**PSGR, a Novel Prostate-specific Gene with Homology to a G Protein-coupled Receptor, Is Overexpressed in Prostate Cancer**

Linda L. Xu, Bennett G. Stackhouse, Kim Florence, Wei Zhang, Naga Shanmugam, Isabell A. Sesterhenn, Zhiqiang Zou, Vasantha Srikanth, Meena Augustus, Viktor Roschke, Kenneth Carter, David G. McLeod, Judw Moul, Dan Soppett, and Shiv Srivastava


Abstract

**PSGR**, a novel prostate tissue-specific gene with homology to the G protein-coupled odorant receptor gene family, has been identified. Here we report the characteristics of the predicted protein sequence of **PSGR** and its prostate tissue specificity and expression profile in human prostate cancers and matched normal tissues. Using multiple tissue Northern blots from over 50 different tissues, **PSGR** expression was restricted to human prostate tissues. Paired normal and tumor specimens from 52 primary prostate cancers, obtained by laser capture microdissection or manual microdissection, were analyzed for **PSGR** expression by semiquantitative and real-time PCR assays. The differential expression of **PSGR** between normal and tumor tissues was highly significant (P < 0.001), and 32 of 52 (62%) matched prostate specimens exhibited tumor-associated overexpression of **PSGR**. Of note, there was very little or no expression of **PSGR** in many normal specimens in comparison with the generally high expression of **PSGR** seen in matched tumor specimens. In **situ** hybridization assays showed restricted **PSGR** expression in the epithelial cells of the normal and tumor tissue sections. Restricted expression of **PSGR** in prostate epithelial cells, overexpression of the **PSGR** in a significant percentage of prostate cancers, and the predicted protein sequence of **PSGR** with seven transmembrane domains provide a foundation for future studies evaluating the potential of **PSGR** as a prostate cancer gene expression marker and the utility of **PSGR** protein as a novel target for developing immunotherapeutic strategies for prostate cancer.

Introduction

CaP is the most common malignancy in men in the United States and the second leading cause of cancer mortality (1). The serum PSA or HK3 test has revolutionized the early detection of CaP (2). Due to the prostatic epithelial cell-specific expression of PSA, it is also of value as a biomarker for disease remission/progression after treatment (2). Although PSA is effective at identifying men who may have prostate cancer, it is often elevated in men with benign prostatic hyperplasia, prostatitis, and other nonmalignant disorders (2). Therefore, identification of additional prostate cancer-specific molecular markers, especially those exhibiting tumor-associated overexpression, is needed to refine the diagnosis as well as prognosis for CaP. It is also recognized that early detection of CaP presents new challenges with respect to predicting the clinical course of the disease for the individual patient (2). The wide spectrum of biological behavior exhibited by prostatic neoplasms calls for the identification of novel biomarkers that may be able to distinguish a slow-growing cancer from a more aggressive cancer with a potential to metastasize (2). Prostate cancer-associated molecular genetic alterations are being unraveled by using various strategies including: (a) analyses of genes commonly involved in human cancer; (b) positional cloning of putative genes on frequently affected chromosome loci in CaP; and (c) gene expression profiling of normal and tumor tissues from individuals with prostate cancer (3–5).

The discovery of additional prostate-specific genes has also resulted in enthusiasm for evaluating their potential utility in the diagnosis and prediction of disease progression of CaP. HK2, another member of the kallikrein gene family, is currently being evaluated for this role in CaP (6). Increased expression of PSMA has been correlated with more aggressive disease (7). CaP-associated expression of PSMA is also being evaluated for imaging of prostate cancer by radiolabeled anti-PSMA monoclonal antibodies (7). Furthermore, promising immunotherapy approaches are being pursued using PSMA peptide (8). Recent gene discovery approaches have led to the identification of several new prostate-specific/abundant genes, such as **NKK3.1** (9), prostate (10), prostate stem cell antigen (11), **TMPRSS2** (12), **STEAP** (13), **PDEF** (14), **PART-1** (15), **HOXB13** (16), **DD3** (17), **PGGEMI** (18), and **PMEPA1** (19), that exhibit diverse characteristics.

