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Molecular Detection of Tumor-Associated Antigens Shared by Human Cutaneous Melanomas and Gliomas

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Both melanocytes and glial cells are derived embryologically from the neural ectoderm. Their malignant transformed counterparts, melanoma and glioma cells, respectively, may share common antigens. Numerous tumor-associated antigens have been identified in melanomas but only a few in gliomas. Using an established reverse transcriptase polymerase chain reaction plus Southern blot assay, we compared the mRNA expression of melanoma-associated antigens (MAAs) of melanomas to brain tumors primarily derived from glial cells. The MAAs studied included tyrosinase (Tyr), tyrosinase-related protein-1 and -2 (TRP-1 and TRP-2), gp100, human melanoma antigen-encoding genes 1 and 3 (MAGE-1 and MAGE-3), and melanotransferrin (p97). Glioblastoma multiforme (n = 21), anaplastic astrocytoma (n = 3), ependymoma (n = 2), meningioma (n = 3), oligodendroglioma (n = 1), and melanoma (n = 12) tumor specimens were assayed for MAA mRNA expression. Glioblastoma multiforme, astrocytoma, and melanoma cell lines were also assayed. We observed that individual MAA mRNAs were expressed in these brain tumors and cell lines at varying frequencies. The melanogenesis-pathway-related MAAs Tyr, TRP-1, TRP-2, and gp100 mRNAs were also expressed at different levels in normal brain tissues but at a much lower frequency than in glioblastoma multiforme and melanoma. MAGE-1 and MAGE-3 mRNA were expressed in different types of tumor specimens and cell lines but never in normal brain tissue. Tumor antigen p97 was expressed in all types of tumors and also in normal brain tissues. These studies demonstrate that melanomas and primary brain tumors express common MAAs and could be exploited in patients with malignant glioma by active specific immunotherapy against these common MAAs. (Am J Pathol 1997, 150:2143–2152)

Glial cells and melanocytes are both derived from the neural ectoderm.[1,2] Many studies have shown that neoplasms derived from these two types of cells, gliomas and melanomas, share many biological properties.[3–6] However, there is limited characterization of tumor antigens in glial-derived tumors. Although melanoma-associated antigens (MAAs) have been well characterized, immunogenic tumor-associated antigens in gliomas have not been well defined. Many of the MAAs assessed in melanomas are related to the melanogenesis pathway. Several of these anti-MAA monoclonal antibodies have been shown to cross-react with gliomas.[4,5] In addition, mRNAs encoding other MAAs such as human MAA melanoma antigen-encoding gene MAGE-1, have been detected in tumors of neural origin such as glioblastomas and neuroblastomas.[3] Melanogenesis-related proteins involved in the synthesis of melanin are inherent characteristics of melanocytes and melanomas, and neural derived tissues of the cochlea and eye have also been reported to express melanin and melanogenesis-related proteins.[7–9]

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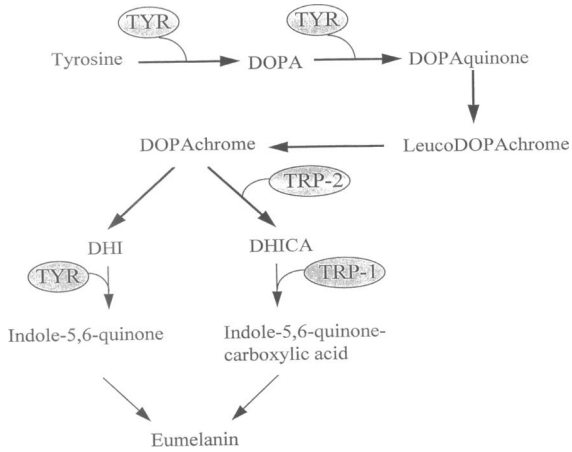

Figure 1. *The schematic of the melanogenesis pathway. DOPA, 3,4-dihydroxyphenylalanine; DHI, 5,6-dihydroxyindole; DHICA, 5,6-dihydroxyindole-2-carboxylic acid.*

These observations have been derived primarily through immunocytochemistry. The melanogenesis pathway is a complex cascade of enzymatic reactions beginning with tyrosine and ending with melanin as the final product (Figure 1). All components of the pathway have not been identified and steps in the pathway are still uncertain at this time. The melanin observed in melanocytes is suggested to be different from neuromelanin in neurons of the substantia nigra, locus ceruleus, and other brain stem cells. The metabolic pathway that leads to neuromelanin remains unknown, although it has been postulated that neuromelanin may be a by-product of norepinephrine and dopamine metabolism commonly associated with neurological functions.

