Differences in steroid 5α-reductase iso-enzymes expression between normal and pathological human prostate tissue

Catherine Iehlé[a], François Radvanyi[b], Sixtina Gil Diez de Medina[b],
L'Houcine Ouafik[c], Henriette Gérard[a], Dominique Chopin[d], Jean-Pierre Raynaud[e],*,
Pierre-Marie Martin[a]

[a]*Laboratoire de Cancérologie Expérimentale, INSERM CJF 93-11, Faculté de Médecine Nord, Bd Pierre Dramard, 13916, Marseille, Cedex 20, France*
[b]*UMR 144, Institut Curie, 26 rue d'Ulm, 75248, Paris, Cedex 05, France*
[c]*INSERM U297, Faculté de Médecine Nord, Bd Pierre Dramard, 13916, Marseille, Cedex 20, France*
[d]*Service d'Urologie, Hôpital Henri Mondor, 94010, Créteil Cedex, France*
[e]*DRI, Université Pierre et Marie Curie, 4 place Jussieu, 75252, Paris, Cedex 05, France*

Abstract

We studied the expression level and cell-specific expression patterns of 5α-reductase (5α-R) types 1 and 2 iso-enzymes in human hyperplastic and malignant prostate tissue by semi-quantitative RT-PCR and in situ hybridisation analyses. In situ hybridisation established that 5α-R1 mRNA is preferentially expressed by epithelial cells and little expressed by stromal cells whereas 5α-R2 mRNA is expressed by both epithelium and stroma. Semi-quantitative RT-PCR has been performed on total RNA from different zones of normal prostate, BPH tissues and liver. We found that 5α-R1 and 5α-R2 mRNAs expression was near the same in all zones of normal prostate. In BPH tissue, 5α-R1 and 5α-R2 mRNAs expression was slightly but significantly increased, when it was compared to the levels recorded for normal prostate. In cancer samples, 5α-R1 mRNA expression was higher than in normal and hyperplastic prostate but the level of 5α-R2 mRNA was not statistically different from that observed in the different zones of normal prostate. In liver, 5α-R2 mRNA level was similar to that measured in BPH but 5α-R1 mRNA expression was ten times higher. The increase observed in 5α-R isoenzymes expression in BPH tissue could play an important role in the pathogenesis and/or maintenance of the disease. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: 5α-reductase; Prostate; Stroma; Epithelium; ISH; RT-PCR

1. Introduction

Androgens and, in particular, dihydrotestosterone (DHT), play a key role in the differentiation and growth of the human prostate. DHT is synthesised from testosterone by two distinct membrane-bound steroid 5α-reductase (5α-R) isoenzymes—denoted type 1 (5α-R1) and type 2 (5α-R2) [1,2]—that are encoded by separate genes and have dissimilar characteristics, tissue distribution and response to pharmacological agents [3–7]. A pseudogene for 5αR1 has also been identified [8]. 5α-R1, which maps to chromosome 5, has a neutral basic pH optimum, is found in skin [9] and liver, and is present in individuals with genetic 5α-R deficiency, whereas 5α-R2, which maps to chromosome 2, has an acidic pH optimum and is expressed at high levels in the prostate. The encoding gene of 5α-R2 is mutated in 5α-R deficiency.

Abbreviations: 5α-R, steroid 5α-reductase (3-oxo-5α-steroid NADP + 4-ene-oxido-reductase); BPH, benign prostatic hyperplasia; CaP, prostate cancer; RT-PCR, reverse transcription followed by polymerase chain reaction; ISH, in situ hybridisation.

* Corresponding author. Tel.: + 33-1-4427-3065; fax: + 33-1-4427-7467.

