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# ESTABLISHMENT AND CHARACTERIZATION OF AN UVEAL-MELANOMA CELL LINE

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A human uveal melanoma cell line (92-1) was established from a primary uveal melanoma, and has now been maintained in culture for over  $2\frac{1}{2}$  years. Light microscopy of the cultured cells demonstrated extremely pleiomorphic 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 melanomaassociated 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- $\alpha$  or - $\gamma$  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 wellcharacterized uveal melanoma cell line can be used in future studies. © 1995 Wiley-Liss, Inc.

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-beteb and HMB-45 revealed a malignant melanoma consisting of epitheloid 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.

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 uvealmelanoma 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

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  $\mu$ g/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% CO<sub>2</sub>. 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 × 10<sup>4</sup>/cm<sup>2</sup>), 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 ( $\pm 4$  mm<sup>3</sup>) 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 180 g 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% CO<sub>2</sub>.

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 superotemporally 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

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Culture medium. For growing melanoma cells, RPMI 1640 (GIBCO) was supplemented with penicillin-streptomycin (100 IU/ml and 100  $\mu$ g/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,  $1.25 \times 10^5$ 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 propidiumiodide 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 phasecontrast 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 0.1% 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% bacto-agar at 60°C and dehydrated in a graded series of ethanol. The cells were then embedded in epoxy resin LX-112 (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  $24 \times 24$ -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 centromerespecific probes for accurate analysis, as described in Kluin-Nelemans et al. (1994). The probes were labelled by nicktranslation 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  $\times$  SSC at 42°C followed by 0.2  $\times$  SSC at 60°C, and finally with  $4 \times SSC$  with 0.05% Tween-20. Visualization of the hybridized probe was made possible by using fluoresceinisothiocyanate(FITC)-conjugated avidin (Vector, Peterborough, UK). The signals were amplified with biotinilated goat anti-avidin followed by 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.

hesion 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 cytospots. After harvesting of the cultured cells by trypsinization, cells were attached to glass slides using a Shandon cytocentrifuge. These cytospin 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 cytospot. 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 cellsorter 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  $1 \times 10^5$  cells were then incubated with 100-µl dilutions of the

TABLE I – MAbs USED FOR IMMUNOHISTOLOGY AND FACS SCAN ANALYSIS

Specificity	Antibody	Reference		
HLA antigens				
HLA-A,B,C,	W6/32	Barnstable <i>et al</i> ., 1978		
B2-microglobulin	BBM1	Brodsky et al., 1979		
β2-microglobulin HLA-A2, Aw69	BB7.2	Parham and Brodsky, 1981		
HLA-A3	GAP-A3	Berger et al., 1982		
HLA-Bw4	116/5/28	Gelsthorpe, Shef- field, UK		
HLA-Bw6	SFR-8-B6	Radka et al., 1982		
HLA-DR	B8.11.2	Rebai et al., 1983		
HLA-DQ	SPV-L3	Spits et al., 1984		
HLA-DP	B7/21.2	Watson et al., 1983		
Melanoma-associ- ated antigens		a a construction of the second groups of the second s		
100/7 kDa	NKI/beteb	Vennegoor <i>et al.</i> , 1988		
100/7 kDa Adhesion mol-	HMB-45	Gown et al., 1986		
ecules				
ICAM-1	CL.203	Maio et al., 1989		
CD44	NKI-P2	Pals <i>et al.</i> , 1989		
Integrin sub-units	1 J 1 1 1 2	1 dið Cl dl., 1909		
$\alpha 1$	TS2/7	Hemler et al., 1984		
α1 α2	A1.43	Bröcker <i>et al.</i> , 1985; Klein <i>et al.</i> , 1991		
α3	J143	Santoso et al., 1989		
α4	HP2/1	Sanchez-Madrid et al., 1986		
α5	NKI-SAM1	Van de Wiel-Van Kemenade <i>et al.</i> , 1992		
α6	MT78	Klein et al., 1990		
αv	13C2	Davies et al., 1989		
β1	4B4	Morimoto <i>et al.</i> , 1985		
β2	CLB54	Keizer et al., 1988		
ανβ3	23C6	Davies et al., 1989		
β4	3E1	Hessle et al., 1984		
ανβ3 β4 ανβ5	P1F6	Wayner <i>et al.</i> , 1991		
Controls				
CD3	Leu4	Ledbetter <i>et al.</i> , 1981		
HLA-A,B,C	W6/32	Barnstable <i>et al.</i> , 1978		

