A human uveal melanoma cell line (92-1) was established from a primary uveal melanoma, and has now been maintained in culture for over 2½ years. Light microscopy of the cultured cells demonstrated extremely pleomorphic cells with large prominent nucleoli. Cell proliferation was determined with a non-radioactive propidium-iodide assay and indicated an in vitro doubling time of approximately 58 hr. Furthermore, the cell line was characterized by cytogenetic analysis, electron microscopy, immunocytochemistry and Northern blotting for HLA and c-myc mRNA analysis. Cytogenetic analysis revealed numerical abnormalities of chromosome 8 and structural abnormalities of chromosome 6. By electron microscopy, different stages of melanosome development were observed. Immunocytochemical analysis demonstrated expression of the melanoma-associated antigen gp100. Expression analysis of HLA antigens revealed a very low level of, in particular, the HLA-B locus products, which could be induced by interferon-a or -y treatment. Likewise, Northern-blot experiments revealed decreased levels of HLA-B mRNA as compared with HLA-A. In addition, high levels of c-myc expression were observed. The phenotypic characteristics of the cultured cells indicate that we have established an uveal melanoma cell line. This now well-characterized uveal melanoma cell line can be used in future studies.

The first reported attempt to culture uveal-melanoma cells was made by Kirby (1929), who succeeded in growing macrophage-like cells from an uveal melanoma for a 10-day period. Most subsequent reports concerned relatively short-term studies (Weitzmann, 1939; Vrabec, 1948; Barishak et al., 1960), while the development of continuous cell lines of uveal melanoma remained elusive (Vrba, 1974; Felberg et al., 1982). Albert et al. (1982) described the establishment of 6 continuous cell lines derived from choroidal and ciliary-body melanomas. Since that time, other uveal-melanoma cell lines have been developed (Kan-Mitchell et al., 1989). However, detailed reports about the establishment and characterization of uveal-melanoma cell lines are still scarce. We here describe the establishment and characteristics of such a cell line. In order to be able to use it in experiments on cell differentiation and in immunological studies, we studied cell proliferation, morphology, cytogenetics, and the expression of immunologically relevant antigens such as HLA-class-I and -II molecules and integrins.

MATERIAL AND METHODS

Clinical and histopathologic findings

The patient was a 76-year-old woman referred to the Department of Ophthalmology of the University Hospital Leiden, with a large tumor in the right orbit. She had no previous history of malignancy. On examination, the visual acuity of the right eye was 0 and the best corrected visual acuity of the left eye was 0.3. The right eyeball had been displaced superonasally by a large tumor, and vessel growth was observed throughout the eyeball. With exception of a cortical cataract, the left eye showed no abnormalities. Computed tomography of the right orbit revealed a small and deformed eyeball with tumor outgrowth into orbital structures. The tumor mass included the optic nerve, the inferior straight muscle, the lateral straight muscle, and the superior straight muscle. Histologic examination of tumor biopsies revealed a poorly differentiated malignant tumor. Immunohistochemistry of paraffin-embedded biopsy material revealed reactivity with monoclonal antibodies (MAbs) against the melanoma-associated antigen gp100 (HMB-45) and against vimentin. These findings strongly suggested the presence of a malignant melanoma. In order to achieve debulking of the tumor, orbital exenteration was performed.

Immediately after exenteration, the tumor was dissected. One part of the tumor was prepared for primary culture as described below, a second part was snap-frozen and stored at −70°C until sectioning for immunohistochemistry. The remainder of the tumor was processed for histopathological examination (paraffin-embedded tissue sections stained with hematoxylin and eosin). Histopathology and immunohistochemistry with MAbS NKI-betab and HMB-45 revealed a malignant melanoma consisting of epithelioid cells, most probably originating from the uvea, with extensive extra-bulbar growth.

Patient HLA typing was performed on peripheral-blood lymphocytes and revealed the presence of the following alleles: A2, A3, B44 (Bw4), and B51 (Bw4). Class-II antigens were not determined.

