Autocrine Regulation of Human Prostate Carcinoma Cell Proliferation by Somatostatin through the Modulation of the SH2 Domain Containing Protein Tyrosine Phosphatase (SHP)-1

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The present study was intended to gain additional information on the growth regulation of prostate by somatostatin (SRIF) and the intracellular events involved. The human prostate adenocarcinoma cell lines PC-3 and LNCaP produce SRIF and express subtypes 2 and 5 of SRIF receptors. The secretion of SRIF is related to the proliferative status of these cells; an inverse relationship exists between cell proliferation and the amount of secreted SRIF. Moreover, the growth of PC-3 cells is inhibited by SRIF overexpression and increased by blockage of endogenous SRIF. Coincident with the increase in SRIF secretion, the activity and levels of the SH2 domain containing protein tyrosine phosphatase (SHP)-1, present in PC-3 cells are augmented, but the effect can be partially prevented by neutralization of secreted endogenously SRIF. The activity of SHP-1 is also stimulated by the SRIF analog RC160. Overexpression of SHP-1 induces inhibition of PC-3 cell growth. SHP-1 is also present in normal prostate, benign prostatic hyperplasia, prostatic intraepithelial neoplasia, and well differentiated adenocarcinoma. In contrast, no signal is detected in poorly differentiated prostate cancer. These findings demonstrate that SRIF inhibits PC-3 and LNCaP cell proliferation through an autocrine/paracrine SRIF loop. This effect could be mediated by activation of the tyrosine phosphatase SHP-1 detected in these cells as well as in human prostate and prostate cancer. (J Clin Endocrinol Metab 87: 915–926, 2002)

PROSTATE CANCER IS one of the most common malignancies among men in the Western world and is a major health problem in many industrialized countries. As the tumor is initially androgen dependent in the majority of cases, endocrine manipulation is a first-line therapy for metastatic and locally advanced cancer that often achieves remission or stabilization of the disease (1). However, this period of remission is invariably followed by tumor relapse, and the treatment options available, based on cytotoxic chemotherapy, antiparasitic agents, or aromatase inhibitors, are only palliative. Patients with metastatic prostate cancer develop an androgen refractory phenotype that will lead to disease progression and eventual death (2). Bearing in mind the need to develop new therapies, it has been demonstrated that the prostate is not exclusively dependent on androgens, but also on additional factors of paramount importance that maintain normal prostate function and play a role in the development of pathological conditions. In this sense, the importance of peptide hormones, growth factors, autocrine-paracrine regulatory loops and stromal-epithelial interactions is now widely recognized (3).

Increasing interest has developed in recent years in the role of somatostatin (SRIF) in prostate cancer. SRIF analogs have successfully been used to inhibit tumor growth in experimental prostatic tumors in animals (4, 5); however, the results obtained in clinical trials are a subject of controversy. Although Figg et al. (6) did not find a response in patients with metastatic hormone refractory prostate cancer treated with the SRIF analog somatuline, Maulard et al. (7) demonstrated therapeutic benefit in patients with hormone refractory prostate cancer using the same analog. Conversely, Logothetis et al. (8) observed that SMS 201–995, other SRIF analog, stimulated prostatic tumor growth in refractory prostatic carcinoma.

What is undeniable is that the peptide SRIF is a powerful inhibitor of a wide range of biological activities, including hormone secretion and cell proliferation (9). In this sense, SRIF and analogs affect the growth of various normal and tumor cells (10, 11). This effect may involve indirect mechanisms through the inhibition of the synthesis and secretion of growth factors and hormones. It has been reported that SRIF and analogs inhibit the release of pituitary GH and PRL, and these hormones facilitate prostatic cancer growth (12). On the other hand, both in vivo and in vitro studies provide solid evidence for the existence of a direct antiproliferative effect of SRIF and analogs, which is exerted through specific SRIF receptors on normal and neoplastic cells (10). Five sub-
types of SRIF receptors (sst1–sst5) have been cloned; all structurally are typical G protein-coupled receptors, and are linked to different signal transduction pathways, including adenylate cyclase, ion conduction channels, and tyrosine phosphatases (9, 13, 14). There is also emerging evidence for the hypothesis that SRIF may act as an autocrine/paracrine regulatory factor. In fact, a variety of normal cells, endocrine and lymphoid cells included, that synthesize endogenous SRIF are known to express SRIF receptors (15–17). However, it has not been documented yet whether SRIF could play a negative autocrine role in such cells.

