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Laboratory Investigation

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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 oligodendroglial 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 .

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[19]. To date, fourty-seven CT gene families, including 93 genes have been described[5,13,18,26]. The CT genes have been demonstrated to induce spontaneous cellular and/or humoral immune responses[7,11]. Most of CT genes are mainly located on the X-chromosome and being used as targets in several vaccination trials[24]. Recently, the expression analysis of seven CT genes showed that 60% of astrocytomas expressed at least one CT genes[15]. 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 oligodendroglial 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 hour after excision. The part of tumor was fixed in formalin and embedded in paraffin for conventional H&E-stained histologic

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sections. The other part of tumor was snap-frozen in liquid nitrogen and microscopically checked for the presence of neoplastic tissue and the amount of contaminating non-neoplastic tissue in cryostat sections before use for reverse-transcription polymerase chain reaction (RT-PCR). The use of RNA material derived from human subjects has been approved by the Institutional Review Board of our Medical Center. The diagnosis of brain tumors was confirmed in all cases by pathologist.

Table 1. Expression of cancer-testis genes by human central nervous system tumors

Tumor	SCP-1	SSX-2	SSX-4	HOM-TES-85	MAGE-E1	SOX-6
Meningioma	0/26	0/26	1/26	1/26	22/26	9/26
Glioblastoma	1/7	2/7	0/7	0/7	5/7	6/7
Astrocytoma	0/4	0/4	0/4	0/4	2/4	4/4
Anaplastic As	0/3	0/3	0/3	0/3	2/3	3/3
Oligodendroglial tumor	0/3	0/3	0/3	0/3	3/3	2/3
Ependymoma	1/2	0/2	0/2	0/2	1/2	2/2
PNET	0/2	0/2	0/2	0/2	1/2	2/2
Craniopharyngioma	1/2	0/2	0/2	0/2	2/2	1/2
Ganglioglioma	1/1	1/1	0/1	0/1	1/1	1/1
Schwannomas	2/6	0/6	0/6	0/6	4/6	5/6
Pituitary adenoma	0/1	1/1	1/1	0/1	0/1	1/1
Hemangioblastoma	0/1	0/1	0/1	0/1	0/1	0/1

As : astrocytoma, PNET : primitive neuroectodermal tumor

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 CTC AGG CT-3'; *MAGE-E1* antisense, 5'-TCT CTC TCT CCT CCT CGC TGC-3'; *SOX-6* sense, 5'-GAT GCC ATC AAC TCC ACA GC-3'; *SOX-6* antisense, 5'-GCT GCA GAG CCA TTC 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'-TTT TGG GTC CAG ATC TCT CGT G-3'; *SSX-4* sense, 5'-AAA TCG TCT ATG TGT ATA TGA AGC T-3'; *SSX-4* antisense, 5'-GGG TCG CTG ATC TCT TCA TAA-3'; *HOM-TES-85* sense, 5'-GGA GAG GCT ACT CAA GAT GCA GAA GC-3'; *HOM-TES-85* antisense, 5'-CTG AGT GAC TAT GAG ATC TCT CTG AGT-3'; *GAPDH* sense, 5'-GGT GAA GGT CGG TGT GAA CG-3'; *GAPDH* antisense, 5'-GGT AGG AAC ACG GAA GGC CA-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 *SSX-2* (29%, 2/7) and *SCP-1* (14%, 1/7). However, four astrocytomas, three anaplastic astrocytomas and three oligodendroglial 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[8,9,20], SPANX[27,28], TRAG-3[6], BAGE[1], OY-TES-1[14], CT17[18], NY-BR-3[10], SCP-1[21], and SOX-6[22,23] have been reported. To date, fourty-seven CT gene families, including

