Expression of MAGE and GAGE in High-Grade Brain Tumors: A Potential Target for Specific Immunotherapy and Diagnostic Markers

Deborah L. Scarcella, C. W. Chow, Michael F. Gonzales, Catherine Economou, Francis Brasseur, and David M. Ashley

Departments of Haematology and Oncology [D. L. S., C. E., D. M. A.] and Anatomical Pathology [C. W. C.], Royal Children’s Hospital, and Departments of Anatomical Pathology [M. F. G.] and Surgery [D. M. A.], Royal Melbourne Hospital, Parkville 3052, Melbourne, Victoria, Australia; and Ludwig Institute for Cancer Research, Brussels Branch, and Cellular Genetics Unit, Université Catholique de Louvain, B-1200 Brussels, Belgium [F. B.]

ABSTRACT

The mRNA expression of the tumor-associated antigens MAGE and GAGE was examined in 60 high-grade brain tumors. This analysis was performed by using reverse transcription-PCR, Southern blotting, and sequencing. It was demonstrated that, of the eight GAGE genes, GAGE-2 and -7 were expressed in five of seven normal brains. Four groups of tumors—adult glioblastoma multiforme (n = 20), pediatric glioblastoma multiforme (n = 9), medulloblastomas (n = 15), and ependymomas (n = 14)—were analyzed for mRNA expression. The following frequencies were observed: MAGE-1, 0, 0, 13, and 0%, respectively; MAGE-2, 5, 11, 60, and 57%; MAGE-3 & -6, 0, 0, 13, and 0%; GAGE-1, 65, 11, 13, and 43%; and GAGE-3–6 and -8: 75, 78, 47, and 93%, respectively. Two unclassified tumors expressed GAGE-3–6 and -8 only. The absence of GAGE-1 expression in normal brain, its relatively high frequency of expression in high-grade brain tumors, and its unique 3’ sequence, suggest it may represent a useful target for specific immunotherapy. The detection method of reverse transcription-PCR and Southern blotting may also be useful for rapid screening of biopsy specimens both for diagnostic purposes and to determine a patient’s eligibility for specific immunotherapy.

INTRODUCTION

Despite advances in surgery, radiotherapy, and chemotherapy, high-grade brain tumors continue to carry a very poor prognosis. Consequently, there is a need to find new therapeutic modalities that specifically target brain tumors.

Several approaches to cancer immunotherapy are under investigation in brain tumors. One such approach is the use of active immunotherapy. Previous studies (1, 2) using animal models of brain tumors have demonstrated that vaccinations of autologous tumor cells that have been genetically modified are able to protect mice against intracranial tumor challenge and prolong survival in mice with established intracranial tumors.

However, there may be several barriers to these approaches in human patients. These include the technical difficulties in preparing vaccines from tumor specimens and the risk of causing experimental allergic encephalomyelitis because it is often difficult to separate normal brain from tumor tissue (3, 4). It has been shown that nonhuman primates and guinea pigs immunized with human GBM1 tissue developed experimental allergic encephalomyelitis that was lethal (5). Therefore, the targeting of a tumor-associated antigen may represent a reasonable initial approach to human active immunotherapy for high-grade brain tumors.

Several tumor-associated antigens have now be identified, and these are coded by the MAGE (6, 7), BAGE (8), and GAGE (9) families of genes. Although these antigens were originally identified in melanoma, they have since been found to be present in a variety of tumor types, including lung and bladder carcinomas, sarcomas, and head and neck tumors (e.g., Refs. 8, 9, and 10). Lack of expression of MAGE and GAGE in a large panel of normal adult tissues, including stomach, lung, breast, colon, skin and uterus, fetal liver, and brain has been demonstrated previously (7, 9). The only normal tissues in which expression of these genes has been observed was testis (9) and placenta for MAGE-3 and -4 only (7).

Because melanocytes and glial cells are both derived from the neuroectoderm, it has been hypothesized that glial tumors also express one or more of these genes. However, studies examining the expression of these genes in glioma are limited. Chi et al. (11) examined the expression of MAGE-1 and -3 in 21 GBM specimens by RT-PCR and Southern blotting and demonstrated frequencies of expression of 38 and 33%, respectively.

We have analyzed the expression of MAGE-1, -2, -3 and -6.
and the GAGE family of genes in a panel of GBMs from adult and pediatric patients as well as medulloblastomas, ependymomas, and two other high-grade brain tumors using the sensitive method of RT-PCR and Southern blotting. A panel of normal brain specimens was also included for analysis of GAGE expression.

