Molecular Characterization of the Melanocyte Lineage-specific Antigen gp100*

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The glycoproteins recognized by monoclonal antibody (mAb) NKI-beteb are among the best diagnostic markers for human melanoma because their expression is restricted to melanocytic cells. Recently, we isolated a cDNA clone, termed gp100-cl, which confers immunoreactivity not only to mAb NKI-beteb, but also to two other mAbs used to diagnose malignant melanoma, HMB-50 and HMB-45. In this report, we demonstrate that gp100-cl cDNA encodes glycoproteins of 100 kDa (gp100) and 10 kDa (gp10) which are recognized by these mAbs in human melanoma cells. The translation product deduced from the open reading frame present in gp100-cl cDNA is highly homologous to another melanocyte-specific protein, Pmel17. Nucleotide sequence analysis of genomic DNA indicates that the transcripts corresponding to gp100 and Pmel17 cDNAs originate from a single gene via alternative splicing. In all normal and malignant melanocytic cells analyzed, gp100 and Pmel17 RNAs are simultaneously expressed.

Melanoma is a neoplasm that originates from melanocytes, pigment-producing cells in the skin. Melanoma is a relatively immunogenic tumor, as demonstrated by the presence of both cytotoxic T lymphocytes (CTL)¹ (Knuth et al., 1992) and antibodies (Mates et al., 1983) reacting with melanoma cells in patients. Characterization of the antigens recognized revealed that they include both tumor-specific antigens (van der Bruggen et al., 1991) and the melanocyte differentiation antigens tyrosinase (Bricliard et al., 1993) and gp75 (Vijayasaradhi et al., 1990). Both these differentiation antigens are localized in a distinct cellular organelle, the melanosome, and are involved in the synthesis of the pigment melanin (Hearing and Tsukamoto, 1991). To understand the potential role of immunological events in the pathogenesis and clinical course of melanoma, it is important to identify more of these antigens. This will not only result in the identification of potential targets for immune responses against melanoma, it may also lead to the identification of antigens involved in melanocyte differentiation and possibly transformation.

Many monoclonal antibodies have been raised against melanoma cells. Clinical and pathological analyses using these mAbs have led to the description of different steps in the transformation and progression of melanoma (reviewed by Ruiter et al. (1990)). Some of these mAbs define important markers in the initial diagnosis of melanoma, while others define melanoma progression antigens. Most of the antigens are expressed by both melanoma cells and melanocytes and are probably expressed during melanocyte differentiation. The melanocyte lineage-specific antigens recognized by mAb NKI-beteb are among the best diagnostic markers for human melanoma (Vennegoor et al., 1988). NKI-beteb reacts with melanoma cells throughout tumor development and does not cross-react with other tumor cell types or normal cells, except for cells of the melanocytic lineage. The antigens recognized by NKI-beteb are glycoproteins of approximately 10 kDa (gp10) and 100 kDa (gp100).

MATERIALS AND METHODS

Cells and Monoclonal Antibodies—The melanoma cell lines Mel-2a, M14, MEWO, and BLM (Adema et al., 1993) and the uveal melanoma cell line Mel 202 (Ksander et al., 1991) have been described. Isolation of human melanocytess from breast or foreskin was performed by the method of Elsinger and Marko (1982) with modifications by (Smit et al., 1993). NKI-beteb and HMB-60 have been described previously (Vennegoor et al., 1988; Vogel and Esclamado, 1988). HMB-45 was purchased from Enzo Biochem.

DNA Constructs, Transfections, and Immunofluorescence—gp100-cl cDNA was cloned in both orientations (pSVLgp100+ and pSVLgp100−) in the Smal site of the eukaryotic expression vector pSVL (Pharmacia Biotech Inc.). pSVL contains the SV40 late promoter and polyadenylation site, as well as the SV40 origin of replication, allowing a very high copy number during transient expression in COS-7 cells. For the construction of the 3′ truncated gp100 transcription unit pSVLgp100+ (ABS), we deleted the sequence between the BglII site in the 3′ part of gp100-cl cDNA and the SstI site in the vector. The resulting construct encodes a truncated gp100 protein in which the carboxyl-terminal 133 amino acids of gp100 are replaced by 4 amino acids (Arg-Ile-Gln-Thr) encoded by vector sequences. Transient expression of the constructs in COS-7 cells was performed by using 40 μg/ml Lipofectin reagent from Life Technologies, Inc. (Felgner et al., 1987) and 7.5 μg of DNA. Transfected COS-7 cells were prepared for immunofluo-
rescence as described previously (Adema et al., 1993) and examined using a confocal laser scanning microscope at 488 nm (Bio-Rad MRC 600).