E-mail address: jean-pierre.raynaud@admp6.jussieu.fr (J.P. Raynaud)

Several teams have suggested that the two 5α-R iso-enzymes are present in human prostate tissue [10–13] but, although the expression of 5α-R2 has long been widely accepted and is known to be inhibited by specific drugs like finasteride, that of 5α-R1 has been controversial until now. In normal and hyperplastic tissue, immunoblotting experiments identified the 5α-R2 isoenzyme only [14], although Northern blot analysis established the presence of the mRNAs of both isoenzymes [1,14,15] and kinetic studies on normal tissue revealed that 5α-R activity is higher at an acidic (5.0) than neutral (7.0) pH [14]. However, very recently, Northern blot analysis and reverse transcription followed by polymerase chain reaction (RT-PCR) on isolated stromal and epithelial fractions have shown that the prostate stroma expresses both 5α-R isoforms whereas the prostate epithelium expresses 5α-R1 only [16]. Cultured epithelial cells expressed only 5α-R in line with our earlier observations on the DU145 human epithelial prostate cancer cell line [17], recently confirmed by others [18].

Selectivity of inhibition can be used to suggest the presence of one or both 5α-R isoforms, but cannot yield information on sub-tissular isoenzyme localisation. For this reason, to determine the cellular expression pattern of both isoenzymes and to quantify their expression level, in the present study, we have used in situ hybridisation (ISH) and RT-PCR on human prostate tissue.

2. Materials and methods

2.1. Prostate tissue processing

Prostate tissue from patients undergoing surgery or biopsy for benign prostate hyperplasia (BPH) or prostate cancer was immediately frozen and kept in liquid nitrogen. A representative sample was taken from each tissue for histopathological and immunohistochemical assessment.

2.2. In situ hybridization

Cryosections (10 μm) of human prostate tissue were mounted on slides coated with 0.5% gelatine (wt/vol) 0.05% chromium potassium sulphate (wt/vol) and prepared for hybridisation to RNA probes as described by Ouafik et al. [19]. Radiolabeled riboprobes were prepared using 35 S-CTP (800 Ci/mmol) (Amersham, UK) and T3 or T7 RNA polymerases (Stratagene, La Jolla, CA, USA). 5α-R cDNA (nucleotides 1-2107 (1)) and 5α-R2 cDNA (nucleotides 224–584 (2)) were sub-cloned into pBluescript Il SK + vector (Stratagene, La Jolla, CA, USA) and used as templates for riboprobe synthesis.

In situ hybridisation was performed as already described [19] for 24 h in a moist chamber under unsealed silane-treated coverslips at 56°C using 0.5–1 × 10^6 cpm probe for each slide. Sections were washed twice for 30 min in 2 × SSC at room temperature, incubated for 30 min at 30°C in 2 × SSC containing 10 μg/ml RNase A (Eurogentec, Belgium), washed twice for 30 min in 2 × SSC and finally immersed in water for a few seconds. Sections were dehydrated by immersion in 100% ethanol, air dried and exposed to Hyperfilm (Amersham, UK) at room temperature for 2–3 days to estimate emulsion exposure times.

For resolution at the cellular level, sections were exposed to Ilford K5 autoradiography emulsion for 1 month at 40°C, developed and stained with hematoxylin and eosin.

2.3. Reverse transcription—polymerase chain reaction (RT-PCR)

Total RNA from human tissues was prepared according to Chirgwin et al. [20]. The level of mRNA was determined by semi-quantitative RT-PCR by comparison with an internal control: TBP, an ubiquitous transcription factor [21].

RNA was reverse transcribed and amplified according to Gil Diez de Medina et al. [22] using the following primers: 5α-R1 forward 5′-GGTTTTGGCTTGTGGTTAACA-3′, 5α-R1 reverse 5′-CAAAATAGTTGGC TGCAGTTAC-3′, 5α-R2-forward 5′-TACACAGACATACGGTTTAGC-3′, 5α-R2reverse 5′-CTTGTGGAATCCTGTAGCTGA3′, TBP-forward 5′-AGTGAAGAA CAGTCCAGACTG-3′, TBP-reverse 5′-CCAGGAAATAACTCTGGCTCAT-3′. The two primers of each pair were chosen in two different exons. The number of cycles was chosen to be in the exponential part of the PCR reactions (26 cycles for 5α-R1 and 24 for 5α-R2). The amplification reactions were performed with an initial cycle of 95°C for 5 min prior to the addition of Hi-Taq DNA thermostable polymerase (Bioprobe). Each cycle was as follows: 94°C for 1 min, 57°C for 1 min and 72°C for 1 min and 20 s. These cycles were followed by a final incubation step at 72°C for 10 min.