The presence of SRIF receptor in human prostate is evident, but contradictory results were obtained about the distribution and expression of receptor subtypes and their relation to different pathological situations (18–22). No studies have been performed to date to clarify the identity of the receptor subtypes and mechanism(s) through which SRIF directly affects cell proliferation. Interestingly, we recently identified the presence of SH2 domain containing protein tyrosine phosphatase (SHP)-1 in rat prostate (23). Other recent reports suggest that SHP-1 may participate in the negative regulation of cellular proliferation by SRIF (24–26). Therefore, a better understanding of the mechanisms underlying this direct inhibitory action would shed new light on the involvement of SRIF in the etiology of prostate cancer and could foster the development of new cancer therapies.

In this report we provide evidence that SRIF is expressed and secreted by PC-3 and LNCaP cells, and that it regulates prostatic cell growth through an autocrine loop. Moreover, we identify SHP-1 in human prostatic cancer cell lines as well as in human prostate and prostate cancer. This enzyme is activated by SRIF in PC-3 cells, and thus it may be involved in the antiproliferative effect of SRIF on the prostate.

**Subjects and Methods**

**Experimental subjects**

The human material selected for this study was obtained from transrectal needle biopsies and routine surgical specimens of radical cystectomy, transurethral resection, and radical prostatectomy. The tissues were used in the experiments after approval by the local ethical committee. Normal adult prostate (2 cases), benign prostatic hyperplasia (7 cases), high grade prostatic intraepithelial neoplasia (PIN) without coexistent adenocarcinoma (3 cases), low grade adenocarcinoma (Gleason score 7 or less; 12 cases), and high grade adenocarcinoma (Gleason score 8 or 9; 8 cases), with or without evidence of neuroendocrine differentiation on light microscopy were not included in the study.

**Cell culture and growth assay**

PC-3 and LNCaP human prostatic carcinoma cell lines were cultured in RPMI 1640 medium (Life Technologies, Inc., Gaithersburg, MD) containing 10% (PC-3) or 2% (LNCaP) FBS and antibiotics. Cell growth was measured by cell counting using a model Cassy 1 (Scha¨rfe System, Coulter, Hialeah, FL), after treatment of cells with 0.05% trypsin and inorganic phosphate released per min at 30 C from radiolabeled substrate. SRIF Regulation of Prostate Carcinoma

**Immunoprecipitation and PTP assay**

Cells were washed and solubilized with 50 mmol/liter Tris-HCl buffer (pH 7.5) containing 140 mmol/liter NaCl, 1 mmol/liter EDTA, 0.3 mg/ml soybean trypsin inhibitor, and 0.1 mmol/liter phenylmethylsulfonylfluoride (buffer A) in the presence of 1.5% 3-[3-cholamidopropyl(dimethylammonio)]-1-propanesulfonate and 0.5 mmol/liter sodium orthovanadate. The mixture was gently agitated for 30 min at 4 C and then centrifuged at 18,500 × g for 20 min. Solubilized proteins (400–600 µg) were incubated for 2 h at 4 C with an anti-SHP-1 protein antibody (Santa Cruz Biotechnology, Inc., Santa Cruz, CA) prebound to Sepha-

**Immunoblotting**

Soluble proteins (50 µg) were resolved through 7.5% SDS-polyacrylamide gels, transferred to a nitrocellulose membrane, and immunoblotted with primary antibodies. Immunoreactive proteins were visualized by the ECL immunodetection system (Pierce Chemical Co., Rockford, IL) with horseradish peroxidase-conjugated secondary antibodies and was quantified by the Image Scion computer program (Scion Corp., Frederick, MD).

**Immunocytochemistry**

Cells were grown in four-well multiwell plates on crystal slides (25,000 cell/cm²) for immunocytochemical detection of SRIF or SHP-1. Cells were fixed for 15 min in 0.1 mol/liter phosphate buffer (pH 7.4) with 4% paraformaldehyde. Cell membranes were permeabilized with 0.05% Triton X-100 in PBS. The slides were blocked with 1% gelatin in PBS and then incubated overnight with monoclonal anti-SRF (Chemicon Co., Temecula, CA) or monoclonal anti-SHP-1 antibodies (Santa Cruz Biotechnology, Inc.). SRIF was detected with biotinylated secondary antibody and avidin-biotin/horseradish peroxidase complex. SHP-1 was detected with antimouse IgG horseradish peroxidase-conjugated antibodies. The color was developed with diaminobenzidine substrate. Finally, slides were washed in PBS, dehydrated in a graded series of ethanol, cleared in xylene, and mounted with Canada balsam. Slides were analyzed under the microscope and photographed.

