Expression of Cancer Testis Genes in Human Brain Tumors[1]

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ABSTRACT

Cancer-testis (CT) genes are expressed in a variety of human cancers but not in normal tissues, except for testis tissue, and represent promising targets for immunotherapeutic and gene therapeutic approaches. Because little is known about their composite expression in human brain tumors, we investigated the expression of seven CT genes (MAGE-3, NY-ESO-1, HOM-MEL-40/SSX-2, SSX-1, SSX-4,HOM-TES-14/SCP-1, and HOM-TES-85) in 88 human brain tumor specimens. Meningiomas expressed only HOM-TES-14/SCP-1 (18% of meningiomas were HOM-TES-14/SCP-1 positive) and did not express any other CT genes. One ependymoma was negative for all CT genes tested. SSX-4 was the only CT gene expressed in oligodendrogliomas (2 of 5 cases), and it was also expressed in oligoastrocytomas (3 of 4 cases) and astrocytomas (10 of 37 cases). Astrocytomas were most frequently positive for HOM-TES-14/SCP-1 (40%) and SSX-4 (27%), followed by HOM-TES-85 (13%), SSX-2 (11%), and MAGE-3 (7%). Whereas MAGE-3 was detected only in grade IV astrocytomas, the expression of the other CT genes showed no clear correlation with histological grade. Of 39 astrocytomas, 60% expressed at least one CT gene, 21% expressed two CT genes, and 8% coexpressed three CT genes of the seven CT genes investigated. We conclude that a majority of oligoastrocytomas and astrocytomas might be amenable to specific immunotherapeutic interventions. However, the identification of additional tumor-specific antigens with a frequent expression in gliomas is warranted to allow for the development of widely applicable polyvalent glioma vaccines.

INTRODUCTION

A variety of immunotherapeutic and gene therapeutic strategies have been pursued in patients with malignant brain tumors to improve on results obtained with surgery, radiotherapy, and chemotherapy (1, 2). A prerequisite for the success of tumor-specific therapeutic strategies is the existence and identification of genes that are either exclusively or preferentially expressed in malignant tissues compared with normal tissues.

According to their expression pattern and the specificity of the immune responses they evoke, antigens expressed by human tumors can be classified into different groups (3). These include the so-called "shared tumor antigens;" the differentiation antigens (including the idiotypes of B-cell lymphomas); the products of viral, mutated, differentially spliced, overexpressed and amplified genes; and the common autoantigens expressed by the malignant cells of a tumor. With respect to gliomas, differentiation antigens that are also expressed by normal brain cells, *e.g.*, the melanogenesis pathway-related differentiation antigens tyrosinase and tyrosinase-related proteins 1 and 2, as well as gp100 and gp75 (4), would be of only limited value because normal brain cells could become the target of a immune attack. This leaves the shared tumor antigens as the most valuable targets for immunotherapeutic approaches in gliomas.

It is enigmatic that all of the shared tumor antigens in humans that have been molecularly defined to date by cellular and serological techniques (5, 6) have in common their expression spectrum, which is restricted to different types of cancers and normal testis. Therefore the term CTAs[3] has been coined for them, and the term CT genes has been coined for their encoding genes (7). The group of CTAs includes the CTL-reactive MAGE (8), BAGE (9), and GAGE (10) families as well as HOM-MEL-40/SSX-2, the other SSX family members (11), NY-ESO-1 (12), HOM-TES-14/SCP-1 (13), and HOM-TES-85,[4] all of which have been defined using SEREX, the serological identification of antigens by recombinant expression cloning (14).

Whereas the expression frequencies of many CTAs in a variety of neoplasms have been determined, little is known about their composite expression in human brain tumors. To investigate as broad a spectrum of CT genes as possible despite the limited amount of cDNA available from each tumor, members of the known CTA families included in this survey had to be selected based on known correlated expression patterns [*e.g.*, NY-ESO-1 (7) and LAGE-1 (15)] and/or relatedness of the respective genes and gene families [*e.g.*, the MAGE family and

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[3] The abbreviations used are: CTA, cancer testis antigen; CT, cancer testis; RT-PCR, reverse transcription-PCR.
[4] U. Sahin, Ö. Tûreci, C. Zwick, T. Eberle, M. Zuber, C. Villena-Heinsen, and M. Pfreundschuh. A novel tumor-associated leucine-zipper protein targeting to sites of gene transcription and splicing, submitted for publication.

