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Immunology

Identification of a meiosis-specific protein as a member of the class of cancer/testis antigens

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ABSTRACT Little is known about the function of human cancer/testis antigens (CTAs), such as MAGE, BAGE, GAGE, HOM-MEL-40, and NY-ESO-1, the expression of which is restricted to human malignancies and testis. When screening a cDNA expression library enriched for testis-specific representative long transcripts for reactivity with high-titered IgG antibodies from the serum of a patient with renal cell carcinoma, one repeatedly detected antigen, designated HOM-TES-14, turned out to be encoded by the synaptonemal complex protein 1 (SCP-1) gene. SCP-1 is known to be selectively expressed during the meiotic prophase of spermatocytes and is involved in the pairing of homologous chromosomes, an essential step for the generation of haploid cells in meiosis I. Investigation of a broad spectrum of normal and malignant tissues revealed expression of SCP-1 transcripts and antigen selectively in a variety of neoplastic tissues and tumor cell lines. Immunofluorescence microscopy analysis with specific antiserum showed a cell cycle phase-independent nuclear expression of SCP-1 protein in cancer cells. SCP-1 differs from other members of the class of CTA by its localization on chromosome 1 and its frequent expression in malignant gliomas, breast, renal cell, and ovarian cancer. The aberrant expression of SCP-1 in tumors might contribute to their genomic instability and suggests that the functional role of other CTA might also relate to meiosis.

The identification of genes that are selectively expressed in cancer and code for proteins inducing a specific immune response in the tumor-bearing host is the prerequisite for specific immunotherapeutic approaches to cancer. By using cytotoxic T lymphocyte- and antibody-based approaches, several human tumor antigens have been defined that might be suitable targets for specific cancer vaccination (1, 2). The difficulties in establishing cytotoxic T lymphocyte clones with specificity to human neoplasms are the major reason why—with the exception of RAGE (renal cell cancer-associated antigen) (3)—all T cell-defined human tumor antigens have been originally described in malignant melanoma. Immunotherapeutic approaches to human neoplasms other than melanoma are restricted to the nonmelanoma cases that express the melanoma-defined antigens. To overcome this limitation, we recently established SEREX (serological analysis of antigens by recombinant expression cloning) that allows for the unbiased search of tumor antigens that elicit IgG antibody responses in the autologous tumor patients (4). With this approach, we have identified multiple tumor antigens in different human neoplasms. Among others, we detected MAGE-1 (melanoma-associated antigen) and MAGE4a transcripts, demonstrating that at least some of the serologically identified antigens are also targets for cytotoxic T cells. The analysis of the expression pattern of SEREX antigens revealed that a group of tumor antigens, such as HOM-MEL-40, NY-ESO-1, and several other (unpublished results) antigens, are selectively expressed in a variety of human neoplasms but not in normal tissues except for testis. This characteristic expression spectrum has also been described for the T cell-defined MAGE, BAGE, and GAGE gene products and has led to the designation of the term cancer/testis antigens (CTAs; ref. 5). Besides differentiation antigens such as Melan A/MART-1, tyrosinase, gp100/Pmel17, and gp75 (6–11), CTA respresent a second major class of molecularly defined human tumor antigens. Although the function of many differentiation antigens is known, the function of all the CTAs has resisted scrutiny.

The selective expression of CTAs in a broad spectrum of neoplasms makes them ideal candidates for specific cancer immunotherapy. However, because the known CTA are expressed in only a small spectrum of human cancers and in only a fraction of cases of a given tumor type, the identification of additional tumor antigens is badly needed. The fact that all CTAs are also expressed at high levels in testis prompted us to screen a testis expression library enriched for specific transcripts instead of a cDNA library derived from a tumor. This approach has led to the identification of several more CTAs. One of these antigens, HOM-TES-14, is encoded by synaptonemal complex protein 1 (SCP-1), a gene specifically expressed in the meiotic prophase of spermatocytes. The SCP-1 protein is involved in the meiotic chromosome synapsis of sperm cells.

MATERIALS AND METHODS

Sera, Tissues, and Cell Lines. The study had been approved of by the local ethical review board ("Ethikkommission der Ärztekammer des Saarlandes"). Recombinant DNA work was done with the official permission and according to the rules of the state government of Saarland. Sera and tumor tissues were obtained during routine diagnostic or therapeutic procedures and were stored at −80°C until use. Normal tissues were collected from autopsies of tumor-free patients.