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, ad-

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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; Dakopatts, Glostrup, 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- $\alpha$  and - $\gamma$ . 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 interferonalpha or -gamma. Uveal-melanoma cells were seeded in 6-well plates in numbers of  $3 \times 10^5$ /well in a total volume of 1.5 ml of standard medium. Twenty-four hours after seeding, cells were incubated with rIFN- $\alpha$  or rIFN- $\gamma$  at concentrations of 50, 200, and 500 IU/ml. Control cultures consisted of cells incubated without cytokines. 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- $\alpha$  (specific activity 1.66  $\times$  10<sup>8</sup> IU/mg protein) was kindly supplied by Schering Plough (Amstelveen, The Netherlands). Recombinant human interferon gamma, rIFN-y (specific activity  $2 \times 10^7$  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 total RNA, Northern blotting, preparation and radiolabelling of the probes was carried out by standard techniques (Versteeg et al., 1989). Hybridizations were performed with probes radiolabeled by the random-primer method (Feinberg and Vogelstein, 1983). The following probes were used: c-myc, a ClaI/EcoRI fragment of a human genomic clone spanning most of exon 3 (Colby et al., 1983); class-I-HLA-A locus: a 1.3-kb PvuII/XbaI fragment of a genomic A2 clone, spanning the 3'-untranslated region (Koller et al., 1984); class-I-HLA-B locus: a 358-bp PstI/PvuII fragment of a B8 cDNA clone, spanning the 3'-untranslated region (Koller et al., 1984). The cDNA of human elongation factor (HEF) was used as a control probe (Peltenburg et al., 1993). All hybridizations were performed at 62.5°C.

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 58 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 pleiomorphic 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 (Figure 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).