Table 1 Expression of cancer testis genes by tissues other than human brain tumors

Tissue	SSX-1	SSX-2	SSX-4	SCP-1	TS85	ESO-1	MAGE-3
Normal human tissues							
Testis	+	+	+	+	+	+	+
Brain							
Frontal cortex	–	–	–	–	–	–	–
White matter	–	–	–	–	–	–	–
Cerebellum	–	–	–	–	–	–	–
Bladder	–	–	–	–	–	–	–
Breast	–	–	–	–	–	–	–
Colon	–	–	–	–	–	–	–
Kidney	–	–	–	–	–	–	–
Liver	–	–	–	–	–	–	–
Lung	–	–	–	–	–	–	–
Lymph node	–	–	–	–	–	–	–
Muscle	–	–	–	–	–	–	–
Ovary	–	–	–	–	–	–	–
Peripheral blood lymphocytes	–	–	–	–	–	–	–
Prostate	–	–	–	–	–	–	–
Rectum	–	–	–	–	–	–	–
Skin	–	–	–	–	–	–	–
Spleen	–	–	–	–	–	–	–
Stomach	–	–	–	–	–	–	–
Tonsil	–	–	–	–	–	–	–
Uterus (endometrium)	–	–	–	–	–	–	–
Malignant human tissues							
Bladder cancer	2/9	4/9	2/9			4/5	
Breast cancer	5/67	5/67	10/67	9/33	0/25	10/33	7/51
Colorectal cancer	3/58	7/58	9/58	0/32	2/21	0/16	
Endometrial cancer	1/8	1/8	1/8	0/7			
Gastric cancer	0/3	0/3	0/3	1/14		0/16	
Head & neck cancer	3/14	5/14	4/14		0/2		
Leiomyosarcoma		0/8	0/8	0/8			
Leukemia	0/31	0/31	0/31	0/11			
Lung cancer	1/24	4/24	1/24	1/14		2/12	
Lymphoma	0/11	4/11	0/11			0/10	
Malignant melanoma	10/37	13/37	10/37	4/28	8/22	23/67	12/23
Ovarian cancer	0/12	0/12	6/12	0/3		2/8	
Prostatic cancer	0/5	1/5	0/5	0/12	0/5	4/16	
Renal cancer	0/22	1/22	0/22	3/36		0/10	
Seminoma	0/3	0/3	0/3	0/2	5/13	0/1	
Synovial sarcoma	0/4	2/4	1/4	0/3	0/2		
Thyroid cancer	0/6	0/6	0/6	0/5		2/5	

related genes such as CT7 (12) or DAM (16)]. Besides NY-ESO-1, we chose MAGE-3 as a representative for the MAGE group of genes because it has been reported to be the most commonly expressed of all MAGE genes in cancer. In addition, SSX-1, SSX-2, and SSX-4 (the most commonly expressed members of the SSX gene family), SCP-1, and HOM-TES-85 were included in the study panel. HOM-TES-85 is a new M_r 40,000 protein CTA that was identified by screening a cDNA bank enriched for testis-specific transcripts with the serum of an allogeneic patient with seminoma.[4] Our results show that the majority of aggressive malignant human brain tumors express at least one of the shared tumor antigens, thus rendering many patients eligible for trials of tumor-specific strategies.

MATERIALS AND METHODS

Tissues and Cell Lines. This study was approved by the local ethical review board ("Ethikkommission der Ärztekammer des Saarlandes"). Recombinant DNA work was done with official permission and in accordance with the rules of the state government of Saarland. Tumor tissues were obtained during routine diagnostic or therapeutic procedures at the University of Saarland Medical School (Homburg, Germany) and the Universitätsklinikum Essen (Essen, Germany). Brain tumor samples used for RT-PCR analysis were checked microscopically for the presence of neoplastic tissue and the absence of contaminating normal brain tissue. WHO brain tumor classification and the Daumas-Dupont/SAMS grading of astrocytomas were used for histological diagnosis. Normal tissues were collected from autopsies of tumor-free patients.

RT-PCR. Total cellular RNA was extracted from frozen tissue specimens using guanidium-isothiocyanate for denaturation followed by an acidic phenol extraction and isopropanol precipitation (17). Total RNA (4 μg) was primed with an oligo$(dT)_{18}$ oligonucleotide and reverse-transcribed with Superscript II (Life Technologies, Inc., Eggenstein, Germany) according to the manufacturer's instructions. cDNA thus obtained was tested for integrity by amplification of β-actin transcripts in a