**SRIF RIA**

Cells were cultured for 48 h in RPMI 1640 medium supplemented with FBS (3500 cells/cm²). After this time the medium was changed, and cells were cultured in three experimental groups: RPMI without FBS (0%); with insulin (50 ng/ml), transferrin (50 ng/ml), and sodium selenite (50 pg/ml; ITS); or with FBS. Pooled culture media were collected and analyzed with trilfluoroacetic acid and concentrated using Sep-Pak C₁₈ cartridges (Waters Corp., Les Ulis, France). The adsorbed peptides were eluted with 80% acetonitrile/0.1% trifluoroacetic acid. The eluates were evaporated under vacuum. The dried samples were analyzed for immunoreactivity. SRIF-like immunoreactivity was measured by RIA with rabbit polyclonal antibody (provided by Dr. E. Arilla, University of Alcalá, Alcalá, Spain), the radioligand [¹²⁵I]-¹¹Tyr]SRIF and the standard SRIF-14 as previously described (27). This assay detects SRIF-14 and molecular forms extended at the amino-terminus of SRIF-14, including SRIF-28 and pro-SRIF.

**RT-PCR**

Total RNA was extracted with the Ultraspec RNA method and treated with deoxyribonuclease I. First strand cDNA synthesis was carried out at 39 C for 2 h using Moloney murine leukemia virus (Life Technologies, Inc.). Aliquots of the first strand reactions were used as templates for subsequent PCR using Tag polymerase (Ecogen, Barcelona, Spain). The nucleotide sequences of the sense and antisense primers for SRIF and the five human SRIF receptor subtypes were: SRIF: sense, 5’-TCTAGCTAAGCTTCCGACTCC-3’; and antisense, 5’-TCAATTTCTTAATCAGGTC-3’; sst1: sense, 5’-GACACATGCTATGCC-3’; and antisense, 5’-GGGTGTGCATCTCAGC-3’; sst2: sense, 5’-TGACAGCTAGGACGAGCAG-3’; and antisense, 5’-GCAAGAGAGAGAGAGGA-3’; sst3: sense, 5’-TCTACTGCTTGCTACCTG-3’; and antisense, 5’-GAGCCCCAAGAAAGCGAC-
Analysis of the SRIF and sst expression in PC-3 cells obtained by selection with G418 (600 \mu g/ml).

We measured whether PC-3 translates SRIF mRNA into peptide. Material was fixed in 10% formalin for less than 24 h and embedded in paraffin following routine methods. Sections (5 \mu m thick) were cut from the paraffin blocks and applied to positively charged slides. The material was subjected to 25, 30, and 35 PCR amplification cycles to provide relative quantification of specific sstr subtype cDNAs. The assay revealed that PC-3 cells exclusively express sst-5 and sst-2, failing to detect the expression of sst-1, sst-3, and sst-4 (Fig. 1A).

**Effects of serum depletion on SRIF secretion**

To learn whether a relationship exists between cell growth and SRIF secretion, we evaluated the ability of different cultured conditions to alter PC-3 SRIF production. PC-3 cells were cultured in the presence of serum for 2 d, then the medium was removed, and the cells were cultured in serum-free medium with or without ITS for 4 d. PC-3 cells were counted daily during the 4 d of culture to monitor cell growth. The results reported in Fig. 2A show that serum deprivation increased SRIF secretion; a maximum increase of 2.5-fold over control level occurred 3 d after serum withdrawal. This increase was prevented by the addition of ITS (a basal medium supplement, the composition of which is indicated in Subjects and Methods) to serum-free RPMI. In these conditions, SRIF secretion did not vary significantly during the 4-d culture period, and it was very similar to that obtained on d 0. Serum withdrawal was also associated with a dramatic reduction of PC-3 cell growth (Fig. 2B). However, the presence of ITS increased the growth of PC-3 cells to levels comparable to those obtained in the presence of serum. To further support the serum deprivation-induced production of SRIF in PC-3 cells, we next examined the expression of prepro-SRIF mRNA by RT-PCR. As shown in Fig. 2C, the level of prepro-SRIF transcripts was clearly up-regulated after 24 and 72 h of PC-3 cell culture in serum-free medium. The results obtained prompted us to analyze whether the synthesis and secretion of SRIF could be a common factor in other prostatic cell lines. LNCaP, an androgen-sensitive human prostatic cell line, also produced and secreted SRIF, although the level of this peptide in LNCaP was higher than that in PC3 cells (234.6 ± 33.5 fmol/10^6 cells) than control medium (levels not detected) that had not been exposed to cells. Using antibodies against SRIF, immunocytochemical analysis revealed the specific expression of the peptide in PC-3 cells (Fig. 1B).

