MAGE-E1, a New Member of the Melanoma-associated Antigen Gene Family and its Expression in Human Glioma

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ABSTRACT

To unearth glioma-specific genes in human glioblastoma, the serial analysis of gene expression technique was applied to a primary glioblastoma, using cultured human astrocytes as a normal control. Among the top 147 most-expressed tags in glioblastoma, we found a tag, TTATGGTAT, that originated from an unidentified gene and which was not detected in human astrocyte cultures. Real-time quantitative reverse transcription-PCR showed that MAGE-E1 expression was 2.6- to 15-fold enriched in glioblastoma relative to human astrocytes. Expressed sequence tags containing this tag were homologous to the melanoma-associated antigen gene (MAGE) family, and this new cDNA, named MAGE-E1, was cloned by the 5’-rapid amplification of cDNA ends technique. Three alternatively spliced variants (MAGE-E1a-c) were found, and deduced amino acid sequence showed that MAGE-E1a and -Eb shared the MAGE-conserved region, whereas -Ec did not. This suggests that although MAGE-E1c is expressed from one of the MAGE family, it has distinct functions from other members. Tissue distribution analysis showed that MAGE-E1 was distinct from other MAGES. MAGE-E1 expression was detected only in brain and ovary among normal tissues. Interestingly, MAGE-E1a and/or -Eb were specifically expressed in glioma cells among cancer cells. These results indicate that MAGE-E1 is a novel and glioma-specific member of MAGE family.

INTRODUCTION

The prognosis of malignant glioma treatment in human patients is distressingly bad, and the 5-year survival rate of patients after surgery is only 8% in Japan. New therapeutic means to cure glioma, including immunotherapy and gene therapy, are being developed; glioma-specific genes to be used for these new therapies have been sought but without any success. Although genes for GFAP and vimentin are known to be expressed in glioma cells and used for cell typing, they are also expressed in normal astrocytes and are not exclusive for glioma. Glioma-specific genes, like α-fetoprotein for hepatocellular carcinoma or MAGE genes for melanoma, should provide us with a tool for diagnosis and a target molecule for treatment of malignant gliomas. Such genes should also be useful for understanding the pathogenesis and progression of gliomas.

Recent progress in molecular biology provides us with tools to uncover tissue- or cell type-specific genes. SAGE is one such technique, and it enables us to detect all expressed genes, including unknown ones, in a given tissue and to quantify the relative expression level of each gene (1). Thus, it is possible to pick up specifically up-regulated genes from an entire gene pool by comparing SAGE profiles between various cancers and normal tissues (2). In SAGE, each mRNA is distinguished by a short nucleotide fragment (~14 base, called a “tag”), which is cleaved from a single particular position of the mRNA. As tags are collectively subcloned and sequenced, thousands of mRNAs can be analyzed in a relatively short time. The original gene of each tag can be identified by searching databases, and the level of the expression can be quantified as the frequency of the appearance of the tag. The great advantage of the technique is that tags from unidentified genes can also be quantified, which was impossible using other techniques such as Northern blotting. In the present study, we performed SAGE experiments to search glioblastoma-specific genes in a glioblastoma sample and discovered expression of a novel gene, a new member of MAGE family, which is up-regulated in glioblastoma as compared with normal tissue.

MAGE is a gene family that encodes melanoma antigens. MAGE-A1 was originally isolated from the melanoma genomic DNA coding an antigen recognized by CTLs (3). Many genes homologous to MAGE-A1 have been determined on Xq 28, Xq21.3, Xq26, and Xp11.23 (4–8) and are classified as MAGE-A, -B, -C, and -D, respectively. Previous studies have described the characteristics of the MAGE-A subfamily as follows: (a) they were not expressed in normal cells except for testis and placenta; and (b) some antigens coded by the gene family are presented by the human leukocyte antigen (9). These findings suggest that immunoreaction against MAGE proteins are expected to affect only the tumor cells but not normal cells and/or tissues, because testis and placenta lack human leukocyte antigen expression. Immunotherapy with MAGE-A peptides showed tumor regression without significant side effects in melanoma patients who were vaccinated with the MAGE peptide (10).