PCR products were analysed in duplicate on 8% polyacrylamide gels, fixed in 7% acetic acid and vacuum-dried. Autoradiograms were quantified with a Molecular Dynamics 300 Phosphorlmager (Molecular Dynamics, Sunnyvale, CA, USA).

Table 1
Comparative analysis of mRNA levels for 5α-reductases type 1 and 2 in normal prostate and BPH tissue. The levels of the the different mRNAs were compared with that of TATA binding protein (TBP) by RT-PCR (±S.D.)

	Samples (n)	5αR1/TBP	5αR2/TBP
Normal prostate			
PZ[a]	6	0.158 ± 0.059	0.802 ± 0.292
TZ[b]	9	0.133 ± 0.069	1.075 ± 0.727
CZ[c]	4	0.157 ± 0.065	0.924 ± 0.028
BPH	17	0.224 ± 0.059	1.527 ± 0.413
Prostate cancer	10	0.285 ± 0.095	0.563 ± 0.298
Other tissues			
Liver	1	2.25	1.8
Striated muscle	1	0.17	0
Lung	1	0.18	0.13

[a] PZ: peripheral zone;.
[b] TZ: transitinal zone;.
[c] CZ: central zone.

3. Results

3.1. High sensitivity detection of 5α-R isoforms in human prostate tissue by semi-quantitative RT-PCR

The presence of 5α-R isoenzymes in the human prostate was first investigated by means of semi-quantitative RT-PCR. 5α-R1 and 5α-R2 mRNAs could be detected in all zones of normal prostate (peripheral, transitional and central) and in all BPH and prostate cancer samples. The levels of both 5α-R mRNAs in the different zones of normal prostate were very similar (Table 1).

In BPH tissues, 5α-R1 and 5α-R2 mRNAs expression was slightly but significantly (Mann–Whitney test) increased ($P < 0.05$), in particular when it was compared to the levels recorded for the transitional zone of normal prostate (Fig.1).

In cancer samples, 5α-R1 mRNA expression was significantly (Mann–Whitney test) higher than in normal prostate ($P < 0.05$) but the level of 5α-R2 mRNA was not statistically different from that observed in the different zones of normal prostate.

Other human tissues have also been investigated (Table 1). As expected, both mRNAs were found in liver with 5α-R1 being expressed at very high levels and 5α-R2 at a level similar to that observed in BPH. Little or no expression of 5α-R2 was found in striated muscle and lung, whereas 5α-R1 mRNA level in these tissues was similar to that of normal prostate.

3.2. Expression of 5α-R mRNA isoforms in human prostate epithelial and stromal cells as determined by in situ hybridisation

In BPH and prostate cancer specimen, hybridisation with antisense 5α-R1 riboprobe revealed large numbers of silver grains in many epithelial cells but comparatively few in isolated stromal cells (Figs. 2A and 3A). Hybridisation with sense 5α-R1 riboprobe only gave a low background signal with no cell type specificity (Figs. 2B and 3B). Hybridisation with antisense 5α-R2 riboprobe revealed labelling of comparable intensity in stromal cells (Figs. 2E and 3E) and some epithelial cells (Figs. 2C and 3C). Hybridisation with sense 5α-R2 riboprobe gave no specific signal (Figs. 2D, 2F, 3D and 3F).

