

Expression of Sulfotransferase 1E1 in Human Prostate as Studied by In Situ Hybridization and Immunocytochemistry

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BACKGROUND. Estrogen is recognized to play a role in the development and function of the prostate. Estrogen sulfotransferase (EST) 1E1 catalyzes the sulfoconjugation of estrogen and is thus involved in the metabolism of estrogen. We have recently shown that EST 1E1 is highly expressed in male mouse reproductive organs, including prostate. It appeared of interest to study the expression of EST 1E1 in human prostate.

METHODS. EST 1E1 mRNA and protein expression was evaluated in benign prostatic hyperplasia (BPH) using in situ hybridization and immunocytochemistry, respectively.

RESULTS. EST 1E1 mRNA and protein were found to be expressed in epithelial cells bordering alveola lumen (luminal cells) as well as stroma cells.

CONCLUSION. The enzyme EST may play a physiological role in regulating local estrogen levels in human prostate. *Prostate* 67: 405–409, 2007. © 2006 Wiley-Liss, Inc.

KEY WORDS: sulfotransferase 1E1; benign prostate hyperplasia; estrogen; in situ hybridization; immunocytochemistry

INTRODUCTION

Androgens are essential for the development, growth, and maintenance of the prostate, and are involved in the prostate pathogenesis including carcinogenesis. Furthermore, it was reported that estrogens also have great impacts on the prostate growth and differentiation [1]. Recently, we have shown that administration of estradiol (E2) to castrated rats during 3 weeks induced hypertrophy of epithelial cells as well as an increase in nuclear immunostaining for androgen receptor (AR) [2]. Estrogen might also be involved in the development of prostate cancer [1,3].

The conversion of the inactive adrenal precursors dehydroepiandrosterone (DHEA) and DHEA sulfate to E2 and dihydrotestosterone (DHT) has been shown to occur in several tissues [4,5]. Human prostate has been shown to express all the enzymes involved in the conversion of DHEA to testosterone (T) and DHT [6–8].

Very recently, we have also reported in human prostate the expression of enzymes involved in estrogen synthesis: aromatase which transforms androstenedione to estrone (E1) and T to E2 and 17 β -hydroxysteroid dehydrogenase (17 β -HSD) type 1, 7,

and 12 which are all involved in the conversion of E1 into E2 [9]. Estrogen sulfotransferase (EST) converts estrogens into their sulfates (E1-S1, E2-S). The inactivated sulfated estrogens can be transformed into the active estrogens by the sulfatase pathway involving the enzyme steroid sulfatase which converts E1S into E1 and E2S to E2 [10]. In the mouse, using quantitative RealTime PCR (Q-RT-PCR), we have recently shown that EST 1E1 mRNA was highly expressed in male reproductive organs including the prostate and very poorly expressed in female reproductive organs such as mammary gland and uterus [11]. The role of the EST might be to protect male tissues against estrogenic action. It has also been recently reported that EST and

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steroid sulfatase could be detected by immunocytochemistry in a high percentage of human prostate cancer tissues, but not in non-neoplastic and benign prostate hyperplasia (BPH) tissues [12].

In the present study, we examined the expression and localization of EST 1E1 in BPH using a combination of in situ hybridization (ISH) and immunocytochemistry.

MATERIALS AND METHODS

Tissue Preparation

This study was approved by the institutional board at Laval University Medical Center. All the patients signed informed consent forms before participation in this research project.

Prostatic tissue was obtained from 10 patients with symptomatic prostatic benign hyperplasia (BPH), undergoing transurethral prostatectomy (age of the patients, between 60 and 80 years). The specimens were immediately fixed by immersion in 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). After 24 hr fixation, the specimens were embedded in paraffin or frozen at -70°C .