To date, many MAAs, including melanogenesis-related proteins, have been demonstrated to be antigenic in humans by cytotoxic T cell and antibody assays.[10–14] Several of these MAAs are currently being investigated as target antigens for active specific immunotherapy against human melanoma.[15–17] On the other hand, there have been only a limited number of antigenic antigens identified in glial tumors. The identification of common MAAs in melanoma and glioma may be potentially useful in the latter as target antigens for active specific immunotherapy. Cancer vaccines for melanoma treatment has shown some promising therapeutic benefit in controlling tumor progression.[15–17] Although surgery and radiotherapy are the primary approaches for treating patients with glial tumors, their efficacy for controlling or preventing recurrent disease has been disappointing. Adjuvant therapies such as active specific immunotherapy that are less debilitating to the patient need to be developed to prevent or stabilize recurrent disease. To develop these therapeutics, however, tumor antigen targets need to be characterized in these brain tumors.

Due to the similar embryological origin of melanocytes and glial cells, we examined mRNA expression of common MAAs in their respective tumors. Molecular diagnosis of cancer by reverse transcriptase polymerase chain reaction (RT-PCR) has recently been shown to be a rapid, efficient method to examine expression of genes with minimal amounts of tissue.[18–21] The development of RT-PCR has significantly aided cancer diagnosis and characterization of gene expression with a limited amount of tissue. Analysis of brain tumor gene expression needs to be studied to design potential new therapeutics and analyze tumor gene expression profiles during tumor progression. The majority of the molecular studies to date on brain tumors have been focused on DNA rearrangements, oncogenes, and tumor suppressor gene activation.[22–24]

In this study, we assessed four major melanogenesis markers, tyrosinase (Tyr), tyrosinase-related protein (TRP)-1, TRP-2, and melanoma antigen (gp100), and three tumor-associated antigens, MAGE-1, MAGE-3, and melanotransferrin (p97), as the MAA markers in a RT-PCR plus Southern blot analysis of brain tumor specimens and glioma cell lines. Tyr is the key enzyme in the initial segment of the melanin synthesis pathway.[25,26] TRP-1 and TRP-2 are involved in the later steps of melanin synthesis.[27–29] gp100, related to the Pmel-17 gene family is associated with the melanosomal-related matrix.[30,31] The tumor-associated antigens MAGE-1 and MAGE-3 are immunogenic in humans, and their function is unknown.[12,13] They are expressed in melanomas and many other types of tumors of different embryological origin and have not been found in normal tissues except testes and placenta.[32,33] p97 is present in most melanomas, and has been shown to be expressed in other tumors and fetal tissues.[34,35] This study demonstrates that gliomas and melanomas express common MAA genes in which a subset of these markers is part of the melanogenesis pathway.

Materials and Methods

Cell Lines and Tumor Specimens

Human melanoma cell lines M12, M24, and M101 were established and characterized at the John Wayne Cancer Institute.[18,36] Human glioblastoma multiforme cell lines U-87 MG and U-118 MG and astrocytoma lines CCF-STTG1 and SW1088 were obtained from American Type Culture Collection (Rockville, MD). Human glioblastoma multiforme lines RC1909 and RC1913 as well as the brain tumor

Table 1. *Oligonucleotide Primers of Melanoma Molecular Markers and β-Actin Gene*