Construction of the Subtractive cDNA Expression Library. For the construction of the subtractive cDNA expression library, a modification of the recently described suppression subtractive hybridization (SSH) technique (12) was used. In brief, a pool of testis-specific cDNA fragments were amplified by the SSH technique. These cDNA fragments were used to capture their long counterparts from a cDNA phagemid library. Finally, cDNA inserts excised from captured phagemids were cloned into λ phage vectors and used for expression

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Abbreviations: CTA, cancer/testis antigen; MAGE, melanoma-associated antigen; SCP-1, synaptonemal complex protein 1; SEREX, serological analysis of antigens by recombinant expression cloning; PBMC, peripheral blood mononuclear cell.

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screening. For the construction of testis-specific cDNA, 2 μg of mRNA derived from two testicular tissue specimens was used as a tester probe. The driver cDNA probe was synthesized from mRNA derived from 10 healthy tissues (colon, stomach, brain, resting and activated peripheral blood mononuclear cells (PBMCs), skeletal muscle, liver, kidney, lung, and skin). Suppression subtractive hybridization PCR was performed as described (12) after hybridization of tester and driver cDNA. The construction of the phagemid library was performed as described (4) with 5 μg of mRNA derived from the same testis samples. The cDNA was cloned into Lambda-ZAP Express phages (Stratagene), resulting in a library with 4.0×10^6 primary clones. Single-stranded pBK-CMV phagemid DNA was extracted after *in vivo* mass excision of the phage expression library. The excised single-stranded phagemid cDNA library was hybridized on nitrocellulose membranes (Schleicher & Schüll, Heidelberg) blotted with the testis cDNA derived from the suppression PCR. After hybridization the nitrocellulose membranes were washed and phagemids bound to immobilized cDNA were eluted. Double-stranded cDNA inserts were synthesized by using thermostable polymerase from *Pyrococcus furiosus* (Stratagene) and (Reverse, Universe) flanking vector-specific primers. The double-stranded cDNA inserts were excised by restriction enzyme digestion from the phagemids and were religated into precut dephosphorylated λ ZAP Express vector. The ligation product was packaged into λ phage particles and used for transfection and library amplification.

Immunoscreening of Transfectants. The immunoscreening for the detection of clones reactive with IgG antibodies in the 1:100 diluted serum from a 62-year-old female patient with renal cell cancer (taken at the time of operation) was performed as described (13). Visualization of reactive clones was performed by incubation with an anti-human Fc alkaline phosphatase-conjugated antibody (Dianova, Hamburg, Germany) and developed with 5-bromo-4-chloro-3-indolyl phosphate and nitroblue tetrazolium according to the manufacturer's instructions (Biomol, Hamburg, Germany). For the screening of additional sera, phages from positive clones were mixed with nonreactive phages of the cDNA library as internal negative controls at a ratio of 1:10 and used to transfect bacteria. IgG antibodies in 1:100 diluted *Escherichia coli*-absorbed sera were detected with the immunoscreening assay described above.

Sequence Analysis of Identified Antigens. Positive clones were subcloned to monoclonality and submitted to *in vivo* excision (14) of pBK-CMV phagemids (15). The nucleotide sequence of cDNA inserts was determined by using a Sequenase 2.0 kit (United States Biochemical). Sequencing was performed according to the manufacturer's instructions starting with the vector-specific primers. Insert-specific primers were designed as the sequencing proceeded. Sequence alignments were performed with DNASIS (Pharmacia Biotech) and BLAST (16) software on EMBL, GenBank, and Prosite (17) databases.

Northern Blot Analysis. RNA from tumors and normal tissues were used for Northern blots (18). Integrity of RNA was checked by electrophoresis in formaline/Mops gels. Gels with 10 μg of RNA per lane were blotted onto nylon membranes, prehybridized, and subsequently incubated with the specific ^{32}P-labeled SCP-1 cDNA probe (bp 2,715–3,264) overnight at 42°C in hybridization solution (50% formamide/6× SSC/5× Denhardt's solution/0.2% SDS). The membranes were then washed at progressively higher stringency, with the final wash in 1× SSC/0.2% SDS at 65°C. Autoradiography was conducted at −70°C for up to 7 days by using Kodak X-Omat-AR film and intensifying screen. Thereafter the filters were stripped and rehybridized with glyceraldehyde phosphate dehydrogenase to prove RNA integrity and assess loading of equal amounts of RNA.