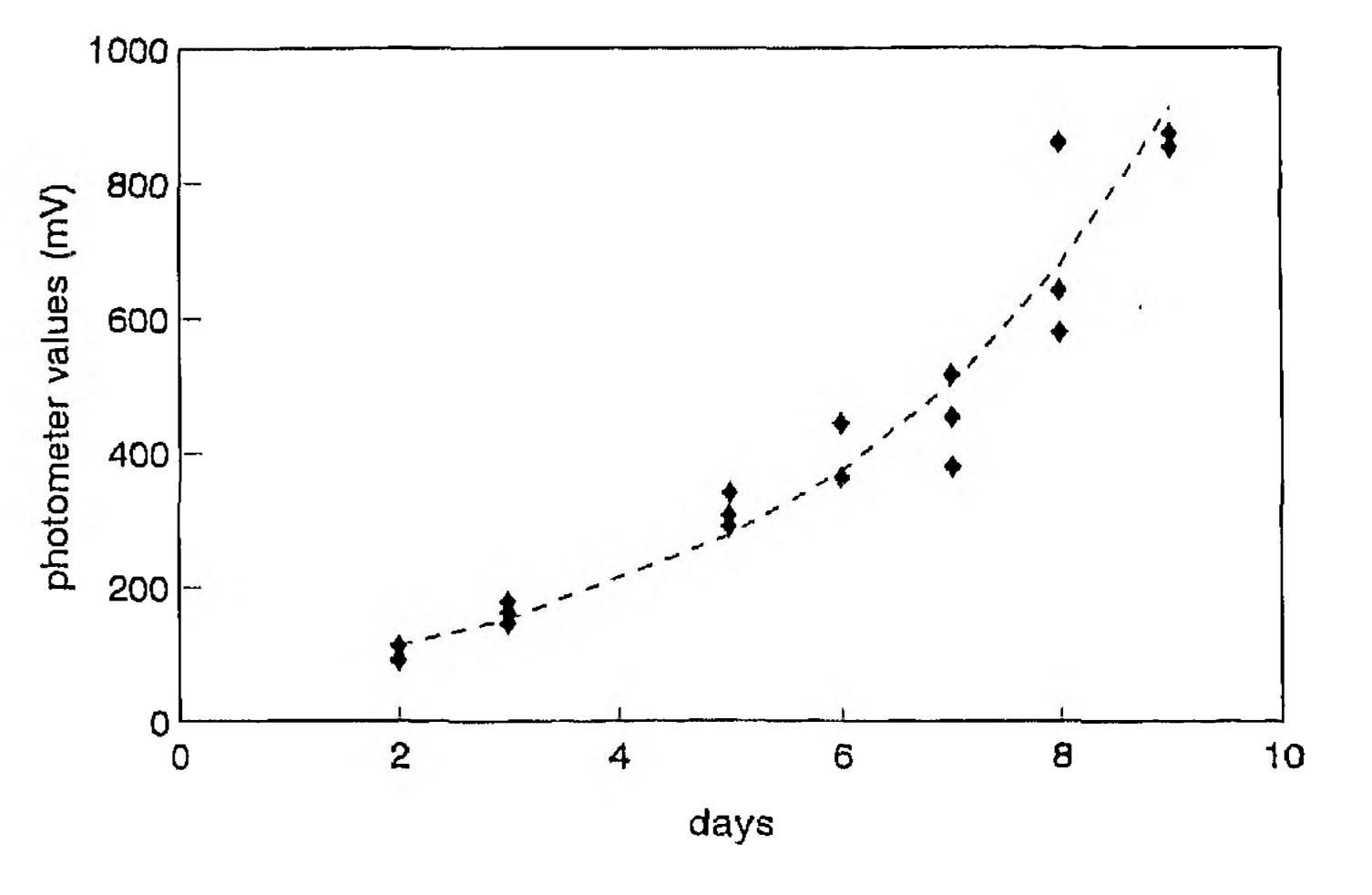
### Cytogenetics

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 ocular melanoma (Horsman and White, 1993), was not found in the material analyzed. The complete karyotype of cell line 92-1 is: 47, -X, der(X) t(X;6)(q28;p11), +8, +8, der(17) t(6;17)(p11; 25). Application of FISH using centromere- and chromosomespecific DNA probes not only identified all of the derivative metaphase chromosomes but also the chromosome-8 aneu-