Gene	Primer sequences	Size (bp)
β-Actin	5′ CCT TCC TGG GCA TGG AGT CCT G 3′ GGA GCA ATG ATC TTG ATC TTC	201
Tyr	5′ TTG GCA GAT TGT CTG TAG CC 3′ AGG CAT TGT GCA TGC TGC TT	284
TRP-1	5′ AGA GAT GAT CGG GAG GTC TG 3′ CTG TGC CAT GTG AGA AAA GC	425
TRP-2	5′ GAG GTG CGA GCC GAC ACA AG 3′ CGG TGC CAG GTA ACA AAT GC	476
gp100	5′ TGG ACC TTG CCC ATC TGG CTC TTG G 3′ TGC CCA TCT GTG GTG CCT GGA ACT G	534
MAGE-1	5′ CGG CCG AAG GAA CCT GAC CCA G 3′ GCT GGA ACC CTC ACT GGG TTG CC	421
MAGE-3	5′ GAA GCC GGC CCA GGC TCG 3′ GGA GTC CTC ATA GGA TTG GCT CC	423
p97	5′ TAC CTG GTG GAG AGC GGC CGC CTC 3′ AGC GTC TTC CCA TCC GTG T	286

specimens were obtained from patients undergoing treatment at Virginia Commonwealth University. Cell lines were maintained as monolayer cultures in RPMI 1640 supplemented with 10% heat-inactivated fetal calf serum (Gemini, Calabasas, CA), penicillin, and streptomycin. Total RNA was extracted from cells when cultures reached 70 to 80% confluency.

Brain tumor specimens studied were from surgically resected tissue and were immediately frozen in the operating room and stored at −80°C until used. Other specimens from these operations were assessed by pathologists to determine the type and grade of tumors. Pathologically defined normal brain tissues from patients undergoing operation for a non-tumor-related problem were studied for comparison. These brain specimens were taken from the frontal cortex and temporal lobe of the brain. Pathologically defined melanoma specimens, along with normal breast and lung tissues from cancer patients were also assessed for comparison. Normal breast and lung tissues were used in this study as negative RT-PCR controls.

Normal male and female volunteer donor blood was used for negative controls. Ten milliliters of blood was collected in sodium-citrate-containing tubes and centrifuged using a hypotonic density gradient solution (Dot Kit, National Genetics Institute, Los Angeles, CA).[19,20] Total peripheral blood lymphocytes (PBLs) in the blood were used for RNA isolation.

RNA Preparation

Total cellular RNA was extracted using Tri-Reagent according to the manufacturer's protocol (Molecular Research Center, Cincinnati, OH) as previously described.[19,20] All specimens were kept on ice during processing. The tissues were minced and lysed in 1 ml of Tri-Reagent. The nucleic acid extracts were washed once with 75% ethanol, vacuum-dried, and resuspended in 10 mmol/L Tris/HCl with 1 mmol/L EDTA solution (pH 7.4). The total amount of RNA and its purity were assessed by ultraviolet spectrophotometry. The integrity of mRNA was assessed by RT-PCR of β-actin expression and analyzed by gel electrophoresis plus ethidium bromide staining. All RNA extraction was carried out in a designated sterile laminar flow hood with RNAse-free labware. Separate rooms and building facilities were designated for tissue processing, RNA isolation, RT-PCR set-up, and Southern blot analysis to avoid post-PCR contamination.

Oligonucleotide Primers and cDNA Probes

Oligonucleotide primers were synthesized and purified at the National Genetics Institute. Optimal primer sequences were designed with the Oligo Primer Analysis Software, version 5.0, by National Biomedical Systems, Plymouth, MN (Table 1).

