Uveal and Cutaneous Melanoma: Shared Expression Characteristics of Melanoma-Associated Antigens

Leonie C. van Dinten,¹ Nicolen Pul,¹ A. Frans van Nieuwpoort,² Coby J. Out,² Martine J. Jager,³ and Peter J. van den Elsen¹,⁴

PURPOSE. Downregulation of melanoma-associated antigens (MAAs), against which natural cytolytic T lymphocytes (CTLs) exist in humans, is one of the mechanisms that aids in evasion of immune surveillance. In view of putative re-expression strategies for MAAs during immunotherapy, this study was conducted to investigate MAA silencing in malignant melanoma.

METHODS. The expression of the MAA Melan-A/MART-1 was analyzed in 10 uveal and 10 cutaneous patient-derived melanoma cell lines by Western blot analysis and RT-PCR. Expression characteristics of four other MAAs—Tyr, Tyrp1, Dct, and gp100/Pmel17—were analyzed by RT-PCR. DNA methylation patterns at the Melan-A/MART-1 promoter region were investigated by methylation-sensitive restriction enzyme digestion and subsequent Southern blot analysis. Exogenous promoter activity was assessed in all 20 melanoma cell lines to correlate the DNA methylation patterns with Melan-A/MART-1 expression.

RESULTS. MAA expression was observed in 15 of the 20 melanoma cell lines. Furthermore, there is a direct correlation between DNA methylation patterns at the Melan-A/MART-1 promoter region, exogenous Melan-A/MART-1 promoter activity, and Melan-A/MART-1 protein expression. These data reveal the division of patient-derived melanoma cell lines into two distinct subsets, which are identical for both uveal and cutaneous tumor types.

CONCLUSIONS. The authors propose a categorization of melanoma cell lines into two different panels based on shared MAA-expression characteristics: panel I, MAA-expressing cell lines, and panel II, MAA-deficient cell lines. This categorization can be used to obtain knowledge about the regulation of MAA-expression and for further research concerning MAA-based immunotherapy. (Invest Ophthalmol Vis Sci. 2005;46: 24–30) DOI:10.1167/iovs.04-0961

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Uveal and cutaneous melanoma are highly malignant diseases that are difficult to treat. One of the novel lines of treatment is immunotherapy, which targets melanoma-associated antigens (MAAs). However, in many cases, immunoselection of antigen-negative tumor cells and silencing of MAAs occurs, resulting in evasion of immune surveillance and tumor escape (for a review, see Ref. 5). In the light of the improved efficacy of immunotherapeutic approaches, we evaluated MAA expression in a panel of uveal and cutaneous melanoma cell lines, with the future prospect of developing strategies that can modulate MAA expression, to obtain optimal immunorecognition.

Melanoma-associated antigens (MAAs) are highly immunogenic human antigens that are recognized by cytotoxic T lymphocytes (CTLs). Both ocular and skin melanomas are derived from normal melanocytes that originate from the neural crest, although the melanocytes of the eye are functionally and morphologically distinct. Both types of melanomas carry a wide range of MAAs, including Melan-A/MART-1, gp100, tyrosinase (Tyr), tyrosinase-related protein 1 (Tyrp1), dopachrome tautomerase (Dct), and Melan-A/MART-1. At the same time, these tumors carry low immunogenic melanoma-specific antigens (expressed only in tumor tissue), including members of the MAGE family. The expression of these melanoma-specific antigens by melanoma cells in primary tumor tissue and in cultured tumor cells has been described to be variable for both ocular and skin melanoma, whereas the MAAs are more ubiquitously expressed (reviewed in Refs. 7,19). One of the important questions that awaits clarification is whether ocular and skin melanomas express similar or distinct MAAs. We therefore analyzed MAA expression in multiple uveal and cutaneous melanoma cell lines and focused on one of the MAAs, Melan-A/MART-1.

Currently, little is known about the function of Melan-A/MART-1 in melanocyte development and differentiation. Melan-A/MART-1 is expressed in normal immature melanocytes and in the majority (≥90%) of fresh melanoma tumors and melanoma cell lines, but it is not expressed in other cells and other tumors. Because of the high percentage of melanoma tumors that show Melan-A/MART-1 expression and due to its immunogenicity, this antigen is one of the targets for cellular immunotherapy against malignant melanoma. However, when immunoselection of antigen-negative tumor cells and silencing of MAAs occurs, their absence in the tumor cells impairs immune recognition by antigen-specific host CTLs, thereby reducing the efficacy of the immunotherapy. Unfortunately, up to now, attempts to correlate Melan-A/MART-1 expression and tumor staging have yielded conflicting results. Nevertheless, its expression can be used as a predictive factor for the selection of patients eligible for Melan-A/MART-1-based immunotherapy.