Table 2 Expression of cancer testis genes by human meningiomas

No.	Diagnosis	Patient's code	SSX-1	SSX-2	SSX-4	SCP-1	TS85	ESO-1	MAGE-3
1	Meningioma	733	−	−	−	−	−	−	−
2	Meningioma	735	−		−	−	−	−	−
3	Meningioma	737	−	−	−	−	−	−	−
4	Meningioma	740	−	−	−	−	−	−	−
5	Meningioma	741	−	−	−	−	−	−	−
6	Meningioma (anaplasia)	695	−	−	−	+	−	−	−
7	Meningioma	192	−	−	−	−			
8	Meningioma	680	−	−	−	−			
9	Meningioma	1210	−	−	−	−			
10	Meningioma	1612	−	−	−	−			
11	Meningioma	1694	−	−	−	+			
12	Meningioma	63/97	−	−	−	−			
13	Meningioma	99/97	−	−	−	−			
14	Meningioma	173/97	−	−	−	−			
15	Meningioma	239/97	−	−	−	−			
16	Meningioma	312/97	−	−	−	−			
17	Meningioma	447/97	−	−	−	−			
18	Meningioma	1129/97	−	−	−	−			
19	Meningioma	1168/97	−	−	−	+			
20	Meningioma	1214/97	−	−	−	−			
21	Meningioma	1400/97	−	−	−	−			
22	Meningioma	1563/97	−	−	−	+			
23	Meningioma	1879/97	−	−	−	−			
24	Meningioma	040/98	−	−	−	+			
25	Meningioma	094/98	−	−	−	−			
26	Meningioma	101/98	−	−	−	−			
27	Meningioma	102/98	−	−	−	−			
28	Meningioma	137/98	−	−	−	−			
29	Meningioma	229/98	−	−	−	−			
30	Meningioma	294/98	−	−	−	−			
31	Meningioma	367/98	−	−	−	−			
32	Meningioma	678/98	−	−	−	−			
33	Meningioma	689/98	−	−	−	+			
34	Meningioma	786/98	−	−	−	−			
35	Meningioma	797/98	−	−	−	−			
36	Meningioma	928/98	−	−	−	+			
37	Meningioma	1214/98	−	−	−	−			
38	Meningioma	1510/98	−	−	−	−			

25-cycle PCR reaction as described elsewhere (18). For PCR analysis of the expression of individual CTA gene transcripts, 1 μg of first-strand cDNA was amplified with transcript-specific oligonucleotides (10 gmol) using 2 units of AmpliTaq Gold (Perkin Elmer, Weiterstadt, Germany), 10 nmol of each deoxynucleotide triphosphate (dATP, dTTP, dCTP, and dGTP), and 1.67 mM $MgCl_2$ in a 30-μl reaction. The primers (MWG Biotech, Ebersberg, Germany) for the respective CT genes have been reported previously (19, 20) and were as follows: (*a*) SX-1 5′ (5′-CTAAAGCATCAGAGAAGAGAAGC) and SX-1 3′ (5′-AGATCTCTTATTAATCTTCTCAGAAA) primers, annealing temperature 56°C; (*b*) SSX-2 5′ (5′-GTGCTCAAATACCAGAGAAGATC) and SSX-2 3′ (5′-TTTTGGGTCCAGATCTCTCGTG) primers, annealing temperature 67°C; (*c*) SSX-4 5′ (5′-AAATCGTCTATGTGTATATGAAGCT) and SSX-4 3′ (5′-GGGTCGCTGATCTCTTCATAA) primers, annealing temperature 60°C; (*d*) SCP-1 5′ (5′-GTACAGCAGAAAGCAAGCAACTGAATG) and SCP-1 3′ (5′-GAAGGAACTGCTTTAGAATCCAATTTCC) primers, annealing temperature 60°C; (*e*) HOM-TES-85 5′ (5′-GGAGAGGCTACTCAAGATGCAGAAGC) and HOM-TES-85 3′ (5′-CTGAGTGACTATGAGATCTCTCTGAGT) primers, annealing temperature 60°C; (*f*) NY-ESO-1 5′ (5′-CACACAGGATCCATGGATGCTGCAGATCCGG) and NY-ESO-1 3′ (5′-CACACAAAGCTTGGCTTAGCGCCTCTGCCCTG) primers, annealing temperature 60°C; and (*g*) MAGE-3 5′ (5′-TGGAGGACCAGAGGCCCCC) and MAGE-3 3′ (5′-GGACGATTATCAGGAGGCCTGC) primers, annealing temperature 63°C.

Amplification was performed in a TRIO-Thermoblock (Biometra, Göttingen, Germany). After a 12-min activation of AmpliTaq Gold polymerase at 94°C for a hot start induction, three cycles of PCR were performed with 1 min at the respective annealing temperature as indicated above, 2 min at 72°C, and 1 min at 94°C, with a final elongation step at 72°C for 8 min. A 15-μl aliquot of each reaction was size-fractionated on a 2% agarose gel, visualized by ethidium bromide staining, and assessed for expected size.

RESULTS

Study Population and Validity of the Experimental Approach. In total, 88 tumor specimens were investigated for the expression of the following seven CT genes: (*a*) MAGE-3; (*b*) HOM-MEL-40/SSX-2; (*c*) SSX-1; (*d*) SSX-4; (*e*) HOM-

TES-14/SCP-1; (*f*) HOM-TES-85; and (*g*) NY-ESO-1. There were 38 meningiomas, 1 ependymoma, and 1 pilocytic astrocytoma. Five cases had been diagnosed as oligodendrogliomas, 4 cases had been diagnosed as oligoastrocytomas, and 39 cases had been diagnosed as astrocytomas of different grades (Table 1). Due to the limited amounts of cDNA available, not all specimens could be tested for expression of the entire CT gene panel included in this study. For example, after SCP-1 was the only CT gene found to be expressed in six meningiomas from which sufficient material was available for extensive testing, 32 additional meningiomas from which only very small amounts of tissue were available were tested only for this CT gene and the SSX family.