The results clearly demonstrate that SRIF is produced and secreted by PC-3 cells, so we next determined the presence of specific SRIF receptors. We isolated total RNA from PC-3 cells and performed RT-PCR reactions using specific pairs of primers for sst-1, -2, -3, -4, and -5. Identical samples were subjected to 25, 30, and 35 PCR amplification cycles to provide relative quantification of specific sstr subtype cDNAs. The assay revealed that PC-3 cells exclusively express sst-5 and sst-2, failing to detect the expression of sst-1, sst-3, and sst-4 (Fig. 1A).

**Transfection of SHP-1 and SRIF in PC-3 cells**

The human SHP-1 cDNA was cloned into the pcDNA1 neo expression vector (Invitrogen, San Diego, CA). PC-3 cells were stably transfected using DOTAP reagent (Roche Molecular Biochemicals, Indianapolis, IN) with 4 \mu g SHP-1 in pcDNA1 neo vector. Stable colonies were selected with G418 (600 \mu g/ml) and subjected to 25, 30, and 35 PCR amplification cycles to provide relative quantification of specific sstr subtype cDNAs. The assay revealed that PC-3 cells exclusively express sst-5 and sst-2, failing to detect the expression of sst-1, sst-3, and sst-4 (Fig. 1A).

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**Statistical analysis**

Statistical comparisons between experimental groups were performed using t-test. P < 0.05 was considered significant.

**Results**

**Analysis of the SRIF and sst expression in PC-3 cells**

In search of regulatory mechanisms responsible for the control of prostatic cell proliferation, we evaluated whether SRIF could be expressed and secreted by PC-3 cells in an autocrine fashion. RT-PCR of PC-3 total RNA with SRIF-specific primers resulted in a single product, 370 bp in size, that corresponds to the expected size of an RT-PCR product derived from prepro-SRIF mRNA (Fig. 1A). As a negative control, the PCR was carried out with water instead of cDNA (Fig. 1A, C_DNA). To exclude the possibility that genomic DNA was amplified, a cDNA reaction was performed without reverse transcriptase (Fig. 1A, C_DNA-free). PC-3 culture supernatants were tested for SRIF immunoreactivity to determine whether PC-3 translates SRIF mRNA into peptide. Medium that overlaid PC-3 cell cultures for 48 h (conditioned medium) contained a significantly greater amount of SRIF (103.5 ± 18 fmol/10^6 cells) than control medium (levels not detected) that had not been exposed to cells. Using antibodies against SRIF, immunocytochemical analysis revealed the specific expression of the peptide in PC-3 cells (Fig. 1B).

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Effect of SRIF on PC-3 and LNCaP cell proliferation

We then explored whether variations in the amount of SRIF present in the culture medium could modify prostatic cell proliferation. We first evaluated the effect of addition of an anti-SRIF antibody to cell culture medium. As observed in Fig. 4A, neutralization of endogenously produced SRIF resulted in the stimulation of PC-3 cell growth of about 35%. For LNCaP cells, a stimulation of 15% was observed.

Conversely, SRIF overexpression decreased the proliferation of PC-3 cells by 45% compared with that of control cells after transfection of PC-3 cells with SRIF expression vector (Fig. 4B). These results show that SRIF, produced by human prostatic cancer cells lines and acting locally, may be involved in the tight regulation of cell proliferation.

SRIF activates SHP-1 in PC-3 cells

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Immunoblotting experiments were undertaken to examine the expression of SHP-1 in PC-3 cells and LNCaP cells (Fig. 5). In both cases, specific monoclonal anti-SHP-1 antibodies revealed a single band with an apparent molecular mass of 66 kDa. It is clear that the expression of SHP-1 in LNCaP was higher than that in PC-3 cells. In contrast, the ubiquitously expressed PTPs, SHP-2 and PTP 1B, were detected at similar levels in PC-3 and LNCaP cells (Fig. 5). The expression of SHP-1 in these cell lines was confirmed by immunocytochemical analysis (Fig. 10, C and D).

After we determined that PC-3 cells contained SHP-1, we investigated the effects of endogenous SRIF on both its protein level and its activity. Immunoprecipitation of SHP-1 with anti-SHP-1 antibodies revealed that after 3 d of culture without serum, SHP-1 activity was increased by 3-fold in PC-3 cells compared with control cells cultured in the presence of serum (Fig. 6A). This increase was prevented at least in part by addition of an anti-SRIF antibody in serum-free medium. Furthermore, the level of SHP-1 protein was also increased when cells were cultured in the absence of serum.

