Expression of Cancer-Testis Genes in Brain Tumors

**Objective**: Cancer-testis (CT) genes are considered promising candidates for immunotherapeutic approaches. The aim of this study was to investigate which CT genes should be targeted in immunotherapy for brain tumors.

**Methods**: We investigated the expression of 6 CT genes (MAGE-E1, SOX-6, SCP-1, SSX-2, SSX-4, and HOM-TES-85) using reverse-transcription polymerase chain reaction in 26 meningiomas and 32 other various brain tumor specimens, obtained from the patients during tumor surgery from 2000 to 2005.

**Results**: The most frequently expressed CT genes of meningiomas were MAGE-E1, which were found in 22/26 (85%) meningioma samples, followed by SOX-6 (9/26 or 35%). Glioblastomas were most frequently expressed SOX-6 (6/7 or 86%), MAGE-E1 (5/7 or 71%), followed by SSX-2 (2/7 or 29%) and SCP-1 (1/7 or 14%). However, 4 astrocytomas, 3 anaplastic astrocytomas, and 3 oligodendrogial tumors only expressed MAGE-E1 and SOX-6. Schwannomas also expressed SOX-6 (5/6 or 83%), MAGE-E1 (4/6 or 67%), and SCP-1 (2/6 or 33%).

**Conclusion**: The data presented here suggest that MAGE-E1 and SOX-6 genes are expressed in a high percentage of human central nervous system tumors, which implies the CT genes could be the potential targets of immunotherapy for human central nervous system tumors.

**KEY WORDS**: Cancer-testis gene - Brain tumor - MAGE-E1 - SOX-6 - SCP-1 - SSX-2 - SSX-4 - HOM-TES-85

**INTRODUCTION**

Cancer-testis (CT) genes are potentially suitable targets for tumor vaccines of human cancers because of their high immunogenicity and their relatively restricted normal tissue distribution except for the testis. To date, forty-seven CT gene families, including 93 genes have been described. The CT genes have been demonstrated to induce spontaneous cellular and/or humoral immune responses. Most of CT genes are mainly located on the X-chromosome and being used as targets in several vaccination trials. Recently, the expression analysis of seven CT genes showed that 60% of astrocytomas expressed at least one CT genes. However, the expression of CT genes in the various human central nervous system tumors is little known. In this study, we analyzed the frequency for the expression of six CT genes in 26 meningiomas and 32 other various brain tumor specimens, using reverse-transcription polymerase chain reaction. In this study, we examined the expression of six CT genes (MAGE-E1, SOX-6, SCP-1, SSX-2, SSX-4, and HOM-TES-85) in 26 meningiomas and 32 other various brain tumors as a preliminary study as well as the possibility of the potential targets of immunotherapy.