Reverse Transcription–Coupled PCR (RT-PCR). cDNA was obtained by extraction of total cellular RNA, priming with a dT_{18} oligonucleotide, and reverse transcription with Superscript reverse transcriptase (GIBCO/Life Technologies). Integrity of the cDNA was proven by amplification of β-actin transcripts in a 30-cycle PCR. Presence of SCP-1 transcripts was studied with a specific pair of primers (sense, 5′-GTACAGCAGAAAGCAAGCAACTGAATG-3′; antisense, 5′-GAAGGAACTGCTTTAGAATCCAATTTCC-3′). Specific PCR products were checked by electrophoresis. The predicted size of the amplified fragment was 564 bp for SCP-1. Authenticity of amplification products was also proved by alkaline blotting of products after electrophoresis and subsequent hybridization with a radioactive labeled internal oligonucleotide.

Southern Blot Analysis. Southern blot analysis was performed as described (19) with *Hae*III-digested (Boehringer Mannheim) DNA extracted from testis and peripheral blood lymphocytes. Equal loading of samples was checked by staining with ethidium bromide and visualization of DNA under UV light. Hybridization with the [^{32}P]dCTP radiolabeled full-length SCP-1 cDNA was carried out in 6× SSC/4× Denhardt's solution/0.5% SDS. Washes and autoradiography were performed as described for Northern blot analysis.

Western Blot Analysis. Ten micrograms of lysate per lane were mixed with 2× SDS sample buffer (0.1 M Tris·HCl, pH 6.8/0.2 M DTT/4% SDS/0.2% bromophenol blue/20% glycerol), electrophoresed in 12% SDS/PAGE gels, and then blotted to nylon membranes (Schleicher & Schüll) by semi-dry transfer (Bio-Rad). After blocking nonspecific binding with 5% low-fat milk in TBS for 1 h, the membranes were incubated with the 1:400 diluted rabbit anti-SCP-1 antiserum (ref. 20; provided by C. Heyting, Agricultural University, Wageningen, The Netherlands). The blots were then incubated for 1 h with alkaline phosphatase-conjugated goat anti-human IgG (Dianova). After each incubation step, the membrane was washed extensively with TBS/0.01% Tween. Positive reactions were visualized with 5-bromo-4-chloro-3-indolyl phosphate and nitroblue tetrazolium. All analyses were done in duplicate.

Immunocytology. Cells from the SCP-1$^+$ CHENG glioma cell line and freshly isolated adherent human monocytes as a negative control were cultured on culture slides (Falcon product 254118) for 2 days and fixed with 2% (vol/vol) paraformaldehyde/PBS. Slides were incubated with rabbit anti-SCP-1 antiserum diluted 1:200 in 10% (vol/vol) fetal calf serum/PBS for 1 h. Preimmune rabbit serum served as a negative control. After 1 h, the slides were washed and incubated with goat anti-rabbit-CY3 antibody (Amersham). Cells were washed with PBS and overlaid with mounting medium containing 4′,6-diamidino-2-phenylindole (Boehringer Mannheim) for counterstaining of nuclei. Staining patterns were analyzed by using fluorescence microscopy.

RESULTS

Establishment of a cDNA Expression Library Enriched for Testis-Specific Clones. A pool of cDNA fragments specifically expressed in human testis was amplified by using the suppression subtractive hybridization technique. The efficacy of the enrichment of testis-specific transcripts was proven by using amplified cDNA as a probe for Northern blot hybridization with RNA extracted from different normal tissues. A strong hybridization signal was detected with testis RNA and much weaker signals with RNA derived from the normal tissues that had been used for the generation of driver mRNA (data not shown). These cDNA fragments were used for capturing their long counterparts by hybridization with the single-stranded phagemid library constructed by using the same tester mRNA as a template. Captured cDNA was double-stranded and

ligated into the predigested ZAP Express vector, resulting in a phage expression library of 400,000 recombinants.