In the present study, we demonstrate that the novel MAGE-related gene, MAGE-E1, is up-regulated in malignant glioma. Distribution within normal tissues indicates that MAGE-E1 is a unique member of MAGE gene family that is specifically expressed in brain and ovary.

MATERIALS AND METHODS

Materials. Primary glioblastoma (GB-1–8) tissue block was obtained surgically from adult patients. A human glioma cell line, U87 MG, was cultured with DMEM (Sigma Chemical Co., St. Louis, MO) containing 10% fetal bovine serum (Life Technologies, Inc., Rockville, MD). Total RNA was extracted by the guanidine isothiocyanate-CsCl gradient method (11). Poly(A) RNA fractions of GB-1 and U87 MG were obtained with an mRNA Isolation Kit (Stratagene, La Jolla, CA). Cultured human astrocytes (HA-1) were prepared as reported previously (12). Poly(A) RNA fraction was obtained from HA-1 with a QuickPrep mRNA Purification Kit (Amersham Pharmacia Biotech, Uppsala, Sweden). Total RNAs of lung cancer (non-small cell lung cancer), stomach cancer, colon cancer, and hepatocellular carcinoma were provided by Dr. Shunichiro Okumura (Kansai Medical University, Osaka, Japan).

SAGE. SAGE was performed with 2.5 μg of the poly(A) RNA according to the original method (1). Tags were analyzed by SAGE software provided by Dr. Kenneth W. Kinzler (Johns Hopkins University, Baltimore, MD). The

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3 The abbreviations used are: GFAP, glial fibrillary acidic protein; MAGE, melanoma-associated antigen gene; SAGE, serial analysis of gene expression; RT-PCR, reverse transcription-PCR; EST, expressed sequence tag; RACE, rapid amplification of cDNA ends; CGAP, Cancer Genome Anatomy Project; VEGF, vascular endothelial growth factor; HSP-70, heat shock protein 70.
original gene of each tag was identified by searching the GenBank database (version 107.0). SAGE profiles reported in the Cancer Genome Anatomy Project (CGAP) were obtained through Internet from the web site at http://www.ncbi.nlm.nih.gov/SAGE.

Real-time Quantitative RT-PCR. Real-time quantitative RT-PCR was performed with total RNA from four primary glioblastomas (GB-2,-3,-4,-6) and human astrocyte cultures (HA-1) with TaqMan Reverse Transcription Reagents and the TaqMan PCR Core Reagent Kit (Perkin-Elmer, Brunchburg, NJ) following the instruction manual. 5'-CCAGCTTCTCTTCCGGATC-3' (F1 primer) and 5'-GTAAACGTGATACCAAAACATG-3' (R1 primer) were designed to amplify the 3'-half region of an EST, AB028447, containing the tag sequence of MAGE-E1. TaqMan probe for detection of MAGE-E1, 5'-CCGGCTTCTctcGCggAGGT-3' (MAGE-E1 probe) was designed to detect a sequence within the F1 primer and the R1 primer (Fig. 1A).

Human &-actin primers and probe were purchased from Perkin-Elmer. The reverse-transcribed sample from GB-6 was diluted (1:2, 1:4, 1:8, and 1:16) to construct the standard curve. Experiments were run in duplicate, and the average value of the threshold cycle (Ct) was calculated for MAGE-E1 and &-actin, respectively. PCR reaction and the resulting relative increases in reporter fluorescent dye emission were analyzed by the 7700 sequence detector (Perkin-Elmer, Brunchburg, NJ).

