf serum (FCS), glutamine, and antibiotics. The cells were plated at high density and after 24 hours the medium containing nonadherent cells was replaced with fresh medium. The pleural fluid of patient Me-41 was also cultured in F10 medium supplemented with 10 ng/ml epidermal growth factor (EGF; Collaborative Research Inc., Lexington, MA) or 10 ng EGF and 0.4 μg/ml hydrocortisone (HC).

When a continuously growing culture was established, cells were routinely subcultured two or three times a week by trypsinization. These three cell lines were the first three we isolated from a panel of 17 human malignant mesothelioma cell lines [15]. The investigations reported here were done after stable establishment of the cell lines between 3 and 6 months after sampling of material. The lines have been frozen and thawed a number of times and have been subcloned later on. Cytogenetic analysis and intermediate filament expression were studied again 1 year later and the findings were consistent with earlier studies.
Cytology and Transmission Electron Microscopy

For diagnosis, routine cytology was applied as described previously [2]. For transmission electron microscopy, the sediments of pleural effusions were fixed in phosphate-buffered 4% paraformaldehyde (pH 7.2) and 1% glutaraldehyde, postfixed in 1% (w/v) OsO₄ in 0.1 M phosphate buffer (pH 7.2) for 12 hours at 4°C, rinsed in the same buffer, acetone dehydrated, and Epon embedded. Cell lines cultured on Melinech plastic (I.C.I., Rotterdam, the Netherlands) were similarly prepared for routine transmission electron microscopy.

Cytogenetics

For cytogenetic studies of cells in pleural fluid, the effusion was collected in 50-ml tubes containing heparin and 1–2 μg Colcemid per tube (GIBCO, Paisley, United Kingdom). After 30–60 minutes, metaphases were harvested following standard cytogenetic procedures.

For cytogenetic studies of cell lines, metaphase cells were obtained by shaking the culture flasks containing exponentially growing cell cultures. The supernatant was collected in centrifugation tubes and Colcemid was added for 15 minutes. The metaphases were then harvested as in the direct method. RFA-, QFQ-, and CTC-banding techniques were used, and the karyotype was established according to the ISCN (1985).

Growth Kinetics

Before the growth kinetic assays were performed, the cells were cultured for at least 10 days in F10 medium with 15% FCS and EGF, EGF plus HC, or HC only. Cells were cultured sixfold (4000 cells/culture) in the appropriate media in a 96-well tissue-culture plate. At 18 hours before harvesting 3H-thymidine (0.4 μCi/culture) was added and the samples were harvested on days 2, 3, 4, 5, and 6, respectively.

Immunocytochemistry of Intermediate Filament Proteins

The intermediate filament protein expression patterns of the three cell lines were assayed by indirect immunofluorescence and Western blotting. Monoclonal antibodies and antisera that recognize various subsets of cytokeratins, vimentin, desmin, and controls were used. The specificity of the antibodies is given in Table 1, together with references to their preparation and characterization [16–20]. The numerical designation of the cytokeratins refers to the catalog of Moll et al. [21]. The indirect immunofluorescence technique was performed as described previously [16].

Gel Electrophoresis and Immunoblotting Assays

Cells were harvested from 75-cm² culture flasks, spun down using a MSE Minor centrifuge at 1200 g for 5 minutes, washed once with PBS, and stored at −20°C until use. Gel electrophoresis and immunoblotting were done essentially as described by Broers et al. [20].