Screening and Sequence Analysis. The 1:100 diluted serum from a 62-year-old female patient with renal cell cancer was used to screen 200,000 recombinants from this library. Five primary positive clones coding for three transcripts were found. Three clones HOM-TES-6, HOM-TES-7, and HOM-TES-14 turned out to be identical to SCP-1. HOM-TES-6 contains nucleotides 726–2,401, HOM-TES-7 represents a fragment from nucleotides 147 to 2,728, and HOM-TES-14 contains the sequence from nucleotides 634 to 2,462. Thus, these clones covered nearly the full length of the SCP-1 transcript. Alignment and sequence analysis confirmed the expected ORF of 2,928 nucleotides. We observed single base differences to the published nucleotide sequence of human SCP-1 with consequences for the predicted amino acid sequence. At amino acid position 208, arginine was replaced with glycine (AGG → GGG), histidine 225 was replaced with phenylalanine (CAT → TTT), and glycine 226 was replaced with glutamine (GGG → GAG).

Expression Spectrum of HOM-TES-14 and Southern Blot Analysis. The expression of HOM-TES-14 in normal tissues was analyzed by Northern blot hybridization and amplification of the respective products from dT_{18}-primed cDNA with specific primers. For tumor tissues expression, analysis was performed by RT-PCR and for selected samples Northern blot analysis was also done. We detected no expression in any normal adult tissue except for a high-level expression in testis (Table 1, Figs. 1 and 2). A considerable number of tumors of various histological types expressed HOM-TES-14 (Table 2 and Fig. 2). The highest proportion of positive tumors was found among gliomas (40%) and breast carcinomas (27%). A less frequent expression was detected in colon carcinomas (16%) and melanomas (14%). SCP-1 transcripts were also detectable in some cases of renal cell (3 of 36), gastric (1 of 10), and lung (1 of 14) carcinomas. In contrast, no expression was found in a large number of colon and prostate carcinomas or in lymphomas.

To compare the HOM-TES-14 transcript levels in testis and tumor tissues, RNA from testis and selected tumor samples that had been shown to express HOM-TES-14 by RT-PCR were analyzed by Northern blotting. In all neoplasms, HOM-TES-14 expression levels were lower than in testis (Fig. 1). However, the transcripts levels in tumors varied considerably, with the highest levels of expression in breast cancers. Besides a band at the position of 3.5–4.0 kb, compatible with the full-length sequence of SCP-1 including a poly(A) tail, autoradiography of Northern blots revealed an additional band at approximately 1.2 kb.

Southern Blot Analysis. Southern blot analysis showed a banding pattern suggestive for a gene family rather than for a single gene (Fig. 3). To analyze whether the HOM-TES-14 mRNA expression in the tumor samples and cell lines resulted in the production of the respective protein, we performed a Western blot analysis of selected tumor specimens and cell lines (20) with a specific anti-SCP-1 rabbit serum (21). The rabbit serum specifically detected a major band migrating at 110 kDa in lysates from glioma cell lines, tumors, and testis but not from any other normal tissue (Fig. 4). Besides this major band, additional bands migrating at 120 kDa and 80 were recognized, indicating the expression of splice variants, post-translational modifications, or cross-reactive nonrelated proteins in the tumor cells. Despite the significant lower transcript expression levels in cancer cells, the amounts of immunoreactive protein in the tumors and cell lines were at least equal to the amounts expressed in testis, suggesting a higher stability of the SCP-1 protein in tumors.

Immunocytology. Indirect immunofluorescence of a SCP-1^{+} glioma cell line revealed a specific staining pattern of the cells (Fig. 5) with a punctate staining pattern almost exclusively in the nuclei. Reactivity was observed in nearly all interphase nuclei and in mitotic cells, indicating that expression of the SCP-1 protein is not cell-cycle-specific in the malignant cells.