MATERIALS AND METHODS

Controls: Normal Brains and Cell Lines. Six normal brain specimens were snap-frozen in liquid nitrogen and stored at -80°C. Three were from adult brains, two were from fetal brain, and one was from adult cerebellum. A sample of the normal human brain 5′-STRETCH PLUS cDNA library (Clontech, Palo Alto, CA) was kindly donated by Dr. Nigel Parker (Royal Children’s Hospital, Melbourne, Victoria, Australia). The D566 (human GBM) cell line was obtained from Dr. Darel Bigner (Duke University Medical Center, Durham, NC) and maintained in zinc option medium (Life Technologies, Inc., Gaithersburg, MD) supplemented with 10% heat-inactivated FCS (Trace Biosciences, Castle Hill, New South Wales, Australia), and the MEL-1 (human melanoma) cell line was maintained in RPMI 1640 (Life Technologies) supplemented with 10% heat-inactivated FCS. The MEL-1 cell line was obtained from Dr. Joe Trapani (Austin Research Institute, Melbourne, Victoria, Australia).

Tumor Specimens. Brain tumor specimens from adult patients were snap-frozen in liquid nitrogen after surgical resection, and those from pediatric patients were embedded and frozen in OCT. All specimens were stored at -80°C. Classification of tumor type and grade was made by two independent pathologists in a blinded fashion in accordance with the WHO histological typing of central nervous system tumors (12).

RNA Isolation and cDNA Synthesis. Total RNA was isolated from brain tumor specimens and cell lines using the acid guanidium thiocyanate-phenol-chloroform method (13), quantitated by UV spectrophotometry and stored at -80°C in 100% ethanol and 75 mM sodium acetate pH6. An aliquot containing 1 μg of RNA was removed and centrifuged, and the dried pellet was resuspended in 20 μl of the reverse transcription mix, which contained the following: 1× reaction buffer [50 mM Tris-HCl (pH 8.3), 75 mM KCl, 3 mM MgCl2, and 10 mM DTT], 1 μM each dNTP (Promega, Madison, WI), 1 nmol pd(N)6 random primer (Pharmacia Biotech, Uppsala, Sweden), 9.2 units of RNasin (Promega), and 200 units of Moloney murine leukemia virus reverse transcriptase RNase H minus (Promega). The reaction was incubated at 37°C for 60 min and then stopped and stored at -20°C.

For normal brain specimens, total RNA was extracted using the guanidinium thiocyanate/cesium chloride method (14), and then 2 μg were reverse-transcribed as described previously (15).

PCR. Sequences of oligonucleotide primers for PCR amplification and expected product sizes are listed in Table 1. β2-microglobulin was used to assess the integrity of the mRNA. PCR for all genes, except GAGE-1, -2, and -7 and GAGE-3–6 and -8, was performed in a 20-μl reaction volume containing the following: 1 μl of cDNA (or 2.5 μl for normal brain), 1× reaction buffer [10 mM Tris-HCl (pH 8.3) and 50 mM KCl], 1.5 mM or 2 mM MgCl2 (depending on primer pair), 0.25 mM each dNTP (Promega), 10 pmol each primer, and 1 unit of AmpliTaq DNA polymerase (Perkin-Elmer Corp., Norwalk, CT). For amplification of GAGE-1, -2, and -7 or GAGE-3–6 and -8, primer and dNTP concentrations were reduced to 8 pmol and 0.1 mM, respectively. The reaction mixture was overlaid with mineral oil. An initial denaturation step was performed at 95°C for 3 min. Amplification for 30 cycles then followed: 94°C for 1 min and 72°C for 2 min for MAGE-1 and β2-microglobulin; 94°C for 1 min, 62°C for 1 min, and 72°C for 1 min for MAGE-2; 94°C for 1 min, 68°C for 1 min, and 72°C for 2 min for MAGE-3 and -6 (for individual gene amplification); 94°C for 1 min, 64°C for 1 min, and 72°C for 2 min for MAGE-3 and -6 (for simultaneous amplification of both genes); 94°C for 1 min, 56°C for 2 min, and 72°C for 2 min for GAGE-1 and GAGE-1, -2, and -7; and 94°C for 1 min, 58°C for 2 min, and 72°C for 2 min for GAGE-3–6 and -8. The reaction was completed by a final extension at 72°C for 10 min or 15 min for GAGE PCRs. All PCRs included a MAGE- or GAGE-positive cell line and a genomic DNA sample as controls.