Only tumor specimens that had been assessed for cDNA integrity by amplification of an 800-bp β-actin product were investigated. To exclude false positive PCR products due to small amounts of contaminating DNA in the RNA preparation, the individual primer sets were chosen for sequences that correspond to sequences located in different exons. Under the experimental conditions, DNA generated no PCR product. Each RT-PCR experiment was done in triplicate using the same poly(dT)-primed cDNA sample together with appropriate controls.

As can be seen from Table 2, all seven CT genes under investigation were not expressed in normal brain or in other normal tissues except for testis. In contrast, they were expressed at various frequencies in different human neoplasms, with melanomas showing the most frequent expression of CT genes in tissues other than gliomas.

Representative examples of RT-PCR results from human brain tumors are shown in Fig. 1. Intensities of PCR products were found to be heterogeneous, and some specimens yielded only faint amplicon bands. These were scored positive only if the result could be reproduced by a repeated RNA extraction and specific PCR from the same tumor specimen. Cases with very low transcript levels that were not reproducibly positive were not regarded as positive. For example, the faint SCP-1 bands of case 20 and case 3 of Table 1 (*Lanes 2* and *3* from the *left* in the SCP-1 gel in Fig. 1) could not be reproduced convincingly; therefore, these two cases were considered to be negative for the expression of the respective CT gene.

Expression of Individual CT Genes in Human Brain Tumors. As can be seen in Tables 1 and 3, NY-ESO-1 was negative in all 88 brain tumor specimens tested, and SSX-1 was weakly expressed in only two grade IV astrocytomas. An intermediate expression frequency was observed for SSX-2 and HOM-TES-85, which were expressed in oligoastrocytomas and astrocytomas, whereas MAGE-3 was only expressed in 2 of 29 astrocytomas, both of which were grade IV. The CT gene most frequently expressed in the brain tumors investigated in this study was SCP-1. SCP-1 was the only CT gene to be expressed in meningiomas (18%) and was also found in the only pilocytic astrocytoma tested, in three of four oligoastrocytomas, and in 38% of the astrocytomas. SCP-1 was followed by SSX-4, which was the only CT gene to be expressed in oligodendrogliomas and was found in three of four oligoastrocytomas and in 26% of the astrocytomas, respectively.

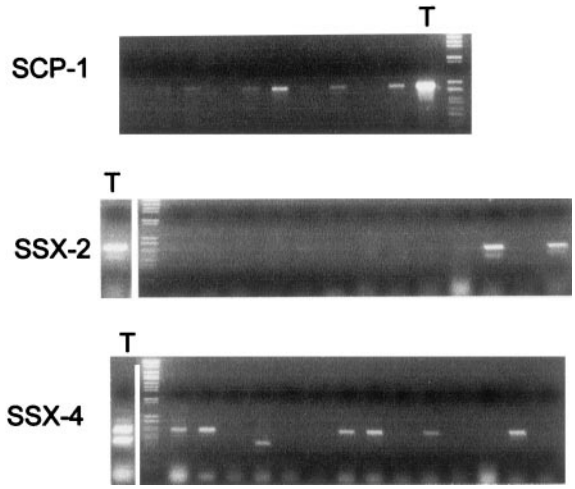

Fig. 1 RT-PCR for the expression of the CT genes SCP-1, SSX-2, and SSX-4 in human brain tumors. An equal amount of testis RNA was used as a representative positive control. *Top*, expression of SCP-1 by (from *left* to *right* with case numbers as indicated in Tables 1 and 3) astrocytoma II (case 12 of Table 1), astrocytoma IV (case 20, Table 1), oligodendroglioma (case 3, Table 1), oligoastrocytoma (case 10, Table 1), oligoastrocytoma (case 11, Table 1), astrocytoma IV (case 34, Table 1), meningioma (case 1, Table 3), astrocytoma IV (case 26, Table 1), astrocytoma IV (case 18, Table 1), astrocytoma IV (case 31, Table 1), and testis (*T*) markers. *Middle*, expression of SSX-2 by testis (*T*) markers, oligoastrocytoma (case 10, Table 1), oligodendroglioma (case 5, Table 1), astrocytoma IV (case 20, Table 1), oligodendroglioma (case 3, Table 1), astrocytoma II (case 12, Table 1), astrocytoma IV (case 33, Table 1), oligoastrocytoma (case 8, Table 1), oligodendroglioma (case 6, Table 1), astrocytoma IV (case 19, Table 1), meningioma (case 1, Table 3), oligoastrocytoma (case 11, Table 1), anaplastic meningioma (case 6, Table 3), and oligoastrocytoma (case 11, Table 1). *Bottom*, expression of SSX-4 by testis (*T*) markers, astrocytoma IV (case 29, Table 1), astrocytoma II (case 16, Table 3), oligodendroglioma (case 7, Table 1), oligodendroglioma (case 5, Table 1), astrocytoma IV (case 18, Table 1), oligodendroglioma (case 3, Table 1), astrocytoma IV (case 33, Table 1), oligoastrocytoma (case 8, Table 1), astrocytoma II (case 15, Table 1), astrocytoma II (case 13, Table 1), astrocytoma IV (case 22, Table 1), meningioma (case 1, Table 3), oligoastrocytoma (case 11, Table 1), and anaplastic meningioma (case 6, Table 3).