RESULTS

Isolation of the Cell Lines

Two continuously growing cell lines called Mero-14 and Mero-25 were isolated in F10 medium with 15% FCS from the effusion and autopsy material of patients Me-14 and Me-25, respectively. The pleural effusion of patient Me-41 was cultured in F10
<table>
<thead>
<tr>
<th>Antibody</th>
<th>IFP recognized</th>
<th>Mero-14</th>
<th>Mero-25</th>
<th>Mero-41</th>
<th>References</th>
</tr>
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<tbody>
<tr>
<td>pKer</td>
<td>Several cytokeratins</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>[16]</td>
</tr>
<tr>
<td>RCK102</td>
<td>Cytokeratins 5 and 8</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>[16]</td>
</tr>
<tr>
<td>RGE53</td>
<td>Cytokeratin 18</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>[17]</td>
</tr>
<tr>
<td>RCK106</td>
<td>Cytokeratin 18</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>[17]</td>
</tr>
<tr>
<td>RCK105</td>
<td>Cytokeratin 7</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>[18]</td>
</tr>
<tr>
<td>LP2K</td>
<td>Cytokeratin 19</td>
<td>+ (5% of the cells)</td>
<td>+</td>
<td>+</td>
<td>[19]</td>
</tr>
<tr>
<td>RKSE60</td>
<td>Cytokeratin 10</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>[12]</td>
</tr>
<tr>
<td>pVim</td>
<td>Vimentin</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>[12]</td>
</tr>
<tr>
<td>RV202</td>
<td>Vimentin</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>[18]</td>
</tr>
<tr>
<td>RD301</td>
<td>Desmin</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>[20]</td>
</tr>
<tr>
<td>FITC conjugated rabbit antimouse IgG</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>FITC conjugated goat antirabbit IgG</td>
<td>-</td>
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</tbody>
</table>

medium with 15% FCS, but also in F10 medium with supplementation of EGF plus HC or EGF only. In this case, a continuously growing cell line (Mero-41) was only obtained with F10 medium supplemented with EGF plus HC.

**Histologic, Cytologic, and Ultrastructural Characteristics of Patient Material and Cell Lines**

Diagnosis of malignant mesothelioma was established by cytology, histology and/or electron microscopy. Clinical findings supported this diagnosis in the three investigated patients.

Histology revealed that patient Me-14 had an epithelial-type malignant mesothelioma. At the autopsy of patient Me-25, the pleura and the pericard were found to be diffusely infiltrated by cells with a spindle-shaped morphology as well as by strands of cells with an epithelial appearance (biphasic pattern). Transmission electron microscopy also revealed ultrastructural features characteristic of malignant mesothelioma, that is, the tumor cells showed long, slender villi, glycogen granules, and extensive intermediate-sized filament arrays.

The highly cellular pleural effusion derived from patient Me-41 contained several morulae of tumor cells with a collagenous core. Tumor cells expressed the epithelial membrane antigen (EMA), which is considered a marker for malignancy in serous effusions [2]. Transmission electron microscopy showed the same ultrastructural features characteristic of the mesothelial nature of the tumor cells as described above.

Cell line Mero-14 consisted of spindle-shaped cells with polymorphic and hyperchromatic nuclei, with prominent and atypical nucleioli. Ultrastructurally, the cells displayed pseudopodiae, but slender villi were not observed. The cytoplasm contained some disperse glycogen granules, numerous polyosomes, some Golgi membranes, and several fat droplets. An extensive perinuclear intermediate filament network was present in addition to a terminal web of intermediate filaments.
Cell line Mero-25 displayed large polymorphic nuclei with a coarse chromatin pattern. Ultrastructurally, the cells with an epithelial appearance formed several slender villi projecting from the surface. Some tight junctions were seen at contiguous areas. The cytoplasm contained small numbers of disperse glycogen granules and numerous polysomes in addition to several Golgi membranes and a few fat droplets. Both dense and fine bundles of perinuclear intermediate filaments were present.

Cell line Mero-41 consisted of epithelial-like cells with large polymorphic nuclei. Occasionally, prominent nucleoli were found within the nucleus. Ultrastructurally, the cells showed a few villous structures and the cytoplasm contained several fat droplets and numerous polysomes, but only a few Golgi membranes. Both dense and fine bundles of intermediate filaments were found. Multinucleate cells occurred in all cell lines.

Cytogenetic Characteristics
Metaphases obtained directly from effusions showed a mosaicism consisting of cells with a normal karyotype as well as highly abnormal cells with high numbers of chromosomes and a number of structural abnormalities. The marker chromosomes were characteristic of a given line and were sometimes found in multiple copies in all abnormal cells.

In the case of the cell lines, all metaphase cells were abnormal and showed the same markers as the effusion cells of the patients of origin (Table 2). Possible identification of the marker chromosomes is given in Table 3. Occasionally, minor progressive changes of a marker were observed; for example, the marker 3q− in Me-14 effusion cells was replaced by i(3p) in the cell line Mero-14.

