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Analysis of Pmel17/gp100 expression in primary human tissue specimens: implications for melanoma immuno- and gene-therapy

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Abstract Pmel17/gp100-encoded tumor-associated antigens are recognized by cytotoxic T lymphocytes in melanoma patients and may represent attractive target antigens for immuno- and gene-therapeutic strategies. An important prerequisite for identifcation and monitoring of melanoma pateints that could potentially benefit from Pmel17/gpl00-based immuno- and gene-therapies is the detailed knowledge of Pmel17/gpl00 expression in vivo. Immunophenotyping is considerably hampered by the different immunoreactivities of Pmel17/gpl00-reactive antibodies. Therefore, we analyzed an extended series of different primary normal and malignant human tumor specimens for Pmel17/gpl00 expression at the mRNA level. Transcripts were detectable in all malignant melanoma tissue specimens representing all stages of tumor progression, with significant levels even in early and amelanotic melanoma lesions. In contrast, normal melanocytes exhibited significantly less Pmel17/gpl00 mRNA in vivo, as determined by comparative in situ hybridization. Tissue specimens from the retina and substantia nigra also contained Pmel17/gpl00 mRNA, whereas other normal and malignant human tissues were negative. As determined by comparative in situ hybridisation and HMB-45 immunostaining, even tumor tissue lacking Pmel17/gpl00 immunoreactivity contained Pmel17/gp100 transcripts. Our results indicate a melanocytic-cell-lineage-restricted expression of Pmel17/gpl00 with significant transcript levels in all stages of melanoma progression, including early and amelanotic melanoma lesions, and a significantly differential expression between melanoma cells and normal melanocytes in vivo. Owing to its higher sensitivity, phenotyping of individual tumor specimens by mRNA expression analysis seems to be more valuable than phenotyping by immunostaining.

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Introduction

In melanoma patients, cytolytic T cells (CTL) are able to mediate an immune response to autologous tumor cells either spontaneously or with an appropriate immunostimulatory manipulation. Anti-melanoma CTL have been derived from tumor-infiltrating lymphocyte populations cultured from melanoma metastases and lymphocytes of draining lymph nodes (reviewed in [9]), generated from peripheral blood of melanoma patients [30, 33, 36], and induced from peripheral blood of normal patients [5, 11, 39]. In addition to lysing tumor cells directly, CTL may secrete lymphokines, such as tumor necrosis factor and interferon γ, that may also contribute to the overall antitumor effect (reviewed in [28]).

Autologous tumor cells are recognized by CTL via cell-surface expression of tumor-associated antigens consisting of short peptides, usually 9–l0 residues long, presented at the cell surface in the context of class I MHC molecules. Enhanced expression of tumor-associated antigens may increase immunogenicity of tumor cells, while elimination of genes coding for these antigens may lead to antigen-loss variants possessing diminished or absent immunogenicity (reviewed in [8]). Several genes encoding different potentially immunogenic melanoma-associated antigens (MAA) have been described thus far. These include antigens encoded by the MAGE, BAGE, and GAGE gene families, which are expressed in human tumors of various histological types but are not expressed in normal tissue, with the exception of testis [6, 18, 37, 38]. A second group comprises differentiation antigens encoded by tyrosinase, Melan-A/MART-1, TRP-1 and TRP-2 which are restricted in their expression to the melanocytic cell lineage [10, 15, 23, 42, 43, 44]. A third group may represent tumor-specific mutated proteins encoded by β-catenin, MUM-1, and CDK4 [16, 32, 43] and a fourth group may contain melanoma antigens encoded by ubiquitously expressed genes such as p15 [31].

Recently, Pmel17 and its highly homologous splice variant gp100 have been identified to encode several epitopes recognized by melanoma-specific CTL [3, 4, 17, 22, 24]. Pmel17/gp100-encoded epitopes seem to represent attractive target antigens for the development of immunotherapeutic strategies for several reasons. First, adoptive transfer of Pmel17/gp100-reactive CTL lines resulted in significant tumor regression when infused into autologous patients in combination with interleukin-2 (IL-2) [22, 24]. Second, Pmel17/gp100-encoded epitopes may represent shared tumor antigens recognized by CTL derived from different melanoma patients [17, 24]. Third, Pmel17/gp100-encoded epitopes are presented in the context of HLA-A2, which is present in 49% of Caucasian individuals [4, 17, 22, 24]. Fourth, mice immunized with recombinant Pmel17/gp100 cDNA-containing adenoviral vectors were protected from subsequent murine melanoma challenge [46]. Fifth, intradermal vaccination of HLA-A2$^+$ melanoma patients with Pmel17/gp100-derived peptides led to objective tumor regression when administered in combination with systemic granulocyte/macrophage-colony-stimulating factor [20].