Expression of CT Genes According to Histological Subtype. Thirty-eight meningiomas were completely negative for the CT genes tested, with the exception of seven cases that expressed HOM-TES-14/SCP-1. No expression of any CTA was detected in the single ependymoma studied, and SCP-1 was the only CT gene expressed in a pilocytic astrocytoma. In oligodendrogliomas, the only CT gene to be expressed was SSX-4, which was positive in two of five cases. Together with SCP-1, SSX-4 was also the prevailing CT gene expressed in the four oligoastrocytomas studied, with both CT genes being positive in three of four cases. Two oligoastrocytomas expressed HOM-TES-85, and one oligoastrocytoma expressed SSX-2.

Astrocytomas of histological grades II—IV most frequently expressed SCP-1 (15 of 39 cases) and SSX-4 (10 of 38 cases), followed by HOM-TES-85 (4 of 33 cases), SSX-2 (4 of 36 cases), and MAGE-3 (2 of 29 cases). The expression pattern of the seven CT genes in astrocytomas of grades II—IV does not show a clear

Table 3 Expression of cancer testis genes by human brain tumors other than meningiomas

No.	Diagnosis	Patient's code	SSX-1	SSX-2	SSX-4	SCP-1	TS85	ESO-1	MAGE-3
1	Ependymoma	215	−		−	−	−		
2	Pilocytic astrocytoma	R59	−	−	−	+	−		−
3	Oligodendroglioma	R70	−	−	−	−	−	−	−
4	Oligodendroglioma	R71	−	−	+	−		−	
5	Oligodendroglioma	R90	−	−	+	−		−	
6	Oligodendroglioma	R82	−	−	−	−	−		−
7	Oligodendroglioma	120	−	−	−	−	−		−
8	Oligoastrocytoma	R21	−	−	+	+	+	−	−
9	Oligoastrocytoma	R28	−		−	+	−		−
10	Oligoastrocytoma	R56	−	−	+	−	+	−	−
11	Oligoastrocytoma	731	−	+	+	+	−	−	−
12	Astrocytoma II	R42	−	−	−	−	+	−	−
13	Astrocytoma II	469	−	−	+	+	+		
14	Astrocytoma II	647	−	+	−	−	−	−	−
15	Astrocytoma II	677	−	+	−	−			
16	Astrocytoma II	706	−	−	+	−	−	−	−
17	Astrocytoma III	G27	−	−	−	−		−	−
18	Astrocytoma IV	46	−		−	−	−		
19	Astrocytoma IV	47	−	−	−	−			
20	Astrocytoma IV	84	−	−	−	−			
21	Astrocytoma IV	85	+	−	−	+	−	−	−
22	Astrocytoma IV	86	−	−	−	−	−	−	−
23	Astrocytoma IV	90	−	+	−	−	−	−	
24	Astrocytoma IV	91	−		+	−	−		−
25	Astrocytoma IV	93	−	−	+	+			
26	Astrocytoma IV	121	−	−		+			
27	Astrocytoma IV	208	−		+	−	−	−	
28	Astrocytoma IV	219	−	−	−	−	−		
29	Astrocytoma IV	705	−	−	+	−	−	−	+
30	Astrocytoma IV	718	−	−	−	−	−	−	−
31	Astrocytoma IV	726	−	−	−	+	−	−	−
32	Astrocytoma IV	R34	−	−	−	+	−		−
33	Astrocytoma IV	R35	−	−	+	+	+	−	−
34	Astrocytoma IV	R79	+	+	+	+	+	−	+
35	Astrocytoma IV	R96	−	−	−	+	−		−
36	Astrocytoma IV	89	−	−	−	+	−	−	−
37	Astrocytoma IV	95	−	−	−	+	−	−	−
38	Astrocytoma IV	210	−	−	−	−	−	−	−
39	Astrocytoma IV	664	−	−	+	+	−	−	−
40	Astrocytoma IV	317/99	−	−	−	+	−	−	−
41	Astrocytoma IV	386/99	−	−	−	−	−	−	−
42	Astrocytoma IV	492/99	−	−	−	+	−	−	−
43	Astrocytoma IV	611/99	−	−	−	−	−	−	−
44	Astrocytoma IV	613/99	−	−	−	−	−	−	−
45	Astrocytoma IV	937/99	−	−	−	−	−	−	−
46	Astrocytoma IV	1164/99	−	−	−	−	−	−	−
47	Astrocytoma IV	1254/99	−	−	−	−	−	−	−
48	Astrocytoma IV	1280/99	−	−	−	−	−	−	−
49	Astrocytoma IV	1460/99	−	−	−	−	−	−	−
50	Astrocytoma IV	HA	−	−	+	+	−	−	−

correlation of differentiation or anaplasia with the frequency of CT gene expression nor an association of a histological subtype with a given CT gene; however, MAGE-3 expression was found only in grade IV astrocytomas in this series.