In this report, we describe the discovery of a new prostate-specific gene, **PSGR**, a member of the G-protein coupled OR family that maps to chromosome 11p15. The most striking aspects of **PSGR** characterization are its highly prostate tissue-specific expression and its tumor-associated overexpression. The predicted protein sequence of the **PSGR** revealed seven transmembrane-spanning domains with homology to G protein-coupled receptor odorant receptors. Tumor-associated overexpression and the prostate tissue-specific nature of **PSGR**, a G protein-coupled **trans**-membrane receptor, suggest its potential as a novel target for immunotherapeutic strategies of prostate cancer.

Materials and Methods

**Identification of **PSGR. **PSGR** was identified as a prostate tissue-specific cDNA, HPRAI during a search of the expressed sequence tag database at Human Genome Sciences using approaches that previously led to the identification of **NKK3.1** (9).
Analysis of Tissue-specific Expression of PSGR Using MTN Blots. Multiple tissue Northern blots containing mRNA samples from 23 human tissues and Master dot blots containing mRNA samples from 50 different human tissues (Clontech) were hybridized with the [α-32P]dCTP-labeled 513-bp PSGR cDNA fragment, which was amplified from a PSGR cDNA clone using primers 5'-GCCACCTGGTGTAGTTGATCCC-3' (sense) and 5'-GACACGATGGATGCACAGACATTG-3' (antisense). As an internal control, GAPDH probe was used to hybridize all of the blots.

Prostate Tissue Specimens, Pathological Evaluation, and Microdissection. Matched prostate cancer and normal tissues were derived from radical prostatectomy specimens from 52 prostate cancer patients treated at Walter Reed Army Medical Center (under an institutional review board-approved protocol). All radical prostatectomy patients in this study are enrolled in the Department of Defense Center for Prostate Disease Research Multicenter Longitudinal Prostate Cancer Database. The genitourinary pathologist (I. A. S.) was present in the operating room to immediately accept the prostate gland from the urologists. If a palpable tumor was present, the surface overlying it was painted with black ink, and a wedge from the center was immediately embedded in Tissue-tek OCT (Miles Inc. Diagnostics Division, Elkhart, IN) and frozen at −70°C. Sextant 14-gauge true cut biopsies were also obtained on each case and processed similarly. Sextant biopsies include apex, mid, and base of the right and left lobes of the prostate performed ex vivo. The volume of biopsy specimens was about 1 × 0.5 × 0.5 cm3. Ten-μm frozen sections were prepared and archived at −70°C. One set of slides was stained with H&E and read by the pathologist (I. A. S.) to define tumor cells. The frozen sections on slides were dissected using laser captured microdissection (35 pairs) according to the protocol provided by the manufacturer (Arcturus Engineering, Mountain View, CA) or manual microdissection (17 pairs). Total RNA was prepared from harvested normal and tumor prostate epithelial cells as described previously (20). The expression of PSGR was correlated with clinicopathological features of the patients.

RT-PCR Assay. Total RNA (100 ng) was reverse transcribed into cDNA with a RNA PCR kit (Perkin-Elmer, Foster, CA), and one-tenth of the reverse-transcribed product from each sample was used for PCR to amplify PSGR and a housekeeping gene, GAPDH. The primer sequences for amplification of PSGR were the same as those described above. The conditions of PCR for each gene were optimized to analyze the amplified product in the linear range of amplification by adjusting amplification cycles for each set of primers. The optimized PCR condition for amplifying PSGR was 36–42 cycles of 94°C for 30 s, 68°C for 30 s, 72°C for 30 s. The expression of GAPDH was used as an internal control for RNA input (20). Expression of GAPDH and PSGR was analyzed simultaneously with the same batch of cDNA. Other controls for the RT-PCR assays included PCR amplification of the RNA samples without reverse transcription with NIH image processing software. The results of the quantitation of bands and expressed as fold change in expression.