Positive staining by both antisense-strand probes was heterogeneously distributed over the sections. Stromal labelling of 5α-R2 was confined to small cell clusters which were at times distributed around the epithelial cells. Epithelial labelling by 5α-R1 or 5α-R2 isoform probe was restricted to certain populations of cells only. Table 2 summarises the results obtained with the two riboprobes on the prostatic tissues examined.
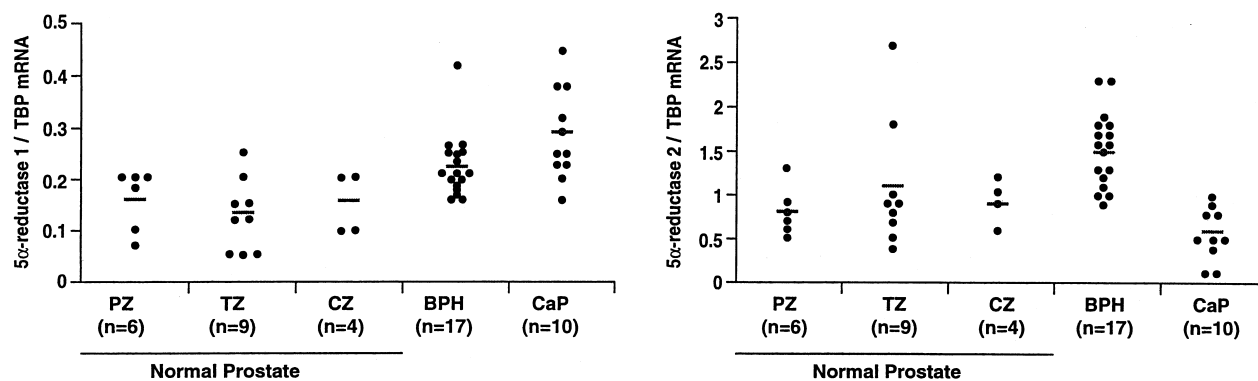

Fig. 1. 5α-Rl and 5α-R2 mRNA expression in normal prostate, BPH and prostate cancer tissues using RT-PCR. Reverse transcription and amplification were performed as described under Materials and methods. PZ: peripheral zone, TZ: transitional zone, CZ: central zone, (−): mean.
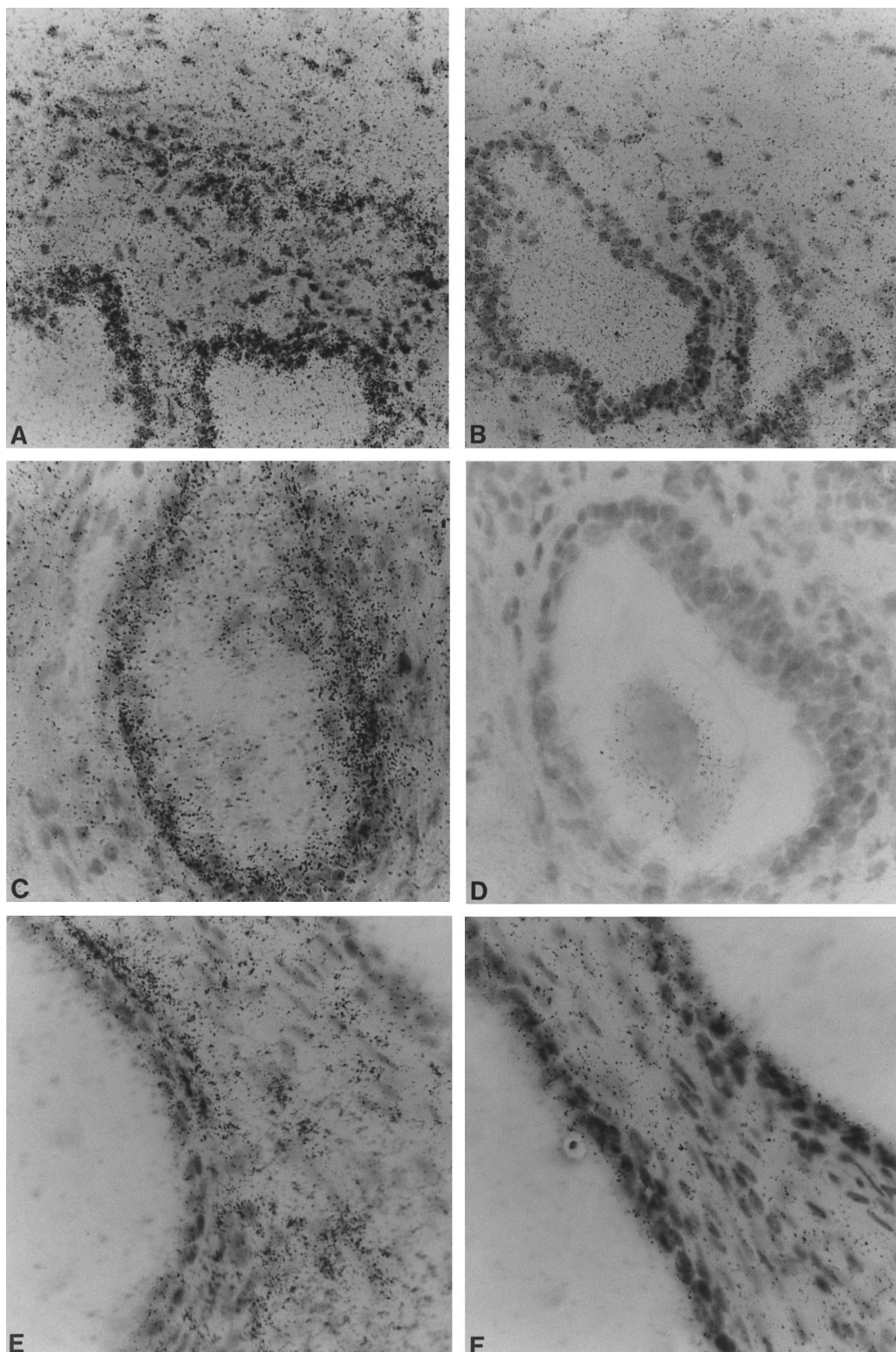
Fig. 2. Hybridisation of 5α-reductase type 1 or type 2 sense- and antisense-strand (35 S)RNA probes to BPH tissues. (A) Section from BPH4 specimen hybridised with the type 1 antisense-strand probe (×250); (B) Same field on another section of the same specimen hybridised with the type 1 sense-strand probe (×250); (C) Section from a different BPH specimen (BPH2) hybridised with the type 2 antisense-strand probe (×400);

