Abstract

PSP94 (β microseminoprotein, β MSP) is one of the three major proteins secreted by the normal human prostate gland. Using reverse transcriptase polymerase chain reaction (RT-PCR) and Southern blotting, PSP94 transcripts were shown in human endometrium, myometrium, ovary, breast, placenta and in the human endometrial cancer cell lines KLE and AN3 CA. Primers used in these studies were specific for human prostate PSP94, and were derived from its flanking non-coding regions. The results were confirmed by sequence analysis of two independently derived clones from normal human breast tissues and the other two from KLE cells respectively. The sequences were identical with the coding sequence of human prostate PSP94 cDNA. Using RNA from the endometrial tissues, two different transcripts of ~487 bp, equivalent to prostate PSP94 and ~381 bp, corresponding to prostate PSP57, its alternately spliced form, were amplified by RT-PCR. Human ovary, breast, placenta and endometrial cancer cell lines (KLE, AN3 CA), however, showed only the full length, ~487 bp, PSP94 transcript. We further demonstrated by in situ hybridization that PSP94 mRNA is expressed specifically in the glandular epithelial cells, and not in the stroma of both the human endometrial and breast tissues. Further, using image analysis of in situ hybridization data, the levels of PSP94 mRNA in the cycling endometrial tissues and in breast confirmed the differential levels of expression in the cycling endometrium (P<0·005). This study distinctly demonstrated significant expression of PSP94 mRNA in human uterine, breast and other female reproductive tissues as well in the endometrial cancer cell lines, suggesting that it may have a role in these tissues as a local autocrine paracrine factor.


Introduction


The amino acid (Johansson et al. 1984, Seidah et al. 1984, Akiyama et al. 1985) and DNA sequences (Mbikay et al. 1987, Green et al. 1990) for PSP94 show no similarity with other known proteins. The PSP94 gene, however, has been shown to be similar in its upstream region to that of prostate-specific antigen (PSA) and prostatic acid phosphatase (PAP) (Ochiai et al. 1995), with respect to the presence of putative transcription regulatory elements for ubiquitous transcription factors, with the consensus sequences (Faisst & Meyer 1992). The significance of these elements in none of these genes is known.

The investigations concerning the expression of PSP94 gene so far primarily include the prostate from human (Mbikay et al. 1987, Green et al. 1990, Ochiai et al. 1995), primate (Mbikay et al. 1988, Nolet et al. 1991), pig and rat (Fernlund et al. 1994, 1996). Although a clear function for PSP94 has not been identified, its reduced expression in prostate cancer suggests it to be a prognostic indicator.
in prostate carcinoma (Dube et al. 1987, Doctor et al. 1986, Brar et al. 1988, Hyakutake et al. 1993, Liu et al. 1993, Kammer et al. 1993). It has also been suggested as an apoptosis-based anti-tumor agent for hormone refractory prostate cancer (Garde et al. 1999). Recently we have identified a binding protein for PSP94 in prostate cancer cell lines (LNCaP, PC3) and prostate tissues (Yang et al. 1998a). The regulation of the expression of PSP94 and its binding protein in LNCaP cells has been suggested to be partially regulated by an autocrine pathway (Yang et al. 1998b).

PSP94 has also been found in comparable concentrations in serum of men and women (Abrahamsson et al. 1989). However, in female tissues, the presence of this protein has been controversial (Dube et al. 1987, Mbikay et al. 1987, Weiher et al. 1990, Ohkubo et al. 1995) and the site of synthesis of this protein has not been identified. It has previously been demonstrated that the immunoreactivity does not necessarily reflect the site of synthesis (Han et al. 1987). Hence, it was significant to demonstrate the local synthesis of PSP94 in female urogenital system to determine its involvement in regulation of growth and reproduction. We thus aimed here to establish the expression of PSP94 mRNA in various human non-malignant female reproductive tissues, breast and in a number of established endometrial cancer cell lines.