Southern Blotting. The entire PCR was electrophoresed on a 1.5 or 2% agarose gel and transferred onto a Hybond-N+ membrane (Amersham Life Sciences, Cleveland, OH) in 0.4 M NaOH. The DNA was fixed to the membrane in 0.4 M NaOH and then washed in 5× SSC. Membranes were prehybridized in 0.5 M sodium phosphate (pH 7.2), 7% SDS, and 1.5 mM EDTA for 45 min. Sequences for probes used for the mRNA detection of all antigens and β2-microglobulin are listed in Table 1. GAGE-1, -2, and -7 and GAGE-3–6 and -8 PCR products were probed with the GAGE-1-8 probe and GAGE-1 PCR products with the GAGE-1-specific probe. All probes were labeled at their 5′ end with T4 polynucleotide kinase (New England Bio-labs, Beverly, MA) and [γ-32P]ATP (DuPont, Wilmington, DE) and added directly to the prehybridization solution, and membranes were hybridized overnight at 37°C. Membranes were washed in 2× SSC at room temperature for 5 min and then two washes in 0.2× SSC-0.1% SDS at 37°C for 15 min each.

Sequencing. To verify MAGE- and GAGE-specific sequences of amplified products, sequencing was performed on two to three specimens from each tumor type and on positive control cell lines for each gene amplified. PCR was performed as described above except that reaction volume was increased to 100 μl, and amplification was increased to 40 cycles to increase the amount of amplicon. PCR products were then purified in one of two ways: (a) agarose gel electrophoresis, isolation of bands of interest, and DNA purification with the BRESAclean DNA purification kit (Bresatec, Adelaide, South Australia, Australia); or (b) direct purification with the PCR product presequencing kit (Amersham). Sequencing was performed by the dideoxy-chain termination method using the T7 Sequenase version 2.0 kit (Amersham). Samples were sequenced with the sense or antisense primer used in the PCR. Sequences were entered into the BLAST sequence program (www.ncbi.nlm.nih.gov/ BLAST/) to search for sequence homology.

RESULTS

Sixty brain tumor specimens were analyzed for gene expression by RT-PCR using PCR primers designed to give either
cDNA-specific bands (MAGE-1, -3, & -6; all GAGE primer pairs; and \( \beta_2 \)-microglobulin) or cDNA bands distinguishable from genomic DNA (MAGE-2, -3, and -6). All PCR products were then probed with internal specific sequence oligonucleotides, and two to three specimens for each tumor type were sequenced for each gene. The panel of specimens consisted of 29 GBMs (20 adult and 9 pediatric), 15 medulloblastomas, 14 ependymomas, and 2 other high-grade brain tumors. The histology of all tumors was typical for that tumor type with two exceptions, GBM028 and GBM031. GBM028 is a GBM arising from a preexisting desmoplastic infantile ganglioglioma, and GBM031 is a GBM that appears to have a dysembryoplastic neuroepithelial tumor-like focus. Two other tumors, the histologies of which were not consistent with that for GBM, medulloblastoma, or ependymoma, were also included. BT02 and BT03 are unclassified high-grade brain tumors, possibly atypical teratoid/rhabdoid tumors. We also examined a panel of seven normal brains for antigen mRNA expression. The expression patterns for all specimens are summarized in Tables 2 and 3, and representative autoradiographs are shown in Figs. 1 and 2. Unless otherwise indicated, all data for analysis of MAGE-3&6 expression were obtained using the MAGE-3/6F/BLE-5 primer pair for simultaneous gene amplification.