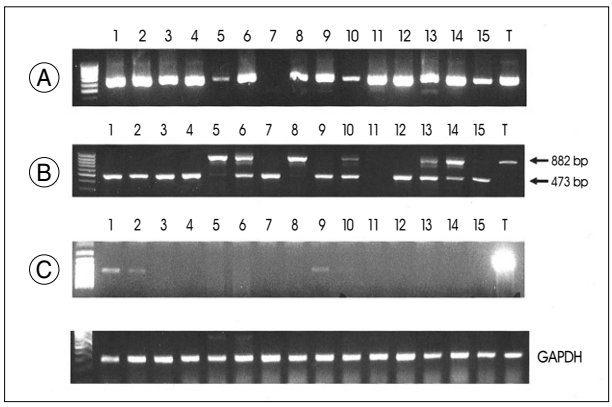

Fig. 1. Reverse transcription-polymerase chain reaction for the expression of the Cancer-testis genes SOX-6, MAGE-E1, and SCP-1 in human central nervous system tumors. An equal amount of testis RNA was used as a representative positive control. A : expression of SOX-6 by testis (T) markers, meningiomas (1, 2), glioblastomas (3, 4) astrocytoma (5), anaplastic astrocytoma (6), hemangioblastoma (7), oligodendroglial tumors (8, 9), ependymoma (10), primitive neuroectodermal tumor (PNET) (11), craniopharyngioma (12), ganglioglioma (13), schwannoma (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), glioblastomas (3, 4), astrocytoma (5, 6), anaplastic astrocytoma (7), oligodendroglial tumors (8, 9), ependymoma (10), hemangioblastoma (11), PNET (12), craniopharyngioma (13), ganglioglioma (14), and schwannoma (15). C : expression of SCP-1 by testis (T) markers, glioblastoma (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).

93 genes have been recently described[5,13,17,26].

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 tumors[22,23]. 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 tumors[15]. 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 high 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.

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

1. Boel P, Wildmann C, Sensi ML, Brasseur R, Renauld JC, Coulie P, et al : BAGE : a new gene encoding an antigen recognized on human melanomas by cytolytic T lymphocytes. **Immunity 2** : 167-175, 1995
2. Boon T, van der Bruggen P : Human tumor antigens recognized by T lymphocytes. **J Exp Med 183** : 725-729, 1996
3. Brinkmann U, Vasmatzis G, Lee B, Pastan I : Novel genes in the PAGE and GAGE family of tumor antigens found by homology walking in the dbEST database. **Cancer Res 59** : 1445-1448, 1999
4. Chen YT, Scanlan MJ, Sahin U, Türeci O, Gure AO, Tsang S, et al : A testicular antigen aberrantly expressed in human cancers detected by autologous antibody screening. **Proc Natl Acad Sci U S A 94** : 1914-1918, 1997
5. Chen YT, Scanlan MJ, Venditti CA, Chua R, Theiler G, Stevenson BJ, et al : Identification of cancer/testis-antigen genes by massively parallel signature sequencing. **Proc Natl Acad Sci U S A 102** : 7940-7945, 2005
6. Feller AJ, Duan Z, Penson R, Toh HC, Seiden MV : TRAG-3, a novel cancer/testis antigen, is overexpressed in the majority of melanoma cell lines and malignant melanoma. **Anticancer Res 20** : 4147-4151, 2000
7. Goodyear O, Piper K, Khan N, Starczynski J, Mahendra P, Pratt G, et al : CD8+ T cells specific for cancer germline gene antigens are found in many patients with multiple myeloma, and their frequency correlates with disease burden. **Blood 106** : 4217-4224, 2005
8. Gure AO, Türeci O, Sahin U, Tsang S, Scanlan MJ, Jäger E, et al : SSX : a multigene family with several members transcribed in normal testis and human cancer. **Int J Cancer 72** : 965-971, 1997