Materials and Methods

Cell lines and cell cultures

All human endometrial epithelial adenocarcinoma cell lines were from the American Type Cell Culture (ATCC) and maintained in the recommended culture mediums. The epithelial cell line used was RL95–2 (ATCC CRL–1671), the (morphologically characterized) epithelial-like cell lines were KLE (ATCC CRL–1622), HEC–1-A (ATCC HTB–112) and AN3 CA (ATCC HTB–111).

Tissue collection

All the tissues were immediately frozen in liquid nitrogen and stored at −80 °C for RNA extractions. The in situ hybridizations (ISH) and immunohistochemistry (IHC) studies were done on the archival surgical material routinely submitted for pathological studies and did not require patient consents. The uterine, ovarian and cervical non-malignant specimens were from patients in their pre-menopausal years, undergoing biopsies or surgeries and were procured from the London Health Science Centre, London, ON, Canada. The uterine biopsies or hysterectomies were performed for gynecological reasons such as benign uterine leiomyomas, not endometrial in origin. The placental and prostate benign prostatic hyperplasia (BPH) specimens were from the St Joseph’s Health Centre, London, ON, Canada. All the samples were the remaining materials from the pathological diagnosis and involved informed patients consents. The normal breast tissues were obtained from the Tumor Tissue Bank (London Regional Cancer Centre, London, ON, Canada), with the consent of the Tumor Bank Steering committee.

Oligonucleotide primers

Human prostate PSP94 cDNA specific primers were derived from the flanking 5′ and 3′ non-coding regions; PR1 (5′ TACTGATAGGCTAGGCTAC 3′) was complementary to a sequence near the poly-A tail of the sense strand of PSP94 cDNA (Mbikay et al. 1987, Green et al. 1990) and PR2 (5′ TGCTTATCACAATGAGTTTC TCTGGGCG 3′) represents the first 29 nucleotides of PSP94 cDNA sequence (Green et al. 1990). The exon III specific probe consisted of the sequences (5′ GTCCACCTCCCGAG 3′), complementary to the PSP94 cDNA sequence (158–176) and the exon IV (5′ TCCATTACGTACAGACAGGTCTC 3′) complementary to (326–348) (Mbikay et al. 1987). The primers specific for β-actin were used as the internal controls. All primers were synthesized by Procyn Biopharma Inc. (London, ON, Canada).

RNA isolation

Total RNA was isolated from all the tissues and cell lines (1–5 × 10⁶ cells, ~90% confluent) using the TRIzol reagent (Gibco BRL, Burlington, ON, Canada) as per manufacturer’s instructions. The integrity of the RNA was checked on ethidium bromide stained 1.5% denaturing gels (data not shown) and quantified spectrophotometrically.

Reverse transcriptase polymerase chain reaction

The reverse transcriptase polymerase chain reaction (R.T–PCR) was performed according to Xuan et al. (1995) with slight modifications using thin-walled tubes (Bio-Rad, Mississauga, ON, Canada) and was not quantitative in nature. The first strand cDNA synthesis was conducted with 3 µg RNA and 1 µg PR1 primer with 50 units of reverse transcriptase (Pharmacia, Uppsala, Sweden). The PCR was performed with 100 pmol PR1 and PR2 primers respectively, using 2.5 units Taq polymerase (Pharmacia). The thermocycle program for PCR consisted of 20 cycles with 30 s at 95 °C, 30 s at 60 °C, 60 s at 72 °C and followed by one cycle for 10 min at 72 °C. Portions (10 µl) of PCR products were run on 6% PAGE or 1.5%-agarose gels and visualized with ethidium bromide staining. Positive controls contained RNA from prostate (BPH) tissues and negative controls did not contain any RNA. One µg RNA from each tissue was used for RT–PCR under similar conditions, with the primers specific for β-actin.
The RT-PCR products were cloned using TA cloning Kit from Invitrogen using pCR 2-1 vector, as per manufacturer’s instructions (Invitrogen, Carlsbad, CA, USA), using INVt F’ One Shot cells for transformation. The screening of the recombinant clones was done by partial restriction mapping. Two independent positive clones were fully sequenced from the normal breast tissues and the KLE cell lines at the sequencing facility, Robarts Research Institute, London ON, Canada, and Mobix Core Facility, McMaster University, Hamilton, ON, Canada, respectively. The alignment of DNA sequences was performed using the Wisconsin Sequence Analysis package from GCG Inc.