Culture method

Feeder layer. The mouse fibroblast strain, 3T3, was used as a feeder layer for primary culture of the melanoma cells. The 3T3 cells were maintained in culture in Ham’s F10 medium supplemented with penicillin-streptomycin (100 IU/ml and 100 μg/ml, Gibco, Paisley, UK), L-glutamine (1 mM, Gibco) and fetal bovine serum (FBS, 10%, HyClone, UT) at 37°C in a humidified incubator with 5% CO2. Three hours before the onset of the primary culture, the 3T3 cells were detached by a 0.01% trypsin solution, counted, re-suspended in culture medium and irradiated with a total dose of 3,000 rad (cobalt-60 source). Thereafter, the 3T3 cells were inoculated into the Petri dishes (1 x 105/cm2), which were placed in an incubator in order to allow attachment to the plastic surface before the melanoma cells were added.

Preparation of a single-cell suspension. The tumor tissue (± 4 mm3) was suspended in complete culture medium (described below) and minced with scalpels to a fine suspension. Thereafter the suspension was repeatedly forced through a 21-gauge needle attached to a syringe. The resulting suspension was centrifuged at 180g for 5 min at room temperature, after which the cells were re-suspended in fresh culture medium and inoculated into 4 Petri dishes (6 cm, TC, Greiner, Alphen a/d Rijn, The Netherlands) containing a feeder layer (see above). The dishes were placed at 37°C in a humidified incubator with 5% CO2.

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Culture medium. For growing melanoma cells, RPMI 1640 (GIBCO) was supplemented with penicillin-streptomycin (100 IU/ml and 100 μg/ml), L-glutamine (3 mM), and FBS (10%). The complete growth medium was renewed twice weekly.

Characterization of the cell line

Cell proliferation. For proliferation experiments, 1.25 × 10⁵ tumor cells were seeded in 4 ml of complete growth medium in 6-cm Petri dishes and allowed to attach over a 24-hr period. The cultures were then renewed once with fresh culture medium. Between day 2 and day 9 after plating, the attached and detached cells from triplicate cultures were harvested and stored at 4°C until use in the proliferation assay at day 10. Cell proliferation was determined in a non-radioactive propidium-iodide assay (PI assay), as described by Bruning and Kardol, (1983), based upon the measurement of total nuclear DNA.

Cell morphology. Cells were examined and repeatedly photographed as they grew in vitro by using an inverted microscope (Axiovert 10, Zeiss, Jena, Germany), equipped with phase-contrast lenses. Since it is difficult to characterize the morphology of cultured cells according to the Callender classification, more descriptive names were used, i.e., uni-, bi-, tri- and multipolar.

For transmission-electron-microscopic examination, cultured cells were fixed in 1.0% glutaraldehyde in 0.14 M cacodylate buffer (pH 7.4, 300 mOsmol) for 30 min at room temperature and then scraped from the plastic. The cells were pelleted, washed twice by re-suspension in phosphate-buffered Ringer solution, and post-fixed in 1% osmium tetroxide in phosphate buffer (pH 7.3, 330 mOsmol) for 30 min at 4°C. After rinsing, the cells were pelleted in 2% baco-agar at 60°C and dehydrated in a graded series of ethanol. The cells were then embedded in epoxy resin (LadK, Ladd, Burlington, VT) and polymerized for 60 hr at 60°C. Ultra-thin sections (60 nm) were cut on an ultramicrotome (Reichert Om U3, Vienna, Austria), collected on copper grids, stained with uranyl acetate and lead hydroxide and examined in a Philips (Eindhoven, The Netherlands) EM 410 LS transmission-electron microscope.

Cytogenetics. After several passages, cells were cultured on 24 × 24-mm coverslips in RPMI-1640 medium supplemented with 10% FBS. In order to obtain good-quality chromosome preparations, synchronization was obtained (Webber and Garson, 1983). Routine GTG-banding was performed according to standard techniques (Seabright, 1971).