In Situ Hybridization

In preliminary experiments, no demonstrative labeling could be obtained when ISH was performed on paraffin sections. We then used frozen sections (10 μm) mounted onto glass slides. The vector used for production of the cRNA probe was constructed by insertion into a pCR-Blunt II-TOPO (Invitrogen, Ontario, Canada) of a cDNA fragment of 767 bp of human EST 1E1 (Genebank No. NM_005420). The cDNA fragment located at position 205–971 was obtained by amplification using the polymerase chain reaction (PCR). Antisense and sense ^{35}S -labeled cRNA probes were prepared by in vitro transcription with the sp6 and the T7 RNA polymerase promoters, respectively. ISH with the antisense and sense probes was performed as previously described [13]. Briefly, the sections were prehybridized at room temperature in a humid chamber for 2 hr in 450 μl /slide of a prehybridization containing 50% formamide, 5 \times SSPE (1SXXPE being 0.1 M NaCl, 10 mM NaH_2PO_4 , pH 7.4, 0.8 mM EDTA), 5 \times Denhart's buffer, yeast-t-RNA 200 $\mu\text{g}/\text{ml}$, denatured salmon testis DNA (Sigma, St. Louis, MO), 200 $\mu\text{g}/\text{ml}$ Poly A (Boehringer-Mannheim, Montreal, Canada), and 4% dextran sulphate. After prehybridization treatment, 100 μl hybridization mixture (prehybridization buffer containing 10 mM dithiothreitol and the ^{35}S -labeled cRNA probe at a concentration of 10^6 cpm/ml) was spotted on each slide, sealed under a coverslip, and incubated at 60°C overnight (15–20 hr) in a humid chamber.

After hybridization, coverslips were removed and slides were rinsed in 2 \times SSC at room temperature for 30 min. Sections were then digested by RNase A (20 $\mu\text{g}/\text{ml}$ in 2 \times SSC) at 37°C for 30 min, rinsed in decreasing concentrations of SSC (2CSSC and 1 \times SSC) for 30 min at room temperature, washed in 0.5 \times SSC for 30 min at 37°C , followed by 90 min at room temperature in 0.5 \times SSC, and finally for 30 min at room temperature in 0.1 \times SSC. The sections were then exposed to Hyperfilm (Kodak, Rochester, NY) for 4–5 days and subsequently coated with liquid photographic emulsion (Kodak-NBT2; diluted 1:1 with water). Slides were exposed for 14 days, developed in Dektol developer (Kodak) for 2 min, and fixed in rapid fixer (Kodak) for 4 min. Thereafter, the sections were rinsed and stained with hematoxylin and eosin.

Immunocytochemistry

To develop antibodies to EST 1E1, we selected the peptide sequence located at amino acid position 157–285 of the human EST 1E1. This peptide sequence was overproduced in *E. coli* BL-21 using PET23a expression vector (EMD Biosciences, San Diego, CA). The purified protein was subsequently diluted in phosphate saline buffer containing 50% complete Freund's adjuvant (concentration: 3 mg/ml) and injected sc with 1 ml at multiple sites on four New Zealand rabbits. The animals were treated twice with the same amounts of proteins in 50% of incomplete Freund's adjuvant at 1 month intervals. Antisera were analyzed by immunoblot using HEK-293 cells non-transfected and stably transfected with EST 1E1, as negative and positive controls, respectively. The proteins were separated by 12% SDS-PAGE and transferred onto a nitrocellulose gel for analysis with the protein A-purified antibody to EST 1E1 (diluted 1: 6,000). Horse anti-rabbit IgG antibody conjugated with horseradish peroxidase (Amersham Biosciences, Inc., Baie d'Urfé) was used as secondary antibody (dilution: 1: 10,000) and the resulting immunocomplexes were then visualized using enhanced chemiluminescence kit (Perkin Elmer Life Science) and exposed on a X-OMAT blue film for 30 sec. As shown in Figure 1, the antibodies react only with the overexpressed protein. The experiment was conducted in an animal facility approved by the Canadian Council on Animal Care (CCAC; Ottawa, Ontario, Canada) and the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC; Rockville, MD). The study was performed in accordance with the CCAC Guide for Care and Use of Experiment Animals.