In this study, we investigated Melan-A/MART-1 expression in 10 uveal melanoma cell lines and 10 cutaneous melanoma cell lines (Table 1). Based on Melan-A/MART-1 protein and mRNA expression, the melanoma cell lines could be divided into two distinct groups: Melan-A/MART-1–protein–expressing and Melan-A/MART-1–protein–deficient cell lines. Interestingly,
malignant melanomas expressing Melan-A/MART-1 also expressed the MAAs Tyr, Tyrp1, Dct, and gp100/Pmel17, whereas cells that lacked expression of Melan-A/MART-1 failed to express these additional MAAs. Moreover, we showed distinct Melan-A/MART-1 DNA methylation patterns that correlated with the typical Melan-A/MART-1 protein/mRNA expression patterns as observed in the two groups of melanoma cell lines.

In conclusion, our results demonstrate the division of patient-derived uveal and cutaneous melanoma cell lines into two panels on the basis of MAA expression characteristics, Melan-A/MART-1 DNA methylation patterns, and Melan-A/MART-1 transcriptional activity. More important, comparable MAA expression patterns were observed in both ocular and skin melanomas, rendering both types of malignant melanoma suitable for identical immunotherapeutic approaches.

**MATERIAL AND METHODS**

**Cell Lines, Melan-A/MART-1 Protein Analysis, and RT-PCR**

Patient characterization and origin of the uveal melanoma cell lines 92-1, 92-2, Mel270, Mel285, Mel290, OCM1, OCM3, OMM1, OMM1.3, and OMM1.5,25 and the cutaneous melanoma cell lines 136.2, 453B, 513E, IGR39D,26 MA, MO,27 Mu89, Mu96, and EW 28 have been isolated from melanoma cell lines Mu96, IGR39D, and MA. The following primer pairs were used for the generation of various promoter constructs in the sense and antisense (A) orientation (see Fig. 3A):

<table>
<thead>
<tr>
<th>Cell Lines Derivation</th>
<th>Cutaneous Melanoma Cell Lines Derivation</th>
</tr>
</thead>
<tbody>
<tr>
<td>92-1†</td>
<td>Primary 136.2 Metastasis</td>
</tr>
<tr>
<td>92-2†</td>
<td>92-1 453A0† Primary</td>
</tr>
<tr>
<td>Mel270§</td>
<td>Primary 453B§ Primary</td>
</tr>
<tr>
<td>Mel285§</td>
<td>Primary 513D Metastasis</td>
</tr>
<tr>
<td>Mel290§</td>
<td>Primary EW Metastasis</td>
</tr>
<tr>
<td>OCM1§</td>
<td>Primary IGR39D Primary</td>
</tr>
<tr>
<td>OCM3§</td>
<td>Primary MA Metastasis</td>
</tr>
<tr>
<td>OMM1§ Metastasis</td>
<td>OMM1.3§ Metastasis Mu89§ Metastasis</td>
</tr>
<tr>
<td>OMM1.5§ Metastasis</td>
<td>OMM1.5§ Metastasis Mu96§ Metastasis</td>
</tr>
</tbody>
</table>

* Primary, primary tumor mass.
† 92-2 is a tissue culture-derived cell line from 92-1.
§ OMM1.3 and OMM1.5 are liver metastases from the Mel270 primary tumour.
|| Mu96 is a tissue culture-derived cell line from Mu89.

For the generation of the various Melan-A/MART-1 promoter reporter constructs, PCRs were performed on genomic DNA that had been digested with HindIII alone, HindII and NruI, HindIII and HpalII, or HindIII and MspI. Digestions were performed in a total volume of 50 μL, using 10 μg (melanocytes) or 20 μg (melanomas) of DNA and 40 to 50 U of each restriction enzyme. Samples were incubated for 5 hours at 37°C before they were loaded onto 0.8% agarose gels. Gels were run 0/n in 1X TBE (89 mM Tris-base, 89 mM boric acid, and 2 mM EDTA) at 30 to 40 V.