Due to the complexity and the large number of structural aberrations that have already been described for ocular tumors, we also utilized fluorescence in situ hybridization (FISH) with chromosome-specific library probes and centromere-specific probes for accurate analysis, as described in Kluin-Nellemans et al. (1994). The probes were labeled by nick-translation with Biotin-16-dUTP (Boehringer, Ingelheim am Rhein, Germany). The chromosome-specific library probes were pre-annealed with 50-fold human cot-1-DNA to suppress non-unique sequences. Slides were denatured separately from the probe at 80°C. Hybridization was performed at 37°C for 48 hr, after which the slides were washed consecutively with 50% formamide/2 × SSC at 42°C followed by 0.2 × SSC at 60°C, and finally with 4 × SSC with 0.05% Tween-20. Visualization of the hybridized probe was made possible by using fluorescein-isothiocyanate (FITC)-conjugated avidin (Vector, Peterborough, UK). The signals were amplified with biotinilated goat anti-avidin by using another layer of FITC-avidin. The slides were then embedded in Vectashield anti-fade medium (Vector), with 4,6-diamidino-2-phenylindole dihydrochloride (DAPI), or propidium iodide as counterstain.

Slides were examined with a Zeiss Axioskop-H fluorescence microscope fitted with a triple pass filter.

Antigen expression. In determining the expression of different molecules (HLA antigens, melanoma-associated antigens, adhesion molecules, c-myc) the following techniques were applied: (i) immunocytochemistry. A 3-step standard indirect immunoperoxidase technique (Ten Berge et al., 1993) was used for determining the expression of HLA antigens and intracellular melanoma-associated antigens on frozen sections of the original tumor and on cytopsots. After harvesting of the cultured cells by trypsinization, cells were attached to glass slides using a Shandon cytocentrifuge. These cytopsin preparations were then air-dried and fixed for 10 min in acetone at room temperature and stored at −70°C until required for staining. All slides were examined by light microscopy, magnification ×400. Immunoreactivity was scored by counting the number of positive cells out of a total of 500 cells in each cytopsot. Intensity of the staining was coded as follows: −, no positive cells; +, majority of positive cells staining weakly; ++, majority of positive cells staining intermediately; ++++, majority of positive cells staining strongly. The MAbs used in our study are listed in Table I. (ii) Fluorescence-activated cell-sorter analysis (FACS analysis): this was performed in order to determine the expression of HLA antigens and adhesion molecules on the cell surface. After the cultured cells were harvested by trypsinization, they were washed in PBS; 0.5 to 1 × 10⁶ cells were then incubated with 100-μl dilutions of the