Survey of Anti-SCP-1 Serum Reactivity. To get an impression of the frequency of anti-SCP-1 antibodies, the sera of 15 healthy controls, 31 patients with renal cancer, and six patients with breast cancer were tested in the phage assay for IgG

Table 1. RT-PCR and Northern blot analysis of HOM-TES-14 expression in normal tissues

Tissue	Expression
Brain	–
Frontal cortex	–
White matter	–
Bladder	–
Breast	–
Colon	–
Kidney	–
Liver	–
Lung	–
Lymph node	–
Muscle	–
Ovary	–
Peripheral blood lymphocytes (PBLs)	–
PBLs (activated)	–
Prostate	–
Rectum	–
Skin	–
Spleen	–
Stomach	–
Testis	+
Thyroid gland	–
Tonsil	–
Uterus (endometrium)	–

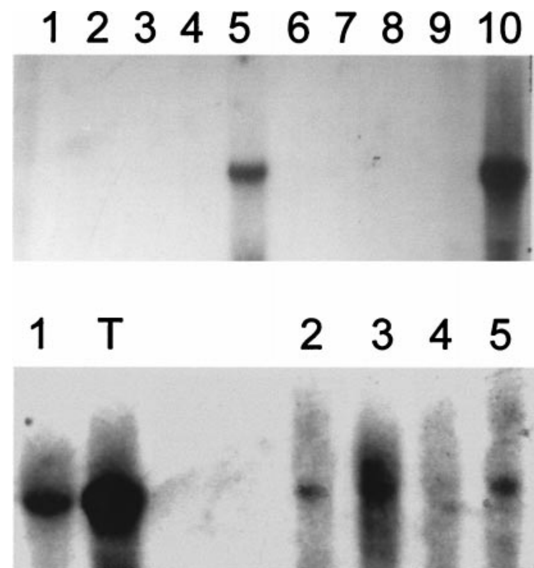

FIG. 1. Northern blot analysis of SCP-1 expression. Expression in normal tissues and tumor samples was tested. No expression is detectable in normal tissues (*Upper*) except for a high level expression in testis. Lanes: 1, brain; 2, PBMCs; 3, mitogen-stimulated PBMCs; 4, kidney; 5, testis; 6, spleen; 7, lung; 8, skeletal muscle; 9, liver; 10, testis. All lanes except lane 5 (5 μg) were loaded with 10 μg of total RNA. (*Lower*) Selected tumor tissues, proven to be positive for SCP-1 by specific RT-PCR, were retested by Northern blotting using 10 μg of total RNA, demonstrating significant transcripts levels, which were lower than the abundant transcript amounts detected in testis (lane T). Lanes: 1, breast cancer; 2, gastric cancer; 3, ovarian cancer; 4 and 5, renal cell carcinoma.

reactivity with SCP-1. Although all healthy controls were negative, 3 of 6 patients with breast cancer (50%) and 1 of 31 patient with renal cancer (3%) had serum antibody response to recombinant SCP-1 protein.

DISCUSSION

A prerequisite for a broader application of antigen-specific immunotherapy is the molecular definition of antigens that are specifically expressed in the commonly occurring neoplasms, e.g., breast, lung, prostate, or colorectal carcinoma. The recognition that members of the MAGE, BAGE, GAGE, HOM-MEL-40, and NY-ESO-1 gene families form a class of tumor antigens with restricted expression confined to cancer and testis has let to the designation CTA. This characteristic expression profile suggested testicular tissue as a prime candidate source for the identification of additional CTA by SEREX. To further increase the yield of unidentified CTAs, we constructed a testis cDNA expression library enriched for specific clones by using a cDNA subtraction strategy that ensures the representation of long cDNA inserts and of rare differentially expressed transcripts. Because the recently described suppression subtractive hybridization (12) yields short cDNA fragments that were not well suited for SEREX (results not shown), we modified the original protocol and used the pool of testis-specific short cDNA fragments to capture the representative long cDNA clones from a conventional testis expression library. These captured cDNA inserts were used to construct an expression library that proved to be suitable for the immunoscreening by SEREX.

When screening this library with a serum from a renal cell carcinoma patient, we identified HOM-TES-14, which turned out to be encoded by the gene for SCP-1. Although library screening with cDNA probes had failed to identify additional SCP-1-related genes (22, 23), our Southern blot analysis suggests that SCP-1 belongs to a family of related genes. We confirmed previous reports (24) that human SCP-1 is not expressed in normal tissues except for testis. Although murine SCP-1 had been reported to be expressed in mouse brain (25), we could not detect the human transcript in three cDNA samples derived from the cortex and white matter of human frontal brain. However, we cannot definitely exclude that human SCP-1 might be expressed in other particular areas of brain.