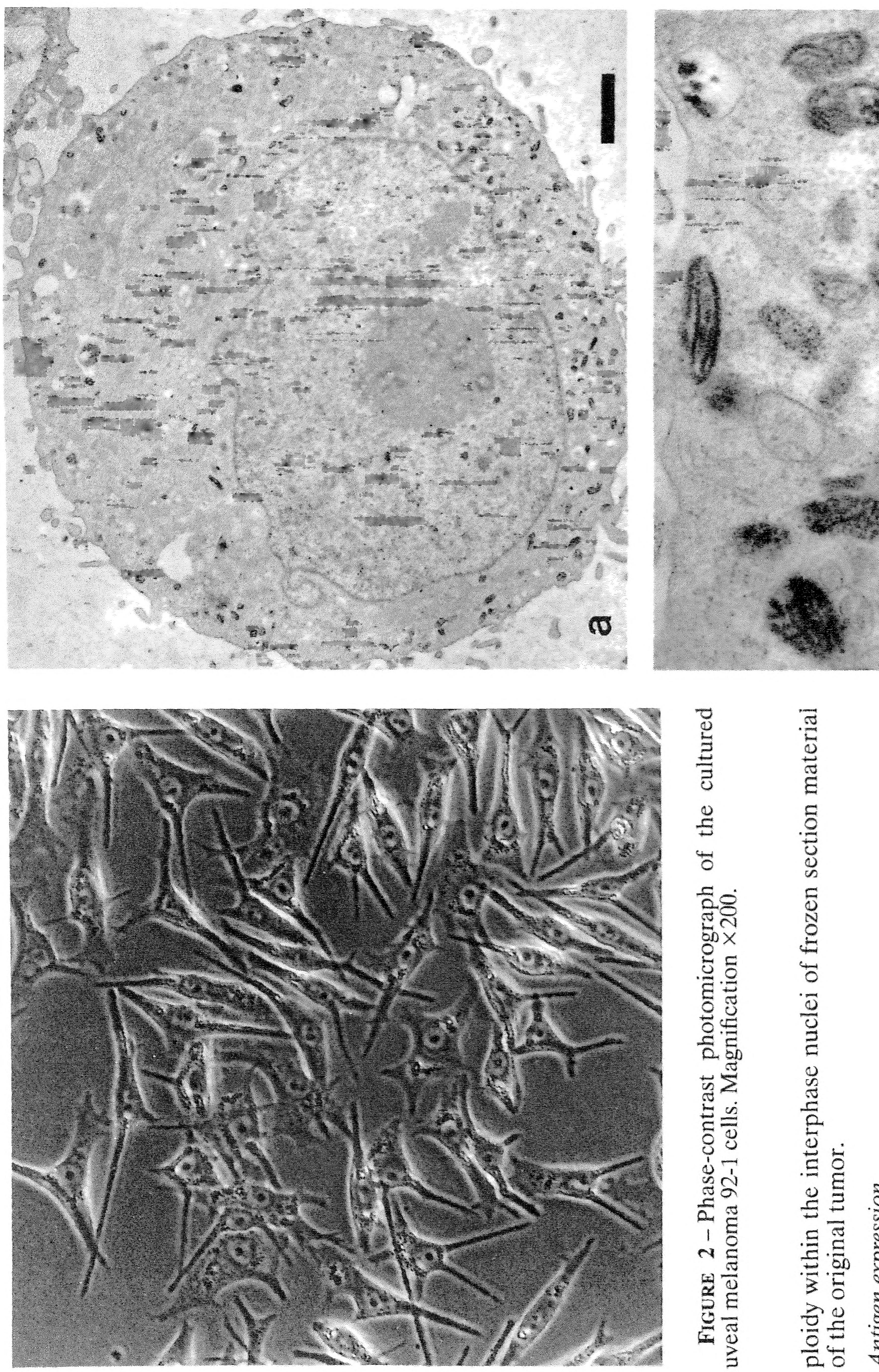
### RESULTS

### 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



**FIGURE 1** – Growth rate of the cultured 92-1 cells as determined in a non-radioative propidium-iodide assay (see "Material and Methods"). The photometer values represent the total amount of nuclear DNA. From the obtained data ( $\blacklozenge$ ) a growth curve was determined by exponential regression (dotted line).



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FIGURE 3 – Electron photomicrographs of 92-1 cells. (a) Culured cell showing a prominent nucleolus, diffuse nuclear chromain and cytoplasmic melanosomes (scale bar,  $2 \mu$ ). (b) An area of ytoplasm showing different stages of melanosome development scale bar,  $0.3 \mu$ ).

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*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  $\beta_2$ -microglobulin. Expression of the HLA-class-II determinants DR, DP, and DQ was not observed. MAbs directed against the polymorphic HLA-class-II determinants DR, DP, and DQ was not observed. MAbs directed against the polymorphic HLA-class-II determinants DR, DP, and DQ was not observed. MAbs directed against the polymorphic HLA-class-II determinants DR, DP, and DQ was not observed. MAbs directed against the polymorphic HLA-class-II determinants A2, A3, Bw4, and Bw6 were applied. Not all cultured cells showed expression of 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's 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- $\alpha$  or - $\gamma$  (Fig. 4). In order to compare HLA-antigen expression *in vitro* with expression *in vitro* with expression *in vitro* with expression *in vitro* for HLA-antigen expression *in vitro* with expression *in vitro* for the conducted by incubating the cell cultures for 2 days with IFN- $\alpha$  or - $\gamma$  (Fig. II).