The value of MAA as targets in immuno- and gene-therapeutic strategies not only depends on the immunogenicity of MHC-presented peptide sequences but also on their expression pattern in vivo. Tumor-cell-specific expression with considerable amounts of MAA in each tumor cell, highly conserved expression in all stages of tumor progression, even in early melanoma lesions, and differential expression between melanoma cells and their normal counterparts are also important determinants. By using different Pmel17/gp100-reactive mAb, immunohistochemistry for analysis of Pmel17/gp100 expression in vivo revealed significantly differing results [19, 40] including false positive results in epithelial tissues and tumors [7, 26]. Furthermore, immunoreactivity to Pmel17/gp100 is dependent on posttranslational modifications that are not required for MAA presentation by MHC [2, 21], and the inhomogeneous distribution of HMB-45 immunoreactivity in individual tissue samples suggested that Pmel17/gp100 represents a minor favorable MAA for immunotherapeutic strategies as compared with other MAA exhibiting a more homogeneous distribution [12]. Thus, immunoreactivity may not necessarily reflect the expression of Pmel17/gp100 and Pmel17/gp100-encoded MAA in vivo and a detailed expression analysis at the mRNA level may give significant additional information to immunohistochemical studies.

The presence of significant transcript levels in melanoma cell lines and epidermal melanocytes in vitro [1–3, 25, 41] has led to the consideration that Pmel17/gp100 may represent a tissue differentiation antigen with high expression levels in melanoma cells and also in normal melanocytes. However, the expression pattern of Pmel17/gp100 transcript levels in normal and malignant human tissues in vivo has not been thoroughly investigated thus far. In the present study, we have analyzed the expression pattern of Pmel17/gp100 at the mRNA level in an extended series of primary malignant human melanoma tissue specimens representing all stages of melanoma tumor progression and compared it to that observed in normal cultured and uncultured melanocytic cells and in a variety of normal and malignant primary human tissue specimens of different origin.

Materials and methods

Tissue specimen collection

Human tissue samples were obtained from freshly excised surgical specimens or during pathological dissection (substantia nigra, fetal brain). A representative portion of each tissue sample was removed from the center of the lesion, fixed in 10% buffered formalin (pH 7.0), and embedded in paraffin wax for histological examination. The rest of the specimen was immediately frozen in liquid nitrogen and subjected to RNA extraction and in situ hybridisation analysis. Histopathological diagnosis and classification of all tissue specimens were performed independently by two of the authors. The specimens included 52 primary and metastatic cutaneous malignant melanoma tissue specimens representing all stages of tumor progression, 2 uveal melanoma specimens, 15 junctional nevocellular nevus tissue samples, 8 dermal nevocellular nevus tissue samples, 5 normal skin specimens (obtained from patients undergoing plastic skin surgery), and a variety of normal and malignant human tissue samples of different origin. The tissue samples analyzed are listed in Table l.

Cultured cell lines

Human melanoma cell lines HT-144 (established from non-pigmented melanoma, metastasis to subcutaneous tissue), SK-Mel-2 (pigmented melanoma, metastasis to skin), and SK-Mel-28 (pigmented malignant melanoma) were obtained from the ATCC (Rockville, Md.) and cultured as monolayers in McCoy’s 5a medium plus fetal calf serum (FCS) and antibiotics and in Eagle’s minimal essential medium plus FCS and antibiotics respectively. Melanoma cell lines WM35 (primary melanoma, radial growth phase) and WM115 (primary melanoma, vertical growth phase) were kindly provided by Dr. Meenhard Herlyn (The Wistar Institute, Philadelphia, Pa.) and cultured as monolayers in MCDB 153/Leibovitz’s L-15 medium plus FCS and insulin. Normal human epidermal melanocytes (NHEM) were obtained from PromoCell (Heidelberg, Germany) and cultured in melanocyte growth medium.