Reverse Transcriptase Polymerase Chain Reaction

An aliquot containing 1 μg of total RNA was mixed with diethyl pyrocarbonate-treated double-distilled water to bring the volume to 11.5 μl. The samples were placed at 70°C for 5 minutes and then chilled on ice. The RT reaction mixture consisted of 4 μl of first-strand RT buffer (USB, Cleveland, OH), 2 μl of 10 mmol/L deoxynucleotide triphosphate mixture (dNTPs), 20 U of RNAsin, 500 ng of oligo dT_{15} primer, and 200 U of Moloney murine leukemia virus

Table 2. *mRNA Expression of Molecular Markers in Glioma Cell Lines*

Cell line	Type	Tyr	TRP-1	TRP-2	gp100	MAGE-1	MAGE-3	p97
RC1909	G	+	+	−	−	−	−	−
RC1913	G	−	+	−	−	−	−	−
CCF-STTG1	A	−	+	+	+	−	+	+
SW1088	A	+	+	+	+	+	+	+
U-87 MG	G	+	+	+	+	+	+	+
U-118 MG	G	+	+	−	+	+	+	+
% Positive		67%	100%	50%	67%	50%	67%	67%

Analysis of cell lines (1 μg of RNA) was by RT-PCR and Southern blot. A, astrocytoma cell line; G, glioblastoma multiforme cell line.

reverse transcriptase (Promega, Madison, WI). The RT mixture was added to the samples to a final volume of 20 μl, and the reaction was incubated at 37°C for 2 hours, heated to 95°C for 5 minutes, and then chilled on ice. All RT reactions were carried out with oligo dT priming.

The PCR mixture was prepared as previously described.[18–20] Briefly, the mixture consisted of 10 μl of 10X thermophilic DNA polymerase reaction buffer, 8 μl of 10 mmol/L dNTP, 6 μl of $MgCl_2$ (25 mmol/L), 100 pmol/L each primer, 5 U of Ampli*Taq* DNA polymerase (Promega), and 20 μl of RT mixture. Double-distilled water was added to bring the reaction volume to 100 μl. The PCR program was set as follows: 1 cycle of 95°C for 5 minutes; 35 cycles of 95°C for 1 minute, 55°C for 1 minute (for all primers except p97 at 65°C), and 72°C for 1 minute; and final extension at 72°C for 10 minutes. PCR was performed in an OmniGene temperature cycler (Hybaid, Middlesex, England).

Automated Southern Blot Analysis

The RT-PCR cDNA products were first run on 2% agarose gel and then alkaline denatured and transferred onto nitrocellulose membrane with 20X standard saline citrate (SSC) buffer as previously described.[18,19] Specific cDNA probes for Tyr, TRP-1, TRP-2, gp100, MAGE-1, MAGE-3, and p97 were developed from corresponding RT-PCR cDNA products and labeled with digoxigenin.[18] Southern blotting was performed using automated Southern blot instrumentations under standard operation procedures (National Genetics Institute). The use of an automated Southern blot procedure allows analysis of multiple specimens and controls under highly stringent quality-controlled conditions at the same time. Southern blot analysis is very essential in verifying the RT-PCR cDNA product and can enhance sensitivity. For each Southern blot run, negative controls included RT-PCR performed with all reagents except for mRNA and two normal PBL samples that were carried throughout the entire RT-PCR procedure. Positive controls included two positive melanoma cell lines. The blots were scanned on an electronic imager and verified by at least two readers for positive and negative results. Any discrepancies, questionable blots, and false positives were repeated for verification.

Results

Melanoma cell lines, melanoma specimens, glioma lines, and brain tumor specimens were assayed for expression of MAA mRNA. We have demonstrated the expression of the MAA in melanoma specimens (to be published).[18] In this study, we assessed melanomas to provide a representative analysis for comparison to brain tumor cell lines and tumor specimens. Melanoma specimens were run at the same time and with the identical conditions as the brain tumor specimens for assay comparison purposes. As negative controls, mRNA of PBLs from 15 normal donors were assessed for the MAA. None of these normal PBLs was positive for the MAA molecular markers after Southern blotting, although there was an occasional related weak positive band for gp100 (<2%) observed near the gp100 RT-PCR cDNA band.