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### 20 IFN-alpha IFN-gamma 100 9 \* 3 N + J + I + Y • • • • • • • • • • • • . . . . . . . . . . A DESCRIPTION OF A DESC 1 2 1 1 3 1 4 3 5 1 . 2 2 8 9 8 8 9 4 1 8 . . . . . . . . . . . 9 \* 2 \* 3 + 1 + 1 + . . . . . . . . . . . . . \* \* \* \* \* \* \* \* \* \* . . . . . . . . . • • • • • • • • • • • 6 / 4 / 5 / 1 / 1 / 1 . R S & L - E F E & P . 80 с × 1 и с 1 и .) у х \* · · · · · · · · · · · \*\*\*\*\*\*\*\*\*\* 5 F F F F F F F F F F F . 8 1 2 4 2 4 3 1 3 3 5 1 5 1 1 4 4 5 3 . \* \* \* \* \* \* \* \* \* \* г 5 5 к 5 г на 35 э 2 2 2 7 7 7 7 7 9 1 . . . . . . . . . . . 5 ) | F a a J x a y \* • • • • • • • • • , , , <sup>1</sup>, , , , , , , , , , , . 60 9 7 \* 3 3 3 7 × L , 1 1 1 1 1 1 1 5 1 5 7 F 5 F F F F F F F . 1 5 7 7 7 8 9 8 3 7 2 2 3 1 2 1 2 3 4 . . . . . . . . . . . \* \* \* \* \* \* \* \* \* \* \* \* \* \* \* \* \* \* \* . 40 2 4 7 4 7 1 2 8 7 # \* \* \* + + + > > + 1 4 1 4 5 1 7 4 5 5 . 9 5 5 7 J 1 5 F 1 7 - **7 1 3 7 3 1 5 5** 5 5 .