(D) Same field on another section of a BPH2 specimen hybridised with the type 2 sense-strand probe (×400); (E) Section from BPH1 specimen hybridised with the type 2 antisense-strand probe (×400); (F) Same field on another section of the same specimen hybridised with the type 2 sense-strand probe (×400). Exposure time was 10 days for (A) and (B), 50 days for (C) and (D) and 25 days for (E) and (F).
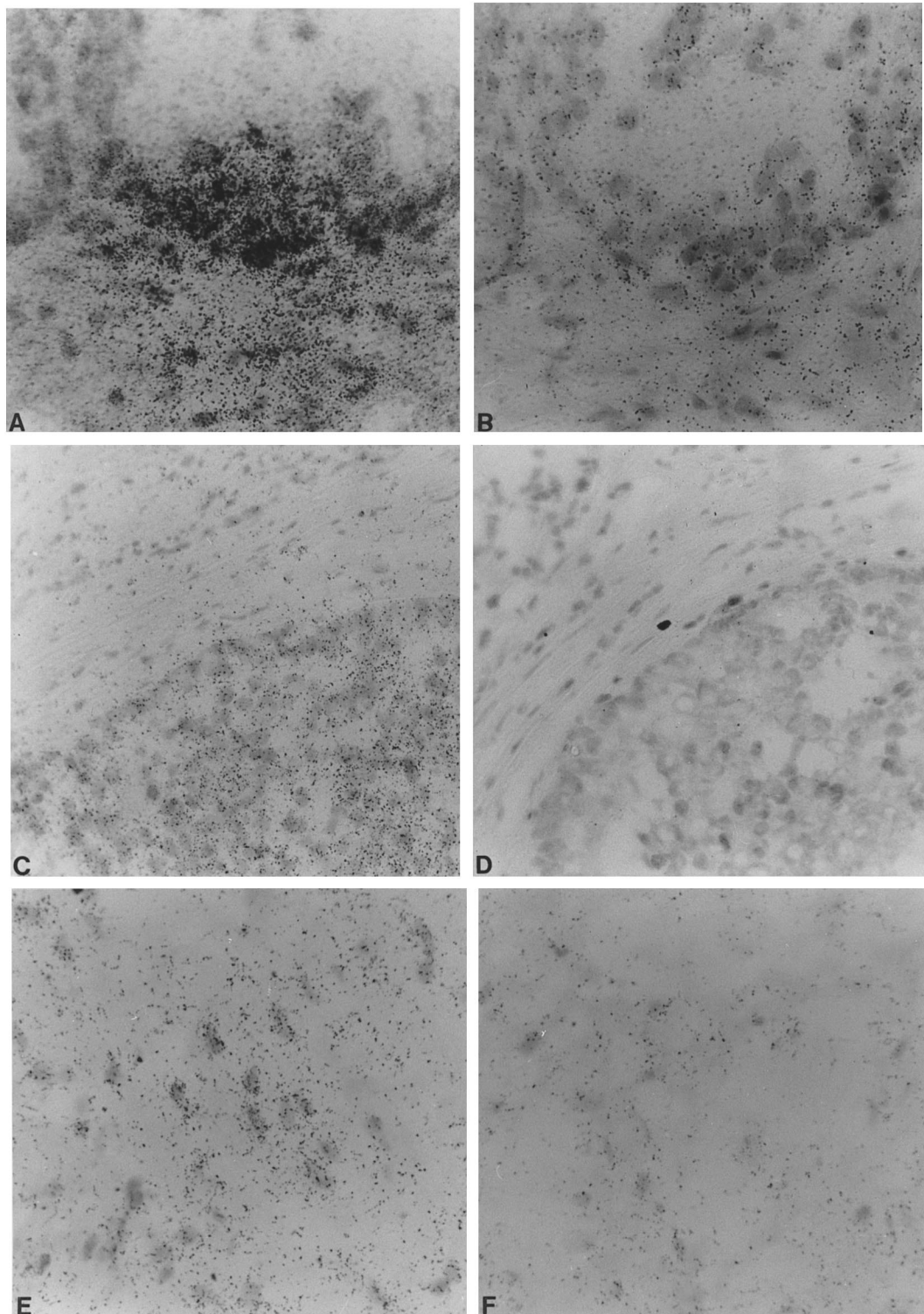
Fig. 3. Hybridisation of 5a-reductase type 1 or type 2 sense- and antisense-strand (35 S)RNA probes to prostate cancer tissues.