FIG. 1. Expression of SRIF and sst mRNA (A) and immunocytochemical detection of SRIF in PC-3 cells (B). Cells were cultured in RPMI 1640 medium with 10% FBS for 2 d. RT-PCR analysis of prepro-SRIF mRNA and sst1-sst5 mRNA was performed. PCR products were 370 bp for SRIF, 284 bp for sst2, and 472 bp for sst5. CDNA-free is a control with absence of genomic DNA; Cneg is a negative control. The figure is representative of three experiments. The positions of the DNA size molecular mass markers are shown (B). SRIF was immunostained as described in Subjects and Methods. A typical field is shown (×32; right panel). The primary antibody was omitted in a negative control (×16; left panel).
(Fig. 6B), and this increase was reduced in the presence of anti-SRIF antibodies. Thus, serum withdrawal was associated with an increase in both SHP-1 protein level and activity, and neutralization of endogenous SRIF reversed these effects, suggesting that serum deprivation-induced SHP-1 activation in PC-3 cells may be due at least in part to SRIF. In addition, when PC-3 cells were transiently transfected with...
SRIF vector, the increase in SRIF levels affected both SHP-1 activity (Fig. 7A) and SHP-1 protein level (Fig. 7B), which were increased by 182% and 244%, respectively.

To confirm the role of SRIF in the activation of SHP-1, we also analyzed the effect of the stable analog of SRIF, RC160, on SHP-1 activity in PC-3 cells. Cells were incubated in the presence of $10^{-8}$ mol/liter RC160 for various times, after which they were solubilized, and SHP-1 activity was measured in SHP-1 immunoprecipitates. As shown in Fig. 8, SHP-1 activity increased upon treatment with RC160. Stimulation of SHP-1 activity was maximal after 5 min of RC160 exposure and was maintained up to 20 min. The stimulation of SHP-1 activity by RC160 was also dose dependent. A maximal increase was observed with $10^{-8}$ mol/liter RC160 (46.5% over a basal value of 100) and was maintained for higher doses of RC160.

Stable expression of SHP-1 induced inhibition of PC-3 cell growth

To obtain direct evidence of the role of SHP-1 in the regulation of PC-3 cell proliferation, SHP-1 or empty vector was stably expressed in PC-3 cells. Two clones expressing high levels of SHP-1 (CSH 11 and CSH12) and one clone expressing empty vector (PS12) were selected (Fig. 9A). As shown in Fig. 9B, the growth of PC-3 cells overexpressing SHP-1 was reduced compared with that of control cells and cells expressing empty vector. After 6 d of culture, the proliferation of the two clones overexpressing SHP-1 was reduced by 35% and 45%, respectively.

SHP-1 is present in human prostate

SHP-1 granular and cytoplasmic immunostaining was detected in normal, hyperplastic, and neoplastic glands of the prostate with different intensities and distributions (Table 1). In normal and hyperplastic glands the immunostaining was restricted to the luminal side of duct and acinar cells (Fig. 10A). In PIN (Fig. 10B) and well differentiated adenocarcinoma (Fig. 10, E and F), SHP-1 antibodies also immunostained the cytoplasm of neoplastic cells, but the staining was diffuse and did not show this polar arrangement observed in benign tissue. High grade adenocarcinomas, represented by cases of Gleason scores 8–10, were all negative (Fig. 10, G and H). Lymphoid cells in both prostatic specimens and controls displayed an intense and diffuse cytoplasmic positivity.
Discussion

The mechanisms underlying prostate tumoral growth are still poorly understood. Since the early work demonstrating the presence of SRIF-like proteins in the human prostate gland (28), a large body of evidence has indicated a prominent role of SRIF in the regulation of prostate growth. However, the mechanism(s) through which SRIF directly affects cell proliferation and the signaling pathways involved still need to be clearly defined.

It is well known that prostatic carcinoma expresses ssts, but conflicting data exist about the expression of subtypes sst2 and sst5. Although sst2 was not found in primary pros-
tate cancer specimens using in situ hybridization and RT-PCR (18, 19), this subtype was revealed in metastases of hormone refractory prostatic adenocarcinoma (20), in orthotopic PC-3 tumors and their metastases (21), and in Dunning rat model R-3327 AT-1 (22). In agreement with this latter set of reports, we detected sst2 and sst5 RNA in PC-3 cells. These cells are derived from metastatic, rather than primary, tumors. More recently, Halmos et al. (29) defined the incidence and properties of sst in patients with organ-confined and locally advanced prostate cancer. They found that the incidence of ssts on prostate cancer did not seem to be affected by tumor progression. SstI and sst5 mRNA were widely distributed, whereas sst2 was rarely detected. However, further analysis of the pattern of ssts expression is needed in a large cohort of patients, with reference to stage and grade of tumors, to know whether the loss or gain of an SRIF receptor subtype may be associated with the progression of prostate cancer.