9. Gure AO, Wei IJ, Old LJ, Chen YT : The SSX gene family : characterization of 9 complete genes. **Int J Cancer 101** : 448-453, 2002
10. Jäger D, Unkelbach M, Frei C, Bert F, Scanlan MJ, Jäger E, et al : Identification of tumor-restricted antigens NY-BR-1, SCP-1, and a new cancer/testis-like antigen NW-BR-3 by serological screening of a testicular library with breast cancer serum. **Cancer Immun 2** : 5, 2002
11. Korangy F, Ormandy LA, Bleck JS, Klempnauer J, Wilkens L, Manns MP, et al : Spontaneous tumor-specific humoral and cellular immune responses to NY-ESO-1 in hepatocellular carcinoma. **Clin Cancer Res 10** : 4332-4341, 2004
12. Lethé B, Lucas S, Michaux L, De Smet C, Godelaine D, Serrano A, et al : LAGE-1, a new gene with tumor specificity. **Int J Cancer 76** : 903-908, 1998
13. Monji M, Nakatsura T, Senju S, Yoshitake Y, Sawatsubashi M, Shinohara M, et al : Identification of a novel human cancer/testis antigen, KM-HN-1, recognized by cellular and humoral immune responses. **Clin Cancer Res 10** : 6047-6057, 2004
14. Ono T, Kurashige T, Harada N, Noguchi Y, Saika T, Niikawa N, et al : Identification of proacrosin binding protein sp32 precursor as a human cancer/testis antigen. **Proc Natl Acad Sci U S A 98** : 3282-3287, 2001
15. Sahin U, Koslowski M, Tureci O, Eberle T, Zwick C, Romeike B, et al : Expression of cancer testis genes in human brain tumors. **Clin Cancer Res 6** : 3916-3922, 2000
16. Sasaki M, Nakahira K, Kawano Y, Katakura H, Yoshimine T, Shimizu K, et al : MAGE-E1, a new member of the melanoma-associated antigen gene family and its expression in human glioma. **Cancer Res 61** : 4809-4814, 2001
17. Scanlan MJ, Gordon CM, Williamson B, Lee SY, Chen YT, Stockert E, et al : Identification of cancer/testis genes by database mining and mRNA expression analysis. **Int J Cancer 98** : 485-492, 2002
18. Scanlan MJ, Simpson AJ, Old LJ : The cancer/testis genes : review, standardization, and commentary. **Cancer Immun 4** : 1, 2004
19. Simpson AJ, Caballero OL, Jungbluth A, Chen YT, Old LJ : Cancer/testis antigens, gametogenesis and cancer. **Nat Rev Cancer 5** : 615-625, 2005
20. Tureci O, Sahin U, Schobert I, Koslowski M, Scmitt H, Schild HJ, et al : The SSX-2 gene, which is involved in the t (X;18) translocation of synovial sarcomas, codes for the human tumor antigen HOM-MEL-40. **Cancer Res 56** : 4766-4772, 1996
21. Tureci O, Sahin U, Zwick C, Koslowski M, Seitz G, Pfreundschuh M : Identification of a meiosis-specific protein as a member of the class of cancer/testis antigens. **Proc Natl Acad Sci U S A 95** : 5211-5216, 1998
22. Ueda R, Iizuka Y, Yoshida K, Kawase T, Kawakami Y, Toda M : Identification of a human glioma antigen, SOX6, recognized by patients' sera. **Oncogene 23** : 1420-1427, 2004
23. Ueda R, Yoshida K, Kawakami Y, Kawase T, Toda M : Immunohistochemical analysis of SOX6 expression in human brain tumors. **Brain Tumor Pathol 21** : 117-120, 2004
24. van Baren N, Bonnet MC, Dreno B, Khammari A, Dorval T, Piperno-Neumann S, et al : Tumoral and immunologic response after vaccination of melanoma patients with an ALVAC virus encoding MAGE antigens recognized by T cells. **J Clin Oncol 23** : 9008-9021, 2005
25. van den Eynde B, Peeters O, De Backer O, Gaugler B, Lucas S, Boon T : A new family of genes coding for an antigen recognized by autologous cytolytic T lymphocytes on a human melanoma. **J Exp Med 182** : 689-698, 1995
26. Wang Z, Zhang Y, Mandal A, Zhang J, Giles FJ, Herr JC, et al : The spermatozoa protein, SLLP1, is a novel cancer-testis antigen in hematologic malignancies. **Clin Cancer Res 10** : 6544-6550, 2004
27. Zendman AJ, Cornelissen IM, Weidle UH, Ruiter DJ, van Muijen GN : CTp11, a novel member of the family of human cancer/testis antigens. **Cancer Res** 59 : 6223-6229, 1999
28. Zendman AJ, Ruiter DJ, Van Muijen GN : Cancer/testis-associated genes : identification, expression profile, and putative function. **J Cell Physiol 194** : 272-288, 2003