Metabolic Labeling, Immunoprecipitation, and V8 Protease Mapping—Immunoprecipitation experiments were performed on metabolically labeled ([35S]methionine/cysteine; Amersham Corp.) cells as described by Vennegoor et al. (1988) using either NKI-beteb or HMB-50 covalently linked to protein A-Sepharose CL-4B beads (Pharmacia). In some experiments, tunicamycin (75 μg/ml, Calbiochem) was added during the prelabeling period and remained present during the metabolic labeling reaction (12.5 min). Immunoprecipitates were analyzed under reducing conditions by SDS-PAGE using 5–17.5% gradient gels. The relative molecular weight of the proteins was determined using co-electrophoresed, prestained markers (Life Technologies, Inc.). Gels were treated with 1× sodium salicylate (pH 5.4) prior to autoradiography (Kodak XAR).

V8 protease mapping was performed using the procedure described by Cleveland et al. (1977). Briefly, gel slices containing the 100-kDa proteins were placed in the wells of a second SDS gel (10%) and overlaid with Staphylococcus aureus V8 protease (2.5 μg/sample, Miles Laboratories). After electrophoresis, gels were treated as described above.

Molecular Cloning of Part of the gp100/Pmel 17 Gene—Part of the gp100/Pmel 17 gene was amplified by PCR on human genomic DNA isolated from peripheral blood lymphocytes using the following primers: 1497/1516 and 1839/1857 (see above) as described (Adema and Baas, 1991). The reaction products were subsequently amplified using a nested set of primers containing an additional EcoRI site (underlined) (5’-TATCTAGAATTCTGCACAGATGCAG-3’ and 5’-TATCTAGAATTCTGCAAGATGCCAGCCATG-3’) and cloned into the EcoRI site of pUC18.

RNA Isolation and Analysis—Total RNA was isolated using the guanidine thiocyanate/cesium chloride procedure (Chirgwin et al., 1979). cDNA was prepared using the GeneAmp RNA PCR kit (Perkin-Elmer, and PCR analysis was performed for 35 cycles in the presence of 3 mM MgCl2 using primers 1497/1516 and 1839/1857 (see above) as described (Adema and Baas, 1991). As probes, we used either a gp100-specific exon/exon junction oligonucleotide (5’-CTTCTCCAGGAGCGACAAGATGCCAGCCATG-3’) or a Pmel17-specific oligonucleotide (5’-TGTGAGAAGATCCACAGACATGATG-3’). The PCR products were subsequently digested using a nested set of primers containing an additional EcoRI site (underlined) (5’-TATCTAGAATTCTGCAAGATGCCAGCCATG-3’ and 5’-TATCTAGAATTCTGCAAGATGCCAGCCATG-3’) and cloned into the EcoRI site of pUC18.

RESULTS

Expression of gp100-c1 cDNA in Non-pigmented COS-7 Cells

Results in Immunoreactivity with mAbs NKI-beteb, HMB-50, and HMB-45—Expression of gp100-c1 cDNA in gp100-negative BL M melanoma cells results in immunoreactivity with the melanocyte lineage-specific mAbs NKI-beteb, HMB-50, and HMB-45 (Adema et al., 1993). To determine whether expression of gp100-c1 cDNA in non-melanocytic cells also results in immunoreactivity with these mAbs, we performed transient expression experiments in COS-7 cells (monkey kidney fibroblasts) with constructs containing gp100-c1 cDNA in the coding or non-coding orientation. As shown in Fig. 1, only COS-7 cells transfected with the construct containing the cDNA in the coding orientation (COS-7/pSVLgp100+) reacted with all three mAbs. These data demonstrate that immunoreactivity with NKI-beteb, HMB-50, and HMB-45 after expression of gp100-c1 cDNA is not restricted to melanocytic cells. In addition, these data show that the COS expression system can be used for further biochemical characterization of the proteins encoded by gp100-c1 cDNA.