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**TABLE I - MAbs USED FOR IMMUNOHISTOLOGY AND FACS SCAN ANALYSIS**

<table>
<thead>
<tr>
<th>Specificity</th>
<th>Antibody</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLA antigens</td>
<td>HLA-A,B,C</td>
<td>W6/32</td>
</tr>
<tr>
<td>HLA-Bw4</td>
<td>GAP-A3</td>
<td>114/5/28</td>
</tr>
<tr>
<td>HLA-A2, Aw69</td>
<td>B7 T2</td>
<td>Purham and Brodsky, 1981</td>
</tr>
<tr>
<td>HLA-A3</td>
<td>SFR-8B6</td>
<td>Gerber et al., 1982</td>
</tr>
<tr>
<td>HLA-Bw6</td>
<td>B8.11.2</td>
<td>Gelishower, Sheffield, UK</td>
</tr>
<tr>
<td>HLA-DQ</td>
<td>SPV-L3</td>
<td>Radka et al., 1982</td>
</tr>
<tr>
<td>HLA-DP</td>
<td>B7/21.2</td>
<td>Rebai et al., 1983</td>
</tr>
<tr>
<td>Melanoma-associated antigens</td>
<td></td>
<td>Vennegoor et al., 1988</td>
</tr>
<tr>
<td>100/7 kDa</td>
<td>HMB-45</td>
<td>Gown et al., 1986</td>
</tr>
<tr>
<td>Adhesion molecule</td>
<td>ICAM-1</td>
<td>CL-203</td>
</tr>
<tr>
<td>CD44</td>
<td>NKI-P2</td>
<td>Pals et al., 1989</td>
</tr>
<tr>
<td>Integrin sub-units</td>
<td>TS2/7</td>
<td>Humler et al., 1984</td>
</tr>
<tr>
<td>CD1</td>
<td>A1.43</td>
<td>Bröcker et al., 1985; Klein et al., 1991</td>
</tr>
<tr>
<td>CD2</td>
<td>J143</td>
<td>Santos et al., 1989</td>
</tr>
<tr>
<td>CD4</td>
<td>HP2/1</td>
<td>Sanchez-Madrid et al., 1986</td>
</tr>
<tr>
<td>CD5</td>
<td>NKI-SAM1</td>
<td>Van de Wiel-Van Kemenade et al., 1992</td>
</tr>
<tr>
<td>CD6</td>
<td>MT78</td>
<td>Klein et al., 1990</td>
</tr>
<tr>
<td>CD7</td>
<td>13C2</td>
<td>Davies et al., 1989</td>
</tr>
<tr>
<td>CD8</td>
<td>4B4</td>
<td>Morimoto et al., 1985</td>
</tr>
<tr>
<td>CD12</td>
<td>CLB54</td>
<td>Keizer et al., 1988</td>
</tr>
<tr>
<td>CD16</td>
<td>23C6</td>
<td>Davies et al., 1989</td>
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<tr>
<td>CD19</td>
<td>3E1</td>
<td>Hessle et al., 1984</td>
</tr>
<tr>
<td>CD20</td>
<td>P1F6</td>
<td>Wayner et al., 1991</td>
</tr>
<tr>
<td>Controls</td>
<td>CD3</td>
<td>Leu4</td>
</tr>
<tr>
<td>HLA-A,B,C</td>
<td>W6/32</td>
<td>Barnstable et al., 1978</td>
</tr>
</tbody>
</table>
primary MABs in PBS/BSA 1% for 1 hr. After incubation with the primary antibody, the cells were washed in PBS/BSA 1% followed by incubation with a 1:50 dilution of FITC rabbit anti-mouse immunoglobulin (RAM/FITC; Dakopats, Gios­
trup, Denmark) for 30 min. Both incubations were performed on ice. After 2 more washes, the samples were stored in the dark at 4°C for one night. The fluorescence measurements were performed with a FACScan II (Becton Dickinson, San Jose, CA). Background fluorescence was determined by incubating the cells with a non-reactive primary antibody (anti-CD3) and RAM/FITC. All experiments were performed in duplicate dishes. The MABs are listed in Table I. (iii) Antigen modification by IFN-α and -γ. Since we observed a discrepancy between patient HLA typing and the expression of HLA-Bw4 on the cultured cells, we investigated whether expression of HLA-Bw4 could be induced by treatment with interferon-alpha or -gamma. Uveal-melanoma cells were seeded in 6-well plates in numbers of 3 × 10⁶/well in a total volume of 1 ml of standard medium. Twenty-four hours after seeding, the cells were incubated with rIFN-α or rIFN-γ at concentrations of 50, 200, and 500 IU/ml. Control cultures consisted of cells incubated with culture medium only. After 2 days of incubation, the cells were harvested by trypsinization, and cytospin preparations were made. Staining and scoring of the cytospots were performed as described above. Recombinant human interferon-alpha-2b, rIFN-α (specific activity 1.66 × 10⁶ IU/mg protein) was kindly supplied by Schering Plough (Amstelveen, The Netherlands). Recombinant human interferon gamma, rIFN-γ (specific activity 2 × 10⁸ IU/mg protein) was kindly provided by Dr. S. Osanto (Leiden University Hospital, The Netherlands). (iv) Northern blotting and hybridizations. Northern blotting was used in order to determine the level of expression of the HLA-A and B loci, and the oncogene c-myc. Skin-melanoma cell lines IGR39D and 634 were used as reference. Isolation of HLA-A and B loci, and the oncogene c-myc. Skin-melanoma cells were cultured in 24-well plates in numbers of 3 × 10⁶/well in a total volume of 1.5 ml of standard culture conditions. After 10 weeks, only one Petri dish was left, due to detachment of the tumor cells in the other culture. Because of the low cell density, cells were transferred to a smaller, 6-well plate. From that time (4 months after plating), the cells started to grow; after 8 months the cells had been transferred 8 times, and some development were present (Fig. 3b). Application of FISH using centromere- and chromosome-specific DNA probes not only identified all of the derivative metaphase chromosomes but also the chromosome-8 aneu-