The present study reveals that SRIF is produced by PC-3 and LNCaP cells and that this peptide is an important negative regulator of the cell proliferation of these cell lines. We show that SRIF secretion is related to the proliferative status of prostatic cells, as culture conditions that increase SRIF secretion, such as serum withdrawal, induce a decrease in PC-3 and LNCaP cell growth. Despite the opposite effect of ITS, a basal medium supplement, on PC-3 and LNCaP cell growth, the inverse relationship between cell proliferation and SRIF secretion is kept. In addition, SRIF overexpression...
inhibits PC-3 cell growth. Conversely, blockage of endogenous SRIF by specific antibodies results in an increase in PC-3 and LNCaP cell growth. Taken together, these data suggest the existence of an autocrine/paracrine SRIF loop, inhibitory for cell proliferation of these human prostatic cancer cell lines. The activity of this loop could be negatively regulated, in a cell-specific manner, by factors present in serum, as is demonstrated by the addition of ITS to basal medium. In this sense, Tang et al. (30) reported that in the absence of serum, PC-3 cells show an initial phase of growth, followed by a

**TABLE 1. Immunohistochemical distribution of SHP-1 in normal and pathological human prostate**

<table>
<thead>
<tr>
<th>Specimen</th>
<th>SHP-1 immunostaining</th>
<th>Location</th>
<th>Pattern</th>
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</thead>
<tbody>
<tr>
<td>Normal prostate (n = 2)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ducts</td>
<td>Positive</td>
<td>Apical cytoplasmic</td>
<td>Granular</td>
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<tr>
<td>Glands</td>
<td>Positive</td>
<td>Diffuse cytoplasmic</td>
<td>Nongranular</td>
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<tr>
<td>Hyperplastic prostate (n = 7)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Hyperplastic glands</td>
<td>Positive</td>
<td>Apical cytoplasmic</td>
<td>Granular</td>
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<tr>
<td>Atrophic glands</td>
<td>Positive</td>
<td>Diffuse cytoplasmic</td>
<td>Nongranular</td>
</tr>
<tr>
<td>Post-atrophic glands</td>
<td>Positive</td>
<td>Diffuse cytoplasmic</td>
<td>Nongranular</td>
</tr>
<tr>
<td>Basal cell hyperplasia</td>
<td>Negative</td>
<td>Apical cytoplasmic</td>
<td></td>
</tr>
<tr>
<td>Transitional metaplasia in ducts</td>
<td>Intensely positive</td>
<td>Diffuse cytoplasmic</td>
<td>Nongranular</td>
</tr>
<tr>
<td>Inflammatory cells</td>
<td>Intensely positive</td>
<td>Cytoplasmic</td>
<td>Nongranular</td>
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<tr>
<td>High grade PIN</td>
<td></td>
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<tr>
<td>PIN associated with cancer</td>
<td>Positive</td>
<td>Diffuse cytoplasmic</td>
<td>Nongranular</td>
</tr>
<tr>
<td>PIN unassociated with cancer (n = 3)</td>
<td>Positive</td>
<td>Diffuse cytoplasmic</td>
<td>Nongranular</td>
</tr>
<tr>
<td>Prostate carcinoma (n = 22)</td>
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<td></td>
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<td>Well differentiated (n = 12)</td>
<td>Positive</td>
<td>Diffuse cytoplasmic</td>
<td>Nongranular</td>
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<td>Microglandular areas</td>
<td>Positive</td>
<td>Diffuse cytoplasmic</td>
<td>Nongranular</td>
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<tr>
<td>Cribriform areas</td>
<td>Weakly positive</td>
<td>Diffuse cytoplasmic</td>
<td>Nongranular</td>
</tr>
<tr>
<td>Poorly differentiated (n = 8)</td>
<td>Negative</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ductal adenocarcinoma (n = 2)</td>
<td>Positive</td>
<td>Diffuse cytoplasmic</td>
<td>Nongranular</td>
</tr>
</tbody>
</table>

PIN, Prostatic intraepithelial neoplasia.