Results

PSGR Is a New Member of G Protein-coupled Olfactory Receptors. Analysis of the 1474-bp PSGR cDNA sequence (Fig. 1A), previously named as HPRAJ, revealed an ORF of 963 bp nucleotides encoding a 320-amino acid protein with a predicted molecular mass of 35.4 kDa. The PSGR ORF revealed intriguing homology (~50% identity and ~70% similarity) to the G protein-coupled odorant receptor family. Specifically, the analysis of PSGR with DnaStar software showed the closest similarity of PSGR to a human OR gene, HPF110R, which was mapped to the β-globin gene cluster on chromosome 11p15.5 (Fig. 1B; Ref. 21). Furthermore, PSGR ORF exhibited almost complete identity to a rat cDNA sequence (accession number AF079864) in the NCBI GenBank.5 A protein motif search using ProfileScan6 indicated the existence of seven trans-membrane domains between amino acid residues 24–293 that are characteristic of GPCRs. Chromosomal mapping assigned PSGR to 11p15 (data not shown), one of the regions of GPCR clusters.

Prostate Tissue-specific and Epithelial Cell-restricted Expression of PSGR. The distribution of PSGR mRNA in normal human tissues was examined by Northern blot analysis. Of the 23 different human tissue mRNAs analyzed, a ~2.7-kb mRNA transcript specifically hybridizing to the PSGR cDNA was detected only in prostate tissue (Fig. 2A). Two independent experiments revealed identical results. Further analysis of RNA Master blot containing RNA from 50 different human tissues confirmed the prostate tissue specificity of the PSGR gene (data not shown). Among the prostate cancer cell lines analyzed, weak expression of PSGR was detected in LNCaP cells by RT-PCR and Northern hybridization (Fig. 2B). In situ RNA hybridization analysis of PSGR expression in prostate tissue revealed that expression of PSGR was predominantly localized to epithelial cells of the prostate gland (Fig. 2C). Therefore, a comprehensive expression analysis of PSGR in human prostate tissues revealed prostatic epithelial cell-specific expression of the PSGR.

PSGR Expression in Prostate Cancer. Fig. 3 shows a representative semiquantitative RT-PCR analysis of PSGR and GAPDH. The overall expression pattern of the PSGR gene is shown in Table 1. Comparison of PSGR expression between normal and tumor tissues revealed overexpression in 62% of tumor specimens (32 of 52 specimens), decreased expression in 11.5% of specimens (6 of 52 specimens), and no change in 27% of specimens (14 of 52 specimens). The cumulative PSGR expression level in tumor tissues (0.4350 ± 0.4987) are significantly higher (P = 0.001) than that in normal tissues (0.1636 ± 0.2843). Quantitative RT-PCR of eight pairs of tumor and


6 Internet address: http://www.ncbi.nlm.nih.gov/cgi-bin/EMBL/MAST.

PROSTATE-SPECIFIC GPCR
normal tissue analyzed by Taqman procedure revealed tumor-associ-
ated overexpression in 7 tumors consistent with the semiquantitative
RT-PCR (data not shown). As a complement to RT-PCR,
PSGR expression was also analyzed by
in situ
RNA hybridization in selected
specimens. Of the 10 cases analyzed, 7 showed significantly elevated
expression of PSGR in malignant prostate epithelial cells as compared
with adjacent benign cells (Fig. 2
C
). Thus, using three different
methods, we have confirmed overexpression of PSGR in prostate
cancer. Preliminary analysis of PSGR expression did not show a
significant correlation with specific clinicopathological features (such
as pathologic and clinical stage) (data not shown). A larger study of
whole mount prostate specimens is in progress for further investiga-
tion of this issue.

Discussion
Recent gene discovery efforts in prostate cancer have been instru-
mental in the identification numerous new prostate-specific genes.
The biological and biochemical characterization of these genes has a
significant impact in understanding the role of these genes in prostate
biology. Furthermore, potential utilities of prostate-specific molecules

Fig. 1. Characterization of PSGR cDNA sequence. A, nucleotide and predicted amino acid sequence of PSGR. The potential initiation methionine codon and the translation stop
codon are indicated in bold. The transmembrane domains are underlined. B, multiple alignments of the predicted protein sequence of PSGR and other human GPCRs shown as a
phylogenetic tree. The right part shows the GenBank accession numbers and the name of those genes (the accession number of PSGR is AF311306).
as biomarkers of prostate cancer onset/progression and as a cancer vaccine target are obvious in a clinical setting. PSA (or HK3) as a biomarker for prostate cancer is a success story in human cancer, despite certain limitations. The identification of additional biomarkers that are not only prostate specific but are also overexpressed in cancer will be of special interest. Genes such as TMPRSS2, STEAP, PDEF, DD3, and PCGEM1 (12–14, 17–18) represent efforts in this direction.