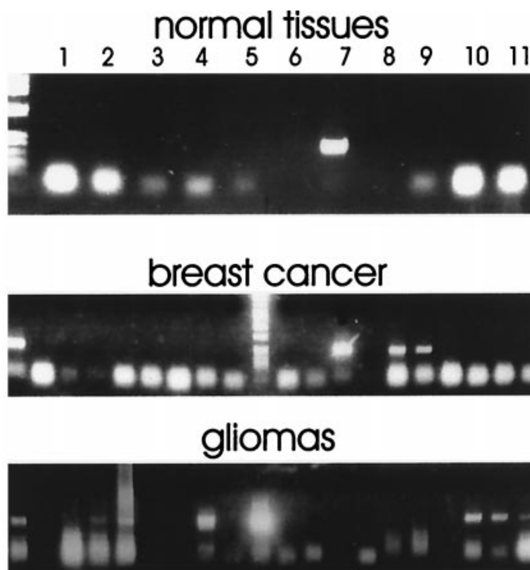

FIG. 2. Expression of SCP-1 analyzed by RT-PCR. SCP-1 expression in normal tissues is restricted to testis. Lanes: 1, PBMCs; 2, mitogen-stimulated PBMCs; 3, breast; 4, brain; 5, liver; 6, spleen; 7, testis; 8, genomic DNA control; 9, skeletal muscle; 10, kidney; 11, lung. In contrast SCP-1 transcripts are detectable with various frequencies in fresh tumor specimens, as shown for selected breast cancer and glioma samples.

Our expression analysis demonstrates the aberrant expression of the meiosis-related SCP-1 gene in a broad spectrum of human neoplasms and establishes SCP-1 encoded HOM-TES-14 as a CTA. HOM-TES-14 differs from the known CTAs by its chromosomal localization and expression pattern. Although nearly all of the hitherto known CTAs have been mapped to the X chromosome, the human SCP-1 gene has been assigned to chromosome 1p12–p13 (23). When compared with the other CTAs, HOM-TES-14 is expressed in only a relatively small proportion of melanomas, but it is detectable in a high proportion of gliomas, breast carcinomas, and also in renal cell carcinomas. No protein was detectable by immunoblot analysis in any of the normal tissues studied. On the other hand, although transcript levels as determined by Northern blot were lower in all neoplasms than in testis, Western blot analysis demonstrated a strong HOM-TES-14 protein expression in the tumors and cell lines, for which transcript expression had been shown by RT-PCR. This suggests a longer half-life of the protein in malignant cells. This assumption is also supported by the immunocytological observation that in contrast to spermatocytes (where SCP-1 is rapidly degraded after the meiotic prophase), the expression of the SCP-1 protein is not limited to a specific phase of the cell cycle.

SCP-1 had been described (24) as a major component of the synaptonemal complex. The synaptonemal complex is a tripartite macromolecular assembly formed between homologous chromosomes during the meiotic prophase (26, 27). It consists of two proteinaceous lateral elements which are formed axially along each of the two homologous chromosomes and a central element. The central and lateral elements are connected by transverse fibers crossing the central element (28). The synaptonemal complex has been conserved almost universally among sexually reproducing eukaryotes (29). The human cDNA encoding one major component of the transverse fibers, SCP-1, has recently been isolated and characterized (22). In the rat SCP-1 expression has been found exclusively in meiotic prophase cells, restricted to zygotene–diplotene spermatocytes (24). SCP-1 disappears when the chromosomes separate during the diplotene stage (28, 30), representing an example of temporary transcriptional regulation operative during meiosis. Meiosis reduces the diploid state to a haploid state that is essential for gametogenesis in mammals. During the prophase of meiosis I, homologous chromosomes condense and pair to segregate and migrate to opposite poles for a balanced division. From cytological and

Table 2. Expression of HOM-TES-14 in malignant tissues

Tumor type	SCP-1 expression, no. positive/no. tested
Melanoma	4 /28
Breast cancer	9 /33
Colorectal carcinoma	0 /32
Prostate cancer	0 /27
Glioma	6 /15
Stomach carcinoma	1 /14
Thyroid cancer	0 /5
Lymphoma or leukemia	0 /14
Lung carcinoma NSCLC	1 /14
Renal cell carcinoma	3 /36
Ovarian carcinoma	0 /3
Seminoma	0 /2
Endometrial carcinoma	0 /7
Sarcoma	0 /4

Expression analysis was performed by RT-PCR with oligonucleotides specific for HOM-TES-14.