The DNA was blotted onto predampened nylon transfer membranes (Hybond N+; Amersham Pharmacia Biotech) in 0.4 M NaOH 0/n at room temperature (RT). Blots were briefly washed in 2X SSC before hybridization in 0.5 M phosphate buffer [pH 7.2], 7% SDS, 10 mM EDTA at 65°C using the appropriate probe. Probes were prepared from the Melan-A/MART-1 promoter region and were purified from agarose gel. DNA (100–200 ng) was labeled by the method of random priming. After 0/n hybridization, blots were washed several times before autoradiography.

**Melan-A/MART-1 Promoter Reporter Assays**

For the generation of the various Melan-A/MART-1 promoter reporter constructs, PCRs were performed on genomic DNA that had been isolated from melanoma cell lines Mu96, IGR39D, and MA. The following primer pairs were used for the generation of various promoter constructs in the sense and antisense (A) orientation (see Fig. 3A): p286 and p286A: sense 5'-GTCGACTGGAAGGATTGTATGAC-3' and antisense 5'-AGGTGAGTATGTAACACTGGAAGGGA-3'; p3018 and p303A: sense 5’-AGCTTCCTCTTGTCTGAGGCGGCGGTCATACGATGTAACACTGGAAGGAGGGA-3' and antisense 5’-TCTTCTCTCTGTCTGAGGCGGCGGTCATACGATGTAACACTGGAAGGAGGGA-3'; p3029 and p3029A: sense 5’-GTCGACTGGAAGGATTGTATGAC-3' and antisense 5’-AGGTGAGTATGTAACACTGGAAGGGA-3'.

**Southern blot analysis**

Genomic DNA was isolated by lysing 10⁶ to 10⁷ cells in 1 mL DNA lysis buffer (100 mM Tris/HCl [pH 8.0], 5 mM EDTA, 0.2% SDS, 200 mM NaCl, 200 μg/mL ProteK) and overnight (0/n) incubation at 56°C. DNA was purified by phenol and subsequent phenol-chloroform-isooamylalcohol extractions and was precipitated with 100% ethanol. Pellets were resuspended in 100 to 200 μL TE (10 mM Tris/HCl [pH 8.0], 1 mM EDTA).

For testing the methylation status of genomic DNA, the DNA was digested with HindIII alone, HindIII and NruI, HindIII and HpalII, or HindIII and MspI. Digestions were performed in a total volume of 50 μL, using 10 μg (melanocytes) or 20 μg (melanomas) of DNA and 40 to 50 U of each restriction enzyme. Samples were incubated for 5 hours at 37°C before they were loaded onto 0.8% agarose gels. Gels were run 0/n in 1X TBE (89 mM Tris-base, 89 mM boric acid, and 2 mM EDTA) at 30 to 40 V.

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The DNA was blotted onto predampened nylon transfer membranes (Hybond N+; Amersham Pharmacia Biotech) in 0.4 M NaOH 0/n at room temperature (RT). Blots were briefly washed in 2X SSC before hybridization in 0.5 M phosphate buffer [pH 7.2], 7% SDS, 10 mM EDTA at 65°C using the appropriate probe. Probes were prepared from the Melan-A/MART-1 promoter region and were purified from agarose gel. DNA (100–200 ng) was labeled by the method of random priming. After 0/n hybridization, blots were washed several times before autoradiography.
from p1839 and p1839A to the Blnl-HindIII digested vectors p1373 and p1373A, respectively.

Transfection assays were performed by using the above-described constructs and construct pRL(β-actin), containing the Renilla gene behind the β-actin promoter. Cells were seeded at 2 × 10^5 cells/10 cm² the day before transfection. For each transfection (in quadruplicate) 4 μg of promoter construct and 0.4 μg of pRL(β-actin) construct were transfected by using the CaCl₂ coprecipitation method. Cells were incubated at 37°C for 2 days before lysis in 200 μL of lysis buffer (Dual-Luciferase Reporter Assay System; Promega) per 10 cm². Of each sample, 10 μL was analyzed for luciferase and Renilla activity using a Victor2, 1420-012 multilabel counter (Wallac, Oy, Finland). Experiments were performed at least twice to confirm reproducibility.