Analysis of the Proteins Encoded by gp100-c1 cDNA—To characterize the proteins encoded by gp100-c1 cDNA, COS-7/pSVLgp100+ cells were metabolically labeled and subjected to immunoprecipitation with NKI-beteb or HMB-50. As shown in Fig. 2, NKI-beteb (panel A) and HMB-50 (panel B) specifically detected proteins of approximately 100 kDa (95–110 kDa) in extracts of COS-7/pSVLgp100+ cells. The molecular mass of these proteins is similar (see also below) to those detected in extracts of metabolically labeled MEWO cells (Fig. 2), which express the antigens endogenously (Vennegoor et al., 1988). Consistent with previous reports (Vennegoor et al., 1988; Vogel and Esclamado, 1988), both mAbs also recognize a protein of 10 kDa in extracts of MEWO cells (Fig. 2, lanes 3 and 7). A protein of the same size reacted with NKI-beteb in COS-7/pSVLgp100+ cells (Fig. 2A, lane 4) and could be discerned with HMB-50 after prolonged exposure (not shown). No specific proteins were immunoprecipitated by either of the mAbs from extracts prepared.
from COS-7 cells transfected with the construct containing the cDNA in the non-coding orientation (Fig. 2). Comparison of the culture medium of metabolically labeled COS-7/pSVLgp100+ cells and MEWO cells revealed that both mAbs also recognized proteins of about 100 kDa (see also below) in the culture medium of these cells (Fig. 3). No proteins of 10 kDa were immunoprecipitated by the mAbs from the culture medium of COS-7/pSVLgp100+ cells, as has been shown for melanoma cells.

To exclude the possibility that the proteins detected by the mAbs are derived from endogenous genes induced after transfection with gp100-c1 cDNA, we performed immunoprecipitation experiments with COS-7 cells expressing a 3' truncated gp100 transcription unit (see "Materials and Methods"). As shown in Fig. 4, proteins of approximately 85 kDa were detected by both mAbs in COS-7 cells expressing this construct, consistent with a deletion of 129 amino acids. This finding provides direct evidence that the 100-kDa protein recognized by NKI-beteb and HMB-50 in COS-7/pSVLgp100+ cells is encoded by gp100-c1 cDNA.

The 100-kDa Protein Encoded by gp100-c1 cDNA Is Identical to gp100—The proteins of about 100 kDa identified by NKI-beteb and HMB-50 in COS-7/pSVLgp100+ cells versus MEWO cells had a slightly different mobility when analyzed by SDS-PAGE (Fig. 2). This difference could be due to altered glycosylation, an event frequently observed in the COS expression system. Analysis of the proteins immunoprecipitated from MEWO cells and COS-7/pSVLgp100+ cells cultured in the presence of the glycosylation inhibitor tunicamycin demonstrated that in both cell types the size of the proteins of about 100 kDa was reduced to two protein bands of 90 and 85 kDa, confirming that the difference in mobility is due to altered glycosylation (not shown).

To provide further evidence that the proteins recognized by NKI-beteb in COS-7/pSVLgp100+ cells and MEWO cells are identical, we performed a V8 protease mapping experiment. As shown in Fig. 5, the same protein fragments were obtained after V8 protease digestion of the major 100-kDa protein isolated from COS-7/pSVLgp100+ cells or MEWO cells. We conclude from these data that gp100-c1 cDNA encodes the melanocyte lineage-specific glycoprotein gp100 recognized by NKI-beteb and HMB-50 in melanoma cells.
gp100 is a Type I Transmembrane Protein Highly Homologous to Pmel17—The nucleotide sequence of gp100-c1 cDNA was determined. It contains 2115 base pairs and terminates in-frame with a poly(A) tract of 15 nucleotides, preceded by the consensus polyadenylation sequence AATAAA (Proudfoot and Brownlee, 1976). An open reading frame extending from position 22 through 2007 is present in gp100-c1 cDNA. This open reading frame starts with an ATG codon within the appropriate sequence context for translation initiation (Kozak, 1987) and predicts a protein of 661 amino acids (Fig. 6). The amino-terminal 20 amino acids fit all criteria for signal sequences, including a potential cleavage site after Ala at position -1 (von Heyne, 1986), implying that mature gp100 contains 641 amino acids (approximately 70 kDa). Based on hydrophobicity plot analysis (Kyte and Doolittle, 1982), a single transmembrane domain bordered by charged residues is present in the carboxyl-terminal part (positions 571–591) of gp100. The predicted cytoplasmic domain is 45 amino acids long. Five putative N-linked glycosylation sites are present, consistent with gp100 being a glycoprotein. Furthermore, a histidine-rich domain (position 162–293), a threonine-rich domain (position 289–407) with repetitive amino acid sequences, and a cysteine-rich domain (position 456–546) are present.