**FIG. 10.** Distribution of SHP-1 in human prostate. Immunohistochemical demonstration of SHP-1 staining in hyperplastic glands covered by double cell layer of benign cells, characteristic of benign prostatic hyperplasia (A). High grade PIN, displaying characteristic papilla without stromal stalks and confined by the basal membrane, presents diffuse cytoplasmic immunostaining in atypical cells (B). C, LNCaP cells. D, PC-3 cells. Well differentiated adenocarcinoma, Gleason score 7 (3 + 4), composed of small glands and some solid cords of atypical cells invading the fibromuscular stroma (E), shows diffuse cytoplasmic immunostaining for SHP-1 (F). Poorly differentiated adenocarcinoma, Gleason score 10 (5 + 5), composed of solid cords of atypical cells diffusely infiltrating the stroma (G), shows negative immunoreaction (H).
phase, starting from d 3–4, in which the number of cells begins to decrease. The initial growth phase could be due to the autocrine production of various growth factors detected in these cells (3). The increase in SRIF secretion (maximum on d 3) that we observed in these cells could counterbalance the positive growth regulatory loops and be responsible for the decrease in cell proliferation. Instead, LNCaP cells keep a relatively constant cell number for 5–6 d after serum withdrawal, coincident with the increase in the SRIF level since first day after serum withdrawal.

It is evident that the presence of SRIF in PC3 and LNCaP epithelial cells revises classic studies in which expression of this peptide in the prostate is limited to neuroendocrine cells (28). However, prior studies have shown that these cell lines consist of multipotent cells capable of both neuroendocrine and epithelial differentiation. In fact, the treatment of cultured prostate cancer cells with hormone-deficient medium induces the acquisition of numerous neuroendocrine characteristics that include the cessation of mitotic activity and the release of some neurosecretory products into the culture medium (31). Assuming that endogenous SRIF inhibits PC3 and LNCaP cell proliferation, the increase in its secretion could be important for the proliferative suppression associated with the neuroendocrine differentiation event. Our group is studying this possibility.

SHP-1, a protein tyrosine phosphatase with two N-terminal Src homology 2 domains that allow binding to phosphotyrosines residues, plays a role in terminating growth factor and cytokine signals by dephosphorylating critical molecules (26, 32–35). SHP-1 is highly expressed in hemopoietic cells (35); however, recent studies revealed SHP-1 is also substantially expressed under the control of an alternative tissue-specific promoter in a variety of nonhemopoietic cells, especially in some malignant epithelial cells (36). However, the precise function and targets of SHP-1 in nonhemopoietic cells are largely unknown. In previous studies we reported the expression of SHP-1 in rat prostate epithelial cells (23), but the presence of this PTP in human prostate has not been previously documented. We report now, for the first time, that SHP-1 is also abundant in human prostatic cancer cells lines and that its activity and protein levels are stimulated by endogenously produced SRIF. Coincident with the increase in SRIF secretion, the results presented here show that serum deprivation increases the activity of SHP-1 as a result at least in part of an increase in SHP-1 expression. This increase is partly caused by SRIF, as it is prevented by the neutralization of endogenously secreted SRIF. The increase in SHP-1 activity is greater than that in SHP-1 levels, suggesting that other factors either present in serum or induced by serum deprivation may modulate the SHP-1 activity, although not its level. Previously, Brevini et al. (37) demonstrated a direct inhibitory effect of SRIF on LNCaP cell proliferation, which could be mediated by the activation of unidentified PTP. Numerous studies reported that SRIF can stimulate PTP activity in other cell types, and all five SRIF receptor subtypes have been shown to stimulate PTP in various transfected cells (9, 13). SHP-1 has been identified as the PTP involved in the SRIF-induced antiproliferative signal (24, 25). In fact, recent studies have revealed that SHP-1 associates with sst2, becomes activated in response to SRIF, and participates in the negative regulation of mitogenic insulin signaling (24, 26). Other studies, however, have suggested that the more widely expressed SHP-2 is the principal component of sst-mediated antiproliferative signaling (38, 39). We demonstrate that RC160 stimulates SHP-1 activity in a time- and dose-dependent manner. This analog binds with high affinity to sst2 and sst5. As we have detected both subtypes in PC-3 cells, we cannot determine which of them is implicated in the activation of SHP-1. Recent reports show that SHP-1 associates with sst2 and is involved in the sst2-mediated up-regulation of p27kip1, leading to cell cycle arrest (40). Moreover, Raully et al. (41) reported that the expression of sst2 in mouse NIH-3T3 fibroblasts generated a negative autocrine loop by stimulating SRIF production and increasing both SHP-1 activity and SHP-1 levels. The antiproliferative effect of SRIF is also mediated by sst5, although the mechanisms regulated by this receptor subtype do not seem to be related to SHP-1 activation, and these results are far from conclusive. In this sense, Cordelier et al. (42) demonstrated that the antiproliferative signal mediated by rat sst5 implicates a cGMP-dependent pathway, whereas Sharma et al. (43) suggest that the antiproliferative signaling via human sst5 leading to growth inhibition is PTP dependent. Thus, it is necessary to determine which SRIF receptor subtype expressed is coupled to SHP-1 in PC-3 cells. Our demonstration that SHP-1 overexpression reduced PC-3 cell growth is an argument in favor of the role of SHP-1 in the negative control of cell growth. Matozaki et al. (44) have also shown that overexpression of SHP-1 is associated with growth inhibition and a decrease in growth factor-induced mitogenesis. Conversely, decreased levels of SHP-1 were associated with increased cell growth and growth factor-mediated cell responses (35). The essential role of SHP-1 as a negative regulator of cell growth is consistent with a marked overexpansion of multiple hemopoietic cell types, which has been observed in motheaten mice characterized by mutations in the SHP-1 gene and loss of SHP-1 activity (45). Our results clearly indicate that SHP-1 is a component involved in the SRIF autocrine inhibitory loop, because when you block secreted SRIF, the PC-3 cell proliferation increases, decreasing the activity and the levels of SHP-1. The overexpression of SRIF causes a decrease in PC-3 cell proliferation and an increase in the activity and levels of SHP-1. However, this cell line expresses other PTPs, such as SHP-2 and PTP1B, which could also mediate the antiproliferative effect of SRIF in the prostate.