MAA mRNA Expression in Melanoma and Glioma Cell Lines

All three representative cutaneous melanoma cell lines assessed (M12, M24, and M101) were positive for each of the MAA markers studied: Tyr, TRP-1, TRP-2, gp100, MAGE-1, MAGE-3, and p97. The six glioma cell lines (four glioblastoma multiforme and two astrocytoma lines) expressed one or more of these MAA markers. The overall expression level of MAA mRNA in glioma lines, however, was lower than that observed for melanomas. TRP-1 was expressed in all six glioma lines, whereas Tyr, gp100, MAGE-3, and p97 were expressed in four (67%) of the lines. TRP-2 and MAGE-1 were expressed in three (50%) of the lines (Table 2). The glioma cell lines were assessed to show relative MAA marker distribution.

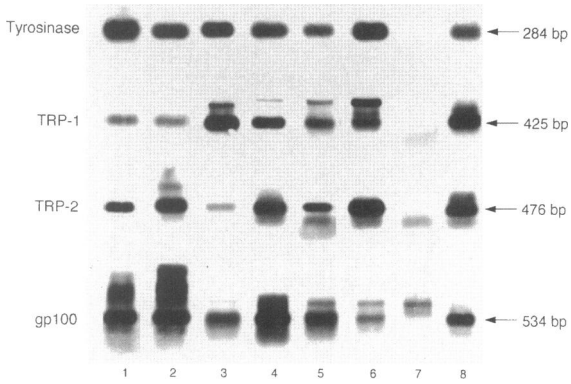

Figure 2. *Representative RT-PCR and Southern blot analysis of MAA (tyrosinase, TRP-1, TRP-2, and gp100) mRNA expression in glioma cell lines and biopsy specimens.* **Lanes 1** *and* **2**, *glioblastoma cell lines;* **lanes 3** *to* **6**, *glioma biopsies positive for MAA mRNA;* **lane 7**, *normal donor PBLs;* **lane 8**, *positive marker Southern blot standard control. Bands on top of the specific cDNA bands for TRP-1, TRP-2, and gp100 are genomic products.*

Representative examples of RT-PCR and Southern blots are shown (Figures 2 and 3). As tumor molecular markers in general are overamplified in established cell lines,[37] we evaluated tumor specimens to get a more realistic assessment of MAA mRNA expression.

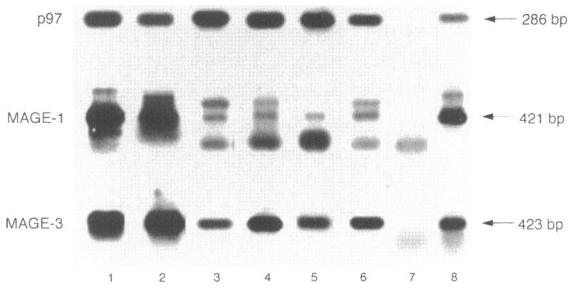

Figure 3. *Representative RT-PCR and Southern blot examples of MAA (MAGE-1, MAGE-3, and p97) mRNA expression in glioma cell lines and biopsy specimens.* **Lanes 1** *and* **2**, *glioblastoma cell lines;* **lanes 3** *to* **6**, *glioma biopsies positive for MAA mRNA;* **lane 7**, *normal donor PBLs;* **lane 8**, *positive marker Southern blot standard control.*

MAA mRNA Expression in Melanoma and Brain Tumor Specimens

Twelve tissue specimens of malignant melanoma were analyzed by RT-PCR plus Southern blot to detect MAA mRNA expression (Table 3). p97 was present in every specimen, followed by Tyr and TRP-2, which were present in all but one specimen (92%). gp100, TRP-1, MAGE-3, and MAGE-1 were expressed in 83, 67, 58, and 42% of the tumor specimens, respectively. All 12 melanoma specimens expressed at least three MAA mRNAs, and 10 (83%) were positive for at least five of the seven MAAs studied.

Thirty brain tumor specimens, which included twenty-one glioblastoma multiforme, three astrocytomas, three meningiomas, two ependymomas, and one oligodendroglioma were assessed for MAA mRNA expression (Tables 4 and 5). RT-PCR plus Southern blot analysis showed that all the MAA mRNAs were expressed in these specimens, although their expression varied among individual tumors (Figures 2 and 3).