**MATERIALS AND METHODS**

**Samples**

Twenty-six meningiomas and thirty-two various brain tumor samples were obtained from patients during surgery in our Medical Center from 2000 to 2005. Of the 32 various brain tumors, there were seven glioblastomas, four astrocytomas, three anaplastic astrocytomas, three oligodendrogial tumors, six schwannomas, two ependymomas, two primitive neuroectodermal tumors, two craniopharyngiomas, one ganglioglioma, one pituitary adenoma, and one hemangioblastoma. The human testis from autopsy used to positive control for CT genes. Biopsy samples were snap-frozen within 1 hr after excision. The part of tumor was fixed...
Reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA was isolated from brain tumor samples by TRizol (Life Technologies, Inc.). Five micrograms of total RNA was reverse transcribed to cDNA using M-MLV reverse transcriptase (Promega Corp., Madison, WI) according to the manufacturer's instructions with oligo (dT) primer. The RT reactions were performed at 42°C for 60 min. Single-stranded cDNA was amplified by PCR with primers for MAGE-E1, SOX-6, SCP-1, SSX-2, SSX-4, and HOM-TES-85, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The primers for the respective CT genes were as follows: MAGE-E1 sense, 5'-CCT GTG CTT TCT TCT AGT TGG CCT TCT CCT CAC GCC TGC-3'; MAGE-E1 antisense, 5'-TCT TCT TCT TCT CTC TCT CACC TTG TGC-3'; SOX-6 sense, 5'-GAT GCC ATC AAC TAA ACA GC-3'; SOX-6 antisense, 5'-GCT GCA GAG CCA ATT GC-3'; SCP-1 sense, 5'-GTA CAG CAG AAA GCA AGC AAC TGA ATG-3'; SCP-1 antisense, 5'-GAA GGA ACT GCT TTA GAA TCC AAT TTC C-3'; SSX-2 sense, 5'-GTG CTC AAA TAC CAG AGA AGA TC-3'; SSX-2 antisense, 5'-TTC TGG GTC CAG ATC TCT GCT G-3'; SSX-4 sense, 5'-AAA TCG TCT ATG TAT ATA TGA AGC T-3'; SSX-4 antisense, 5'-GGG TCG CTG ATC TCT TCA TAA-3'; HOM-TES-85 sense, 5'-GGA GAG GGT ACT CAA GAT GCA GAA GC-3'; HOM-TES-85 antisense, 5'-CTG AGT GAC TAT GAG ATC TCT CGT AGT-3'; GAPDH sense, 5'-GGT GAA GGT CGG TGT GAA CG-3'; GAPDH antisense, 5'-GGT AGG AAC ACG GAA GGC GC-3'. The following PCR conditions were applied: MAGE-E1, SOX-6, and HOM-TES-85, 35 cycles of denaturation at 94°C for 30 s, annealing at 58°C for 30 s, and extension at 72°C for 30 s; SCP-1, 35 cycles of denaturation at 94°C for 30 s, annealing at 60°C for 30 s, and extension at 72°C for 30 s; SSX-2 and SSX-4, 35 cycles of denaturation at 94°C for 30 s, annealing at 63°C for 30 s, and extension at 72°C for 30 s; GAPDH, 18 cycles of denaturation at 94°C for 30 s, annealing at 57°C for 30 s, and extension at 72°C for 30 s. GAPDH was used as an internal control to evaluate relative expression of CT genes.

RESULTS

MAGE-E1 was expressed in 22 cases (85%) of 26 meningiomas and SOX-6 was positive in 9 cases (35%) of 26 meningiomas, and two fibrous meningiomas coexpressed SOX-6 with SSX-4 and MAGE-E1 with HOM-TES-85, respectively. In 7 glioblastomas, SOX-6 were most frequently expressed (86%, 6/7) and MAGE-E1 (71%, 5/7), followed by SOX-2 (29%, 2/7) and SCP-1 (14%, 1/7). However, four astrocytomas, three anaplastic astrocytomas and three oligodendrogial tumors expressed MAGE-E1 and SOX-6 without any other CT genes. Other tumors (ependymomas, PNETs and craniopharyngiomas) predominantly expressed SOX-6 and MAGE-E1 as well. Schwannomas also expressed SOX-6 (83%, 5/6), MAGE-E1 (67%, 4/6), and SCP-1 (33%, 2/6). One hemangioblastoma was negative for all CT genes tested. These results are shown in Table 1 and Fig. 1.

DISCUSSION

CT genes are expressed at different frequencies of a wide range of different types of malignancy, but not in normal tissues except testis, ovary and placenta[20]. This fact makes CT genes especially attractive targets for specific tumor immunotherapy. Many kinds of CT genes, such as MAGE[2], GAGE/PAGE/XAGE[3,25], NY-ESO-1/LAGE[1,4,12], SSX[2,20], SPANX[27,28], TRAG-3[30], BAGE[1], OYTES-1[30], CT17[30], NY-BR-3[30], SCP-1[21], and SOX-6[22,23].
CT genes were expressed in human brain tumors15). There expressed in glioma cells among cancer cells. Some of many meningiomas express only detected only in brain and MAGE blastoma (15).