### Table 1 Oligonucleotide primers and probes for mRNA analysis of antigens and \( \beta_2 \)-microglobulin

<table>
<thead>
<tr>
<th>Gene</th>
<th>Name (direction)</th>
<th>Sequence</th>
<th>Ref.</th>
<th>Product size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAGE-1</td>
<td>CHO-14 (S)</td>
<td>CGGCCGAAGGAACCTGACCAG</td>
<td>10</td>
<td>421</td>
</tr>
<tr>
<td>MAGE-1</td>
<td>CHO-12 (AS)</td>
<td>GCTGGAACCCCTCAGTGCTGC</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>MAGE-2</td>
<td>CDS-9 (S)</td>
<td>AAGTAGGGCCAGGACAGTCC</td>
<td>10</td>
<td>236</td>
</tr>
<tr>
<td>MAGE-2</td>
<td>CDS-7 (AS)</td>
<td>GAAGAGGAAGAAGCCTGCTG</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>MAGE-3</td>
<td>AB1197 (2)</td>
<td>TGGAGGACCAAGGGCCCC</td>
<td>10</td>
<td>725</td>
</tr>
<tr>
<td>MAGE-3</td>
<td>BLE-5 (AS)</td>
<td>GGAGGACCAAGGGCCCGTC</td>
<td>10</td>
<td>805</td>
</tr>
<tr>
<td>MAGE-6</td>
<td>AB1197 (S)</td>
<td>TGGAGGACCAAGGGCCCC</td>
<td>10</td>
<td>727</td>
</tr>
<tr>
<td>MAGE-6</td>
<td>BLE-5 (AS)</td>
<td>GGAGGACCAAGGGCCCGTC</td>
<td>10</td>
<td>805</td>
</tr>
<tr>
<td>MAGE-3 &amp; 6</td>
<td>MAGE-3/6F (S)</td>
<td>GGTGAGGAGGCAAGGTTCTG</td>
<td>759/761</td>
<td></td>
</tr>
<tr>
<td>GAGE-1</td>
<td>VDE-44 (S)</td>
<td>GACCAAGAGCCTAGCTAG</td>
<td>9</td>
<td>352</td>
</tr>
<tr>
<td>GAGE-1</td>
<td>VDE-1R (AS)</td>
<td>GCAATTTGTCATTAAAGCC</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>GAGE-1, 2, and 7</td>
<td>VDE-44 (S)</td>
<td>GACCAAGAGCCTAGCTAG</td>
<td>9</td>
<td>244</td>
</tr>
<tr>
<td>GAGE-1, 2, and 7</td>
<td>VDE-24 (AS)</td>
<td>CCATCAGGACCATCTTCA</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>GAGE-3–6 and 8</td>
<td>VV-1 (S)</td>
<td>GACCAAGGGCCTGATGAC</td>
<td>—⁠*</td>
<td></td>
</tr>
<tr>
<td>GAGE-3–6 and 8</td>
<td>VDE-24 (AS)</td>
<td>CCATCAGGACCATCTTCA</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>( \beta_2 )-microglobulin</td>
<td>( \beta_2MG-F ) (S)</td>
<td>CTCGCGCTACTCCTCTTCTG</td>
<td>18</td>
<td>300</td>
</tr>
<tr>
<td>( \beta_2 )-microglobulin</td>
<td>( \beta_2MG-R ) (AS)</td>
<td>GTTACATGCTTCTGATCCCACCTAA</td>
<td>18</td>
<td></td>
</tr>
</tbody>
</table>

* Direction of primers: S, sense; AS, antisense.

** Table 2 Antigen mRNA expression in normal brain specimens**

<table>
<thead>
<tr>
<th>Specimen</th>
<th>GAGE-1</th>
<th>GAGE-1, -2 and 7</th>
<th>GAGE-3–6 and 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adult brain</td>
<td></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Nb01</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Nb02</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Nb03</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Fetal brain</td>
<td></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Nb04</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Nb05</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Adult cerebellum</td>
<td></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Nb06</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Normal brain 5' STRETCH</td>
<td></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>PLUS cDNA library</td>
<td></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>% positive (n = 7)</td>
<td>0</td>
<td>71</td>
<td>0</td>
</tr>
</tbody>
</table>

* Seven normal brain specimens were analyzed for antigen mRNA expression by RT-PCR (0.05 \( \mu \)g of RNA) and Southern blotting using internal specific sequence probes.

**Molecular Detection of MAGE and GAGE Expression in Normal Brain.** Expression of GAGE genes was detected using three sets of PCR conditions: GAGE-1, GAGE-1, -2, and...
Using the GAGE-1, -2, and -7 PCR conditions, we detected mRNA expression in five of seven normal brain specimens (Fig. 1; Table 2). However, when GAGE-1 only was amplified, none of the specimens were positive for this gene, thus indicating that GAGE-2 and/or -7 only are expressed in normal brain. No expression of GAGE-3–6 or -8 was observed. The normal brain specimens were analyzed for GAGE expression only, as absence of MAGE-1, -2, and -3 expression in normal brain has been reported previously using RT-PCR and Southern blotting (6).