RNA preparation and Northern blot analysis

Total cellular RNA was prepared from tissue specimens by guanidinium thiocyanate extraction and cesium chloride centrifugation [13]. Samples comprising 15 μg total cellular RNA were size-fractionated on 1% agarose, 2 M formaldehyde gels and transferred to nylon membranes by vacuum blotting. Membranes were UV-cross-linked (Stratalinker, Stratagene, Heidelberg, Germany) and prehybridized in the presence of 4×SSPE (1×SSPE is 180 mM NaCl, 8 mM NaH_2PO_4, 1 mM EDTA), 1% sodium dodecyl sulfate (SDS), 5×Denhardt′s solution, 500 μg/ml denatured salmon DNA, and 50% deionized formamide. RNA was hybridized to random-primed [α-^{32}P]dATP-labeled cDNA probes at 42 °C; any residual probe was removed by high-stringency washing, finally twice for 60 min in 0.1×SSC, 0.1% SDS at 65 °C for Pmel17/gp100 and 68 °C for β-actin, and filters were subjected to autoradiography as described [41].

For hybridization, a full-length 2.1-kb Pmel17 cDNA fragment was used, previously cloned by immunoscreening a SK-MEL-28 cDNA library with mouse monoclonal antibody HMB-45 [41]. Control hybridization for RNA integrity and RNA loading was performed

Table 1 Pmel17/gp100 mRNA expression in tissue samples and cell lines

Normal tissue/cell lines	No. of positive/total samples	Tumor tissue or cell line	No. of positive/total samples
Melanocytes (in vitro)	5/5	Melanoma cell lines	5/5
Melanocytes (tumor-surrounding)	++[a]	Junctional nevocellular nevus tissue samples	15/15
Melanocytes (in vivo)	(+)[a]	Dermal nevocellular nevus tissue samples	0/8
		Melanoma tissue samples (inc. amelanotic samples)	52/52
Skin	(+)[b]/5	Squamous cell carcinoma, skin	0/16
		Merkel cell carcinoma	0/1
Retina	1/1	Uveal melanoma	2/2
Kidney	0/3	Renal cell carcinoma	0/33
Lung	0/1	Small cell lung cancer	0/4
		Non small cell lung cancer	0/12
Bronchus	0/1	Bronchial carcinoid	0/1
Colon	0/3	Colon adenocarcinoma	0/15
		Adenomatous colorectal polyp	0/7
Stomach	0/2	Gastric adenocarcinoma	0/15
Liver	0/2	Liver cancer	0/6
Breast	0/2	Breast cancer	0/22
Thyroid	0/1	Medullary thyroid carcinoma	0/3
Adrenals	0/1	Pheochromocytoma	0/1
Lymph node	0/3	Non-Hodgkin's lymphoma	0/5
Fetal brain	0/1	Neuroblastoma	0/1
Substantia nigra	1/1		

[a] Determined by in situ hybridization
[b] Low level

with the 3.6-kb *Hin*dIII fragment of human β-actin sequences (pλHac69) [29].

In situ hybridization

In situ hybridization was performed as described [41]. Briefly, [α-^{35}S]dATP-labeled single-stranded antisense and sense cDNA probes were obtained by reverse transcription of sense and antisense cRNA transcripts generated by in vitro transcription of the full-length 2.1-kb Pmel17 cDNA subcloned in pBluescript II SK$^+$ (Stratagene, La Jolla, Calif.).

Cryosections of 8 µm were incubated with the respective ^{35}S-labeled cDNA probe (1.8×10^4 cpm/µl) at 52 °C for 12 h. Residual radioactive probe was removed by high-stringency washing (50% deionized formamide, 300 mM NaCl, 10 mM TRIS/Cl, 10 mM $NaPO_4$, 5 mM EDTA, and 10 mM dithiothreitol) at 52 °C. After graded dehydration, slides were covered with LM-1 autoradiographic emulsion (Amersham, Braunschweig, Germany) and exposed for 10 days.

Immunohistochemistry

Immunohistochemistry was performed with mAb HMB-45 (DAKO, Hamburg, Germany) and visualized by alkaline phosphatase/anti-alkaline phosphatase staining as described [41].

Results

Pmel17/gp100 mRNA expression in primary human tissue specimens

Pmel17/gp100 mRNA could be detected in all of 52 primary cutaneous malignant melanoma specimens including amelanotic variants and metastatic tumor samples, in both of the 2 uveal melanoma specimens, and in all of 15 junctional nevocellular nevus tissue samples. Primary human tumor specimens derived from a variety of non-melanocytic tissues did not express Pmel17gp100 transcripts at a detectable level (Fig. 1, Table 1). Normal human tissue specimens known to contain melanocytic cells, such as normal skin, retina, and substantia nigra, exhibited Pmel17gp100 transcripts at low levels, whereas other normal human tissue specimens lacked detectable Pmel17/gp100 expression at the mRNA level (Fig. 1, Table 1). These results indicate a melanocytic-cell-lineage-restricted expression of Pmel 17/gp100.