Cloning of MAGE-E1 cDNA. An EST, AB028447, was found to contain a tag sequence, CATGTTTTGGGTAT. To use the sequence information of this EST for the cloning of corresponding cDNA, we first confirmed that the tag sequence, 5'-CCAGCTTCTCTTCCGGATC-3' (F1 primer) and 5'-GTAAACGTGATACCAAAACATG-3' (R1 primer) were designed according to the nucleotide sequence in the region confirmed by the second round of 5'-RACE, a RT-PCR experiment was performed with 1 &g of U87 MG total RNA with 5'-CTCCGGGTTTCTCTGGTCG-3' (F2 primer) and 5'-GGGT-GCTTGTGTCAGAGAGA-3' (R5 primer). Sequences were registered with the DNA Data Bank of Japan database as accession nos. AB040527, AB040528, and AB040529 for MAGE-E1a, -E1b, and -E1c, respectively.

Northern Blot Analysis. Northern blot experiments were done according to standard method (11). Fifty &g of total RNA from U87 MG cell line and a primary glioblastoma (GB-6) were separated on a formaldehyde gel to make a filter. RNA ladder (New England Biolabs, Inc. Beverly, MA) was used as a size marker. Human Multiple Tissue Northern blot (MTN; Clontech, Palo Alto, CA) was purchased to investigate the normal tissue distribution of MAGE-E1.

RT-PCR. One &g of total RNA from five glioblastoma (GB-1,-5,-6,-7, and -8) and other cancers was reverse-transcribed with oligo(dT) primer following the method described previously (11). Samples without reverse transcriptase were prepared for negative control. 5'-CCGTGTCCTTCTCTCACGGT-3' (F3 primer) and 5'-TCTCCTCTCTCTTCCTGCTG-3' (R6 primer) were designed to amplify an 882-bp fragment of MAGE-E1c and a 473-bp fragment of MAGE-E1a and -E1b. The reverse-transcribed product was amplified with F3 primer and R6 primer with the LA-PCR Kit (Takara, Tokyo, Japan) by the following cycling parameters: 30 s at 94°C; 30 s at 55°C; 1 min at 72°C for 25 cycles; and a final extension at 72°C for 3 min. The amplified products were subcloned into the pCR-2.1 vector (Invitrogen, Groningen, Netherlands). The sequence was analyzed by the dyeodeoxy sequencing method.

The 3'-half region of the EST was also confirmed by RT-PCR experiment with F1 primer and R1 primer, which were described above (Fig. 1A). The R1 primer sequence was chosen from the region between the tag and the 3'-end. The PCR product was subcloned into the pCR-2.1 vector and sequenced.

The upstream region of the cDNA was cloned by the 5'-RACE method.

The 1.3-kb fragment of MAGE-E1c was labeled with &g of total RNA from U87 MG cell line and a primary glioblastoma (GB-6) were separated on a formaldehyde gel to make a filter. RNA ladder (New England Biolabs, Inc. Beverly, MA) was used as a size marker. Human Multiple Tissue Northern blot (MTN; Clontech, Palo Alto, CA) was used as a size marker.
RESULTS

Identification of the Genes Specifically Up-Regulated in Glioma.

The SAGE experiment was performed with a primary glioblastoma (GB-1) tissue fragment obtained surgically from a patient. For comparison, cultured human astrocytes (HA-1) obtained from an embryo brain of 14 weeks’ gestation was analyzed in parallel because glioblastoma is defined as an astrocytic tumor according to the WHO classification. Numbers of the tags analyzed for GB-1 and HA-1 were 9,556 and 12,353, respectively. By comparing obtained profiles, genes showing >5-fold increase in GB-1 were selected from the top-147 most abundantly expressed genes in GB-1. Ninety-seven genes were obtained, which included 76 known and 21 unknown genes. Table 1 shows a portion of these genes, which also showed a 5-fold increase in GB-1 over another human astrocytes profile reported in the CGAP. By this selection, 34 known and 18 unknown genes were determined. All of these genes were undetectable in HA-1. In the list of known genes (Table 1A), previously reported glioma-related genes such as VEGF and brain-specific angiogenesis inhibitor 2 were also included. GFAP and vimentin, which have been used as a glioma-specific marker by earlier investigators, are absent from the list because of the high-level expression in normal astrocytes (see Table 2). Some other glioma-related genes such as cyclin-dependent kinase 4 and epidermal growth factor receptor were also found in the profile of GB-1; however, the levels were too low (0.001%) to be selected. Genes which had not been focused on in previous glioma studies are also included in Table 1A. For example, expression of heparin-binding neurite-promoting factor 1 in glioma was not reported previously, and HSP-70 has been reported for other cancers but not for glioma.