Of the 21 glioblastoma specimens analyzed for MAA by RT-PCR plus Southern blot, 7 (33%) were positive for at least five markers, and only 3 specimens were negative for all seven MAAs (Table 4). The melanogenesis-related proteins Tyr, TRP-1, TRP-2, and gp100 mRNA were expressed in 38, 52, 62, and 38% of the glioblastoma specimens, respectively (Table 4). The tumor antigens MAGE-1, MAGE-3, and p97 were present in 38, 33, and 67% of the glioblastoma specimens, respectively. The overall frequency of all of the markers mRNA expression in these glioblastoma specimens was lower than that observed in melanoma specimens.

Table 5 shows the mRNA expression frequency of the markers in the astrocytoma (n = 3), ependymoma (n = 2), meningioma (n = 3), and oligodendroglioma (n = 1) specimens. These tumor specimens were assessed to determine whether other brain tumors of glial

Table 3. *mRNA Expression of Molecular Markers in Metastatic Melanoma Specimens*

Melanoma specimen	Tyr	TRP-1	TRP-2	gp100	MAGE-1	MAGE-3	p97
A	+	+	+	+	+	+	+
B	−	−	+	+	−	−	+
C	+	−	+	+	−	+	+
D	+	+	+	+	+	+	+
E	+	+	+	+	−	−	+
F	+	+	−	+	+	−	+
G	+	−	+	+	−	+	+
H	+	+	+	+	−	−	+
I	+	−	+	−	−	+	+
J	+	+	+	−	−	+	+
K	+	+	+	+	+	−	+
L	+	+	+	+	+	+	+
% Positive	92%	67%	92%	83%	42%	58%	100%

Analysis of 12 melanoma specimens (1 μg of RNA) tested by RT-PCR and Southern blot.

Table 4. *mRNA Expression of Molecular Markers in Glioblastoma Specimens*

Specimen	Tyr	TRP-1	TRP-2	gp100	MAGE-1	MAGE-3	p97
RC1904	−	−	−	−	−	−	−
RC1908	−	−	−	−	−	−	−
RC1934	−	+	−	−	+	+	+
RC1941	−	−	−	−	+	−	+
RC1946	−	−	−	−	+	−	−
RC1951	−	+	+	+	−	−	+
RC1953	−	+	+	+	+	−	+
RC1955	−	+	−	+	−	−	−
RC1967	−	−	−	−	−	−	−
RC1969	+	−	−	−	−	−	+
RC1974	+	−	+	−	+	+	+
RC1977	−	+	+	−	−	−	−
RC1983	−	+	+	+	+	+	+
RC1988	+	+	+	+	−	+	+
RC1995	+	−	+	−	−	−	+
RC2002	+	+	+	−	−	−	+
RC2004	−	+	+	+	−	+	+
RC2027	+	−	+	−	−	−	+
RC2049	−	−	+	+	+	−	+
RC2057	+	+	+	+	−	+	+
RC2060	+	+	+	−	+	+	−
% Positive	38%	52%	62%	38%	38%	33%	67%

Analysis of 21 glioblastoma specimens (1 μg of RNA) tested by RT-PCR and Southern blot.

and nonglial origin express MAA mRNA. All three astrocytoma specimens expressed p97, followed by Tyr, TRP-1, and TRP-2 in two specimens and gp100 and MAGE-1 in one specimen. None of the three astrocytomas were positive for MAGE-3. TRP-1 and p97 were shown in both ependymoma specimens. One ependymoma specimen expressed all of the MAAs except MAGE-1. gp100 and p97 were expressed in all three meningioma specimens, followed by TRP-1 and MAGE-1 in two specimens and TRP-2 and MAGE-3 in one specimen. None of the three meningioma specimens were positive for Tyr. The oligodendroglioma specimen was positive for TRP-2, gp100, MAGE-1, and p97 MAA.