The immunohistochemical localization of EST 1E1 was performed as previously described [8]. Deparaffinized sections were incubated at 4°C with the anti-serum against human EST 1E1 diluted 1:500. Control

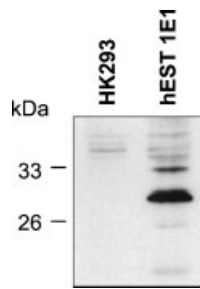


Fig. 1. Western blot analysis of proteins from untransfected or transfected HK293 cells stably expressing human EST 1E1 (Mw: 31 kDa). The antiserum specifically reacts with the overexpressed enzyme.

experiments were performed on adjacent sections by substituting preimmune rabbit serum (1:500) or the antiserum preabsorbed with an excess of the antigen (10^{-6} M). The primary antibodies to EST 1E1 were detected following incubation at room temperature for 4 hr with peroxidase-labeled goat antirabbit γ -globulin (HyClone Laboratories, Inc., Logan, UT) diluted at 1:200. The peroxidase was revealed during incubation with 10 mg of 3,3'-diaminobenzidine in 100 ml of Tris-saline buffer containing 0.03% H_2O_2 . The intensity of staining was controlled under the microscope. Thereafter, the sections were counterstained with hematoxylin.

RESULTS

In Situ Hybridization

After 4–5 days of exposure of the films, specific hybridization signal could be obtained in all the prostate specimens. At the light microscopic level, following hybridization with the antisense cRNA probe, silver grains were detected over the epithelial cells of the prostatic alveoli as well as the stroma cells (Fig. 2A). In consecutive control sections hybridized with the sense probe, only weak diffused labeling could be seen (Fig. 2B). Since frozen sections do not allow to clearly identify the basal cells of the alveoli, it cannot be excluded that labeling occur not only in luminal cells which are clearly labeled but also in the basal cells.

Immunohistochemistry

Using specific antibodies to hEST 1E1, we could localize the enzyme in all the prostate specimens. As shown in Figure 3A, immunolabeling could be detected in the luminal cells of the alveoli, the labeling being both cytoplasmic and nuclear. Light staining was also observed in stroma cells. When we used the antiserum previously immunoabsorbed with hEST 1E1 or pre-immune serum, no reaction could be found (3B).