8), ependymoma (9), anaplastic astrocytoma (10), oligodendroglial glioblastoma (1), schwannoma (2), meningioma (3-6), astrocytoma (7, schwannoma (15). C : expression of SCP-1 by testis (T) markers, composed of MAGE-E1a and/or -E1b (lower bands. 473 bp) and E1c (upper bands, 882 bp), meningiomas (1, 2), glial tissues (3, 4), astrocytoma (5, 6), anaplastic astrocytoma (7), oligodendroglial tumors (8, 9), ependymoma (10), hemangioblastoma (11), PNET (12), schwannoma (13), glioblastoma (14), and pituitary adenoma (15). B : expression of MAGE-E1 by testis (T) markers, composed of MAGE-E1a and/or -E1b (lower bands. 473 bp) and E1c (upper bands, 882 bp), meningiomas (1, 2), glial tissues (3, 4), astrocytoma (5, 6), anaplastic astrocytoma (7), oligodendroglial tumors (8, 9), ependymoma (10), hemangioblastoma (11), PNET (12), schwannoma (13), glioblastoma (14), and schwannoma (15). C : expression of SCP-1 by testis (T) markers, glial tissues (1), schwannoma (2), meningioma (3-6), astrocytoma (7, 8), ependymoma (9), anaplastic astrocytoma (10), oligodendroglial tumor (11, 12), PNET (13), pituitary adenoma (14), and hemangioblastoma (15).

have been reported. To date, forty-seven CT gene families, including 93 genes have been recently described13,17,20).

Immunotherapy for brain tumors is one of the alternative approaches that have been rarely studied in detail in the various brain tumors. To evaluate CT genes for diagnostic and therapeutic approach, accurate information on the CT genes expression pattern in various brain tumors is essential. Among the many CT genes, SOX-6 may be a useful marker for the diagnosis of neuronal and glial brain tumors1,2,13,23). Sasaki et al.16) reported that MAGE-E1 expression was detected only in brain and MAGE-E1 were specifically expressed in glioma cells among cancer cells. Some of many CT genes were expressed in human brain tumors15. Therefore, we examined MAGE-E1, SOX-6, SCP-1, SSX-2, SSX-4, and HOM-TES-85 expression using RT-PCR in various brain tumor tissues and normal testis tissue as positive control.

Our results show a highly expression rate of MAGE-E1 and SOX-6 gene mRNA in meningioma tissues and various brain tumors. In meningioma cases, two fibrous meningiomas coexpressed SOX-6 with SSX-4 and MAGE-E1 with HOM-TES-85, respectively. Sahin et al.15) reported that meningiomas express only SCP-1. From the data of present study, no apparent expression of SCP-1 was detected in the meningiomas. Interestingly, we demonstrate that MAGE-E1 and SOX-6 are commonly expressed in meningiomas. Although the most effective treatment of meningiomas is surgery, adjuvant immunotherapy or chemotherapy could be considered in cases of unresectable or failed radiotherapy. Our preliminary data may provide basic idea for expression of CT genes for meningiomas.

Importantly, the most relevant findings of this study are a high incidence of expression of the MAGE-E1 and SOX-6. MAGE-E1 and SOX-6 in low or high grade glioma and other various benign brain tumors. However, our study only provided qualitative analysis for expression of CT genes and no quantitative results were obtained. Expression of MAGE-E1 and SOX-6 was never detected in normal tissue samples. Previously, MAGE-E1 and SOX-6 were reported glioma-specific CT genes of brain tumors.16,23) In the present study, MAGE-E1 and SOX-6 genes may serve as an attractive target for immunotherapy designed for especially high grade brain tumor.

In conclusion, MAGE-E1 and SOX-6 may make ideal candidate genes for antigen-specific tumor immunotherapy and could become an adjuvant diagnostic tool for brain tumors. Further related studies, such as quantitative RT-PCR, immunohistochemistry, histological grade and clinical outcome with sufficient cases may be necessary.

References