Pmel17/gp100 mRNA expression in different stages of melanoma progression

The panel of melanoma samples analyzed included melanoma in situ and radial-growth-phase melanoma, representing early stages of tumor progression, as well as vertical-growth-phase and metastatic melanoma, representing ad-

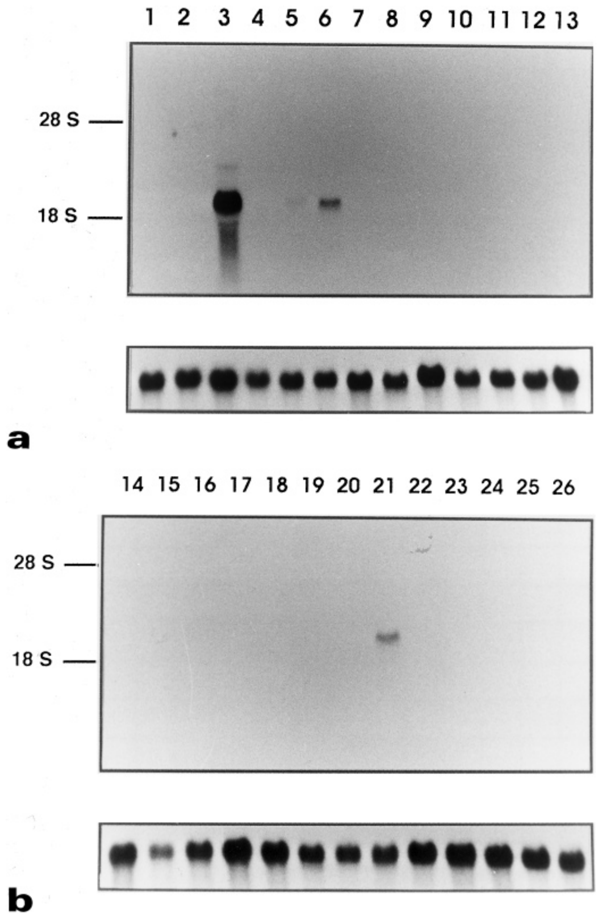

Fig. 1a, b Northern blot analysis. RNA of paired primary normal and malignant human tissue specimens. *Upper panel* RNA was hybridized with a Pmel17 cDNA probe as specified in Materials and methods. The migration positions of 18S and 28S rRNA are indicated. *Lower panel* control of RNA loading and RNA integrity by rehybridization with β-actin cDNA probe. **a** *Lanes*: 1 breast; 2 breast cancer; 3 malignant melanoma; 4 dermal nevocellular nevus; 5 normal skin; 6 cultured melanocytes; 7 kidney; 8 renal cell carcinoma, clear cell type; 9 renal cell carcinoma, chromophilic cell type; 10 lymph node; 11 nodular non-Hodgkin lymphoma; 12 bronchus; 13 bronchial carcinoid: **b** *Lanes*: 14 colon; 15 colon adenocarcinoma; 16 stomach; 17 gastric adenocarcinoma; 18 lung; 19 small-cell lung cancer; 20 non-small-cell lung cancer; 21 retina; 22 neuroblastoma; 23 thyroid; 24 medullary thyroid carcinoma; 25 adrenals; 26 pheochromocytoma

vanced stages of tumor progression. Pmel17/gp100 transcripts were detected in all stages of tumor progression with significant expression levels in melanoma in situ and with high expression levels in the radial and vertical growth phases as well as in metastatic melanoma (Fig. 1, Table 1). Cultured human melanoma cell lines established from different stages of melanoma progression also contain significant amounts of Pmel17/gp100 mRNA. Cell lines WM35 and WM115, established from radial- and vertical-growth-phase melanoma respectively, as well as the melanotic SK-Mel2 and SK-Mel28, and the amelanotic HT144 cell lines, derived from metastatic melanoma, expressed Pmel17/gp100 transcripts at high levels (Table 1).