AN UVEAL-MELANOMA CELL LINE

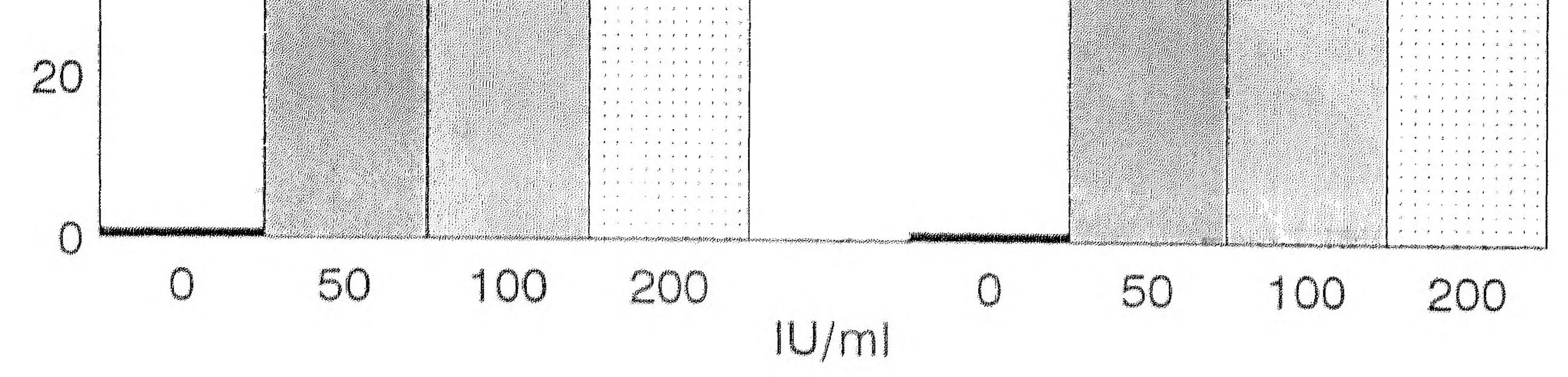
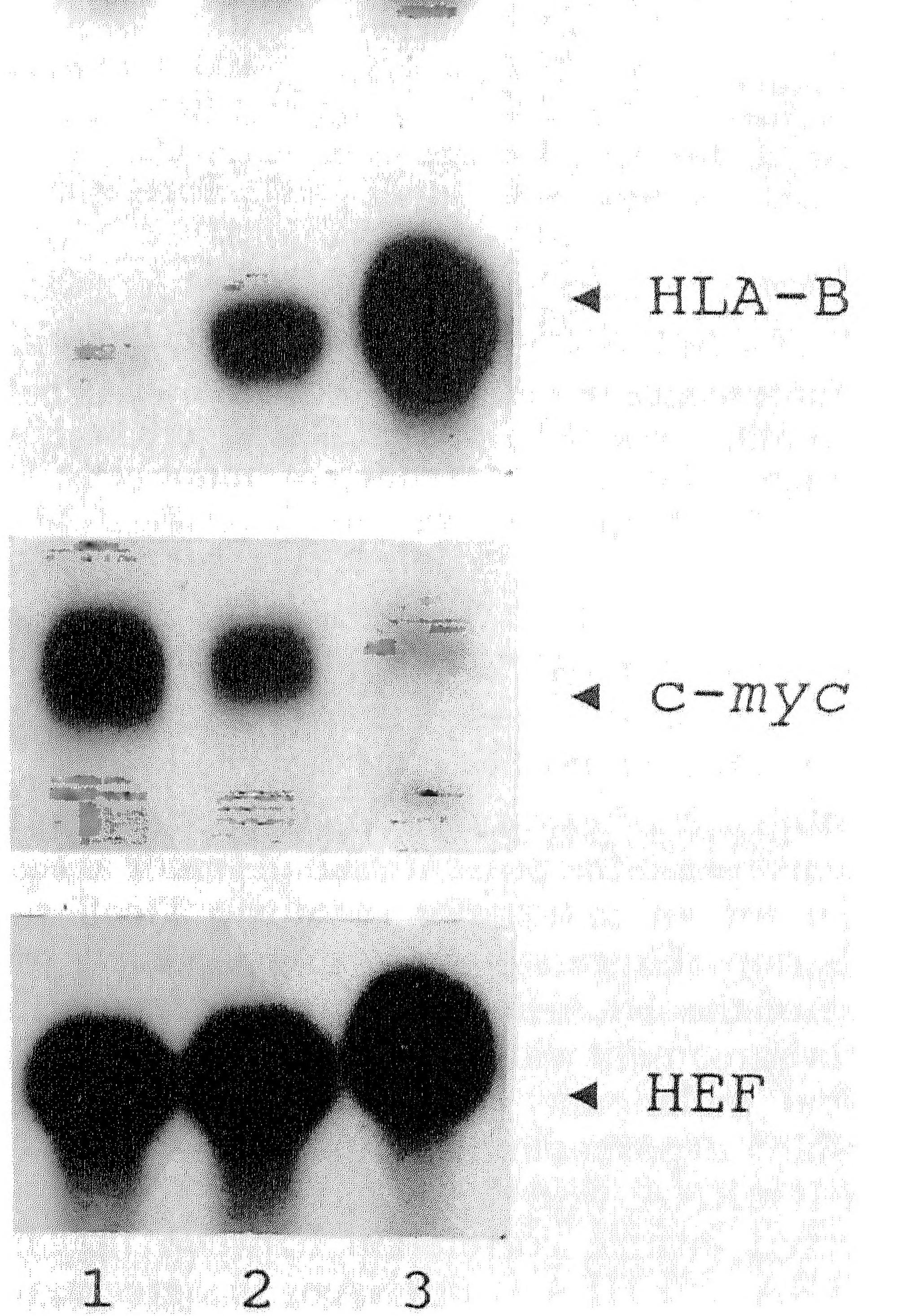


FIGURE 4 – Percentage of positive tumor cells (92-1) with a MAb against the HLA-Bw4 antigens (116/5/28) after 2 days of incuba-tion with 0, 50, 200 or 500 IU/ml interferon-alpha or -gamma.

differences between tumor tissue and cultured cells were observed (Table II). In contrast to the cultured cells, a2 expression was not observed on tumor tissue, whereas the tissue did express the  $\alpha 3$  and  $\alpha 5$  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



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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  $1\frac{1}{2}$ -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  $2\frac{1}{2}$ 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. structural chromosomal abnormalities. Of particular interest 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