In the Me-25 biopsy material cultured for a few days, and in a small percentage of the mitoses from the Me-25 effusion, the karyotype was found to contain 67 chromosomes (range 65–69). However, the cell line Mero-25 contained 135 chromosomes, which was also found in the majority of the effusion cells at diagnosis.

Growth Kinetics
The growth of normal mesothelial cells is promoted by the addition of EGF plus HC, as described by Connell et al. [22]. We could confirm these results in 3H-thymidine incorporation experiments with normal mesothelial cells isolated from the pleural effusion of a noncancerous patient (unpublished results). The growth characteristics of the malignant mesothelioma cell lines Mero-14 and Mero-25 in media with different supplements were analyzed. The addition of either EGF plus HC or HC only inhibited the growth of the malignant mesothelioma cell lines Mero-14 and Mero-25 (Fig. 1). Addition of EGF only increased the proliferation slightly as compared to the growth in medium without EGF supplementation (Fig. 1). The cell line Mero-41 was not tested in this assay. After its establishment, this cell line no longer required EGF and HC for its growth.

Intermediate Filament Protein Expression
In order to support the mesothelial nature of the isolated cell lines and to investigate whether Mero-14, Mero-25 and Mero-41 contained intermediate filament proteins (IFP) typical of mesotheliomas [7], several antibodies to IFP were applied in the indirect immunofluorescence assay and in immunoblotting tests. Table 1 and Figures 2–4 summarize the results of these studies.
Table 2  Cytogenetic data of the mesothelioma cell lines Mero-14, Mero-25, and Mero-41

<table>
<thead>
<tr>
<th>Patient cell line</th>
<th>No. of cells studied</th>
<th>Modal chromosome no. (range)</th>
<th>Number of copies of normal chromosomes</th>
</tr>
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<tbody>
<tr>
<td>Mero-14</td>
<td>13</td>
<td>75(72–78)</td>
<td>0 3 0–1 2 4 2 2 3 2 2 3–4 1 2 3 2 3 4 2 0 1 2 1 2 0–1</td>
</tr>
<tr>
<td>Mero-25</td>
<td>15</td>
<td>135(127–140)</td>
<td>0 2–3 2–3 3–4 3–4 6–8 3–4 6–8 0 6–8 6–8 2–3 3–4 3–4 3–4 4–5 0 3 1–2 0 3–4 0 2–3</td>
</tr>
<tr>
<td>Mero-41</td>
<td>12</td>
<td>72(68–75)</td>
<td>2–3 2 0 2 0 2 4 0 0 3–3 3–4 2 0 2–3 1–2 2–3 2 2–3 3–4 2 3–6 0–2 2 2</td>
</tr>
</tbody>
</table>