Pmel17/gp100 immunoreactivity and transcript expression pattern in situ

We have analyzed the expression pattern of Pmel17/gp100 in individual tumor specimens at the cellular level by in situ hybridization and compared it with HMB-45 immunoreactivity. A small subgroup including primary and metastatic melanoma lesions showed an inhomogeneous distribution with tissue areas or single cells containing very high transcript levels (Fig. 2a). Despite this inhomogeneous distribution, almost all of the metastatic and primary melanoma cells exhibited Pmel17/gp100 transcripts at a detectable level (Fig. 2b). Most of the primary melanoma tissue specimens, however, exhibited a homogeneous distribution of Pmel17/gp100 transcripts at the cellular level even in tissue areas obviously lacking HMB-45 immunoreactivity (Fig. 2c, d). Furthermore, even metastatic melanoma specimens completely lacking HMB-45 immunoreactivity contained significant amounts of Pmel17/gp100 mRNA, as determined by Northern blot analysis (Table 1). These results indicate a differential detection pattern of Pmel17/gp100 expression by means of immunohistochemistry and in situ hybridization/Northern blot analysis.

Pmel17/gp100 mRNA expression in normal and tumor-surrounding melanocytes in vivo

Cultured normal human epidermal melanocytes (NHEM) express Pmel17/gp100 transcripts at levels comparable to those observed in melanoma tissue specimens and cell lines (Fig. 1, Table 1). However, Northern blot analysis revealed only low levels of Pmel17/gp100 mRNA in normal skin (Fig. 1, Table 1). This apparent difference may be due either to the dilution of the mRNA signal by other cell types present in normal skin and/or to low expression levels in normal human epidermal melanocytes in vivo. To address this question, we analyzed Pmel17/gp100 expression of normal melanocytes with that of malignant melanoma cells by comparative in situ hybridization. Figure 3a demonstrates the result for Pmel17/gp100 in situ hybridization of normal skin treated absolutely identically to the malignant melanoma shown in Fig. 2c. Only sporadic Pmel17/gp100 transcript signals were detected in normal epidermal melanocytes in vivo and, if present, signal intensities were significantly less than those obtained in malignant melanoma cells (Fig. 2c). A completely different situation could be observed in melanoma-surrounding melanocytes. As demonstrated by Pmel17/gp100 in situ hybridization, melanoma-surrounding melanocytes contained Pmel17/gp100 mRNA at levels significantly higher than those observed in normal melanocytes in vivo (Fig. 3b, Table 1).