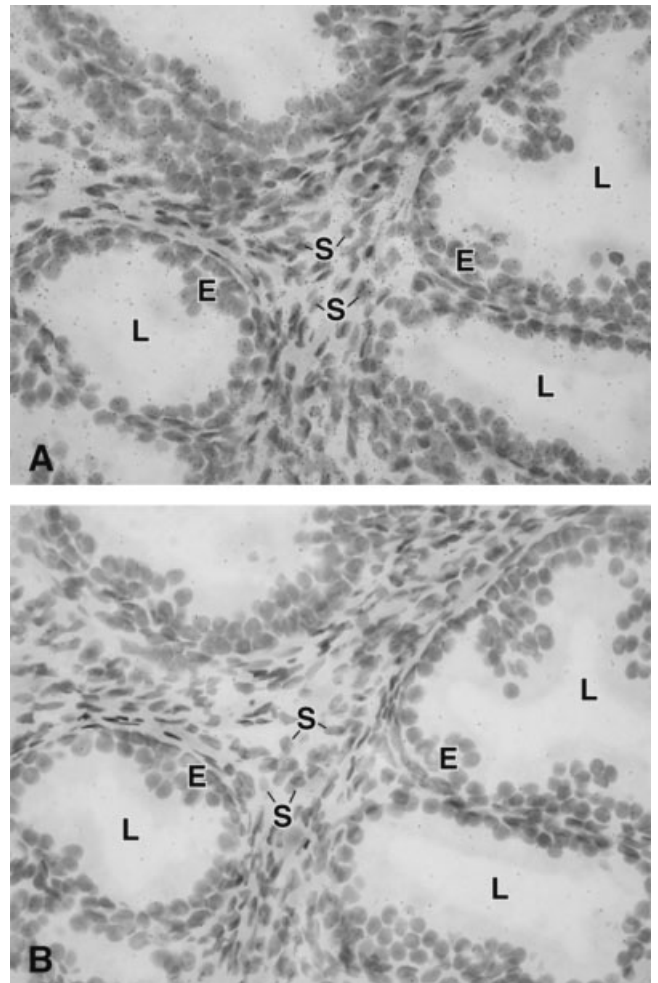


Fig. 2. **A:** Micrograph illustrating the localization of EST 1E1 mRNA in human prostate by ISH. Silver grains are seen over the epithelial (E) cells bordering alveolar lumen (L) as well as stroma (S) cells. **B:** Consecutive section hybridized with the radiolabeled sense probe. Few dispersed grains can be observed. Exposure time: 10 days. 400 \times .

DISCUSSION

Combining two complementary approaches, namely ISH and immunohistochemistry, we have clearly shown that EST 1E1 is expressed in epithelial and stroma cells in BPH tissues. Immunostaining was detected in both nucleus and cytoplasm in the epithelial cells. The function of EST 1E1 in the nuclear compartment remains to be fully explored. We have previously observed that 17-HSD type 12 and 3β -HSD type 1 were also expressed in both the nuclei and cytoplasm in human prostate epithelial cells and guinea pig adrenocortical cells, respectively [9,14].

By RT-PCR, EST 1E1 mRNA has been detected in human prostate [15,16]. In those studies, there was no identification of the prostate cell types expressing the enzyme mRNA. Very recently, Nakamura et al. [12]

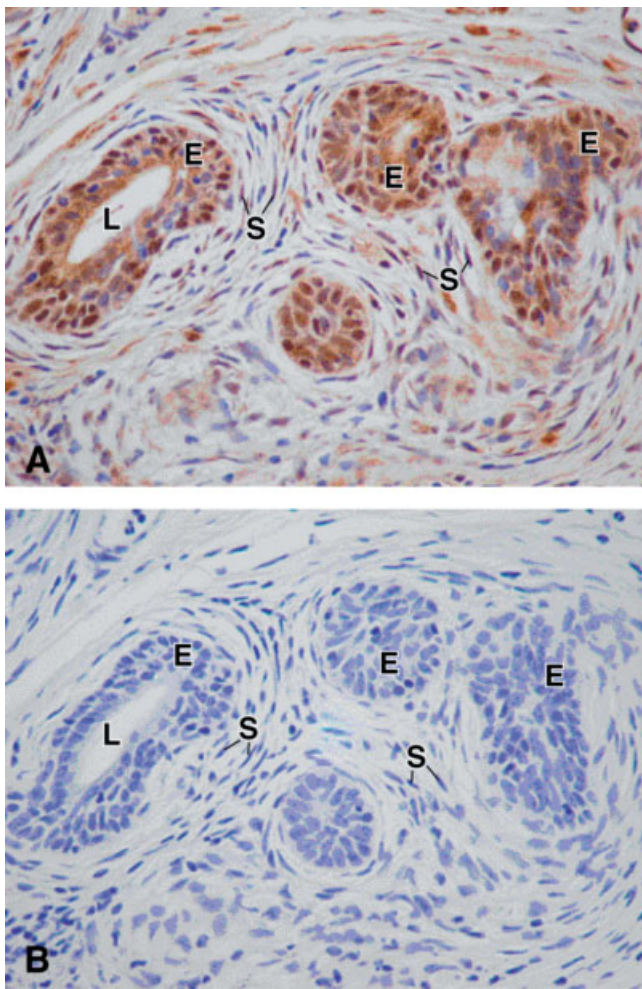


Fig. 3. **A:** Immunostaining for EST 1E1 in human prostate. Positive reaction is observed in the cytoplasm and nuclei in the epithelial cells bordering the alveolar lumen (luminal cells). Stroma cells (S) are also immunolabeled. **B:** Adjacent control section. Immunoabsorption of the antiserum with an excess of antigen (10^{-6} M) has completely prevented immunostaining. L, lumen. 400 \times .

have reported that EST mRNA was expressed in three types of prostate cancer cell lines as studied by RT-PCR analysis. They also found by immunohistochemistry involving use of commercially available polyclonal antibodies to EST that immunoreactive EST could be detected in 75% of prostate cancer cases examined, but not in the non-neoplastic peripheral zones and BPH tissues. This discrepancy between the results obtained by Nakamura et al. [12] and the present results showing expression of EST 1E1 in BPH might be explained by differences in the sensitivity and/or specificity of the antisera used. Since we have obtained the same results with both immunohistochemistry and in situ hybridization, we are quite confident that the enzyme is expressed in both epithelial and stroma cells in BPH tissues.