The 18 tags which could not be identified by the database search are listed in Table 1B. Many of them, 15 out 18, were found in EST sequences; thus the rest, 3 tags, were totally unknown. Among the ESTs found by the search, ESTs containing the tag TTTTGGGTAT sequences; thus the rest, 3 tags, were totally unknown. Among the ESTs found by the search, ESTs containing the tag TTTTGGGTAT showed high homology with MAGE-D1. We decided to focus on the original gene of this tag and named it MAGE-E1. Our SAGE profiles (Table 2, above the dotted line) showed that MAGE-E1 was expressed abundantly in glioblastoma (0.052%) but not detected in human astrocytes. Compared with GFAP and vimentin, MAGE-E1 expression was less abundant but highly specific to glioblastoma.

Our findings are supported by the other SAGE profiles obtained from the CGAP (Table 2, below the dotted line). MAGE-E1 was more abundantly expressed in glioblastoma (0.041%) than in normal adult brain (NB, 0.006%) or human astrocytes (HA, 0.010%). In brain tumor cells, the expression level was high in medulloblastoma (MB, 0.026%) but low in fibrillary astrocytoma (FA, 0.006%). These results indicate that MAGE-E1 is up-regulated in undifferentiated brain tumors.

Because the CGAP profile shows a 4-fold increase of MAGE-E1 expression in glioblastoma in comparison with human astrocyte cultures, real-time quantitative RT-PCR was performed to quantify MAGE-E1 expression in the other four glioblastomas (GB-2, -3, -4, and -6) relative to cultured human astrocytes (HA-1). MAGE-E1 expression was up-regulated in all four tumors, showing >2.6–15-fold enrichment (Table 3). Ct negative control without reverse transcriptase was 40 (data not shown).

