Murine Six-Transmembrane Epithelial Antigen of the Prostate, Prostate Stem Cell Antigen, and Prostate-specific Membrane Antigen: Prostate-specific Cell-Surface Antigens Highly Expressed in Prostate Cancer of Transgenic Adenocarcinoma Mouse Prostate Mice

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ABSTRACT

To identify genes that are differentially up-regulated in prostate cancer of transgenic adenocarcinoma mouse prostate (TRAMP) mice, we subtracted cDNA isolated from mouse kidney and spleen from cDNA isolated from TRAMP-C1 cells, a prostate tumor cell line derived from a TRAMP mouse. Using this strategy, cDNA clones that were homologous to human six-transmembrane epithelial antigen of the prostate (STEAP) and prostate stem cell antigen (PSCA) were isolated. Mouse STEAP (mSteap) is 80% homologous to human STEAP at both the nucleotide and amino acid levels and contains six potential membrane-spanning regions similar to human STEAP. Mouse PSCA (mPsca) shares 65% homology with human PSCA at the nucleotide and amino acid levels. mRNA expression of mSteap and mPsca is largely prostate-specific and highly detected in primary prostate tumors and metastases of TRAMP mice. Both mSteap and mPsca map to chromosome 5. Another known gene coding for mouse prostate-specific membrane antigen (mPsma) is also highly expressed in both primary and metastatic lesions of TRAMP mice. These results indicate that the TRAMP mouse model can be used to effectively identify genes homologous to human prostate-specific genes, thereby allowing for the investigation of their functional roles in prostate cancer. mSteap, mPsca, and mPsma constitute new tools for preventative and/or therapeutic vaccine construction and immune monitoring in the TRAMP mouse model that may provide insights into the treatment of human prostate cancer.

INTRODUCTION

Prostate cancer is the most common malignancy and the second leading cause of cancer death in men in North America. The serum PSA test has revolutionized the early detection of prostate cancer and has resulted in enthusiasm to identify additional novel and increasingly specific markers in prostate cancer. Progress in identifying such markers has been markedly accelerated by recent advances in molecular biology technologies, such as differential display analysis, subtraction approaches, and microarray techniques. These techniques and others have led to the identification of more than 20 genes with prostate-specific/abundant expression (1–20), such as PSMA (3), PSCA (9), and STEAP (12). Given their cancer/prostate-specific distributions, these genes theoretically encode targets suitable for implementation in therapies of prostate cancer. However, the development of such therapies has been severely limited by the absence of an animal model that closely mimics human prostate cancer and expresses the prostate-specific genes under study at physiologically relevant levels.

The recent development of a spontaneous transgenic mouse model of prostate cancer, TRAMP (21), and the isolation of syngeneic transplantable prostate cancer cell lines (TRAMP-C1 and -C2) derived from TRAMP prostate tumors (22) has provided much needed model systems to comprehensively test various experimental prostate cancer therapies. Hence, to identify genes specifically or overexpressed in TRAMP prostate tumors, we used a SSH3 to subtract cDNA derived from mouse kidney and spleen from cDNA isolated from TRAMP-C1 cells. Using this strategy, cDNA clones homologous to human STEAP (12) and PSCA (9) were obtained. mSteap and mPsca have similar tissue distributions as human STEAP and PSCA and are highly expressed in primary and metastatic lesions in TRAMP mice. Another known gene, mPsma (23), was also highly detected in this study in both primary and metastatic cancers of TRAMP mice. These murine prostate-specific genes combined with the TRAMP model may provide a useful animal model system to investigate therapeutic strategies against prostate cancer.

MATERIALS AND METHODS

Cell Lines and Mouse Tissues. The TRAMP-C1 and -C2 cell lines derived from TRAMP mice were maintained in DMEM supplemented with 5% FCS, 5% NuSerum (Collaborative Biomedical Products, Bedford, MA), 5 μg/ml insulin (Sigma Chemical Co.), and 0.01 nM dihydrotestosterone (Sigma Chemical Co.). All of the mouse tissues for RNA analysis were isolated from male C57BL/6 mice.

SSH. TRAMP-C1 cells and mouse tissues were homogenized in Trizol reagent (Life Technologies, Inc.) to isolate total RNA. Poly(A) -RNA was purified from total RNA by using the Oligotex mRNA Mini and Midi kits of Quagen (Chatsworth, CA). SSH was performed with the PCR-Select cDNA Subtraction Kit (Clontech) according to the manufacturer’s protocol. SSH-derived gene fragments were inserted into pCR2.1-TOPO by using a TOPO TA cloning kit (Invitrogen). Transformed Escherichia coli were subjected to blue/white and ampicillin screening. White colonies were picked and stored in 25% glycerol in 96-well plates. Plasmid DNA was prepared, sequenced, and searched for homology against public databases. Secondary protein structure prediction was performed by using the web tool PSORT II.3

Northern Analysis. Nylon membranes with UV-fixed total RNA (5 or 20 μg/lane) from normal mouse tissues and TRAMP-C cells and mouse poly(A)+ Northern blot containing mRNA samples from 12 different tissues (OriGene) were hybridized with the [α-32P]dCTP-labeled cDNA fragments. The cDNA probes used were 260-bp (nucleotide 34-293) mSteap and 424-bp (nucleotide 25-448) mPsca cDNA fragments. As an internal control, β-actin was used to hybridize all of blots.