Southern blot analysis

Southern blotting was performed as previously described (Sambrook et al. 1989), in which DNA was blotted on to a positively charged membrane (Boehringer Mannheim, Laval, Quebec, Canada), using a Trans-Blot SD semi-dry transfer cell (Bio-Rad). Human PSP94 cDNA probe labeled using the DIG Oligo-nucleotide 3’ transfer cell (Bio-Rad). The blot was hybridized with32P-labeled exon IV probe, labeled by random priming using an oligonucleotide primer under similar hybridization conditions at 42 °C. The products from the same set of RT-PCR reactions were run on another gel, under similar conditions and hybridization signals were detected on the Hyperfilm-ECL (Amersham Life Science Inc., Arlington Heights, IL, USA).

Hybridization with DIG-labeled cDNA probe was done overnight at 42 °C in high SDS buffer (SDS 7%; formamidine, 50%; 5 × SSC; 50 mM sodium phosphate, pH 7-0; N-lauroyl-sarcosine, 0-1% (w/v)) and blocking reagent, 2%. CSPD (Boehringer Mannheim) was used as the chemiluminescence substrate. The same blot was stripped and re-probed with DIG-labeled exon IV oligonucleotide probe under similar hybridization conditions at 48 °C. The products from the same set of RT-PCR reactions were run on another gel, under similar conditions and were transferred to a Zeta probe membrane (Bio-Rad). The blot was hybridized with35P-labeled exon IV probe, labeled by random priming using an oligonucleotide labeling kit (Pharmacia) to specific activity of 1 × 10^9 c.p.m. and was exposed overnight to Kodak XAR film (Eastman Kodak, New Haven, CT, USA).

In another set of experiments, the RT-PCR products obtained under similar conditions were transferred to a new positively charged membrane. The blot was hybridized with DIG-labeled exon III probe at 48 °C, under similar conditions and hybridization signals were detected as described earlier.

Preparation of tissue sections

Serial microtome sections (5 µm thick) were mounted on Superfrost Plus slides (Fisher Scientific, Fairlawn, NJ, USA) and prepared for immunohistochemistry (IHC) and in situ hybridizations (ISH) using the method described by Lazowski et al. (1994). The slides were baked for 3–4 days at 45 °C. Adjacent serial sections were used for IHC using rabbit polyclonal anti PSP94 (Baijal-Gupta et al. 1996), raised against native PSP94 or PSP57 antiserum against a synthetic 41-mer peptide (Xuan et al. 1995) or for ISH, as described below.

Preparation and labeling of riboprobe

The 35S UTP-labeled antisense RNA probe was generated from a 483 bp human PSP94 cDNA insert subcloned into Sma I site of the pBluescript vector–KS (Stratagene, La Jolla, CA, USA). The fragments obtained by linearizing this plasmid with restriction enzymes, XbaI and EcoRI, were gel isolated and used for labeling as sense and antisense RNA probes, respectively, using either T3 or T7 promoters as per recommended protocols (RNA Transcription Kit, Promega, Madison, WI, USA). Probes were labeled to an approximate specific activity of 10^9 c.p.m./µg.