Cloning of MAGE-E1 cDNA. To use sequence information of the ESTs found by the search, first we confirmed that the MAGE-E1 tag

```{r}
# We focused on ESTs containing a tag, TTTTGGGTAT, showing high homology with MAGE-D1.

# Known genes
TAAAGAGCCA 0.11% ESTs AA594490
CTCAGTTAAG 0.09% ESTs AA121179
CTAATCCTTT 0.09% ESTs A420977
ACAGGGGTGG 0.08% ESTs AA873072
CCTGTGTGTC 0.08% ESTs T63193
AAGAGCGGCC 0.07% ESTs W28593
AGGGTGGCAG 0.07% No match
TAACAAAGGA 0.06% ESTs AA803277
TTTTGGGTAT 0.05% ESTs ARB2447
CTGCTAGATG 0.05% ESTs AT760380
GCAAGGGTGG 0.05% ESTs AR83856
CCTGTAGTCT 0.05% No match
GCTCTGGGGA 0.04% ESTs T59486
CCAGGTGTCG 0.04% ESTs A922855

Table 2 Expression of MAGE-E1, GFAP, and Vimentin observed in SAGE profiles

<table>
<thead>
<tr>
<th>Gene</th>
<th>Total tags</th>
<th>TTTTTGGGTAT (MAGE-E1)</th>
<th>ACTTTGGCCC (GFAP)</th>
<th>TTCCAATATCGA (Vimentin)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GB-1</td>
<td>9556</td>
<td>0.052%</td>
<td>0.303%</td>
<td>0.115%</td>
</tr>
<tr>
<td>HA-1</td>
<td>12353</td>
<td>Not detected</td>
<td>0.113%</td>
<td>0.105%</td>
</tr>
</tbody>
</table>

a GB, glioblastoma multiforme.

b NB, normal brain.

c HA, human astrocytes.

d FA, fibrillary astrocytoma.

e MB, medulloblastoma.
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A NOVEL MEMBER OF MAGE GENE FAMILY EXPRESSED IN GLIOMA

Table 3 MAGE-E1 expression in glioblastoma relative to cultured human astrocytes detected by real-time quantitative RT-PCR experiment

<table>
<thead>
<tr>
<th></th>
<th>MAGE-E1 C&lt;sub&gt;T&lt;/sub&gt;</th>
<th>MAGE-E1 Average C&lt;sub&gt;T&lt;/sub&gt;</th>
<th>MAGE-E1 Relative to HA&lt;sup&gt;a&lt;/sup&gt;</th>
<th>β-actin Average C&lt;sub&gt;T&lt;/sub&gt;</th>
<th>β-actin Relative to HA&lt;sup&gt;a&lt;/sup&gt;</th>
<th>MAGE-E1/β-actin&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>HA-1</td>
<td>25.20</td>
<td>25.06</td>
<td>25.13</td>
<td>1.0</td>
<td>19.54</td>
<td>1.0</td>
</tr>
<tr>
<td>GB-2&lt;sup&gt;d&lt;/sup&gt;</td>
<td>23.11</td>
<td>23.37</td>
<td>23.24</td>
<td>3.1</td>
<td>21.26</td>
<td>0.39</td>
</tr>
<tr>
<td>GB-3</td>
<td>22.35</td>
<td>22.83</td>
<td>22.59</td>
<td>4.7</td>
<td>20.45</td>
<td>0.61</td>
</tr>
<tr>
<td>GB-4</td>
<td>23.32</td>
<td>23.20</td>
<td>23.26</td>
<td>3.1</td>
<td>19.23</td>
<td>1.2</td>
</tr>
<tr>
<td>GB-6</td>
<td>23.36</td>
<td>22.81</td>
<td>23.09</td>
<td>3.3</td>
<td>22.30</td>
<td>0.22</td>
</tr>
</tbody>
</table>

<sup>a</sup> CT, threshold cycle in real-time quantitative RT-PCR experiment.
<sup>b</sup> HA, human astrocytes.
<sup>c</sup> MAGE-E1 expression level normalized against β-actin.
<sup>d</sup> GB, primary glioblastoma.

Fig. 2. A, structure of the deduced polypeptides coded by MAGE-E1 mRNAs. The deduced polypeptides encoded by MAGE-E1 mRNAs are schematically demonstrated. All of them possess the NH₂-terminal 348 amino acids. MAGE-E1b is identical to MAGE-E1a except for 2 amino acids missing in the COOH terminus. MAGE-E1c lacks a large portion of COOH-terminal half and has a distinct 66-amino acid tail. B, homology of deduced MAGE-E1a polypeptides with other MAGES. Homology search with the database showed that deduced MAGE-E1a polypeptides were highly homologous with other MAGE family members at a region spanning 421–600 amino acids: 78.3% identical to MAGE-D1; 41.1% and 43.5% to MAGE-B1; and 41.4% to MAGE-C1.

had actually originated from the same gene that gave us the ESTs. An intermediate product of the SAGE experiment was amplified with a tag-specific primer and the oligo(dT)-primer by PCR and sequenced (see "Materials and Methods"). The resulting sequence matched the ESTs. Then, 5’-RACE was performed with specific primers designed according to the EST. Finally, RT-PCR cloning covering the entire coding region was performed. Three variants, possibly originated by an alternative splicing, were obtained (MAGE-E1a, -E1b, and -E1c; Fig. 1B). The length of MAGE-E1a, -E1b, and -E1c cDNAs was 2485 bp, 2497 bp and 2997 bp, respectively. MAGE-E1c and -E1b had an additional 12 bp in the 3’-terminal region of -E1a, and -E1c also possessed another 411 bp in the middle.