RT-PCR. All of the RNA used for RT-PCR was treated with DNase. cDNA synthesis was performed by oligodeoxythymidylicate priming. For RT-PCR amplification, the following primer pairs were used: 5′-GGTTGGCG-GAACCGCTACTAT and 5′-GGTAGTATGATGGCAGGCACG for mSteap, 5′-TTCTCCGTGCGCCACCTAC and 5′-GCAGCTCATCCTCCCTTACAT

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2 The abbreviations used are: PSA, prostate-specific antigen; STEAP, six-transmembrane epithelial antigen of the prostate; PSCA, prostate stem cell antigen; PSMA, prostate-specific membrane antigen; TRAMP, transgenic adenocarcinoma mouse prostate; SSH, suppression subtractive hybridization; mPsca, mouse PSCA; mSteap, mouse STEAP; mPsma, mouse PSMA; poly(A), polyadenylate; RT-PCR, reverse transcription-PCR; RACE, rapid amplification of cDNA ends.

http://psort.imsc.u-tokyo.ac.jp/form2.html.
for mPsca, and 5’-GGGAAGATTGTTGATTGGCAAGAT and 5’-GGCTCTCGTCCCTTCCTTCCCTCA for mPsma. Thermal cycling was performed for 35 cycles at 94°C for 30 s, 58°C for 1 min, and 72°C for 1 min.

RACE. RACE reactions were performed to identify full-length mSteap and mPsca following the manufacturer’s instructions by 5’- and 3’- RACE systems (Life Technologies, Inc.). Templates for RACE reactions were RNA isolated from TRAMP-C1 cells and mouse prostate tissues. 5’-RACE were performed with the gene-specific primers 5’-GGATGATATGATGGACGAC-GAC and 5’-GCTGGGTGTGCACTGAACTCG for mSteap and 5’-GTTGGATG-GCACGCTCTATCTCTCA and 5’-GCCGGATGGCGCATGTAA for mPsca. The 3’-RACE reactions were conducted with the gene-specific primers 5’-CTCTGAGACTTGAGACCCGC for mSteap and 5’-GGGCCATTGGACTC-GTGACAGT for mPsca.

Chromosomal Mapping. Chromosomal localization of mSteap and mPsca was determined by using the Mouse/Hamster Radiation Hybrid Panel (Research Genetics, Inc.). mSteap gene products were amplified by PCR with the following primers: 5’-CTCTGAGACTTGAGACCCGC and 3’-CTCTGAGCAGAGCAGG. The primers used for mapping mPsca were 5’-GCACGACGACAGAAACACAG and 5’-GCACGAGTACAGAGTAG-CAGAC. The resulting mapping vectors were processed through a mapping program.

RESULTS

SSHs Identify mSteap and mPsca. In pursuit of genes that are up-regulated in prostate tumors in the TRAMP model, cDNA derived from mouse kidney and spleen was subtracted from cDNA isolated from TRAMP-C1 cells. Extensive screening was performed on a selected set of SSH-derived clones to look for genes that were differentially regulated between tester and driver cDNA. Among the differentially expressed clones, two gene fragments were highly homologous to human STEAP and PSCA, respectively. The full-length cDNA sequences of these two genes were amplified through 5’ and 3’ RACE reactions using RNA from TRAMP-C1 cells and normal prostate tissues as a template. Sequence analysis revealed that a novel 1175-bp cDNA, which did not show significant identity with any known mouse genes, shared 80% homology with human STEAP at both the nucleotide and amino acid levels and, therefore, represented the mSteap (accession no. AF 297098). Similar to human STEAP, mSteap also encodes a predicted 339 amino acid protein with six transmembrane domains without a signal peptide (Ref. 12; Fig. 1).

Fig. 1. Amino acid sequences of mSteap and human STEAP. Shaded regions highlight conserved amino acids. The underlined regions correspond to the putative membrane-spanning domains. No NH2-terminal signal peptide is found, and NH2- and COOH termini are both predicted to be intracellular by use of web tool PSORT II.

Another novel 864-bp cDNA, which shares a 65% identity with human PSCA at both nucleotide and amino acid levels, is mPsca (accession no. AF 319173). Although the protein sequence of mPsca had been reported by Reiter et al. (9), the nucleotide sequence of mPsca could not be found in the public databases. mPsca additionally shares 52% nucleotide and 33% amino acid homology with mouse stem cell antigen 2.