ISH

ISH was performed as described by Lazowski et al. (1994) with some modifications to the hybridization conditions. Tissue sections were hybridized with the35S-UTP labeled antisense RNA probe generated as described above, at a concentration of 10^6 c.p.m./µl in 1 × hybridization buffer (deionized formamide, 50%; NaCl, 0.3 M; Tris–HCl, pH 8, 20 mM; EDTA, 1 mM; Denhardt’s solution, 1 ×; yeast tRNA, 500 µg/ml; denatured ssDNA, 100 µg/ml; SDS, 0.1%; dithiothreitol (DTT), 100 mM; dextran sulphate, 10%) overnight at 68 °C. Control sections were hybridized with35S-UTP labeled sense RNA probe generated from the same cDNA, as described earlier. The specimens were finally exposed to X-ray film BioMax MR (Eastman Kodak) for 18–24 h. The slides were coated with photoemulsion (NTB-3 nuclear track emulsion, Eastman Kodak) and exposed at 4 °C for 2–4 weeks, depending on the autoradiographic intensity of the sections on the X-ray film. The photoemulsion on the sections was developed with D-19 developer (Eastman Kodak), fixed, and the slides were counter-stained with Harris’s hematoxylin and eosin for morphologic characterization and mounted with paramount (Fisher Scientific). The distribution and location of the silver grains were examined by microscopy under dark and light field illumination with several magnifications, using Aristoplan Photomicroscope (Leica, Heerbrugg, Switzerland). Two to three sets of slides from each tissue were studied for reproducibility.

The specificity of ISH was demonstrated by lack of specific autoradiographic signals, when the adjacent tissue sections were hybridized with a35S labeled sense RNA probe or by abolishment of specific hybridization when the
hybridization was performed on adjacent tissue sections that were pretreated with RNase.

**Semiquantitative analysis of ISH data**

The PSP94 mRNA signals on the ISH studies with the endometrial, breast and prostate tissue sections were quantified by the Northern Eclipse Imaging system software (Empix Inc., Mississauga, ON, Canada). The measurements were done in bright field microscopy at 40 × magnifications. The density of the grains in the selected regions of the tissues were denoted in pixel units (background counts were taken into account). The area selected for assessment on each specimen remained constant (1 × 10^4 μm) to eliminate any variability. Prostate (BPH) were considered with highest positive signals in the cytoplasm of the epithelial cells with 5900–8000 pixel counts (=High). Based on the pixel counts in BPH tissues, the signals in other specimens were categorized as medium positive signals with 1000–5000 pixels (=Medium); low positive signals in the perinuclear region with 700–1000 pixels (=Low); negative signals = to the background counts 40–400 pixels.

**Results**

**Southern blot analysis of RT-PCR products**

Using RT-PCR reactions with PSP94-specific primers, and prostate (BPH) as a positive control (Brar et al. 1988), we identified PSP94 mRNA in the biopsy specimens from the endometrium (n=10), myometrium (n=5), ovary (n=5), breast (n=5), placenta (n=3), cervix (n=3) and two different endometrial cancer cell lines on ethidium bromide stained PAGE (data not shown), which included the molecular weight markers (100 bp ladder).

RT-PCR products from the endometrial cell lines, KLE and RL95–2, and from the normal breast tissues were further analyzed by partial restriction mapping. Two clones each from these were sequenced in both directions. The transcripts from the KLE cells and the breast tissues were identical in size (~487 bp) to that of the prostate PSP94 fragment. The DNA sequence alignment using the GCG Sequence Analysis Package confirmed 100% identity of the entire coding region of these transcripts to human prostate PSP94, as reported by Mbikay et al. (1987) and Green et al. (1990). Limited substitutions were seen in the 3′ non-coding region of the gene from the KLE cells. The Genbank Accession number assigned to the nucleotide sequence of cDNA cloned from cell line KLE was U78976. None of the sequences obtained from the RL95–2 from a number of batches (n=4) of RL95–2 cells matched with PSP94 cDNA sequence, suggesting these as the artifacts of the PCR amplification.