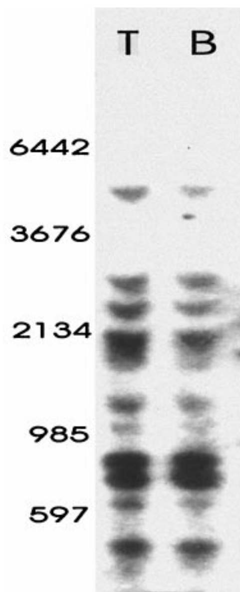

FIG. 3. Southern blot analysis for SCP-1. Hybridization of *Hae*III-digested blotted genomic DNA derived from testis (lane T) and PBMCs (lane B) using radiolabeled SCP-1 cDNA as a probe demonstrated multiple bands suggestive of a gene family rather than a single gene.

genetic data, it has been hypothesized that the function of SCP-1 as a constituent of the transversal element may be the zippering orchestration of the alignment of the two homologous chromosomes during meiosis, promotion of crossing-over events, and ensuring chromosome segregation (31–34).

Thus the observation that HOM-TES-14 is expressed in tumors demonstrates that a meiosis-specific gene can be expressed in nonmeiotic cells. Given the shared characteristics of CTAs, one may hypothesize that other members of the CTA class of antigens may also participate in the fine-tuned sequence of events that make up the process of meiosis. The significance of this aberrant expression in mitotic cancer cells can only be speculated on at this point; a possible hypothesis is that it contributes to numeric chromosomal abnormalities in the malignant cells.

The mechanism of the selective expression of CTAs in cancer and testis is not fully understood. The activation of the human CTA MAGE-1 has been found to be correlated with genome-wide hypomethylation in cancer cells (35) that has also been observed in testicular cells (36). Methylation of CpG

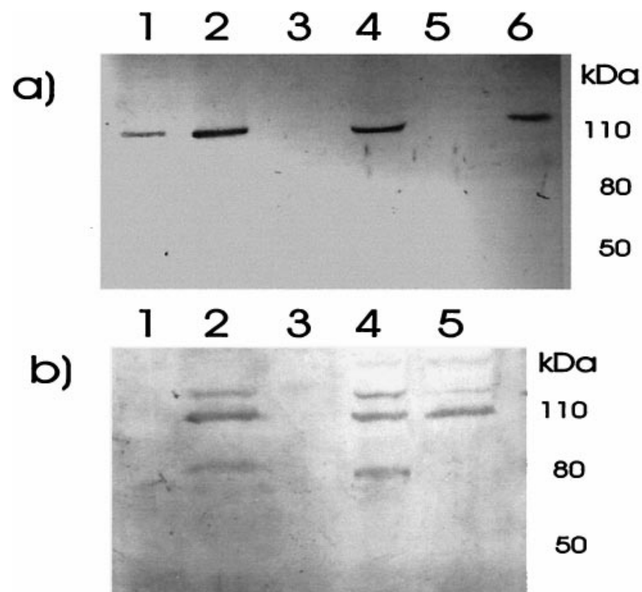

FIG. 4. Western blot analysis of SCP-1 expression. Immunoblot analysis with SCP-1 antiserum was performed to measure protein expression levels in cultured glioma cell lines (*a*) (lanes: 1, T98; 2, Lear; 3, Markus; 4, Cheng; 5, U373; 6, testis) and tissue samples derived from fresh biopsies (*b*) (lanes: 1 and 3, normal breast samples; 2 and 4, breast cancer tissues; 5, testis). A major band migrating at 110 kDa was detected in lysates of testis, in three glioma cell lines, and in the breast tumor samples. Bands were almost equal in intensity, indicating comparable levels of protein expression. Besides this major band, additional bands were detected in some tumor samples migrating at 120 kDa and 80 kDa.