PC-3 is an established cell line and it may not truly represent the in vivo situation. In an attempt to determine whether SHP-1 is expressed in human prostate we investigated the expression and immunolocalization of SHP-1 in normal, benign, and malignant prostatic tissue. We found consistent changes in localization and intracellular distribution of SHP-1, associated with the transition from benign prostate to prostate cancer and also with a variable degree of differentiation within malignant tissue. SHP-1 expression is detected in normal prostate, benign prostatic hyperplasia, high grade PIN, and well differentiated adenocarcinoma. In contrast, no signal is detected in poorly differentiated prostate cancer. This correlated well with our findings that the expression level of SHP-1 is higher in LNCaP than in PC-3 cells. LNCaP is a well differentiated human prostatic cancer
cell line. However, PC-3 is a highly invasive, androgen-independent, and less differentiated human prostatic carcinoma cell line (46). Moreover, the tumors formed by both cell lines after injections in athymic mice are different (47, 48). Tumors formed by PC-3 cells are undifferentiated adenocarcinomas, whereas LNCaP tumors are better differentiated. Thus, the loss of SHP-1 expression may play an important role during the development and progression of prostate cancer. In this sense, SHP-1 can be activated by SRIF and participate in the negative regulation of growth factor signaling. A low expression of SHP-1 has been detected in Burkitt lymphoma (49) and in erythroid progenitors of patients with polycythemia vera, which are hypersensitive to the mitogenic effects of growth factors and cytokines (50). Growth factors and their receptors seem to play an important role in the control of prostate growth. Recent studies have provided evidence that there is a shift toward increased expression and autocrine production of growth factors during the progression of prostate cancer, which represents an adaptation in response to androgen ablation (3). The androgen deprivation could also lead to the development of a negative growth-regulating loop involving SRIF. However, this loop could be deficient due to low expression of SHP-1. Assuming that the incidence of ssts on prostate cancer appears to be unaffected by tumor progression (29), postreceptor signaling defects, such as loss of SHP-1, may play a role in the pathogenesis of prostate cancer by permitting the persistence of signals generated by growth factors. In this sense, Douzi et al. (51) demonstrated that SRIF exerts different effects on human pancreatic cancer cell growth depending upon the presence or absence of SHP-1. The antiproliferative effect of the peptide is not observed when the enzyme is not expressed.

Despite the attention focused in recent years on the efficacy of SRIF to treat prostate cancer, the molecular mechanisms used by this peptide are far from being well documented. We have demonstrated that PC-3 and LNCaP cells synthesize and secrete SRIF, and this peptide, acting through sst2 and/or sst5, inhibits cell proliferation. This antiproliferative effect could be mediated by SHP-1, a PTP present in prostatic cells and regulated, both short and long term, by growth factors. In this sense, Douzi et al. (51) demonstrated that SRIF exerts different effects on human pancreatic cancer cell growth depending upon the presence or absence of SHP-1. The antiproliferative effect of the peptide is not observed when the enzyme is not expressed.

Acknowledgments

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