FIGURE 5 - Northern analysis of RNA isolated from the cutaneous-melanoma cell lines 634 and IGR39D (lanes 1 and 3 respectively), and from the uveal-melanoma cell line 92-1 (lane 2). Hybridizations were performed with HLA-A, HLA-B, c-myc and human elongation factor (HEF) probes.

are the observed abnormalities of chromosomes 6 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

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 NKIbeteb 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 pleiomorphic, have large prominent nucleoli, and do not exhibit contact inhibition. Furthermore, cytogenetic analysis of the cultured cells revealed numerical and

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### TABLE II – INTEGRIN, CD44 AND ICAM-1 EXPRESSION ON UVEAL-MELANOMA CELLS IN VITRO AND IN VIVO

Antigen	Cultured cells MFI <sup>1</sup>	Cryostat sections	
		Positive cells%	Intensity <sup>3</sup>
Control	4.1	0	
α1	4.7	<5	-╂- ╺╂┑
$\alpha^2$	9.5	<1	+-
α2 α3	6.4	100	-++
$\alpha 4$	4.1	< 1	+
α4 α5	5.9	100	~~{~~
αб	4.8	< 1	╺╀╸
αν	24.2	100	+
	19.2	100	<b>∽╂</b> ╼╺ <b>┟</b> ╾
β1 β2	3.8	0	•
$\alpha v \beta 3$	3.9	0	
ανβ3 β4	4.4	0	
$\alpha v \beta 5$	10	1 <b>00</b>	-
$\alpha \nu \beta 5$ CD44	$6\bar{5}4.1$	100	┍ <del>┨╸</del> ╺ <del>┠╸</del>
ICAM-1	56.3	100	<b>-</b>

melanoma. We therefore studied the expression of integrins ICAM-1 and CD44 on the cultured cells as well as on the tumor of origin. The observed differences in integrin expression between tumor tissue and cultured cells are possibly due to the use of different techniques (immunohistochemistry vs. FACS analysis). On the other hand, these differences might represent real changes in expression due to environmental changes, *i.e.*, the *in vitro* culture. The observed pattern of expression of the integrin sub-units on the tumor tissue in the present study is in good agreement with the integrin expression on uveal melanomas in situ as described by Ten Berge et al. (1993). The absence of the  $\alpha\nu\beta\beta$  vitronectin receptor is of particular interest, since in advanced primary cutaneous melanomas and metastases  $\alpha v\beta 3$  is expressed, whereas in primary uveal melanoma expression is absent. Additionally, the absence of  $\alpha 2$  and the presence of  $\alpha 5$  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 21/2 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.

<sup>1</sup>Mean fluorescence intensity on FACS-analysis of cultured 92-1 cells.-<sup>2</sup>Percentage positive tumor cells in cryostat sections of tumor of origin.-<sup>3</sup>Intensity of staining of tumor cells: -, negative; +, positive; ++, strongly positive.

accompanied by changes in cell type (Felberg *et al.*, 1982). Electron microscopy revealed morphological characteristics belonging to either spindle or epitheloid cell types.

HLA-antigen expression on the cultured cells was studied because of its role in the presentation of tumor antigens, which is essential for an anti-tumor cytotoxic T-cell response in immunotherapy. Expression analysis revealed a low Icvel of expression of the HLA-B locus products in particular. This finding is in agreement with a previous study on frozen sections of 24 uveal melanomas (data not shown). Likewise, the Northern-blot experiments revealed differences in HLA-A and HLA-B mRNA, with decreased levels of HLA-B mRNA. The observed inverse correlation between the amounts of c-myc mRNA and HLA-B mRNA is in agreement with the findings by Versteeg et al. (1989) on cell lines derived from primary skin melanoma and skin-melanoma metastases. Adhesion molecules, such as integrins ICAM-1 and CD44, are involved in cell-cell and cell-matrix interactions, and are thought to be important in tumor progression and metastasis (Albelda, 1993). Ten Berge et al. (1993) reported differences in integrin expression between cutaneous melanoma and uveal

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