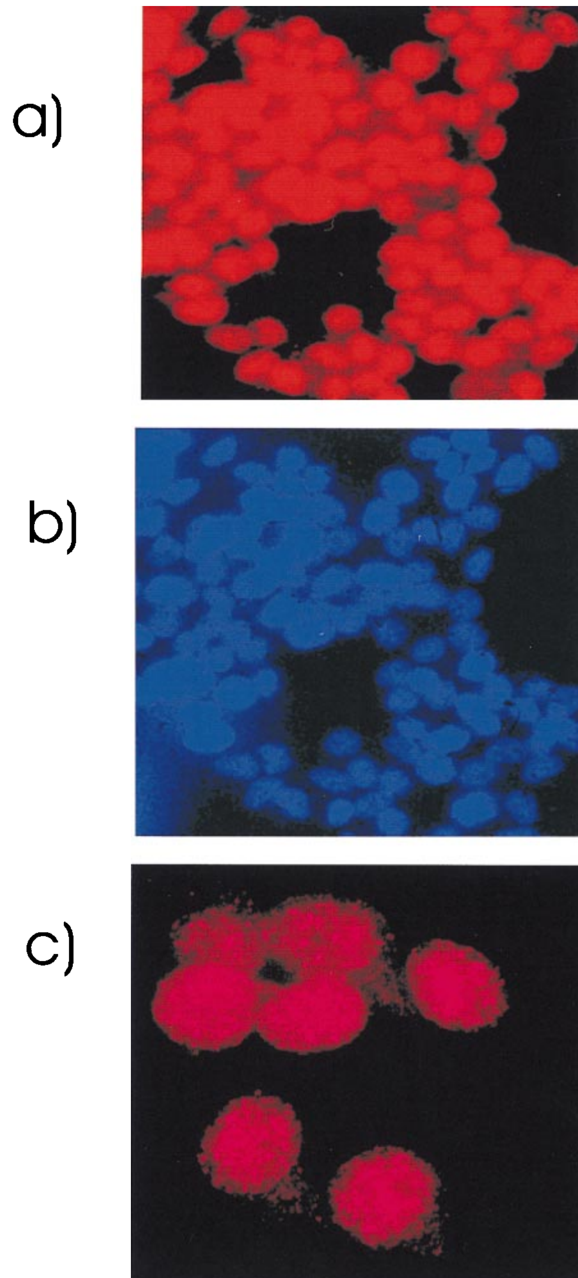

FIG. 5. Immunofluorescence analysis of SCP-1 expression. Indirect immunofluorescence with SCP-1 antiserum (*a*) and counterstaining with 4′,6-diamidino-2-phenylindole (*b*) reveals a nuclear staining with a punctated staining pattern (*c*) in Cheng cells that scored positive for SCP-1 in RT–PCR and in Western blot analysis. This pattern was observed in almost all interphase nuclei, indicating that SCP-1 expression in tumor cells is not restricted to a particular cell cycle phase as observed in sperm cells.

sites within promoters is a mechanism of effective gene silencing. In this regard it is interesting that the 5′ untranslated region of the SCP-1 mRNA contains several CpG sites and demonstrates high homology to a CpG-rich DNA clone identified previously by affinity purification of genomic DNA (gb Z56946) using a methylated DNA binding column (37). Future studies should reveal whether the methylation status of the SCP-1 gene promoter correlates with the observed tissue-specific expression pattern. Two observations indicate that genomic hypomethylation is not the only mechanism responsible for the expression of CTA. (*i*) The frequency of transcript expression varies clearly for individual CTAs in different neoplasms. For certain neoplasms such as colorectal carcinoma for which genomic hypomethylation has been clearly demonstrated, some CTAs (e.g., MAGE-1, BAGE, or HOM-TES-14) are very rarely or even not at all expressed, whereas other members (e.g., MAGE-2 or HOM-MEL-40) are detected more frequently. (*ii*) The fact that SCP-1 is expressed only during the meiotic prophase of spermatocytes indicates that SCP-1 expression is regulated by mechanisms other than or in addition to methylation.

HOM-TES-14 is a human CTA that has been defined screening a cDNA library enriched for testis-specific clones. The high protein expression levels and the high frequency of humoral anti-SCP-1 responses among breast cancer patients suggests that it might be a good candidate for the development of a cancer vaccine particularly for this large patient population. By applying the strategy systematically, it can now be realistically expected that the exploitation of the antigenic repertoire of normal testis will soon provide at least one or even several molecules specifically expressed by a patient's individual tumor and should enable their use as targets for immunotherapeutic interventions not only in malignant melanoma but also in other more frequent human malignancies.

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