TABLE 2. Characteristics and Division of Melanoma Cell Lines into Two Panels

<table>
<thead>
<tr>
<th></th>
<th>Panel I</th>
<th>Panel II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melan-A/MART-1</td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Protein expression</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>mRNA expression</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Methylation pattern</td>
<td>HpaII™/NruI™</td>
<td>HpaII™/NruI™</td>
</tr>
<tr>
<td>Promoter activity</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>MAA mRNA expression</td>
<td>Tyr, Tyrp1*, Dct, gp100/Pmel17</td>
<td>Tyr, Dct, gp100/Pmel17</td>
</tr>
<tr>
<td>Cutaneous melanoma cell lines</td>
<td>136.2, 453A0, 453B, 513D, MA, MO, Mu89</td>
<td>92-1, 92-2, Mel270, OCM1, OCM3, OMM1, OMM1.3, OMM1.5, Mel285, Mel290</td>
</tr>
<tr>
<td>Uveal melanoma cell lines</td>
<td>92-1, 92-2, Mel270, OCM1, OCM3, OMM1, OMM1.3, OMM1.5</td>
<td>No</td>
</tr>
</tbody>
</table>

m, methylated restriction site; u, unmethylated restriction site.
* Minute expression was observed for Tyrp1 in panel II cell lines.

RESULTS

MAA Expression in Uveal and Cutaneous Melanoma Cell Lines

We first evaluated the expression of Melan-A/MART-1 by Western blot analysis in our panel of melanoma cell lines derived from tissue samples of various melanoma patients (Table 1, Fig. 1); normal melanocyte cultures served as a control. β-Actin staining was used as an internal control for equal protein loading. The results of the Western blot analysis (Fig. 1A) showed that Melan-A/MART-1 expression could easily be detected in several melanoma cell lines (cutaneous: 136.2, 453A0, 453B, 513D, MA, MO, and Mu89; uveal: 92-1, 92-2, Mel270, OCM1, OCM3, OMM1, OMM1.3, and OMM1.5), whereas other melanoma cell lines lacked detectable levels of Melan-A/MART-1 protein expression (cutaneous: EW, IGR39D, and Mu96; and uveal: Mel285 and Mel290), in normal skin-derived melanocytes (M0101 and M9228), Melan-A/MART-1 was abundantly expressed (Fig. 1A).

RT-PCR analysis (Fig. 1B) revealed that the Melan-A/MART-1 protein–negative uveal and cutaneous melanoma cell lines were also deficient for Melan-A/MART-1 mRNA synthesis. Furthermore, uveal (not shown) and cutaneous melanocyte cell lines showed strong Melan-A/MART-1 expression. We next evaluated the expression characteristics of other MAAs (Tyr, Tyrp1, Dct, and gp100/Pmel17) by RT-PCR in these melanoma cell lines and melanocyte cell cultures. The results of these analyses showed that cell lines that expressed Melan-A/MART-1 also expressed the other MAAs, whereas cell lines that were deficient for Melan-A/MART-1 expression also lacked expression of Tyr, Dct, and gp100/Pmel17, although minute levels of Tyrp1 were detected in the otherwise negative cell lines (Fig. 1B, Table 2). Similar to the Melan-A/MART-1–expressing melanoma cell lines, uveal (not shown) and cutaneous melanocyte cell cultures (Fig. 1B) showed expression of the other MAAs.

Based on these analyses, a division was made into two different panels of uveal and cutaneous melanoma cell lines: panel I, MAA-expressing cell lines, and panel II, MAA-deficient cell lines. These data are indicative of a process whereby melanocytes transform into different types of tumor cells, which can be distinguished on the basis of MAA expression or deficiency.

Specific DNA methylation Pattern Associated with Expression of Melan-A/MART-1 in Uveal and Cutaneous Melanoma Cell Lines

To characterize further the two subsets of melanoma cell lines, we next investigated whether we could correlate the observed differences in Melan-A/MART-1 protein and mRNA expression with DNA methylation patterns. To analyze the methylation...
status of the Melan-A/MART-1 gene, we performed Southern blot analyses on genomic DNA by using methylation-sensitive restriction enzymes. Figure 2A shows two representative samples of Melan-A/MART-1-expressing and -deficient melanoma cell lines of uveal and cutaneous origin, and two melanocyte cultures derived from the skin. Figures 2B and 2C depict a schematic representation of the Melan-A/MART-1 locus and the region that was investigated, respectively. The latter encompasses the region of the Melan-A/MART-1 gene, which is involved in high promoter activity, and includes the position of the restriction sites and the probe that was used for restriction fragment detection.