**RESULTS**

**Growth of melanoma cells**

For primary culture of the uveal-melanoma cells, a cell suspension was prepared from fresh tissue of the exenterated orbital tumor and inoculated onto a feeder layer. Two weeks after initial plating, uveal-melanoma cells covered 40% and 50% of the surface of 2 out of the 4 original Petri dishes. The first culture passage took place after 4 weeks, when the melanoma cells covered approximately 80% of the Petri dishes. After 10 weeks, only one Petri dish was left, due to detachment of the tumor cells in the other culture. Because of the low cell density, cells were transferred to a smaller, 6-well plate. From that time (4 months after plating), the cells started to grow; after 8 months the cells had been transferred 8 times, and some sub-cultures were frozen and stored. Transfers of cells were undertaken at 1:2 dilution. In the following 4 months, we lost all subsequent sub-cultures due to infection, and exactly one year after initial plating we re-started the culture with frozen cells from the seventh transfer. Presently, 2½ years after initial plating, the uveal-melanoma cells have been passaged approximately 45 times, with transfers at 1:3 or 1:4 dilution.

**Growth rate**

The growth rate stabilized after approximately 10 passages. Cultured cells from passages 30 to 40 were used in the proliferation experiments. The results are shown in Figure 1. From the data obtained, a growth curve was determined by exponential regression. The in vitro doubling time as determined from these regression data was 38 hr.

**Cell morphology**

Light microscopy revealed attachment of cells at day one after initial plating. In the first passage, the majority of cells were bipolar, but tripolar and multipolar (dendritic-like) cells were also observed. In the subsequent passages the relative number of multipolar cells increased, but after about the tenth passage no further changes in cell morphology occurred. In standard culture conditions the cells were extremely pleomorphic and had large prominent nucleoli which often occurred in multiple numbers. Multinucleated and giant cells were also observed, although usually in small numbers. A photomicrograph of the cultured cells is shown in Figure 2.

Electron microscopy of the cultured cells showed high nuclear/cytoplasmic ratios, large prominent nucleoli, and diffuse deposition of chromatin throughout the nucleus (Fig. 3a). The melanocytic origin of the cultured cells was confirmed by the presence of (pre)melanosomes in the cytoplasm using electron microscopy. Different stages of melanosome development were present (Fig. 3b).

**Cytogenetics**

Analysis of GTG-banded metaphases of the 92-1 tumor cells revealed a complex karyotype. The modal chromosome number of the cultured cells was 47. Numerical abnormalities of chromosome 8 (tetrasomy 8) and structural abnormalities of chromosome 6 (tetrasomy 6p, with translocations to a sex chromosome and to chromosome 17) were present. Monosomy of chromosome 3, which has been reported for uveal melano-

**Figure 1** — Growth rate of the cultured 92-1 cells as determined in a non-radioactive propidium-iodide assay (see "Material and Methods"). The photometer values represent the total amount of nuclear DNA. From the obtained data (♦) a growth curve was determined by exponential regression (dotted line).
plody within the interphase nuclei of frozen section material of the original tumor.

Antigen expression

*HLA expression.* Both immunocytochemistry and FACS analysis of the cultured 92-1 melanoma cells revealed expression of the monomorphic HLA-class-I backbone determinant recognized by MAb W6/32 and β2-microglobulin. Expression of the HLA-class-II determinants DR, DP, and DQ was not observed. MAbs directed against the polymorphic HLA-class-I determinants A2, A3, Bw4, and Bw6 were applied. Not all cultured cells showed expression of HLA-A2 (approximately 70% of the cells were positive), while HLA-A3 was expressed on more than 95% of the cells. In agreement with the patient's HLA-typing, the cultured tumor cells were negative for HLA-Bw6. Although HLA typing had shown that the patient was positive for HLA-Bw4, only very low expression of HLA-Bw4 antigens was observed. HLA-Bw4 expression could be induced by incubating the cell cultures for 2 days with IFN-α or -γ (Fig. 4).