Expression of mSteap and mPsca Is Prostate-predominant. The distribution of mPsca and mSteap in normal mouse tissues was examined by Northern blot and RT-PCR analysis. Northern blotting of normal mouse prostate tissue RNA has shown that mSteap is ~1.2 kb (Fig. 2). Small amounts of mSteap mRNA could be detected in kidney and testis after prolonged exposure (3 days versus 1 day for prostate) in mouse poly(A). Northern blot containing mRNA samples from brain, heart, kidney, liver, lung, muscle, skin, small intestine, spleen, stomach, testis, and thymus (data not shown). The mPsca transcript in normal mouse prostate is ~0.9 kb (Fig. 2). mPsca is expressed predominantly in prostate, with a lower level of expression present in kidney, colon, and testis (data not shown). This distribution of mPsca is consistent with that reported by Reiter et al. (9). RT-PCR also confirmed the distribution pattern of mSteap and mPsca analyzed by the Northern blot analysis (Fig. 3). Together, these data indicate that mSteap and mPsca expression is largely prostate-specific.

mSteap and mPsca Are Highly Expressed in Primary and Metastatic Tumors of TRAMP Mice. Human STEAP has been shown to be expressed in human primary tumors and cell lines derived from metastatic lesions of prostate cancers (12), and human PSCA is detected in both primary and metastatic lesions of human prostate cancer (9, 24). Here, we examined the expression of their mouse homologues in TRAMP-C cells and cancer tissues from TRAMP mice. Northern blot and RT-PCR analysis demonstrated that both mSteap and mPsca were expressed in TRAMP-C cells and prostate tumor tissues from TRAMP mice (Fig. 2 and Fig. 4). Furthermore, they were highly detected by RT-PCR in metastatic samples from lymph node, liver, and viscosa of TRAMP mice (Fig. 4). Comparison of mSteap and mPsca expression in normal prostate, prostate tumors, and TRAMP-C cells suggested that mSteap and mPsca were up-regulated in TRAMP prostate tumors and TRAMP-C cells (Fig. 2). However, because the RNA samples derived from normal prostate tissues contain small amounts of message from other tissues nearby the prostate, the expression level of mSteap and mPsca in normal prostate might be underestimated.

mPsma Is Also Detected in Primary and Metastatic Tumors of TRAMP Mice. On the basis of the expression of mPsca and mSteap in TRAMP prostate tumors, we inferred that some other human prostate-specific gene homologues might also be expressed in the TRAMP model. We selected mPsma, which was the only mouse prostate-specific gene we were able to find in GenBank databases by Northern blot and RT-PCR analysis. Total RNA from normal prostate, TRAMP prostate tumor tissues, TRAMP-C1 cells, and TRAMP-C2 cells were analyzed by using mSteap- or mPsca-specific probes. The normal prostate lane contained 20 μg of total RNA, and the other lanes were prepared with 5 μg of total RNA. All of the RNA samples were normalized by ethidium bromide staining and subsequent analysis with an β-actin probe.
presently (accession no. AF026380), mPsma shares 76% nucleotide and 86% amino acid homology with human PSMA (23). RT-PCR using the mPsma specific primers showed that mPsma was expressed in mouse brain, kidney, testis, and prostate tissues (Fig. 3). Similar to mSteap and mPsca, mPsma is also highly detected in TRAMP-C cells, primary cancer tissues, and metastatic samples from lymph node, liver, and viscera of the TRAMP model (Fig. 4).

**mSteap and mPsca Genes Localize to Chromosome 5.** Chromosomal mapping was performed by using radiation hybrid analysis. mSteap localized to Chr05, 20.33 cR from D5Mit331, a region corresponding to the location of human STEAP (7p22.3; Ref. 12) according to the human-mouse map at public databases. mPsca localized to Chr05, 24.08 cR from D5Mit89. This region did not exhibit a clear correlation with the chromosomal location of human PCA (8q24.2; Ref. 9) in the human-mouse map. Because this human-mouse map is not presently completed, further development of this database may provide more information about this issue.

**DISCUSSION**

In search of novel genes preferentially expressed in the prostate tumors of TRAMP mice, we have isolated and characterized mSteap and mPsca using a subtraction approach. Of interest, human STEAP and PSCA were also identified by use of subtractive approaches in the LAPC-4 xenograft model of human prostate cancer (12, 9). mSteap and human STEAP share high homology at both the nucleotide and amino acid levels. The putative six-transmembrane domains are conserved in mSteap and human STEAP. This structure suggests a potential function as a channel, receptor, or transporter protein. Similarly, mPsca is highly homologous to human PSCA at both nucleotide and amino acid levels. Because of this homology, Reiter et al. (9) were able to identify the mPsca cDNA through searching the murine expressed sequence tag (EST) databases with the sequence of human PSCA. At present, the biological function of mouse and human PSCA is not clear. According to its homology to Ly-6 families, PSCA is considered a member of Ly-6 family, which may be involved in signal transduction and cell-cell adhesion (9).