Coexpression of Multiple CT Genes in Human Brain Tumors. Expression of at least one antigen was observed in 7 of 38 (18%) meningiomas, 2 of 5 (40%) oligodendrogliomas, all (100%) oligoastrocytomas, and 23 of 39 (59%) astrocytomas. Expression of more than one CT gene was not observed in meningiomas or oligodendrogliomas but was seen in 3 of 4 (75%) oligoastrocytomas and 8 of 39 (21%) astrocytomas. Coexpression of three CT genes occurred in 2 of 4 (50%) oligoastrocytomas and 3 of 39 (8%) astrocytomas.

DISCUSSION

A wide range of human neoplastic tissues express CT genes (20). Because both glial cells and melanocytes are derived from the neuroectoderm, these two cell types share many biological features, and it is not surprising that they express not only a similar spectrum of differentiation antigens but also of the "shared" or so-called CTAs. With the exception of NY-ESO-1/LAGE-1 (7, 15), which are frequently expressed in mel-

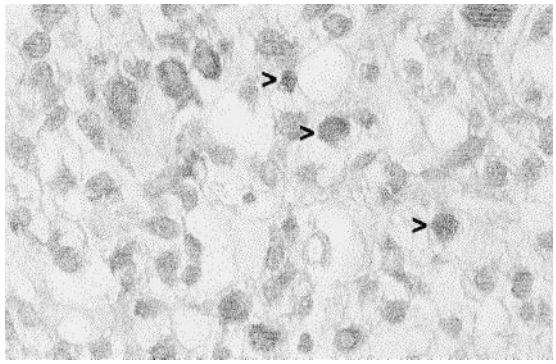

Fig. 2 Immunohistological detection of HOM-TES-14/SCP-1 in an astrocytoma. The tumor cells, especially those of the gemistocytic type, show a fine granular cytoplasmic staining. Some tumor cells also show or exclusively show nuclear reactivity (>). Immunoperoxidase stain (×180).

anomas but not expressed at all in gliomas, all other CT genes described to date are found in both melanomas and malignant brain tumors (8, 9, 11, 13, 16).

Whereas meningiomas express only SCP-1, about half of the gliomas express at least one CTA. Oligoastrocytomas appear to be the type of human brain tumor with the highest frequency of expression of a single CT gene or coexpression of several CT genes. Whereas there was no expression pattern that suggested preferential expression of a given antigen in a particular histological subtype, the expression pattern of oligoastrocytomas bore a greater resemblance to that of astrocytomas than that of oligodendrogliomas. This seems surprising because a common cellular origin has been suggested for oligodendrogliomas and oligoastrocytomas, which is different from the putative cell of origin of astrocytomas (21). Because RT-PCR is a whole-tissue approach, which does not allow conclusions as to the cell of origin of a positive band, it cannot be excluded that the similar expression pattern of astrocytomas and oligoastrocytomas is due to the contribution of cells with astrocytic differentiation within the oligoastrocytomas. Moreover, the absence of expression in certain types of tumors (*e.g.*, the absence of NY-ESO-1 in gastric and colorectal cancers or lymphomas) seems to characterize a given CT gene better than the description of its positive expression pattern (Table 2).

The function of most of the CT genes is unknown. Exceptions are HOM-TES-14/SCP-1, which is involved in meiotic chromosome pairing (13, 22), the SSX genes, which contain a KRAB domain that has recently been shown to have a transcriptional repressor function (23, 24), and HOM-TES-85, a novel member of the leucine zipper proteins, which are involved in DNA binding and gene transcription.[4]

A recently published analysis of the expression of several melanoma-associated antigens (4) investigated the expression of MAGE-1 and MAGE-3 in 21 glioblastoma (astrocytoma grade IV) specimens and a few other types of human brain tumors. The authors observed that MAGE-1 and MAGE-3 genes were expressed in the order of one in three grade IV astrocytomas or glioblastomas. This is considerably higher than the 2 of 25 positive cases we observed in this study and the 0 of 20 positive cases observed by others (25) in a recently published study in grade IV astrocytomas. Whether the high detection rate of MAGE-3 reported in the study by Chi *et al.* (4) is due to a selective effect of small sample numbers, the different primers used, or nonspecific hybridization of internal probes to non-MAGE amplification products remains an open question. Scarcella *et al.* (25) recently reported that GAGE-1, a CT gene that was not included in the present study, was the most frequently expressed (65%) in glioblastoma multiforme.