The panel I melanoma cell lines (Fig. 2A, left panel), show methylation of the HpaII site in the 5' upstream region (lane 2), as they lacked the typical 3640-bp HpaII-HindIII fragment (compare with control digestions on the melanoma cell lines with the HpaII methylation insensitive isoschizomerMspI, lane 3). The observed fragments in lane 2 were the result of partial methylation of upstream HpaII sites. Furthermore, they showed unmethylated DNA at the NruI site in intron 1, where a 786-bp NruI-HindIII fragment was observed (lane 4).

In contrast, the panel II melanoma cell lines (Fig. 2A, middle panel), showed methylation of the intronic NruI site (they lack the 786-bp fragment, lane 4) in all cell lines. Digestion of the genomic DNA with HpaII (lane 2) resulted in a mixture of patterns. For the cutaneous melanoma cell lines (EW, IGR39D), partial methylation was observed, which generated 3640-bp fragment (lane 2), indicating changes in methylation of the upstream region when compared with panel I cell lines. In the panel II uveal melanoma cell lines (Mel285, Mel290), the HpaII site was predominantly methylated. Notably, the methylation pattern for the two melanocyte cell lines, M0101 and M0228 (Fig. 2A, right panel, no isoschizomer control digestion), was similar to that observed in panel I melanoma cell lines: methylation of the HpaII site (lane 2) and an unmethylated NruI site (lane 4). Based on these DNA methylation analyses, showing a contrasting pattern at the intronic NruI site, the division into the two panels of melanoma cell lines was highlighted.

**Exogenous Melan-A/MART-1 Promoter Activity and Endogenous Melan-A/MART-1 Expression Levels**

To test for the possible involvement of DNA methylation in the transcriptional regulation of the Melan-A/MART-1 gene we generated a set of promoter-reporter constructs (Fig. 3A). The smallest construct, p286, contained virtually all the Melan-A/MART-1 exon I sequences, since, as previously established, this exon comprises the Melan-A/MART-1 regulatory sequences.52 The other constructs contained sequences up- and/or downstream of this core promoter region, comprising the intron 1 region (Fig. 3A). The intron 1 region contains the NruI site that was analyzed in the DNA methylation assay, and for which a difference in methylation pattern was observed between all panel I and II melanoma cell lines. Each of these (unmethylated) constructs was first tested in two panel I cell lines (a uveal, 92-2, and a cutaneous, Mu89, melanoma cell line) and a panel II cell line (cutaneous, Mu96) for transcription activation of the luciferase reporter gene in transient transfection assays (Fig. 3B). If DNA methylation is the principle mechanism for Melan-A/MART-1 silencing, promoter activity of the unmethylated exogenous promoter should be observed in both panels of cell lines.

The panel I cell lines (Mu89, 92-2) showed activity of the core promoter construct, p286. Extension of this region with upstream sequences (p1373) resulted in significant downregulation of promoter activity and the intron 1 sequence itself (p1593, containing the NruI site) showed no activity at all. The highest activity was observed for construct p2956 containing the complete 3-kb promoter region, including the intronic NruI site. In contrast, the panel II cell line (Mu96), showed no significant activity for any of the constructs. Together, these
data suggest an important role for the intronic region of the Melan-A/MART-1 gene in promoter activation.

Because the p2956 construct showed the highest activity and contains the region where a difference in DNA methylation pattern was observed, we tested this (unmethylated) construct for promoter activity in the complete panel of cell lines. In both uveal and cutaneous panel I melanoma cell lines (Fig. 3C) p2956 promoter activity was observed, albeit at various levels (3000–183,000 relative luciferase units [RLU]/s). In contrast, panel II cell lines showed no to background levels of p2956 promoter activity (Fig. 3C, <900 RLU/s). The results of these promoter activation studies corroborate the division of melanoma cell lines into two different panels on the basis of MAA expression characteristics.

DISCUSSION

The data presented in this study, show that two subsets of melanoma cell lines can be discerned on the basis of MAA expression: panel I, melanoma cell lines that display MAA expression, and panel II, melanoma cell lines that are deficient in MAA expression (Table 2). The origin of the tumor cell lines (primary or metastatic) was not found to correlate with their classification as a panel I or II cell line (Table 1, 2), in the sense that not all panel II cell lines were derived from metastases nor were all panel I cell lines derived from primary tumor material. Moreover, the uveal melanoma cell lines OMM1.3 and -1.5 are derived from different liver metastases from the same patient as the primary Mel270 uveal melanoma cell line, and these three cell lines showed identical MAA expression characteristics.