Southern blot analysis of the RT-PCR amplified products from various samples, as described earlier, with DIG-labeled human PSP94 cDNA is shown in Fig. 1A. Signals for two different transcripts were detected in the endometrium (lane 1), myometrium (lane 2) and prostate (lane 5) tissues. The higher band corresponded to PSP94 ~ 487 bp (Mbikay et al. 1987, Green et al. 1990), denoted with long arrow and lower band ~381 bp, denoted with short arrow, corresponding to PSP57, the alternately spliced form of PSP94 (Xuan et al. 1995). However, no RT-PCR product was detected in one of the myometrial samples tested. The endometrial tissues were from early secretory (n=3), late secretory (n=3), to late proliferative (n=2) phases of the cycling endometrium. Both PSP94 and PSP57 were identified in about equal intensities in all the endometrial samples analyzed independent of phase of the cycle (data not shown). The RT-PCR assays used here were not quantitative and conclusions as to the apparent variations in levels of expression observed between the samples and also the proportions of the two different transcripts could not be made. The KLE (lane 7) and AN3 CA (lane 8) cell lines showed only ~487 bp transcript (Fig. 1A). No signal was detected with HEC-1-A or

![Figure 1 Southern blot analysis of RT-PCR products electrophoresed from 6% PAGE (not shown). Shown in (A) with DIG-labeled human PSP94 cDNA probe. Positions of PSP94 (long arrow) and PSP57 (short arrow) are indicated, as determined by the molecular weight. The samples were lane 1, endometrium; lane 2, myometrium; lane 3, ovary; lane 4, cervix; lane 5, prostate; lane 6, placenta; lane 7, KLE cells; lane 8, AN3 CA cells; lane 9, negative control without RNA; lane M, not indicated on the blot. Southern blot analysis of the same blot (B) with DIG-labeled exon IV probe. (C) Southern blot analysis of the RT-PCR products transferred to a new membrane from a similar 6% acrylamide gel (not shown) hybridized with DIG-labeled exon III probe. Samples were lane 1, prostate; lane 2, endometrium; lane 3, myometrium; lane 4, ovary; lane 5, KLE cells.](image-url)
RL95–2 (data not shown) cell lines or with negative controls without RNA. The integrity of RNA isolated from the samples was confirmed by RT-PCR with primers for human β-actin (data not shown).

The same blot as shown in Fig. 1A, when stripped and re-probed with DIG-labeled exon IV probe, shows two bands (PSP94 and PSP57) with the endometrium (lane 1), myometrium (lane 2) and prostate (lane 5) and single band (PSP94) with ovary (lane 3), placenta (lane 6), KLE (lane 7) and AN3 CA cells (lane 8) and breast (data not shown), thus confirming PSP94 and PSP57 cDNA signals. Hybridization with 32P-labeled exon IV probe (data not shown) showed similar results as with the DIG probes.

An identical blot, as described earlier, probed with exon III specific probe (Fig. 1C) to confirm the identity of PSP57 transcript, detected only the larger ~487 bp in the prostate (lane 1), endometrium (lane 2) and myometrium (lane 3), suggesting the deletion of the exon III in these tissues similar to prostate (Xuan et al. 1995). The ovary (lane 4) and KLE cells (lane 5), however, showed same results as with the PSP94 cDNA probe described earlier.

### ISH

The samples from the cycling endometrium, early secretory (n=9), late secretory (n=3) and proliferative (n=3) phases, and from the normal breast (n=4) were selected for ISH studies. Prostate (BPH) tissues (n=6) were used as positive controls. The 35S-labeled antisense riboprobe hybridized to the epithelial cells of the endometrial glands (Fig. 2A). Bright field microscopy also shows PSP94 mRNA signals specifically in the epithelial cells of endometrium (Fig. 2E) and in breast (Fig. 2F) with no signals in the stromal cells of these tissues. The specificity of the probe was demonstrated on the adjacent tissue sections by the lack of specific hybridization of the sense riboprobe to the endometrial tissues (Fig. 2C), under identical conditions.

Using the same antisense riboprobe as used for the endometrial specimens (Fig. 2A), significantly greater hybridization signals were observed in the epithelial cells of prostate glands (Fig. 3A) under identical conditions. No signals were detected in the stromal cells. The adjacent tissue sections hybridized with 35S-labeled sense riboprobe displayed no specific signals (Fig. 3C), showing the integrity of the probe. Furthermore, complete abolition of the specific hybridization signals was observed when RNase pretreatment preceded the hybridization with the antisense RNA probe for all the tissues tested, confirming the specificity of the probe.