Molecular Detection of MAGE and GAGE Expression in Brain Tumor Specimens. Because no GAGE-1, -3–6, or -8 or MAGE was detected in normal brains, we examined tumor specimens for expression of these genes. In adult GBM, GAGE was the predominantly expressed gene, with 65% of specimens expressing GAGE-1 and 75% expressing GAGE-3–6 and -8. Little or no expression of the four MAGE genes was observed, with only one specimen expressing MAGE-2. The antigen mRNA expression profile in pediatric GBM was similar to that for adult GBM with respect to MAGE and GAGE-3–6 and -8 expression. However, only 11% of pediatric GBM specimens expressed GAGE-1. GBM025 did not express any of the genes.

The MAGE-3 and -6 expression data presented here for adult GBM specimens was initially determined using two pairs of PCR primers: AB1197/BLE5 and AB1197/MAGE-6R for individual amplification of MAGE-3 and -6, respectively (10). Southern blot results from this analysis showed amplification of a 805-bp genomic DNA band, as expected. However, the cDNA band amplified in the tumor samples appeared slightly larger than expected. The cDNA band in the positive control, D566,
was 725/727-bp, as predicted. We, therefore, sequenced the genomic DNA and cDNA bands from two GBM specimens and the cDNA band from the positive control for both MAGE-3 and -6. As expected, in all cases, the sequence of the 805-bp genomic DNA band was 100% homologous to published MAGE-3 and -6 sequences (7, 16); however, the sequence of the cDNA band from the tumor specimens was not homologous to any human sequence. The sequence of the 725-bp MAGE-3 or 727-bp MAGE-6 cDNA bands from D566 was 100% homologous with published sequences. We then screened the specimens again with a third pair of PCR primers for simultaneous amplification of both MAGE-3 and -6 (MAGE-3/6F/BLE5). Using these primers, no expression of these genes was observed in tumor specimens; however, good amplification was noted in positive controls. In summary, in contrast to a previous report (11), these data confirmed that MAGE-3 and -6 mRNA is not detectable in GBM specimens.

For medulloblastomas and ependymomas, GAGE-3–6 and -8 were more frequently expressed than GAGE-1: a similar profile to that for both adult and pediatric GBM. MAGE-1, -3, and/or -6 were expressed by medulloblastomas only—the only group of tumors examined that expressed any of these three genes. MAGE-2 was expressed at a high frequency by both medulloblastomas (60%) and ependymomas (57%). Of the two other glial tumors examined, both had the same expression profile, in that GAGE-3 and -6 mRNA is not detectable in GBM specimens.

Confirmation of Results. In addition to sequencing MAGE-3 and -6 PCR products amplified from adult GBM specimens, we also sequenced two to three specimens from each tissue group (i.e., normal brain and tumors) for each gene amplified to confirm our Southern blot results. In normal brain, insufficient GAGE-1, -2 and -7 PCR product was obtained for sequencing due to the low expression levels of these genes. In all tumor specimens, however, sequence of all GAGE, MAGE-1, -2, and -3 and -6 (simultaneous gene amplification) PCR products (where amplified) was 100% homologous with published sequences (6, 7, 9, 16, 17), as were sequences of positive control PCR products.

**DISCUSSION**

We have demonstrated the expression of various genes of the MAGE and GAGE families in high-grade brain tumors using the sensitive technique of RT-PCR and Southern blotting. This is also the first observation that these genes are expressed in a normal tissue other than testis or placenta. These observations have important implications for both the development of active immunotherapy and the diagnosis of high-grade brain tumors.

Although the lack of expression of MAGE in normal brain had been demonstrated by RT-PCR and Southern blotting (6), a similar analysis had not been performed for GAGE. The data presented here using RT-PCR and Southern blotting suggest that two of the eight GAGE genes, GAGE-2 and/or -7, are expressed in normal brain, albeit at very low levels. This observation will need further investigation because we were unable to sequence the PCR products for GAGE-1, -2, and -7.