We have previously shown that all the enzymes involved in the biosynthesis of E2 from circulating DHEA are expressed in human prostate epithelial and stroma cells [6,8,9]. Moreover, it has been reported that cultured prostate cancer LNCaP cells could produce E2 [17]. Intraprostatic E2 can also originate from circulating E2 of which the levels remain constant with aging [18,19]. Human EST 1E1 is an enzyme that catalyzes the sulfoconjugation of E1 and E2, at the 3-hydroxyl position [10]. It is expressed in several male and female tissues [15,16]. In the mouse, using quantitative RT-PCR, we have reported that EST 1E1 mRNA was highly expressed in male-specific organs, including prostate, testis, and seminal vesicles, and almost completely absent in female reproductive organs such as ovary, uterus, and mammary gland [11]. By ISH, EST 1E1 was found to be expressed in epithelial cells in mouse prostate [20]. In the mouse testis, EST is exclusively localized to Leydig cells which produce high amounts of androgen and also estrogen, and its expression in these cells appear to be dependent on LH and androgen [10] (Pelletier et al., unpublished data). This sex-specific expression of EST 1E1 suggests that the enzyme may be involved in the inactivation of estrogens which might antagonize androgenic action in androgen-dependent tissues. The hypothesis that EST may play a physiological role in modulating estrogen response in male tissues is supported by the high affinity of EST for estradiol [21,22].

In summary, we report that the enzyme EST 1E1, involved in the sulfoconjugation and then inactivation of estrogen, is expressed in both epithelial and stroma cells in human prostate. The enzyme might play a role in the regulation of estrogen levels in the human prostate.

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REFERENCES

1. Cunha GR, Wang YZ, Hayward SW, Risbridger GP. Estrogenic effects on prostatic differentiation and carcinogenesis. *Reprod Fertil Dev* 2001;13(4):285–296.
2. Pelletier G. Effects of estradiol on prostate epithelial cells in the castrated rat. *J Histochem Cytochem* 2002;50(11):1517–1523.
3. Griffiths K. Estrogens and prostatic disease. *International Prostate Health Council Study Group. Prostate* 2000;45(2):87–100.
4. Labrie F, Luu-The V, Lin S-X, Simard J, Labrie C, El-Alfy M, Pelletier G, Bélanger A. Intracrinology: Role of the family of 17 β -hydroxysteroid dehydrogenases in human physiology and disease. *J Mol Endocrinol* 2000;25:1–16.
5. Martel C, Melner MH, Gagné D, Simard J, Labrie F. Widespread tissue distribution of steroid sulfatase, 3 β -hydroxysteroid