A data base search (Pearson and Lipman, 1988; Altschul et al., 1990) revealed that gp100 is almost identical to Pmel17, another melanocyte-specific protein (Kwon et al., 1991). The amino acid differences between gp100 and Pmel17 consist of substitutions at position 254 (T → C/Pro → Leu) and 577 (C → G/Arg → Pro) and a stretch of 7 amino acids absent in gp100 at position 567 (see also Table II). A single nucleotide difference at position 762 (C → T) does not result in an amino acid substitution. gp100 is also 80% homologous to a putative protein deduced from a partial bovine cDNA clone (RPE-1) (Kim and Wistow, 1992) and 42% homologous to a chicken melanosomal matrix protein, MMP115 (Mochii et al., 1991).

gp100 and Pmel17 Are Encoded by a Single Gene—The most striking difference between gp100 and Pmel17 cDNAs is the in-frame deletion of 21 bp in gp100 cDNA. Possibly, both cDNAs correspond to transcripts generated by alternative splicing of a single primary transcript. To test this hypothesis, we used PCR to analyze the genomic DNA corresponding to the part of the gp100 gene surrounding the putative alternative splice site. Comparison of the nucleotide sequence of this genomic DNA with the sequence of gp100-c1 cDNA revealed the presence of an intron (102 bp) just at the position of the 21-bp insertion in Pmel17 cDNA (Table I). The exon/intron boundaries nicely fit the consensus 5′ donor and 3′ acceptor splice site sequences (Padgett et al., 1986). In the genomic DNA, the sequence comprising the additional 21 bp in Pmel17 cDNA is located directly upstream of this 3′ cleavage site and is preceded by an alternative 3′ acceptor splice site (Table I). Whereas the gp100-specific 3′ acceptor splice site fits the consensus sequence, the Pmel17-specific 3′ acceptor splice site appears to be suboptimal, in that it lacks a pyrimidine-rich region (Table I). Subop-
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The deduced amino acid sequence contains a putative signal peptide (underlined). The first amino acid of mature gp100 is designated +1. Putative TV-linked glycosylation sites are indicated by an asterisk whereas the transmembrane region is underlined.

Expression of gp100 and Pmel17 RNAs in Cells of the Melanocytic Lineage—The finding that gp100 and Pmel17 RNAs arise by alternative splicing of a single primary transcript raises the question of whether this occurs in a regulated manner. Previously, we showed that an RNA species of 2.5 kilobases is the major RNA product detected by gp100-cl cDNA on Northern blots of a B16 melanoma cell line.
comigrate with those detected by HMB-50 and NKI-beteb (Esclamado et al., 1986; Vennegoor et al., 1988). In addition, an
additive enzyme immunoassay revealed an additivity index of
91% for the mixture of HMB-45 and NKI-beteb (Vennegoor et al.,
1988). For our study, HMB-45 could not be obtained in
sufficient amounts to directly analyze the proteins it detects in
COS-7/pSVLgp100+ cells. However, the combined data of
the authors mentioned above and those described herein indicate
that the antigens recognized by HMB-45 are also encoded by
gp100-c1 cDNA.

Proteins Homologous to gp100—A data base search revealed
that gp100 is almost identical with the melanocyte-specific protein
Pmel17. The cDNA encoding Pmel17 was isolated from a λ
gt11 melanocyte cDNA library (Kwon et al., 1987; 1991). The
most striking difference between gp100-c1 and Pmel17 cDNA
consists of an in-frame deletion of 21 bp in gp100-c1 cDNA.
Nucleotide sequence analysis of part of the gene encoding
gp100 demonstrates that both cDNAs correspond to transcripts
originating from a single gene via alternative splicing. A single
5' donor splice site is used in combination with two different,
partially overlapping, 3' acceptor splice sites. No regulated
expression of gp100 and Pmel17 mRNAs in cells of the melanocytic
lineage has been observed; cells either expressed neither
of the mRNAs or both. These data are consistent with previous
results obtained by Kwon et al. (1987, 1991), indicating that the
gene encoding Pmel17, and hence the gp100 gene, is a single-
copy gene that maps to human chromosome 12 (region 12pter-
q21). Three other nucleotide differences have been detected
between gp100 and Pmel17, two of which give rise to an amino
acid substitution. They may represent allelic variations or poly-
morphisms, but we cannot exclude the possibility that they result
from mutations.