Structure of the Predicted Polypeptides and Homology with MAGE Proteins. The three species of transcripts used the same ATG but different stop codons, resulting in different open reading frames (Fig. 2A). All three products share the NH₂-terminal 348 amino acids. MAGE-E1b is identical to MAGE-E1a except for 2 amino acids missing in the COOH terminus. MAGE-E1c lacks a large portion of the COOH-terminal half and has a distinct 66-amino acid tail.

The homology search with on database showed that MAGE-E1a and -E1b were highly homologous with other MAGE family members at a region spanning amino acid 421–600: 78.3% identical and 96.1% similar to MAGE-D1; 41.1% and 76.1% to MAGE-A1; 43.5% and 76.7% to MAGE-B1; and 41.4% and 76.1% to MAGE-C1 (Fig. 2B). MAGE-E1c lacked the conserved domain, suggesting that it has different functions from the members of MAGE gene family.

MAGE-E1 Expression in Normal and Various Cancer Tissues. Northern blot analysis showed that MAGE-E1 mRNA was detected as a single band of about 3 kb in U87 MG cell line and a primary glioblastoma (GB-5; Fig. 3A). The size was consistent with that of cDNAs cloned and sequenced (Fig. 1B). Signals of similar size were observed in human brain and faintly in ovary (Fig. 3B) but not in other tissue, including testis and placenta, in which all other MAGE genes are expressed.

A set of primers for RT-PCR was designed to cover the additional 411 bp of MAGE-E1c. Using these primers, an 882-bp fragment of MAGE-E1c could be distinguished from a 473-bp fragment of MAGE-E1a and -E1b. RT-PCR showed that MAGE-E1a and/or -E1b (Fig. 4, lower bands) were expressed abundantly in glioma, whereas -E1c (Fig. 4, upper bands) was expressed in all tumor samples examined. The expression pattern in cultured human astrocytes (HA-1) was similar to that of glioblastomas (data not shown). Non-specific signals were not observed in the control experiment that was performed without reverse transcriptase (data not shown).

DISCUSSION

In the present study, we focused on the genes up-regulated in glioblastoma (Table 1A). The list of these genes illustrated the character of glioblastoma as a cancer. As previously reported, VEGF elicits angiogenesis in various tumors (13). Up-regulation of this gene is consistent with the character of glioblastoma as a hypervascular tumor. Finding of the genes associated with protein synthesis in the list, such as sec61γ and ribosomal protein L18, may reflect the active growth of glioma cells. Several glioma-related genes were found in the SAGE profile as we expected, however, our results indicate that
they are not the best and exclusive markers for gliomas because of the high-level expression also in normal astrocytes (GFAP and vimentin) or relatively low expression in glioblastoma (CDK-4 and EGFR). The list also included genes which has not been focused on in the previous studies. A previous study has reported that a complex of HSP-70 and antigenic peptides could induce antitumor immunity (14). Our finding of an abundant expression of HSP-70 in glioblastoma suggests that it could be a candidate target molecule for the immunotherapy of glioma. As discussed above, our results provide us with new clues to understand the nature of pathogenesis and progression of gliomas in vivo. However, we should exercise caution in interpreting our experimental results. Although freshly resected primary glioblastoma tissue is the most suitable material to determine the gene expression pattern of gliomas, normal cells such as vascular endothelial cells and reactive astrocytes are inevitably included among the tumor cells. Therefore, genes of interest should be identified and analyzed carefully, and their expression should be assigned to each cell type. Additional study, such as histochemical staining, is necessary to confirm the expression in glioma cells.