### Evaluation of PSP94 mRNA (ISH) signals

Table 1 shows the results of the image analysis of the ISH data on various stages of the cycling endometrium and breast tissues. Prostate (BPH) was used as a positive control. The mRNA quantification from the early secretory phase endometrium showed an average of 3620 pixel counts with a standard deviation (s.d.) of ±944, denoted as medium expression. Relatively lower counts for PSP94 signal were detected in the late secretory phase and no signals in the proliferative phase of the endometrium. The PSP94 mRNA levels in the normal glands of the breast tissues were also in the medium range.

### IHC

The early secretory phase of the endometrium showed low but specific immunoreactivity for PSP94 in the cytoplasm of the epithelial cells of these glands and in the luminal lining (Fig. 2B), with little or no immunoreactivity in the stromal cells. In the prostate, however, the immunoreactivity was significantly higher in the cytoplasm of the epithelial cells and also in the secretions of the glandular lumen (Fig. 3B). The specificity of the antibody was demonstrated by total abolition of the immunostaining of the endometrial (Fig. 2D) and the prostate specimens (Fig. 3D) with immuno-absorbed PSP94 antiserum. No PSP57 protein was detected by IHC using PSP57-specific antibody, either in the endometrium or in the prostate tissues.

### Discussion

In this study we have demonstrated PSP94 transcripts in the human endometrium, myometrium, breast, ovary, placenta and in the endometrial cancer cell lines, KLE and AN3 CA by RT-PCR and Southern blot analysis. Sequencing the RT-PCR products further substantiated these results.

Previous studies on the distribution of this protein in female genital tract include the epithelium of the fallopian tubes, uterine cervix, ovarian follicular secretions (Weiber et al. 1990, Kammer et al. 1990). β MSP has also been detected in the endometrial and breast tissues by IHC (Teni et al. 1992), however its site of synthesis in these tissues is not known. To our knowledge, this is the first evidence for the specific localization of PSP94 mRNA and co-localization of the protein in the same glandular epithelial cells of the human endometrium, albeit at a lower abundance than prostate. These data provided a strong indication for a local site of synthesis of the protein (PSP94) in the endometrium.

Although PSP94 mRNA was detected in the early and late secretory as well in the proliferative phases of the cycling endometrium by RT-PCR, the conclusion regarding the apparent variations in the levels of expression between samples could not be made by this procedure. We quantified the ISH hybridization signals employing a novel computerized image analysis system (Petrik et al. 1999). The use of radioactive probes for ISH analysis

www.endocrinology.org
Figure 2  Dark field (A) photograph of endometrium with $^{35}$S-labeled antisense probe and (C) with sense probe for PSP94. Bright field (E) photograph of endometrium and (F) photograph of breast with $^{35}$S-labeled antisense probe. The PSP94 antisense probe hybridized strongly to the cytoplasm of the epithelial cells, as indicated by the density of the grains in both dark and bright field microscopy. The specificity of hybridization with antisense probe for the endometrium was shown by the lack of specific hybridization of the sense probe (C) for PSP94. Immunohistochemical localization of PSP94 in the endometrium is indicated by pale brown staining in the cytoplasm of the epithelial cells (B). The specificity of the PSP94 antibody is demonstrated by lack of staining in the adjacent section (D) with immuno-absorbed PSP94 antibody. Abbreviations: G, gland; S, stroma.
Figure 3 Dark field photograph of prostate (BPH) with $^{35}$S-labeled antisense probe (A) and with sense probe for PSP94 (C). The PSP94 antisense probe hybridized strongly to the cytoplasm of the epithelial cells, as indicated by the density of silver grains. The specificity of hybridization with antisense probe for the prostate was demonstrated by the lack of hybridization of the sense probe for PSP94 (C). Immunohistochemical localization of PSP94 in the prostate is indicated by brown staining in the cytoplasm of the epithelial cells (B). The specificity of the PSP94 antibody is shown by lack of staining in the adjacent section (D) with immunoabsorbed PSP94 antibody. Abbreviations: G, gland; S, stroma.