Types of markers (number of copies) Unidentified markers

<table>
<thead>
<tr>
<th>Mero-14</th>
<th>1p–(2), 3q–(2), 2p+(2), 1p+(2), 3p–(2), 6p–(2), mar7(1), 9p+(2), 12q+(2), mar16(2), mar22(1)</th>
</tr>
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<tbody>
<tr>
<td>Mero-41</td>
<td>inv del(1)(2), 3p–(1), 3q–(2), mar3(2), Inv(5)(2), 5p+(1), 6p–(3–3), 9p–(3), 17p–(4), 14q–(4), 15q–(1), 17p+(3), 20q+(3), 17q+(1), 21q+(1), 22q+(2)</td>
</tr>
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7
4
1
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<tr>
<th>Marker</th>
<th>Mero-14</th>
<th>Mero-25</th>
<th>Mero-41</th>
</tr>
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<tbody>
<tr>
<td>1p−</td>
<td>der(1) t(1;?,p11,?)</td>
<td>1p−</td>
<td>1qter→1p11: sat</td>
</tr>
<tr>
<td>1q−</td>
<td>t(1;p19q)</td>
<td>inv(1q−)</td>
<td>del(1)</td>
</tr>
<tr>
<td>3p−</td>
<td>del(3)(p23)</td>
<td>del(3)(p12,?)</td>
<td>3q−</td>
</tr>
<tr>
<td>3q−</td>
<td>der(3) t(3;7)(q11;?) or t(3;9)(q11;?)</td>
<td>2p+</td>
<td>der(2)(2;7)(p12,?)</td>
</tr>
<tr>
<td>3p+</td>
<td>der(3) t(1;9)(q11;p21)</td>
<td>3p−</td>
<td>del(3)(p21)</td>
</tr>
<tr>
<td>6p−</td>
<td>der(6)(6;7)(p11,?)</td>
<td>5q−</td>
<td>del(5)(q21;q34)</td>
</tr>
<tr>
<td>mar(7)</td>
<td>t(7p21→cen::7q32→7q11;19p, or q9p</td>
<td>inv(X)</td>
<td>inv(X)(p22q12)</td>
</tr>
<tr>
<td>9p+</td>
<td>dup(9)(p21q22) or HSR</td>
<td>9p−</td>
<td>del(9)(p21)</td>
</tr>
<tr>
<td>12q+</td>
<td>der(12) t(3;12)(q12q23)</td>
<td>?</td>
<td>inv(11)(p11q14)</td>
</tr>
<tr>
<td>mar(16)</td>
<td>?</td>
<td>mar(16)</td>
<td>?</td>
</tr>
<tr>
<td>22q+</td>
<td>der(22) t(22;?)(q13,?)</td>
<td>mar(E)</td>
<td>17q− or 19q+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>mar(14)</td>
<td>17q− or 19q+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>der(20) t(20;21)(q11;q11)</td>
<td>15q−</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20q+</td>
<td>duplication</td>
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Figure 1 $^3$H thymidine incorporation in Mero-14 (left) and Mero-25 (right). Cell lines were cultured in F10 medium with 15% FCS and different supplements. ■ F10 medium with 15% FCS; ○ F10 medium, 15% FCS, and 10 ng/ml EGF; △ F10 medium, 15% FCS, 10 ng/ml EGF, and 0.4 ng/ml HC; ● F10 medium, 15% FCS, and 0.4 µg/ml HC.

When the polyclonal rabbit antiserum to skin keratins was applied to methanol-fixed cells, Mero-14, Mero-25, and Mero-41 all showed filamentous staining reactions, albeit in different patterns and intensities (compare Figs. 2a, b, and c). The same holds true for the broadly cross-reacting monoclonal antibody, RCK102.

When the monoclonal antibodies, each specific for only one cytokeratin polypeptide, were applied to the cell lines, it became obvious that all three cell lines contained cytokeratin 18 and cytokeratin 7 in virtually all cells.

Figure 2 Detection of cytokeratins in Mero-14(a), Mero-25(b), and Mero-41(c) by indirect immunofluorescence using the polyclonal cytokeratin antibody pKer (A–C ×500).
Figure 3  One-dimensional SDS-containing polyacrylamide gel electrophoresis and subsequent protein immunoblotting tests of cytoskeletal preparations from Mero-14 (panel A), Mero-25 (panel B), and Mero-41 (panel C). Lanes 1: Coomassie brilliant blue stained gels; lanes 2: protein blots incubated with RCK106 (anticytokeratin 18); lanes 3: lanes 2, subsequently incubated with RCK 102 (anticytokeratin 5 + 8); the asterisk indicates immunoreactivity with the extra protein band between vimentin and cytokeratin 8; lanes 4: lanes 3, subsequently incubated with RCK 105 (anticytokeratin 7); lanes 5: protein blots incubated with LP2K (anticytokeratin 19); lanes 6: protein blots incubated with RCK105 (anticytokeratin 7). The immunoreaction at the level of actin is probably caused by the presence of breakdown products of cytokeratin 7; lanes 7: protein blots incubated with RV202 (antivimentin). Note that the antibody also reacts with the vimentin breakdown products. Abbreviations: V, vimentin; A, actin; 7, 8, 16, and 19 indicate the corresponding cytokeratin polypeptides.

When monoclonal antibody LP2K, specifically reacting with cytokeratin 19, was applied to Mero-14, only about 5% of the cells were found to show a filamentous staining reaction. Mero-25 and Mero-41 showed a filamentous reaction in virtually all cells present in the culture. Furthermore, all cells showed filamentous staining reactions when incubated with the polyclonal or monoclonal antibody to bovine lens vimentin (pVIM and RV202, respectively), but again typical differences in the distribution patterns and staining intensity were found. No reactions were seen when antibodies to cytokeratin 10 (RKSE 60), desmin (RD301), or only second-step antibodies were applied (see Table 1).