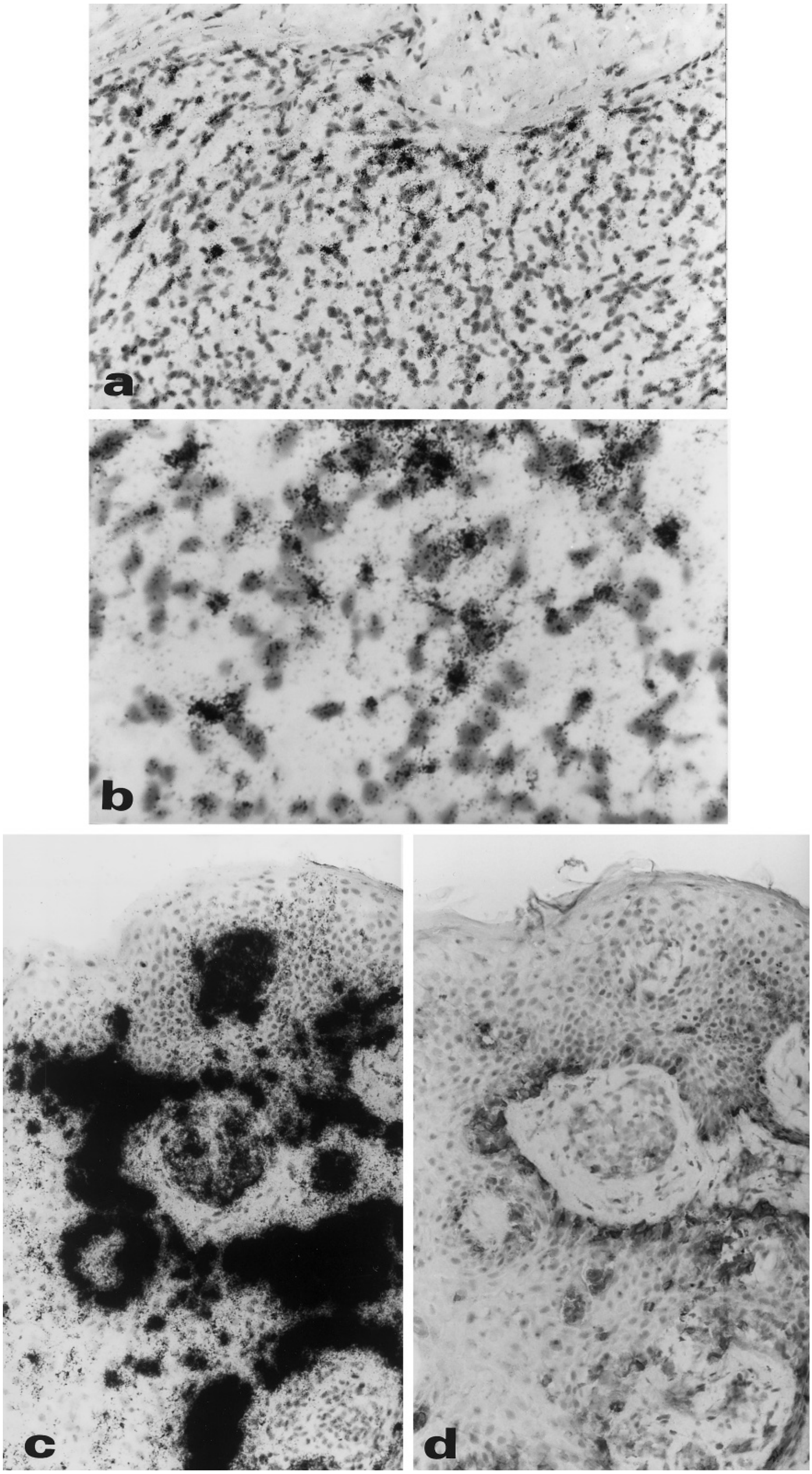

Fig. 2a In situ hybridization of a metastatic melanoma tissue sample representing a subgroup with inhomogeneous Pmel17/gp100 expression. Note single cells containing very high transcript levels. **b** Close-up of the same tissue section as in **a**, note autoradiographic grains overlying almost all melanoma cells. **c**, **d** Comparative in situ hybridization (**c**) and HMB-45 immunohistochemistry (**d**). **c** In situ hybridization of a tissue sample representing the majority of melanoma lesions. Note strong expression in melanoma cells. **d** Comparative HMB-45 immunoreactivity on a serial cryostat section. Note strong expression of Pmel17/gp100 transcripts even in tissue areas lacking detectable HMB-45 immunostaining. **a**×260, **b**×1050, **c, d**×520