Assessment of MAA mRNA in Normal Tissues

For comparison, pathology-defined normal brain (n = 11) tissues were also assessed in this study and were run at the same time as the brain tumors. The melanogenesis-related markers Tyr, TRP-1, TRP-2, and gp100 mRNA were found in some normal brain specimens (Table 6). p97 mRNA was present in four (36%) of the normal brain specimens. However, none of the normal brain tissue samples expressed MAGE-1 or MAGE-3 mRNA. Pathology-defined normal tissues of breast and lung assessed at the same time showed no marker expression.

Discussion

This study demonstrates by molecular analysis that melanoma and primary tumor of the brain cells share common tumor markers. The technique used to identify specific markers is highly specific and sensitive as previously described.[19,20] The advantage of using this molecular detection approach as opposed to other techniques such as biochemical analysis or

Table 5. *mRNA Expression of Molecular Markers in Nonglioma Tumor Specimens*

Specimen	Type	Tyr	TRP-1	TRP-2	gp100	MAGE-1	MAGE-3	p97
RC2016	A	−	−	−	−	+	−	+
RC2031	A	+	+	+	−	−	−	+
RC2034	A	+	+	+	+	−	−	+
RC2029	E	−	+	−	−	−	−	+
RC2023	E	+	+	+	+	−	+	+
RC2022	M	−	+	−	+	+	−	+
RC2012	M	−	−	−	+	−	−	+
RC2011	M	−	+	+	+	+	+	+
RC1957	O	−	−	+	+	+	−	+

Analysis of specimens (1 μg of RNA) tested by RT-PCR and Southern blot. A, astrocytoma; E, ependymoma; M, meningioma; O, oligodendroglioma.

Table 6. *mRNA Expression of Molecular Markers in Normal Brain Specimens*

Specimen	Tyr	TRP-1	TRP-2	gp100	MAGE-1	MAGE-3	p97
A	+	+	+	+	−	−	−
B	−	−	+	−	−	−	−
C	+	+	+	+	−	−	+
D	+	+	+	+	−	−	−
E	−	+	+	+	−	−	−
F	−	−	+	+	−	−	−
G	−	−	−	−	−	−	−
H	−	+	−	−	−	−	−
I	+	+	+	+	−	−	+
J	+	+	+	−	−	−	+
K	−	+	+	−	−	−	+
% Positive	45%	73%	82%	55%	0%	0%	36%

Analysis of specimens (1 μg of RNA) tested by RT-PCR and Southern blot.

antibody staining is that it is less labor intensive and more specific, it can be easily repeated several times on a small amount of tissue, reagents are readily available for all the markers and, most importantly, it requires a very small amount of tissue. Using our established technique, we were able to assess multiple molecular markers that are common for both melanomas and brain tumors. As the embryological origins of both tumor types are common, there was expectancy that expression of some of these molecular markers would be shared. Assessment of molecular markers by RT-PCR of tumors can provide rapid assessment of genotype expression of specific genes. However, no information about expression of these MAAs at the protein level in the cells or the heterogeneity of each MAA expression within a lesion is known. We have used the tumor marker RT-PCR assay to assess metastatic occult tumor cells in blood and various types of tissues successfully.[18–20]

To date, there has not been a comprehensive comparison analysis of antigens in both human melanomas and gliomas. The study is important for several reasons because the identification of common tumor antigens in both tumor types will help in developing active specific immunotherapy protocols such as cancer vaccines.[15] Melanoma has been well studied by our group and others in terms of antigen-specific immune responses induced by active specific immunotherapy.[17,40] The outcome of clinical trials of melanoma vaccines has been encouraging. However, effective treatment of malignant primary tumors of the brain by various treatments, including active specific immunotherapy, is far less developed.[41] Identification of common antigens among gliomas, the most common of brain tumors, and melanomas would be important to address the feasibility of potential therapeutic development against the former. Also, it is important to note that melanomas often eventually kill the host through brain metastases.[42] Greater than 70% of melanoma patients who have expired with advanced disease have metastases to the brain.[43] Therefore, treatment of metastatic melanoma to the brain with active specific immunotherapy against specific antigens will require some knowledge of normal brain tissue expression of these antigens as well.