Here we report novel observations on the identification of a putative GPCR with a remarkable prostate tissue specificity showing tumor-specific overexpression in a large percentage of tumors. The homology of PSGR to the G protein-coupled odorant receptor genes is particularly intriguing because odorant receptors are almost exclusively localized in nasal epithelium. It is important to note that GPCR genes belong to the largest “gene family” in the human genome, and it has been argued that odorant receptors have emerged directly for GPCRs and that any GPCR has the potential to become an OR at a given point in evolution (22). In addition to nasal epithelium, odorant receptor genes have been detected in testis, and their functional

![Fig. 2. PSGR expression by Northern hybridization and in situ hybridization analysis. A, multiple tissue Northern blots were hybridized with an [α-32P]dCTP-labeled PSGR cDNA fragment. A 2.7-kb mRNA transcript specifically hybridizing to PSGR cDNA was detected only in prostate tissue. B, PSGR expression in prostate cancer cell lines. Eight μg of polyadenylated RNA were used to make the Northern blot. The blot was hybridized with [α-32P]dCTP-labeled PSGR probe. The samples are RNA from PC3 cells (Lane 1), LNCaP cells cultured in androgen-free medium for 5 days and then stimulated with R1881 at 10^-8 M for 24 h (Lane 2), and LNCaP cells processed as described for lane 2 but without R1881 stimulation (Lane 3). C, in situ hybridization analysis. A and B show the hybridization signal with PSGR antisense riboprobe in benign and malignant prostate tissues from the same patient. C shows a similarly processed hybridization with sense PSGR riboprobe. The arrow shows the restricted expression of PSGR in glandular epithelial cells.]

![Fig. 3. PSGR expression in primary prostate cancer specimens by RT-PCR. Representative RT-PCR products of tumor and adjacent normal tissues were analyzed on agarose gel. Lanes 1–9 represent PCR results of paired normal and tumor tissues from the same patient. NC, no reverse transcription (control); N, normal prostate tissue; P, prostatic intraepithelial neoplasia; T, prostate tumor tissue.](image)

![Table 1. PSGR expression status in prostate cancer](image)

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of patients/group (%)</th>
<th>Expression score (no. of specimens)</th>
</tr>
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<tbody>
<tr>
<td>T &gt; N</td>
<td>32 (61.5)</td>
<td>1+ (13)</td>
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<tr>
<td></td>
<td></td>
<td>2+ (3)</td>
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<tr>
<td></td>
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<td>3+ (5)</td>
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<tr>
<td></td>
<td></td>
<td>4+ (11)</td>
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<tr>
<td>T &lt; N</td>
<td>6 (11.5)</td>
<td>1– (2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4– (4)</td>
</tr>
<tr>
<td>T = N</td>
<td>14 (26.9)</td>
<td>0 (14)</td>
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future studies will address these issues.

expression also appears to be tissue context specific because we were unable to detect \( \text{PSGR} \) expression in a number of cultured primary prostate cancers or immortalized cell lines and established metastatic prostate cancer cell lines. However, weak expression of \( \text{PSGR} \) was detected only in LNCaP prostate cancer cells. In this regard, it is important to mention that expression of several prostate-specific genes including PSA is often lost during cell culture condition. Another important finding relates to a highly significant overexpression of \( \text{PSGR} \) in prostate cancer cells. Of note also is the restricted expression of \( \text{PSGR} \) in epithelial cells of the prostate gland. In summary, we have demonstrated prostatic epithelial cell-restricted expression of \( \text{PSGR} \), and tumor cells exhibit significantly increased expression of this putative seven-transmembrane GPCR. However, it is not yet clear how \( \text{PSGR} \) overexpression may play a role in the process of tumorigenesis. GPCR may play important roles in cell signaling and cell proliferation (24). Therefore, future experiments will focus on the biological function of \( \text{PSGR} \) in tumorigenesis of CaP. Membrane localization of the \( \text{PSGR} \) protein makes \( \text{PSGR} \) an attractive target for immunotherapy approaches and antibody-based imaging of metastatic prostate cancer. It also will be very important to determine the signals transduced by \( \text{PSGR} \) in the prostate gland, and future studies will address these issues.

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References

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