In order to compare HLA-antigen expression *in vitro* with expression *in vivo*, HLA-antigen expression was determined on frozen tumor-tissue sections obtained from the original tumor. Similarly to the cultured cells, the tumor tissue showed hardly any expression of HLA-Bw4, whereas the tumor vessels were positive. The other HLA-class-I antigens showed no obvious differences between *in vivo* and *in vitro* expression (data not shown).

*HLA-class-I expression and c-myc oncogene.* In order to confirm the low level of expression of the HLA-B products, Northern-blot analysis was performed. Since locus-specific downregulation of HLA-B products by the c-myc oncogene in skin melanoma had been reported (Versteeg *et al.*, 1989), we studied the level of c-myc mRNA as well. The Northern blot, hybridized with a locus-specific HLA-A and HLA-B probe, showed that the 92-1 cells had intermediate levels of both HLA-A and HLA-B mRNA, when compared with the 2 cutaneous-melanoma cell lines 634 and IGR39D (Fig. 5; lanes 2, 1 and 3 respectively). The cell line IGR39D is known to express high levels both of HLA-A and of HLA-B mRNA. By comparing the amounts of mRNA in cell line 92-1 with the amounts of mRNA in IGR39D (visually and by densitometry), we concluded that the level of HLA-B mRNA in cell line 92-1 was lower than that of HLA-A. Of special interest is the expression of c-myc mRNA. A comparison of the amounts of c-myc mRNA and of HLA-B mRNA in the 3 cell lines demonstrated an inverse correlation of these 2 parameters (Fig. 5).

*Expression of melanoma-associated antigens.* Expression of intracellular melanoma-associated antigens was determined by immunocytochemistry. Staining with MAbs NKI/beteb and HMB-45 showed that all cultured cells were positive for these melanocyte-specific antigens (data not shown).

*Expression of adhesion molecules.* Expression of adhesion molecules on the cell surface was determined by FACS analysis. MAbs that recognized various integrin sub-units and the adhesion molecules ICAM-1 and CD44 were used (Table 1). On the cultured cells we observed expression of the integrin sub-units α2, αv, β1 and αvβ5, but not of α1, α3–α6, β2, αvβ3 and β4. The 92-1 cells were strongly positive for the adhesion molecules ICAM-1 and CD44. Expression of these antigens was also determined on frozen tumor-tissue sections. Some
differences between tumor tissue and cultured cells were observed (Table II). In contrast to the cultured cells, α2 expression was not observed on tumor tissue, whereas the tissue did express the α3 and α5 sub-units.

DISCUSSION

The successful development of continuous cell lines of uveal melanomas has only sporadically been described (Albert et al., 1984; Kan-Mitchell et al., 1989), which is quite different from the results with cutaneous melanoma. The reason for this discrepancy is unknown, but it suggests differences in culture requirements between these tumors. In our laboratory, we attempted to culture uveal-melanoma cells from 12 freshly derived tumors over a 1/2-year period. Additionally, we used frozen tumor tissue in order to establish *in vitro* growth of uveal-melanoma cells. Most of these attempts resulted in short-term cultures (data not shown), but as we have stated above, one of the cultures has survived *in vitro* for over 2½ years. However, the exceptional clinical appearance of the original tumor of this particular culture, with massive orbital extension, should be mentioned. Clinical and histopathological features strongly suggested that the tumor of origin was a primary intra-ocular malignant melanoma. Immunohistochemistry and histopathology revealed a malignant melanoma. Clinically, no evidence for a primary cutaneous melanoma was found. The assumption that we were dealing with a tumor of intra-ocular origin was based in particular on the histopathologic findings. The almost total destruction of the eyeball argued against conjunctival origin, since conjunctival melanomas rarely invade the eyeball, especially not the posterior segment.