Because one of the arguments for metastasis is that immune detection is evaded by silencing of tumor antigens, allowing settlement in a distant organ, the finding that Melan-A/MART-1 silencing is not correlated with metastatic behavior presents a paradox. One possible explanation is that these features change (not necessarily sequentially) during the process of transformation of normal cells into often-metastatic cancer cells, as discussed by Bernards and Weinberg.33 This process relies on an apparent random succession of genetic and epigenetic changes that occur relatively early in tumorigenesis and that alter the expression profiles of a myriad of genes. Because of these successive changes in gene expression profiles, individual neoplastic cells acquire selective advantages in the process of tumor progression.33,34 It infers heterogeneity in expression profiles of specific genes in subsets of cells within the original primary tumor cell population.33 It also infers that the expression profiles of specific genes in different tumor metastasis could differ from those of cells in the primary tumor mass, whereas the overall gene-expression patterns may be strikingly similar.33,35

Of interest is the notion that, at the level of DNA encompassing the Melan-A/MART-1 regulatory region, there is a clear difference in DNA methylation pattern of the intronic NruI site, between melanoma cells that express this antigen and cells that are deficient for the antigen. At the same time, cells lacking the Melan-A/MART-1 antigen also failed to support the activity of an exogenous, unmethylated, Melan-A/MART-1 promoter-reporter construct, as demonstrated in transient transfection assays (see Fig. 3). This is in line with previous reports28,32 suggesting that the lack of Melan-A/MART-1 expression is caused by absence of transcription factors involved in Melan-A/MART-1 promoter activation.

Recent work on the Melan-A/MART-1 and Pmel17 promoters36 and on the Tyrp1 and Dct promoters37 showed the involvement of the master regulator of melanocyte development, microphthalmia-associated transcription factor (Mitf38), in their transcriptional regulation. Although Mitf is capable of
activating the exogenous Tyr and Tyrp1 promoters. Mitf is not sufficient to re-induce endogenous expression once these genes have been silenced.39 These data suggest a role for chromatin remodeling in combination with specific transcription factors in the transcriptional regulation of these genes in vivo.39 Our data are in line with these speculations, because differences in DNA methylation patterns are observed in vivo, together with the absence of required transcription factors in panel II cells (Fig. 3). As a whole, our results from the promoter reporter assays and DNA methylation studies indicate the existence of multiple regulatory regions and mechanisms that control MAA expression and point toward a transcription-factor-regulated silencing process during the conversion of normal melanocytes to melanoma cells, with a putative role for chromatin remodeling.

For the analyses described in this article, we used a panel of cell lines in view of future reverse genetic approaches and re-expression strategies for MAAs. Because cell lines are derived from tumor tissue by single cell dilutions and subsequent clonal expansion, panel I cell lines must be derived from MAA-expressing homogeneous or heterogeneous tumor masses. At the same time, panel II cell lines must be derived from MAA-deficient or heterogeneous tumors or must have arisen during expansion in tissue culture. Of the 20 cell lines analyzed, only one panel II cell line, Mu96, arose in tissue culture on high-density growth and TIL immunoselection.28 Cell line 92-2 was also derived from another cell line (92-1) in tissue culture. However, this cell line retained the panel I phenotype (Table 1, 2) but, in contrast to its ancestor, was unable to form metastases in mice (Jager et al., unpublished observation, 2003). Whether cell lines are more prone to undergoing changes in tissue culture when derived from heterogeneous tumor masses than those derived from homogeneous tumor masses can only be speculated on, because the immunophenotype of the original tumor masses could not be traced.

On the whole, our data, summarized in Table 2, show the division of patient-derived melanoma cell lines into two panels, based on MAA expression. At the same time, differences in methylation patterns were observed that have to be confirmed in freshly isolated tumor cells, to investigate the possibility of therapeutic strategies whereby DNA methylation can be limited or blocked in vivo. Finally, a distinction between uveal and cutaneous melanoma cell lines could not be established, indicating that identical transformation processes take place in uveal and cutaneous melanocytes and that a general approach can be used concerning MAA-based immunotherapy strategies.

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