In addition to Pmel17, gp100 was found to be 80% homolo-
gous to the putative protein (RPE1) product encoded by a par-
tial bovine cDNA isolated from retinal pigment epithelium
(RPE) (Kim and Wistow, 1992). Bovine RPE has been shown to
react with HMB-50 (Kim and Wistow, 1992) and human RPE
with HMB-45 (Kapur et al., 1992). Since the RPE1 protein is
80% homologous to gp100 and lacks the 7 amino acids present
in Pmel17 (Table II), it may well represent the bovine homo-
logue of gp100.

Another data base entry showing significant homology (42%)
to gp100 is the melanosomal matrix glycoprotein MMP115 iso-
lated from chicken RPE (Mochii et al., 1991). MMP115 local-
izes, as gp100, in melanosomal vesicles. No function has been
reported for MMP115. The amino-terminal sequence (670
amino acids) of MMP115 is 46% homologous to the correspond-
ing part in gp100. The carboxy-terminal part of MMP115 (84
amino acids) is only 13% homologous to gp100 and does not
contain a transmembrane domain. Strikingly, the homology
between gp100 and MMP115 decreases just at the site of the
7-amino acid insertion in Pmel17 (Table II). Perhaps MMP115
represents a soluble form of the chicken homologue of gp100
still to be discovered in man.

A data base search with the gp100 amino acid sequence or
parts of this sequence did not reveal the presence of any known
functional domain. The spacing of the cysteines in the cysteine-
rich region that determines its tertiary fold is highly conserved
between gp100/Pmel17, RPE1, and MMP115 (Table II) but is
distinct from the ones found in other protein families, e.g. the
integrin (Kishimoto et al., 1989) or nerve growth factor receptor
families (Mallett et al., 1991). Therefore, the cysteine-rich do-
main present in the Pmel17/gp100 family may represent a
novel structural or functional (interaction with other proteins)
domain.

**DISCUSSION**

Herein, we demonstrate that gp100-c1 cDNA encodes the
melanocyte lineage-specific antigens recognized by mAbs NKI-
beteb, HMB-50, and HMB-45, which are valuable diagnostic
markers for melanoma. Expression of gp100-c1 cDNA in COS-7
cells demonstrates that NKI-beteb and HMB-50 recognize the
same proteins of 100 and 10 kDa. Comparison of the 100-kDa
protein in these cells and MEWO melanoma cells by V8 prote-
ase digestion demonstrates that they have the same protein
backbone. The relationship between the 100- and 10-kDa pro-
teins is not clear at present. The 10-kDa protein may be derived
from the 100-kDa protein either by specific proteolytic process-
ing or by degradation. Two findings lend support to the latter
explanation. (a) The amount of 10-kDa proteins relative to the
amount of 100-kDa proteins varies considerably between ex-
periments, and (b) the 10-kDa protein can only be detected
after a labeling period of 2 h while the 100-kDa protein is
already present in the culture medium after 1 h (Esclamado et
al., 1986; results not shown).

In addition to NKI-beteb and HMB-50, HMB-45 also reacts
with gp100-transfected COS-7 cells. HMB-45 has been reported
to immunoprecipitate proteins of 10 kDa from extracts and of
100 kDa from culture medium of melanoma cells, both of which

**FIG. 7.** Expression of gp100 and Pmel17 RNA in cells of the
melanocytic lineage. Reverse transcriptase/PCR was performed on
RNA isolated from MEWO (lanes 2), BLM (lanes 3), Di14 (lanes 4),
Nel2a (lanes 5) cutaneous melanoma cells, the uveal melanoma cells
Nel 202 (lanes 9), neonatal melanocytes (lanes 6 and 7), and adult
(lanes 8) melanocytes. As a control, PCR was performed on gp100-c1
cDNA (lanes 1). The reaction products were analyzed by Southern blot-
ting and hybridization to either a gp100-specific (left panel) or a
Pmel17-specific (right panel) oligonucleotide probe. As a control, both
probes were hybridized to a spot blot containing different amounts (100,
10, and 1 ng) of the Pmel17-specific exon/exon junction. The position of
the DNA species corresponding to Pmel17 and gp100 spliced products as
well as unspliced material and/or contaminating genomic DNA (*) are
indicated. Note that the gp100-specific exon/exon junction probe does
not react with DNA species corresponding to unspliced material or
genomic DNA.