Of the MAGE genes we have analyzed, MAGE-2 showed the highest frequency of expression, with 5, 11, 60, and 57% of adult GBMs, pediatric GBMs, medulloblastomas and ependymomas, respectively, being positive for this gene. MAGE-1, -3, and/or -6 were expressed by medulloblastomas only. These results differ from those of Chi et al. (11), who showed 38 and 33% expression of MAGE-1 and -3, respectively, in GBM and
expression of MAGE-3 in one of two ependymomas but no expression of MAGE-1. We believe that this discrepancy may be explained by nonspecific hybridization of internal probes to non-MAGE amplification products. In this study, we encountered cross-hybridization of the MAGE-3/6 probe and so performed sequencing of PCR products amplified from adult GBM specimens to confirm our Southern blot results. This sequencing of MAGE-3 and -6 PCR products (from individual gene amplification) showed that there was nonspecific amplification and hybridization of MAGE-3 and -6 primers and probes indicating that there was no expression of either of these genes in GBM. This was further confirmed using primers which simultaneously amplified both MAGE-3 and -6.

The high level of expression of MAGE-2 in medulloblastomas and ependymomas, in contrast to GBM, is of interest. These differences in expression patterns may be exploited to differentiate these tumor types where diagnosis is proving difficult.

In contrast to the expression patterns of the four MAGE genes examined, GAGE was expressed in all tumor types with 11–65% expressing GAGE-1 and 47–93% expressing GAGE-3–6 and -8. This high frequency of expression is comparable only to that in neuroblastomas, in which 82% of this group of tumors express one or more GAGE genes (18).

Although the expression of the antigen mRNA was heterogeneous within each tumor group, all genes were expressed in the GBM cell line, D566. Previous studies have demonstrated that transcriptional activation of MAGE-1 and the GAGE family of genes is dependent upon the methylation status of the promoter (19).

There is high sequence homology between GAGE-2 and -7, the 5′ end of GAGE-1 and GAGE-3–6 and -8. GAGE-1 differs from GAGE-2 to -8 in that it has an additional 143-bp exon that encodes 22 COOH-terminal amino acids in the putative GAGE-1 protein. The unique sequence in GAGE-1 may represent a more specific target for immunotherapy for two reasons. First, immunizing against GAGE-3–6 or -8, which are expressed in a larger proportion of tumors, as opposed to GAGE-1, may be problematic as these genes share a high sequence homology with GAGE-2 and -7, which are expressed in normal brain. Second, the additional 22 COOH-terminal amino acids unique to GAGE-1 contains several potential immunoreactive epitopes.

Lethé et al. (15) demonstrated that CTL lysis of melanoma and other tumor cell lines occurs only if MAGE-1 expression exceeds 10% of that found in a control cell line. The feasibility of using GAGE as a target for specific immunotherapy must include a consideration of the level of expression of this gene in tumors examined. When compared with D566, a GBM cell line, the expression level of GAGE in tumors was <1% of that in D566 (data not shown). Thus, further investigation is required to determine whether GAGE would be a useful target for immunotherapy as the level of expression is low, requiring 30 cycles of PCR for detection, and a homogeneous expression within a tumor type has not been ascertained.

The specific diagnosis and grading of astrocytomas is often difficult. Significant inconsistencies in the pathological classification of astrocytomas can occur, often compromising patient management and jeopardizing the interpretations of clinical studies (20, 21). Molecular tools for diagnosis are urgently required. Thus, the pattern of MAGE and GAGE expression may also be potentially useful as a diagnostic marker for high-grade brain tumors. Studies examining the expression of MAGE and GAGE in WHO grades I, II, and III gliomas are currently underway.

Our results show that expression of MAGE-1, -3, and -6 in high grade brain tumors is infrequent. A large percentage of medulloblastomas and ependymomas express MAGE-2. GAGE was expressed by all four tumor types. The absence of GAGE-1 in normal brain, its relatively high frequency of expression in high-grade brain tumors, and its unique 3′ sequence make it attractive as a potential target for specific immunotherapy. The detection method we used here may be used for rapid screening of biopsy specimens both for diagnostic purposes and to determine a patient’s eligibility for such therapy.

ACKNOWLEDGMENTS

We thank Prof. Thierry Boon for his support of this project.

REFERENCES


Expression of MAGE and GAGE in High-Grade Brain Tumors: A Potential Target for Specific Immunotherapy and Diagnostic Markers


Updated version  Access the most recent version of this article at: http://clincancerres.aacrjournals.org/content/5/2/335

Cited articles  This article cites 15 articles, 6 of which you can access for free at: http://clincancerres.aacrjournals.org/content/5/2/335.full.html#ref-list-1

Citing articles  This article has been cited by 12 HighWire-hosted articles. Access the articles at: /content/5/2/335.full.html#related-urls

E-mail alerts  Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions  To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions  To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.