Whereas mRNA levels of CT genes do not strictly correlate with the protein expression, no case of a malignant tumor has been observed to date in which the antigenic protein was absent despite expression of the respective mRNA (13). This also held true for the brain tumors tested for SCP-1 antigen expression in this study: all RT-PCR-positive samples were also positive in immunohistological analysis with a monoclonal anti-SCP-1 antibody, the only antibody to CT genes that is available to us. Immunohistology for HOM-TES-14/SCP-1 showed that in HOM-TES-14/SCP-1-positive cases, virtually all tumor cells express the antigen (Fig. 2).

From the data of our study, it appears that about half of the patients with oligoastrocytomas and astrocytomas would be eligible for specific immunotherapeutic approaches with at least one CTA in ways similar to the ones that are currently being evaluated in malignant melanomas (26–28), which express CTAs at a similar frequency as astrocytomas. Whereas all patients with a tumor expressing a given CTA would be candidates for vaccine strategies using whole antigenic proteins, the percentage of patients eligible for peptide-specific vaccinations would be much lower because it requires antigenic peptides with binding motifs restricted to specific MHC alleles. Therefore, additional antigenic CT genes must be identified for human gliomas, especially if the development of multivalent vaccines for a majority of patients is the goal. Because the expression of a CTA by a tumor is a prerequisite for a strong antibody response against the respective molecule, it makes sense to exploit the expressed B-cell repertoire of glioma patients for the identification of novel CT genes in glioma. Thus, using sera from glioma patients should enhance the chance to identify new CT genes that have resisted discovery to date because such a search would be biased for antibodies with reactivity to antigens with preferential expression in gliomas.

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REFERENCES

1. Ram, Z., Culver, K. W., Oshiro, E. M., Viola, J. J., de Vroom, H. L., Otto, E., Long, Z., Chiang, Y., McGarrity, G. J, Muul, L. M., Katz, D., Blaese, R. M., and Oldfield, E. H. Therapy of malignant brain tumors by intratumoral implantation of retroviral vector-producing cells. Nat. Med., *3:* 1354–1361, 1997.

2. Sawamura, Y., and de Tribolet, N. Immunobiology of brain tumors. J. Neurosurg., *69:* 745–750, 1991.

3. Türeci, Ö., Sahin, U., and Pfreundschuh, M. Serological analysis of human tumor antigens: molecular definition and implications. Mol. Med. Today, *3:* 342–349, 1997.

4. Chi, D. D. J., Merchant, R. E., Conrad, A. J., Garrison, D., Turner, R., Morton, D. L., and Hoon, D. S. B. Molecular detection of tumor-associated antigens shared by human cutaneous melanomas and gliomas. Am. J. Pathol., *150:* 2143–2152, 1997.

5. van den Eynde, B. J., and van der Bruggen, P. T cell defined tumor antigens. Curr. Opin. Immunol., *9:* 684–693, 1997.

6. Sahin, U., Türeci, Ö., and Pfreundschuh, M. Serological identification of human tumor antigens. Curr. Opin. Immunol., *9:* 709–716, 1997.

7. Chen, Y. T., Scanlan M. J., Sahin, U., Türeci, Ö., Güre, A. O., Tsang, S., Williamson, B., Stockert, E., Pfreundschuh, M., and Old, L. J. A testicular antigen aberrantly expressed in human cancers detected by autologous antibody screening. Proc. Natl. Acad. Sci. USA, *94:* 1914–1918, 1997.

8. van der Bruggen, P., Traversari, C., Chomez, P., Lurquin, C., de Plaen, E., van den Eynde, B. J., Knuth, A., and Boon, T. A gene encoding an antigen recognized by cytolytic T lymphocytes on a human melanoma. Science (Washington DC), *254:* 1643–1647, 1991.

9. Boel, P., Wildmann, C., Sensi, M. L., Brasseur, R., Renauld, J. C., Coulie, P., Boon, T., and van der Bruggen, P. BAGE: a new gene encoding an antigen recognized on human melanomas by cytolytic T lymphocytes. Immunity, *2:* 167–175, 1995.

10. van den Eynde, B., Peeters, O., de Backer, O., Gaugler, B., Lucas, S., and 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.

11. Türeci, Ö., Sahin, U., Schobert, I., Koslowski, M., Schmitt, H., Schild, H-J., Stenner, F., Seitz, G., Rammensee, H-G., and Pfreundschuh, M. 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.

12. Chen, Y. T., Güre, A. O., Tsang, S., Stockert, E., Jäger, E., Knuth, A., and Old, L. J. Identification of multiple cancer/testis antigens by allogeneic antibody screening of a melanoma cell line library. Proc. Natl. Acad. Sci. USA, *95:* 6919–6923, 1998.

13. Türeci, Ö., Sahin, U., Zwick, C., Koslowski, M., Seitz, G., and Pfreundschuh, M. Identification of a meiosis-specific protein as a member of the class of cancer/testis antigens. Proc. Natl. Acad. Sci. USA, *95:* 5211–5216, 1998.