Our initial focus of study was on the melanogenesis markers. In the literature, there are indications, primarily by biochemical analysis, that melanogenesis-related proteins are present in normal brain.[8] One purpose of our study was to determine whether neural-derived brain tumors express melanogenesis MAA mRNA. Several of the melanogenesis-related proteins, such as gp100, Tyr, gp75 (TRP-1), MAGE-1, and MAGE-3, have been shown to be antigenic.[10–14,16,28,40] All of the MAAs were found in varying frequencies among the glioblastoma specimens. It was surprising in detecting the melanogenesis cascade of proteins Tyr, TRP-2, TRP-1, and gp100. However, the frequency of Tyr was significantly lower in these glioblastomas *versus* melanomas. TRP-1 and TRP-2 mRNAs were highly expressed in gliomas but less than in melanoma specimens. gp100 expression in glioblastomas *versus* melanomas was comparable to Tyr expression. Other types of primary tumors of the central nervous system were shown to express MAAs. However, the number of specimens was limited.

p97, which was highly expressed in all primary tumors of the brain we studied, was also expressed in 36% of normal brain tissue analyzed. There are limited studies on the overall expression of this marker in tumors.[34,35] Studies suggest that it is not a melanoma-specific protein and has other functions in various tissues.[34] A similar observation has been found with MAGE marker expression in various tumors. Both MAGE-1 and MAGE-3 were expressed to a lesser degree in the glioblastomas than in the melanomas. However, expression of MAGE-1 in glio-

blastomas was not that much less than in melanomas. Overall, MAGE-3 was less expressed than MAGE-1 in gliomas, which was opposite that of melanomas. Interestingly, a similar pattern was found in the other neural tumors. Although MAGE-1 and -3 were discovered in melanomas, they are not melanoma specific, as studies have shown that they are expressed in various frequencies in different carcinomas.[12,13,32,23] Both MAGE-1 and MAGE-3 are immunogenic in humans and can be potential targets for active specific immunotherapy. We observed that no normal tissues of the brain expressed MAGE-1 or -3 marker mRNA.

Previously, studies have demonstrated the presence of Tyr by antibody staining or biochemical analysis in several neural-crest-derived cells such as Schwann cells and the satellite of spinal ganglia.[8] Certain parts of the brain such as the substantia nigra, locus ceruleus, and brain stem are pigmented.[44] The retina of the eye is pigmented and is the site of origin of the ocular melanoma that expresses the melanogenesis marker and other MAA mRNA (unpublished data). Studies have not previously documented the expression of melanogenesis pathway markers such as TRP-1, TRP-2, and gp100 in brain tumors. It has been indicated the pigmentation of the neural tissue is due to the presence of neuromelanin, although the neuromelanin synthesis pathway has not been well established.[44] Catecholaminergic neurons involved in perception, movement, emotion, and memory have pigmentation.[46] The overlap, if any, of the neuromelanin and melanocyte melanin synthesis is not known.

The expression of several MAAs in gliomas and other types of brain tumors offers a rationale for applying current active specific immunotherapy protocols used in treating malignant melanoma to gliomas.[41,47] Currently, we have a melanoma cell vaccine protocol that has been shown to control tumor progression and prolong survival of melanoma patients with advanced-stage disease.[15] Preliminary studies also indicate that it can prevent tumor metastases to the brain.[15] The melanoma cell vaccine contains all of the MAAs described in the present study, and there have been no major adverse effects particularly to the central nervous system in patients treated to date. One could question whether cytotoxic T cells to immunogenic MAAs such as Tyr or gp100 would affect normal tissue. In general, normal nonhemopoietic tissue and particularly the brain do not or weakly express major histocompatibility antigens (MHC) on their cell surfaces.[48,49] Therefore, they would not be expected to be susceptible to attack by MAA-specific MHC-restricted cytotoxic T cells.

Our study demonstrates a rapid system of identifying tumor markers in neural tumors that could be potentially useful in screening for active specific immunotherapy patient candidates. We have also identified potential new known tumor antigens in primary brain tumors that were not previously defined. Glioma patients may benefit from active specific immunotherapy targeting specific MAAs.

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