The deduction that the cultured cells described in the present study are indeed melanoma cells is based on several growth and phenotypical characteristics. In the first place, all cultured cells showed high levels of expression of the melanocyte-lineage-specific antigens recognized by the MAbs NKI-beteb and HMB-45, indicating that the cultured cells are of melanocytic origin. Since normal skin or uveal melanocytes would not grow *in vitro* in a culture medium such as used in the present study without the addition of mitogens and growth factors (Hu et al., 1993), the cultured cells are most probably malignant melanocytes. In addition, the cultured cells are morphologically neoplastic and have malignant growth properties, i.e., they are pleomorphic, have large prominent nucleioli, and do not exhibit contact inhibition. Furthermore, cytogenetic analysis of the cultured cells revealed numerical and structural chromosomal abnormalities. Of particular interest are the observed abnormalities of chromosomes 3 and 8, since previous cytogenetic reports of uveal melanomas have identified numerous chromosomal abnormalities, with the chromosomes 3, 6 and 8 being most frequently involved (Prescher et al., 1990; Sisley et al., 1992). In cutaneous melanoma on the other hand, the chromosomes 1, 6 and 7 especially show cytogenetic abnormalities (Fountain et al., 1990; Schrier, 1992). The present cytogenetic findings support therefore an uveal-melanoma origin of the cultured cells. In addition, the absence of monosomy of chromosome 3 suggests that the tumor originated from the choroid and not from the ciliary body (Sisley et al., 1992).

In light microscopic examination, it is difficult to characterize the morphology of the cultured cells according to the Callender classification. However, when the morphology of the cultured cells in the present study is compared with that of cultured melanoma cells as described by others (Felberg et al., 1982; Albert et al., 1984), the present culture can best be described as spindle and epitheloid cells. That the tumor was defined by histology as an epitheloid cell tumor does not mean that the cultured cells have to be epitheloid cells as well, since the establishment of uveal melanomas in tissue culture can be
Percentage positive tumor cells in cryostat sections of primary skin melanoma and skin-melanoma metastases. Findings by Versteeg et al. (Lond.) 1986, 109, 1817-1826 (1989). The absence of αβ3 vitronectin receptor is of particular interest, since in advanced primary cutaneous melanomas and metastases αβ3 is expressed, whereas in primary uveal melanoma expression is absent. Additionally, the absence of α2 and the presence of α5 expression on the tumor-tissue sections also suggests that the tumor of origin is a primary uveal melanoma rather than a cutaneous melanoma.

From all of the above-mentioned findings we deduce that the cultured cells are indeed uveal melanoma cells. Since the 92-1 cells have been in culture for over 2½ years without senescence, the culture has most probably evolved into a real cell line. Uveal melanoma cell lines may be used in future studies regarding the genetic basis of this tumor, and as a model to test different approaches for immunotherapy. A more detailed study of the presence of melanoma-associated antigens, which might be useful as targets for immunotherapy, and the sensitivity to lysis by cytotoxic cells is in progress.

ACKNOWLEDGEMENTS

We thank Mr. S. van der Burg (Immunohematology and Bloodbank, University Hospital Leiden) for kindly supplying the MAbs against the HLA antigens. We thank Drs. S. Ferrone, C. Figdor, E. Klein and M. Horton for providing the anti-adhesion-molecule antibodies. We are also indebted to Dr. J. van der Meulen (Department of Electron Microscopy, University Hospital Leiden) for performing the transmission-electron microscopy, to Dr. J.L. van Delft for technical assistance and Dr. D. de Wolff-Rouendaal for histopathologic evaluation. We are grateful to Ms. G. Bouma (Immunohematology and Bloodbank, University Hospital Leiden) for assistance in the propidium-iodide proliferation assay, to Mr. W.M. Creyghton for assistance in the FACS experiments, and to Ms. K. Noz and Ms. C. Out (Department of Dermatology, University Hospital Leiden) for kindly providing the 3T3 feeder cells, together with advice on the culture method.

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