14. Sahin, U., Türeci, Ö., Schmitt, H., Cochlovius, B., Johannes, T., Stenner, F., Luo, G., Schobert, I., and Pfreundschuh, M. Human neoplasms elicit multiple immune responses in the autologous host. Proc. Natl. Acad. Sci. USA, *92:* 11810–11813, 1995.

15. Lethe, B., Lucas, S., Michaux, L., de Smet, C., Godelaine, D., Serrano, A., de Plaen, E., and Boon, T. LAGE-1, a new gene with tumor specificity. Int. J. Cancer, *76:* 903–909, 1998.

16. Fleischhauer, K., Gattinoni, L., Dalerba, P., Lauvau, G., Zanaria, E., Dabovic, B., van Endert, P. M., Bordignon, C., and Traversari, C. The DAM gene family encodes a new group of tumor-specific antigens recognized by human leukocyte antigen A2-restricted cytotoxic T lymphocytes. Cancer Res., *58:* 2969–2972, 1998.

17. Chomczynski, P., and Sacchi, N. Single step method of RNA isolation by acid guanidium thiocyanate-phenol-chloroform extraction. Anal. Biochem., *162:* 156–159, 1987.

18. Türeci, Ö., Chen, Y-T., Sahin, U., Güre, A. O., Zwick, C., Villena, C., Tsang, S., Seitz, G., Old, L. J., and Pfreundschuh, M. Expression of SSX genes in human tumors. Int. J. Cancer, *77:* 19–23, 1998.

19. Güre, A. O., Türeci, Ö., Sahin, U., Tsang, S., Scanlan, M., Jäger, E., Knuth, A., Pfreundschuh, M., Old, L. J., and Chen, Y. T. SSX, a multigene family with several members transcribed in normal testis and human cancer. Int. J. Cancer, *72:* 965–971, 1997.

20. Sahin, U., Türeci, Ö., and Pfreundschuh, M. Expression of multiple cancer/testis (CT) antigens in breast cancer and melanoma: basis for polyvalent CT vaccine strategies. Int. J. Cancer, *78:* 387–389, 1998.

21. Kraus, J. A., Koopmann, J., Kaskel, P., Maintz, D., Brandner, S., Schramm, J., Louis, D. N., Wiestler, O. D., and von Deimling, A. Shared allelic losses on chromosomes 1p and 19q suggest a common origin of oligodendroglioma and oligoastrocytoma. J. Neuropathol. Exp. Neurol., *54:* 91–95, 1995.

22. Heyting, C. Synaptonemal complexes: structure and function. Curr. Opin. Cell Biol., *8:* 389–396, 1996.

23. Brett, D., Whitehouse, S., Antonson, P., Shipley, J., Cooper, C., and Goodwon, G. The SYT protein involved in the t(X;18) synovial sarcoma translocation is a transcriptional activator localised in nuclear bodies. Hum. Mol. Genet., *6:* 1559–1564, 1997.

24. Lim, F. I., Soulez, M., Koczan, D., Thiesen, H. J., and Knight, J. C. A KRAB-related domain and a novel transcription repression domain in proteins encoded by SSX genes that are disrupted in human sarcomas. Oncogene, *17:* 2013–2018, 1998.

25. Scarcella, D. L., Chow, C. W., Gonzalez, M. F., Economou, C., Brasseur, F., and Ashley, D. M. Expression of MAGE and GAGE in high-grade brain tumors: a potential target for specific immunotherapy and diagnostic markers. Clin. Cancer Res., *5:* 335–341, 1999.

26. Marchand, M., van Baren, N., Weynants, P., Brichard, V., Dreno, B., Tessier, M-H., Rankin, E., Parmiani, G., Arienti, F., Humblet, Y., Bourlond, A., van Wijck, R., Lienard, D., Beauduin, M., Dietrich, P-Y., Russo, V., Kerger, J., Masucci, G., Jaeger, E., de Greve, J., Atzpodien, J., Brasseur, F., Coulie, P. G., van der Bruggen, P., and Boon, T. Tumor regressions observed in patients with metastatic melanoma treated with an antigenic peptide encoded by gene MAGE-3. Int. J. Cancer, *80:* 219–230, 1999.

27. Nestle, F. O., Alijagic, S., Gilliet, M., Sun, Y., Grabbe, S., Dummer, R., Burg, G., and Schadendorf, D. Vaccination of melanoma patients with peptide- or tumor lysate-pulsed dendritic cells. Nat. Med., *4:* 328–332, 1998.

28. Rosenberg, S. A., Yang, J. C., Schwartzenhuber, D. R., Hwu, P., Marincola, F. M., Topalian, S. L., Restifo, N. P., Dudley, M. E., Schwarz, S. L., Spiess, P. J., Wunderlich, J. R., Parkhurst, M. R., Kawakami, Y., Seipp, C. A., Einhorn, J. H., and White, D. E. Immunologic and therapeutic evaluation of a synthetic peptide vaccine for the treatment of patients with metastatic melanoma. Nat. Med., *4:* 321–327, 1998.

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