ern blots containing RNA isolated from melanocytic cells. Es-
sentially the same results were obtained by Kwon et al. (1987)
using Pmel17-1 cDNA as a probe. However, neither of the
probes discriminate between gp100 and Pmel17 RNAs. There-
fore, we performed a reverse transcriptase/polymerase chain
reaction assay followed by Southern blotting and hybridization
to either a gp100-specific exon/exon junction or a Pmel17-spe-
cific oligonucleotide probe (see "Materials and Methods"). As
shown in Fig. 7, gp100 and Pmel17 spliced products were both
detected in three out of four cutaneous melanoma cells (lanes 2,
4, and 5) and in uveal melanoma cells (lanes 9), as well as in
neonatal (lanes 6 and 7) and adult melanocytes (lanes 8). Both
spliced products were also present in three different primary
melanomas (not shown). No products were detected with either
probe in gp100-negative BLM melanoma cells (lanes 3). These
results demonstrate that in all melanocytic cells examined,
gp100 and Pmel17 RNAs are expressed simultaneously.

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**TABLE II**

**Alignment of the carboxyl-terminal part of members of the gp100/Pmel17 family**

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<th>Pmel17</th>
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<tr>
<td>Gp100 Met</td>
<td>FLCVLYRGSVFTDLQIGESEEAILQAVPS</td>
<td><strong>GEGDAFELTVSCQGGLPKEA</strong></td>
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<td>Pmel17</td>
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<td>RPE1 Asp</td>
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<td>MMP115</td>
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<td>Gp100 Glu</td>
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Melanocyte-specific Antigen gp100: Cell-biological and Oncological Significance—The synthesis of melanin in melanocytes is a multistep process regulated at different levels (reviewed by Hearing and Tsukamoto (1991)). Tyrosinase (EC 1.14.11.1) is the key enzyme in this pathway and catalyzes the initial steps in the cascade of reactions leading to the production of melanin from the amino acid tyrosine. Two other members of the tyrosinase family are TRP-1 or gp75 (Shibahara et al., 1986; Vijayasrardhi et al., 1990) and TRP-2 (Jackson et al., 1992), the latter of which contains 3,4-dihydroxyphenylalanine (DOPA)chrome tautomerase (EC 5.3.2.3) activity (Tsukamoto et al., 1992). The combined data on gp100 and Pmel17 imply that they are also related to this process: 1) both proteins are only expressed in pigmented cells (Kwon et al., 1987; Vennegoor et al., 1988), 2) gp100 is present in melanosomal vesicles (Vennegoor et al., 1988), and 3) an increase in the amount of transcripts derived from the gp100/Pmel17 gene correlates with increasing levels of melanization (Kwon et al., 1987). Preliminary data indicate that Pmel17 also reacts with the anti-gp100 mAbs and is localized in melanosomes. Cumulatively, these data support a role of both gp100 and Pmel17 in melanization, either as enzymes regulating the quality or quantity of melanin synthesis or as structural components of the melanosome. The possibility that these proteins play a role in malignant transformation is not likely, but it cannot be excluded.

Melanoma is a relatively immunogenic tumor, demonstrated by the presence of both CTL (reviewed by Knuth et al., 1992) and antibodies (Mattes et al., 1983) against melanoma cells in patients. The recent finding that some CTL clones react not only with melanoma cells but also with normal melanocytes suggests that tissue-related antigens can also be recognized (Anichini et al., 1993). Moreover, Brichard et al. (1993) showed that a peptide derived from tyrosinase is recognized by a CTL clone. Recently, they have identified gp100-specific tumor-infiltrating lymphocytes in a melanoma patient (Bakker et al., 1994). These data demonstrate that besides its value as a tumor marker, gp100 may also serve as a target for specific immunotherapy against melanoma, provided that no unacceptable side effects are observed against normal tissues harboring pigmented cells.

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**REFERENCES**

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Tsukamoto, K., Jackson, I. J., Urabe, K., Montague, P. M., and Hearing, V. J. (1992) EMBO J. 11, 519