The expression profiles of mSteap and mPsca are similar to the largely prostate-specific expression of their human homologues, STEAP and PSCA belong to a small group of cell-surface antigens that are expressed in prostate cancer and represent potential targets for both antibody-based and T-cell-mediated therapies. This family includes PSA (1), PAP (2), PTCA-1 (5), PSGR (20), PSMA (3), STEAP (12), and PSCA (9). Of this family, PSMA, PTCA-1, PSA, and PAP are secretory proteins that have been found in considerable concentrations in peripheral blood and, thus, represent somewhat less desirable targets for immunotherapy. In contrast, STEAP and PSCA are not secreted. Human STEAP and PSCA are further found to be stably expressed in advanced prostate cancer tissues, and PSCA expression is retained by metastatic tissues of prostate cancer (9, 12, 24). These proteins are potentially good targets for immunotherapy of prostate cancer. Similar to their human homologues, mSteap and mPsca are expressed in primary TRAMP prostate cancer tissues, TRAMP-C cell lines, and metastatic samples from lymph node, liver, and viscera of TRAMP mice.

Human PSMA is one of the best-characterized prostate-specific genes and the first prostate-specific gene used in clinical trials for the immunotherapy of prostate cancer (25, 26). Its mouse homologous gene, mPsma, has been released recently from the GenBank database. mPsma is highly homologous to human PSMA and maps to chromosome 7 (7D1-D2; Ref. 23). We have shown here that mPsma is also expressed in primary prostate cancer and metastatic lesions of TRAMP mice. These results imply that other human prostate-specific and cancer-associated antigens may have their homologues in the TRAMP model, and hence this mouse model may prove particularly useful in evaluating the function of these gene products and therapeutic efficacy of treatments targeting these molecules.

Ideal targets for therapy of prostate cancer should be antigens that are exclusively expressed in nonvital tissues like prostrate, highly expressed in metastatic disease, and accessible to therapeutic modalities. Unfortunately, the majority of defined prostate-specific gene products do not fulfill all of these conditions. Like human STEAP, PSCA, and PSMA we have discussed here, they are not exclusively

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![Fig. 3. RT-PCR analysis of mRNA expression of mSteap, mPsca, and mPsma in normal mouse tissues. DNase-treated total RNA from the indicated tissues was submitted to 35 cycles of RT-PCR amplification with mSteap-, mPsca-, and mPsma-specific primers, respectively. All of the PCR products were visualized on 2% agarose gel stained with ethidium bromide.](image)

![Fig. 4. RT-PCR analysis of mRNA expression of mSteap, mPsca, and mPsma in TRAMP-C cells, primary tumors, and metastatic tumors of TRAMP mice. DNase-treated total RNA was submitted to 35 cycles of RT-PCR amplification with mSteap-, mPsca-, and mPsma-specific primers, respectively. Lanes are: 1, TRAMP-C1; 2, TRAMP-C2; 3 and 4, primary prostate tumors; 5, 6, and 7, liver metastases; 8 and 9, lymph node metastases; and 10, visceral metastases. All of the PCR products were visualized on 2% agarose gel stained with ethidium bromide.](image)
prostate-specific. Low-level human STEAP mRNA is detected in the colon and liver, and its protein is found in the bladder at moderate level. Small amounts of human PSMA mRNA are detected in kidney and small intestine, and human PSMA is expressed in various human tissues (27). Effective therapies against these targets may bear the risk of inducing damage to the normal organs expressing these antigens. Although no autoimmune disease has been observed in PSMA-based immunotherapies in clinical trials, these studies (25, 26) have not reported any consistent and effective immune responses generated against PSMA. Because mSteap, mPsca, and mPsma are expressed in some normal tissues other than prostate, these murine antigens can be used to evaluate such therapeutic side effects. In the case of immunological approaches, the immune system may have ignored or de-velopment to the prostate/cancer-specific genes because they are also expressed in normal prostate. Therefore, these murine prostate-specific antigens combined with the TRAMP model will provide a good model system to investigate various immunotherapeutic strate-gies to treat prostate cancer. In addition, these antigens are murine by nature. Studies with these antigens will be more representative in vivo than approaches using transfected xenogeneic antigens. In summary, mSteap, mPsca, and mPsma are homologous to their human counterparts, largely prostate-specific, and highly expressed in primary and metastatic cancers of TRAMP mice. These murine genes in combination with the TRAMP model will provide an animal system for the evaluation of prostate antigen-directed therapeutic strategies against prostate cancer.

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