Cytoskeletal preparations of the different cell lines were analyzed by one- and two-dimensional gel electrophoresis as well as by immunoblotting assays. The one-dimensional gels of these cytoskeletal preparations revealed protein bands at the molecular weight levels of vimentin, actin, and cytokeratins 7, 8, 18, and 19 (Fig. 3, lanes 1), however, in varying amounts. For example, in Mero-14, the vimentin protein band was most pronounced, while in Mero-25 and Mero-41, this IFP occurred in concentrations comparable to those of the cytokeratins. Two-dimensional gel electrophoresis (Fig. 4 a, g, and j) confirmed these findings.

The individual protein bands were identified using the Western blotting technique in combination with the specific monoclonal antibodies. In all three cell lines, cytokeratins 18, 8, 7, and 19 as well as vimentin were detected with the antibodies.
Figure 4  Two-dimensional gel electrophoresis (a, g, j) and immunoblotting assays of these gels (b–f, h, i, k, l) of cytoskeletal preparations from cell lines Mero-41 (a–f), Mero-14 (g–i), and Mero-25 (j–l). The protein blots were subsequently incubated with RCK106 (b, h, k), RCK102 (c, h, k), RCK102 (d, i, l), RV202 (e, i, l) and LP2K (f, i, l). Abbreviations: v: vimentin; a: actin; 7, 8, 18, and 19 indicate the corresponding cytokeratin polypeptides; NEpHGE, first-dimension nonequilibrium pH gel electrophoresis; SDS, second-dimension SDS polyacrylamide gel electrophoresis.

RCK106, RCK102, RCK105, LP2K, and RV202, respectively (see Fig. 3). RCK102 also reacted with a protein band migrating just below vimentin in the one-dimensional immunoblots of all three cell lines (Fig. 3, lanes 3). This antibody has been shown previously in immunoblotting studies on keratin preparations of human epidermis and from human squamous cell carcinomas to recognize cytokeratin 5 next to cytokeratin 8. Although the immunostained protein band migrates in the position of cytokeratin 5, two-dimensional immunoblotting could not confirm the occurrence of this cytokeratin polypeptide in the mesothelioma cell lines. Two-dimensional immunoblots, which were subsequently incubated with antibodies RCK106, RCK102, RCK105, RV202, and LP2K, confirmed the one-dimensional immunoblotting studies with respect to cytokeratins 18, 8, 7, and 19 (Fig. 4).

DISCUSSION

Three continuously growing cell lines have been derived in vitro from untreated malignant mesothelioma cells from three different patients. The malignant mesothelioma character of the three cases has been demonstrated cytologically, histologically and/or ultrastructurally.

The isolated cell lines show individual cellular morphology and growth charac-
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teristics. Cytomorphologic and ultrastructural studies showed that these cell lines have characteristics consistent with mesothelioma and definitely different from that of adenocarcinomas.

Cytogenetic studies demonstrated that the cell lines shared the same chromosomal aberrations as the primary tumor cells obtained from the patient material and that they thus represented in vitro proliferation of the same tumor. Although no chromosomal aberrations specific for malignant mesothelioma could be identified on the basis of this study, the nature of the abnormalities observed in the cell lines follow the same pattern of monosomy and marker formation as observed in a large series of mesothelioma patients that we have studied: two types of clonal changes were (a) most frequently a hypodiploid/hypotetraploid karyotype characterized by relative loss of chromosomes 4, 6, 13, and 22 or (b) a hyperdiploid karyotype with, among other changes, extra copies of chromosome 7 [A. Hagemeijer et al., manuscript in preparation]. Gibas et al. [6] investigated the karyotype of 14 patients with a malignant mesothelioma and suggested the nonrandom involvement of chromosomes 1, 2, 3, 6, 9, 11, 17, and 22 in this tumor. In the three malignant mesothelioma cell lines described in this study we found structural abnormalities of chromosomes 1, 3, 9, and 22 (Table 3). Other markers were only occasionally present in these cell lines and in other mesotheliomas studied. This may be due to the fact that patients Me-14, Me-41, and Me-25 had not received therapy at the time that the pleural effusions were collected. Two recent publications on cytogenetics of malignant mesothelioma also emphasize the absence of specific changes [23, 24]. Structural abnormalities of chromosome 3 reported by Popescu et al. [23] are consistent with our findings.