Table 1 Qualification of PSP94 mRNA from ISH studies on various tissues by Northern Eclipse image analysis system. Values for signal intensities are given as mean (± s.d.)

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Number of samples</th>
<th>Signal intensity in pixels*/$10^4$ μm</th>
<th>Levels of mRNA expression</th>
<th>P values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prostate</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BPH</td>
<td>6</td>
<td>6077 (949)</td>
<td>High</td>
<td>&lt;0·0001†</td>
</tr>
<tr>
<td>Endometrium</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Early secretory</td>
<td>9</td>
<td>3085 (754)</td>
<td>Medium</td>
<td>&lt;0·005‡</td>
</tr>
<tr>
<td>Late secretory</td>
<td>3</td>
<td>1636 (224)</td>
<td>Low</td>
<td></td>
</tr>
<tr>
<td>Proliferative</td>
<td>3</td>
<td>400 (4)</td>
<td>Nil</td>
<td></td>
</tr>
<tr>
<td>Breast</td>
<td>4</td>
<td>3213 (490)</td>
<td>Medium</td>
<td></td>
</tr>
</tbody>
</table>

*Number of grains measured in pixels.
†Versus endometrium or breast by unpaired t-test.
‡Between the three stages by ANOVA for repeated measures.
facilitated the estimation of the grains in dark or bright field microscopy to provide reliable counts. Significantly higher levels of PSP94 signals were detected in the early secretory phase endometrium as compared with the late secretory phase, with no signals in the proliferative phase of the cycle. The data strongly suggest an association of PSP94 with the cyclic proliferation of the endometrium and its perspective role as a local regulator of uterine function.

Among all the tissues tested, only the endometrial and myometrial tissues showed prevalence of the two transcripts, PSP94, and its splice variant PSP57. However, no PSP57 protein could be detected in either of these tissues, as reported earlier for the prostate (Xuan et al. 1995). The mechanism of its regulation and expression in tissue-/cell-specific manner is not yet determined.

In conclusion, this study indicates significant expression of PSP94 transcript in a number of non-pathological female reproductive tissues and in the endometrial cancer cell lines. Furthermore, PSP94 mRNA was found to be expressed in much higher amounts in the early secretory stage of the cycling endometrium. These data suggest its association with organs highly influenced by the endocrine hormones. Studying the levels of expression of PSP94 gene in the biopsies of the normal female reproductive tissues, from patients under hormonal therapy, and from biopsies from various carcinomas, may further elucidate its significance in normal and tumor biology of the female reproductive system.

Acknowledgements

We thank Dr Steve Power, Department of Obstetrics & Gynecology, and Dr M E Kirk, Department of Pathology, University of Western Ontario, London, ON, Canada for kindly providing the biopsy tissues of all the human female reproductive tissues. We also thank Dr John C Walton, Pathology Department and Dr John Denstedt, Urology Department, St Joseph Hospital, London ON for providing the prostate (BPH) tissues.

Thanks also to Dr Ann F. Chambers, London Regional Cancer Centre, London, ON for a comprehensive review of this manuscript and her constructive comments and to Dr Chandra Panchal, President and C.E.O. and the staff of Procyon Biopharma Inc. London, ON for their suggestions and help in the preparation of the manuscript.

References

Garde S, Busrur VS, Li L, Finkelman MA, Krishan A, Welleham L, Ben-Josef E, Haddad M, Taylor JD, Porter AT & Tang DG 1999 Prostate secretory protein (PSP94) suppresses the growth of androgen-independent prostate cancer cell line (LNCaP) and xenografts by inducing apoptosis. Prostate 38 118–125.


Received 17 June 1999
Revised manuscript received 20 October 1999
Accepted 6 December 1999