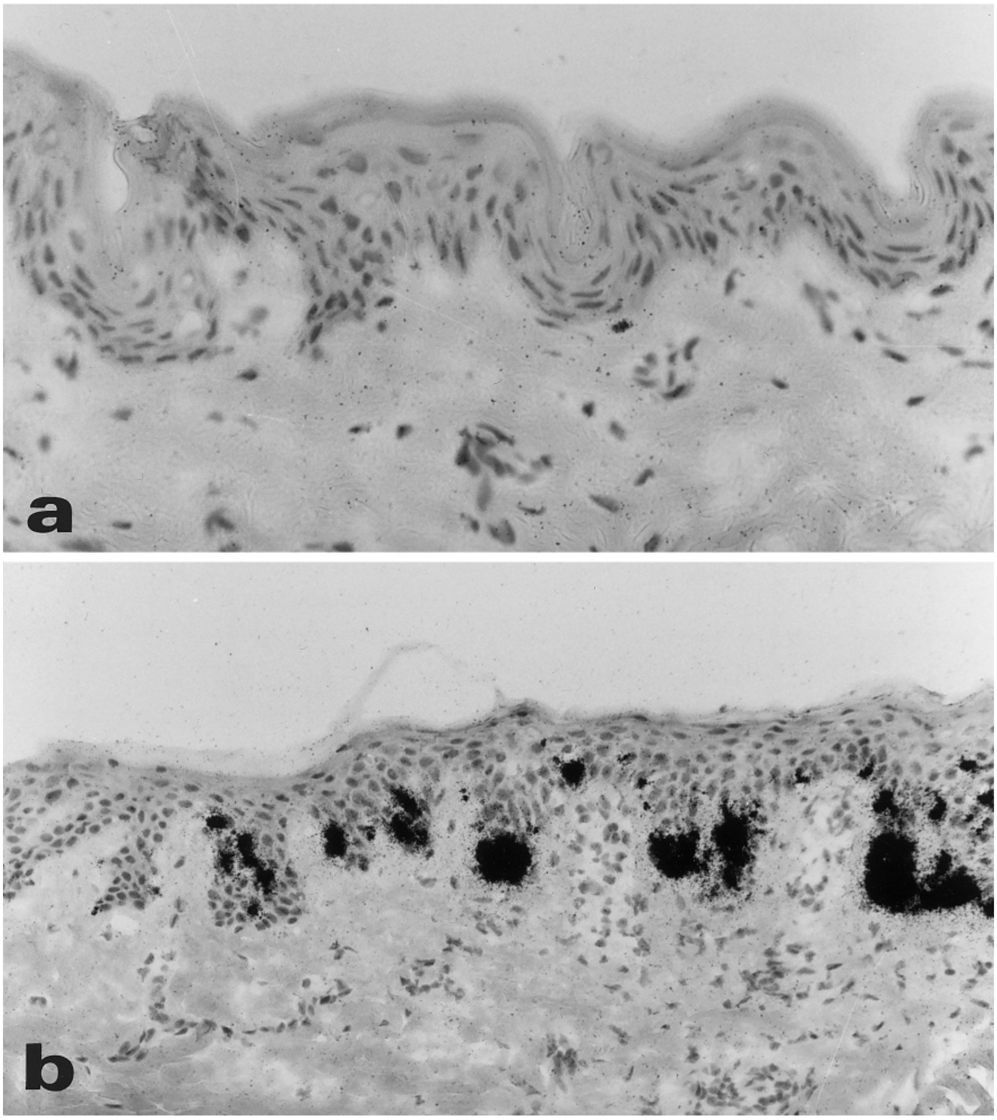

Fig. 3a Comparative in situ hybridization. Primary tissue specimen of normal skin that was treated absolutely identically to the malignant melanoma demonstrated in Fig. 2c. Note only sporadically weak autoradiographic signal overlying normal melanocytes at levels significantly below those obtained in malignant melanoma cells. **b** Tumor-surrounding normal epidermal melanocytes exhibiting significant Pmel17/gp100 transcript level. Note autoradiographic signal decreasing from right to left with increasing distance to the adjacent melanoma lesion. **a**×680, **b**×510

Discussion

Recently we and others have identified Pmel17/gp100 as representing the antigen recognized by melanoma-reactive monoclonal antibodies (mAb) HMB-45, HMB-50, and NKI-beteb [2, 41]. These antibodies are valuable diagnostic markers of malignant melanoma but, because of their heterogeneous staining pattern in melanoma tissue specimens, Pmel17/gp100 has been suggested to represent a minor favorable antigenic target for tumor vaccination as compared to other MAA exhibiting a more homogeneous distribution, for example tyrosinase [12]. However, there are several reasons why immunoreactivity obtained by use of these antibodies may not necessarily reflect the expression pattern of Pmel17/gp100 in vivo. First, the currently available Pmel17/gp100-reactive mAb exhibit significantly different immunoreactivity [19, 40]. Second, Pmel17/gp100 protein is highly glycosylated and the immunoreactivity of these mAb has been suggested to depend on posttranslational modifications [2]; this is supported by the observation of abrogation of HMB-45 immunoreactivity in tissue sections pretreated with neuraminidase [21]. Third, Pmel17/gp100-reactive antibodies may give false positive immunostaining results in breast and other epithelial tissues and tumors [7, 26].

In the present study, we have analyzed the expression of the MAA-encoding gene Pmel17 at the mRNA level in a variety of primary human tissue specimens of different origin (Fig. 1, Table 1). The detection of Pmel17/gp100 transcripts in all primary malignant melanoma specimens, in junctional nevocellular nevus tissue samples, and in subsets of normal epidermal melanocytes, but not in tumor and normal tissue specimens derived from a variety of nonmelanocytic tissues, strongly implies a melanocytic-cell-lineage-restricted expression of Pmel17/gp100. Furthermore, Pmel17/gp100 transcripts were detected in tissue specimens as well as in cell lines established from all stages of tumor progression [14], with significant expression levels in melanoma in situ and with high expression levels in the radial and vertical growth phases as well as in metastatic melanoma. Even amelanotic tissue specimens, as well as amelanotic melanoma cell lines, expressed Pmel17/gp100 transcripts at high levels. Taken together, these results indicate that the expression of Pmel17/gp100 is highly conserved in all stages of melanoma progression with the presence of significant transcript levels even in early and amelanotic melanoma lesions.