Lechner et al. [25] reported that asbestos treatment of normal mesothelial cell cultures induced hypodiploidy and selective loss of chromosomes 11 and 21. In cell lines Mero-14, Mero-25, and Mero-41, however, chromosomes 11 and 21 were present in numbers that might be expected in these cells.

Connell and Rheinwald [22] described the stimulating effect of EGF plus HC supplements on the proliferative capacity of normal mesothelial cells, whereas addition of EGF or HC only did not promote the growth of the normal mesothelial cells. In contrast to the observation in normal mesothelial cells, the growth of the cell lines Mero-14 and Mero-25 was found to be inhibited by the addition of EGF plus HC in similar concentrations. This negative effect appeared to be caused by HC, as the addition of HC only decreased the proliferation of Mero-14 and Mero-25, whereas the addition of EGF slightly increased their growth rate.

Extensive studies on intermediate filament protein expression support the assumption that Mero-14, Mero-25, and Mero-41 are derived from mesotheliomas and have retained this character. First of all, the detection of cytokeratins in all three cell lines proves their epithelial nature. The fact that the cell lines additionally express vimentin also seems to be in support of the mesothelial derivation because normal and malignant mesothelial cells have been shown to be able to coexpress cytokeratins and vimentin in vivo [8]. However, one should realize that many cell types initiate the synthesis of vimentin when brought into tissue culture [26].

Further proof of the mesothelial nature of the three cell lines has come from the study of their individual cytokeratin polypeptides. Blobel et al. [7] showed that mesotheliomas can contain cytokeratins 7, 8, 18, and 19 and occasionally some other cytokeratins. The presence of cytokeratin 5 especially in some of these tumors is noteworthy in this respect because normal mesothelial cells apparently do not express this cytokeratin polypeptide and contain only cytokeratins 7, 8, 18, and 19 [27–30]. Aside from our findings that the cell lines contain cytokeratins 7, 8, 18, and 19, based on immunofluorescence and immunoblotting tests with chain-specific monoclonal cytokeratin antibodies, we were not able to confirm the presence of a cyto-
keratin 5-like protein using two-dimensional gel electrophoresis and immunoblotting.

Connell and Rheinwald [22] found that during rapid growth, normal mesothelial cells reduce cytokeratin levels, elevate vimentin expression, and adopt a fibroblastoid shape. Reduction of the growth rate, on the other hand, would result in increase of the keratin content, decrease of vimentin, and an epithelial phenotype of the cells. In the malignant mesothelioma cell lines Mero-14, Mero-25, and Mero-41, keratin and vimentin expression appeared to be present during rapid growth as well as in confluent cultures. The cell line with the highest vimentin:keratin ratio (Mero-14) had the highest proliferation rate, fibroblastoid-shaped cells, and in transmission electron microscopy, no villi could be distinguished, in contrast to Mero-25 and Mero-41. The observed correlation between the vimentin:cytokeratin ratio, proliferation rate, and phenotype in normal mesothelial cells seems to hold true for malignant mesothelioma cell lines as well. However, in contrast to normal mesothelial cells, the vimentin/cytokeratin level and the phenotype of the investigated malignant mesothelioma cell lines appeared to be typical for each cell line and could not be altered by affecting the growth rate.

In summary, characterization of the cell lines support the assumption that Mero-14, Mero-25, and Mero-41 are derived from malignant mesotheliomas and have retained this character. They exhibit features of mesothelial cells such as ultrastructural organization and intermediate filament protein expression. Their response to growth factors is different from that of benign mesothelial cells, as is the highly abnormal karyotype, and the latter has been associated with malignant mesothelioma [6].

Establishment of tumor cell lines has notably extended the possibilities for cell biologic and genetic investigations of tumors. Malignant mesothelioma is a rare tumor for which viable tissue specimens are difficult to obtain, and the availability of characteristic cell lines is therefore particularly important. To our knowledge, only a few malignant mesothelioma cell lines have been described [31–33]. The use of such cell lines should promote research aiming at the study of the mechanism of oncogenesis of this particular cancer, and development of specific diagnostic tools, which are needed for better diagnosis and safer therapeutic decisions in the clinical management of patients presenting with pleural effusions.

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