Our SAGE profiles and that of the CGAP showed that MAGE-E1 expression in glioblastoma (GB-1) is >4-fold higher than that in cultured human astrocytes (Table 2). Real-time quantitative RT-PCR showed that MAGE-E1 was 2.6–15-fold enriched in glioblastoma relative to human astrocytes. This difference of expression levels of MAGE-E1 in glioblastoma could be used for the development of additional modes of diagnosis and treatment of human patients suffering from malignant gliomas. As MAGE-E1a and -E1b polypeptides have the structures similar to MAGE-A proteins, they might be useful for treatment as target antigens of malignant glioma. Previous reports showed that MAGE-A4 could be detected in the sera of patients with various cancers (15, 16). Although tumor specific markers have been used to diagnose cancer patients, e.g., carcinoembryonic antigen for gastrointestinal cancers, glioma-specific markers have not been identified to date. MAGE-E1a and -E1b polypeptides are candidate molecules for such glioma-specific markers. Quantitative assay of antibody against these peptides in the sera of patients could facilitate continuous monitoring of tumor progression in patients with malignant glioma.

The function of MAGE gene products is still unclear, however, previous reports demonstrated that MAGE genes were expressed in undifferentiated, proliferating cells. MAGE-A1 and -A4 were expressed in testicular cells in the proliferative phase (spermatogonia) and the meiotic phase (spermatocytes) but not in the differentiation phase (spermatids) during spermatogenesis (17). MAGE-A1 expression was also observed during the wound healing of human skin, where skin cells are actively proliferating and migrating (18). The mouse homologues of human MAGE genes were expressed in mouse blastocysts and embryonic stem cells but not in adult tissues except for testis (19). These findings suggest that products of MAGE genes have some functions in the proliferating phase of the cells. MAGE-E1 might also play an important role in the cell proliferation of malignant glioma.

In the present study, we cloned MAGE-E1, the first member of MAGE family expressed predominantly in brain and ovary. Recent studies have also reported novel MAGE genes whose tissue distribution is different from that of MAGE-A, -B, and -C; MAGE-D1 expressed ubiquitously (8) and MAGE-L2 expressed in brain and placenta (20). These observations suggest that MAGE-related genes are distributed in various tissues. Radiation hybrid mapping showed that the MAGE-E1 gene is located on chromosome X like other MAGEs.4 This result indicates that chromosome X may contain other MAGE-related genes expressed in various tissues. Such genes could be isolated by cDNA cloning from various cancer cells. Indeed, MAGE-D1 was isolated from genes activated in multiple myeloma (8) and MAGE-E1, described in the present study, was identified from genes up-regulated in glioblastoma. Our RT-PCR results show that MAGE-E1a and/or -E1b are expressed specifically in glioblastomas, whereas MAGE-E1c is distributed in various tumors. This pattern of -E1c expression is similar to members of classical MAGE family such as -A, -B, and -C, which are widely found in various tumors. Because MAGE-E1c polypeptide lacks the MAGE-conserved domain, it should play a totally unrelated role in tumor cells, unlike other members of MAGE family. MAGE-E1a and -E1b were observed in human astrocytes. This observation suggests that MAGE-E1a and/or E1b possibly could be originated by a brain-specific alternative splice. MAGE-E1 products could have dual functions, one of which is unique in glioblastoma, whereas the other is conserved in various other tumors. Additional studies on the structure and function of MAGE-E1 products should elucidate their biological roles in brain, glioma cells, and other cancer cells.

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REFERENCES


4 Manuscript in preparation.

Fig. 4. MAGE-E1 expression in various cancers. MAGE-E1a and/or -E1b (lower bands) are expressed abundantly in glioma relative to other cancers, whereas E1c (upper bands) is expressed in all tumor samples examined. U87, U87 MG cell line; GB, glioblastoma multiforme; LC, lung cancer; SC, stomach cancer; CC, colon cancer; HCC, hepatocellular carcinoma.
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