Our results also imply that immunoreactivity may not necessarily represent Pmel17/gp100 expression in individual tumor specimens. By comparative in situ hybridization and immunohistochemistry, a differential expression pattern of Pmel17/gp100 was observed at the cellular level in individual tumor specimens. This is most probably due to the higher sensitivity of techniques for detecting Pmel17/gp100 expression at the mRNA level. Thus, most primary melanoma tissue specimens exhibited a homogeneous distribution of Pmel17/gp100 transcripts at the cellular level even in tissue areas obviously lacking immunoreactivity. Even individual melanoma specimens completely lacking immunoreactivity contained significant amounts of Pmel17/gp100 mRNA, as determined by Northern blot analysis. The higher sensitivity of in situ hybridization/Northern blot analysis as compared to immunostaining indicates that Pmel17/gp100 expression in melanoma tissue samples can be analyzed more precisely at the mRNA level. Neither immunostaining nor mRNA analysis can give direct information about the presence of CTL-recognized peptides presented at the cell surface. But in view of the different staining patterns of the currently available Pmel17/gp100-reactive mAb, their glycosylation-dependent immunoreactivity and the glycosylation-independent HLA class I presentation of Pmel17/gp100 encoded peptides, the false positive results obtained by immunostaining, and the higher sensitivity of measurements at the mRNA level, analysis at the transcript level seems to be more valuable for typing of individual tissue specimens than immunophenotyping.

Cultured normal human epidermal melanocytes (NHEM) express Pmel17/gp100 transcripts at levels comparable to those observed in melanoma cells; however, Northern blot analysis of normal skin and comparative in situ hybridization revealed only low – if any – expression in normal human epidermal melanocytes in situ. These results indicate significant differentially quantitative Pmel17 gene expression between melanoma cells and their normal counterparts in vivo, with low transcript levels in normal epidermal melanocytes and high transcript levels in melanoma cells. A possible explanation for the high transcript levels in cultured NHEM is provided by data on the immunoreactivity of cultured NHEM. Normal epidermal melanocytes in vivo are not immunoreactive with mAb HMB-45; however, in cultured NHEM, HMB-45 immunoreactivity is inducible by usually substituted growth factors, for example insulin and epidermal growth factor (EGF) [35]. Similarly, melanoma-surrounding melanocytes also contain significant amounts of Pmel17/gp100 mRNA in vivo (Fig. 3b, Table l) correlating well with the previously described induction of HMB-45 immunoreactivity in melanoma-surrounding, so-called activated, epidermal melanocytes [34]. Thus, it seems reasonable to assume that the high Pmel17/gp100 transcript levels in cultured NHEM and in melanoma-surrounding normal epidermal melanocytes may be induced by growth factors substituted in vitro or by the presence of growth factors that have been implicated in melanoma growth and progression in vivo (i.e. insulin-like growth factor 1 and EGF) (reviewed in [27]).

In view of the low levels of Pmel17/gp100 transcripts in normal melanocytes and the strong and highly conserved expression in all stages of melanoma tumor progression, it is tempting to speculate that Pmel17/gp100 may potentially behave as a tumor-specific antigen in vivo. The inflammatory conditions at the tumor site, with the presence of cytokines and costimulatory molecules in combination with a highly increased Pmel17/gp100 expression in melanoma cells, may help to overcome autoimmune tolerance to Pmel17/gp100. And, indeed, the expression pattern of Pmel17/gp100 conforms well to the pattern of immune responses clinically observed against malignant melanoma or nevocellular nevus lesions. Expression of Pmel17/gp100 in melanoma cells themselves may correlate with spontaneous regression, perilesional vitiligo with Pmel17/gp100 expression in “activated” tumor-surrounding melanocytes, and Pmel17/gp100 expression in nevocellular nevi with the development of so-called Halo-nevi. The recognition of Pmel17/gp100-encoded peptides by CTL lines associated with in vivo tumor regression, the identification of shared Pmel17/gp100-encoded melanoma antigens, the in vivo rejection of melanoma transplants after immunization with adenoviral vectors containing Pmel17/gp100 cDNA, the tumor regression observed in melanoma patients after intradermal vaccination with Pmel17/gp100-derived peptides in combination with GM-CSF, the highly conserved expression of Pmel17/gp100 in all stages of melanoma tumor progression with significant transcript levels even in early lesions and in almost all tumor cells, the quantitatively differential expression of Pmel17/gp100 between melanoma cells and normal melanocytes in vivo, and the presentation of Pmel17/gp100-encoded peptides by HLA-A2 indicate that Pmel17/gp100-encoded MAA could be a promising target for immuno- and/or gene-therapeutic strategies in melanoma patients. Our results also indicate that retinal pigment cells as well as the substantia nigra could represent potential sites of adverse effects and, therefore, should be monitored accurately in patients receiving Pmel17/gp100-targeted immuno- and/or genetherapies.

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