- dehydrogenase/D5-D4 isomerase (3b-HSD), 17b-HSD 5a-reductase and aromatase activities in the rhesus monkey. *Mol Cell Endocrinol* 1994;104:103–111.
6. El-Alfy M, Luu-The V, Huang XF, Berger L, Labrie F, Pelletier G. Localization of type 5 17b-hydroxysteroid dehydrogenase, 3b-hydroxysteroid dehydrogenase and androgen receptor in the human prostate by in situ hybridization and immunocytochemistry. *Endocrinology* 1999;140:1481–1491.
 7. Pelletier G, Luu-The V, Huang XF, Lapointe H, Labrie F. Localization by in situ hybridization of steroid 5a-reductase isozyme gene expression in the human prostate and preputial skin. *J Urol* 1998;160:577–582.
 8. Pelletier G, Luu-The V, Têtu B, Labrie F. Immunocytochemical localization of type 5 17b-hydroxysteroid dehydrogenase in human reproductive tissues. *J Histochem Cytochem* 1999; 47:731–737.
 9. Takase Y, L'Ōvesque MH, Luu-The V, El-Alfy M, Labrie F, Pelletier G. Expression of enzymes involved in estrogen metabolism in human prostate. *J Histochem Cytochem* 2006; 54:911–921.
 10. Song WC. Biochemistry and reproductive endocrinology of estrogen sulfotransferase. *Ann N Y Acad Sci* 2001;948:43–50.
 11. Luu-The V, Pelletier G, Labrie F. Quantitative appreciation of steroidogenic gene expression in mouse tissues: New roles for type 2 5-alpha-reductase, 20-alpha-hydroxysteroid dehydrogenase and estrogen sulfotransferase. *J Steroid Biochem & Mol Biol* 2005;93:269–276.
 12. Nakamura Y, Suzuki T, Fukuda T, Ito A, Endo M, Moriya T, Arai Y, Sasano H. Steroid sulfatase and estrogen sulfotransferase in human prostate cancer. *Prostate* 2006;66(9):1005–1012.
 13. Givalois L, Li S, Pelletier G. Age-related decrease in the hypothalamic CRH mRNA expression is reduced by dehydroepiandrosterone (DHEA) treatment in male and female rats. *Mol Brain Res* 1997;48:107–114.
 14. Dupont É, Luu-The V, Labrie F, Pelletier G. Light microscopic immunocytochemical localization of 3b-hydroxy-5-ene-steroid dehydrogenase/D5-D4 isomerase (3b-HSD) in the gonads and adrenal glands of the guinea pig. *Endocrinology* 1990;126:2906–2909.
 15. Dooley TP, Haldeman-Cahill R, Joiner J, Wilborn TW. Expression profiling of human sulfotransferase and sulfatase gene superfamilies in epithelial tissues and cultured cells. *Biochem Biophys Res Commun* 2000;277(1):236–245.
 16. Miki Y, Nakata T, Suzuki T, Darnel AD, Moriya T, Kaneko C, Hidaka K, Shiotsu Y, Kusaka H, Sasano H. Systemic distribution of steroid sulfatase and estrogen sulfotransferase in human adult and fetal tissues. *J Clin Endocrinol Metab* 2002;87(12):5760–5768.
 17. Harkonen P, Torn S, Kurkela R, Porvari K, Pulkka A, Lindfors A, Isomaa V, Vihko P. Sex hormone metabolism in prostate cancer cells during transition to an androgen-independent state. *J Clin Endocrinol Metab* 2003;88(2):705–712.
 18. Seppelt U. Correlation among prostate stroma, plasma estrogen levels, and urinary estrogen excretion in patients with benign prostatic hypertrophy. *J Clin Endocrinol Metab* 1978;47(6):1230–1235.
 19. Shibata Y, Ito K, Suzuki K, Nakano K, Fukabori Y, Suzuki R, Kawabe Y, Honma S, Yamanaka H. Changes in the endocrine environment of the human prostate transition zone with aging: Simultaneous quantitative analysis of prostatic sex steroids and comparison with human prostatic histological composition. *Prostate* 2000;42(1):45–55.
 20. Pelletier G, Luu-The V, Li S, Ouellet J, Labrie F. Cellular localization of mRNA expression of enzymes involved in the formation and inactivation of hormonal steroids in the mouse prostate. *J Histochem Cytochem* 2004;52(10):1351–1356.
 21. Falany CN, Krasnykh V, Falany JL. Bacterial expression and characterization of a cDNA for human liver estrogen sulfotransferase. *J Steroid Biochem & Mol Biol* 1995;52:529–539.
 22. Petrotchenko EV, Doerflein ME, Kakuta Y, Pedersen LC, Negishi M. Substrate gating confers steroid specificity to estrogen sulfotransferase. *J Biol Chem* 1999;274(42):30019–30022.