(A) Section from CaPl specimen hybridised with the type 1 antisense-strand probe (×400); (B) Same field on another section of the same specimen hybridised with the type 1 sense-strand probe (×400); (C) Section from CaP2 specimen hybridised with the type 2 antisense-strand probe (×250);

(D) Same field on another section of the same specimen hybridised with the type 2 sense-strand probe (×250); (E) Section from a different CaP specimen (CaP3) hybridised with the type 2 antisense-strand probe (×400); (F) Same field on another section of CaP3 specimen hybridised with the type 2 sense-strand probe (×400). Exposure time was 10 days for (A), and (B) and 25 days for (C), (D), (E) and (F).

Table 2
Cell-type-specific mRNA expression of 5α-reductase isoforms in benign prostatic hyperplasia (BPH) and prostate cancer (CaP) specimens using in situ hybridization

	5α-reductase 1		5α-reductase 2	
	Epithelium	Stroma	Epithelium	Stroma
BPH3	ND[a]	ND	+ +	−
BPH4	±	+	−	+ +
BPH5	+ +	−	±	−
BPH6	+ +	−	+	±
BPH7	−	−	−	+
BPH8	+ + +	+ +	−	+
BPH9	+ +	−	+ +	−
BPH10	+	−	−	+
BPH11	−	−	−	−
Number of positive/total PHM cases	6/8	2/8	4/9	5/9
CaP3	+	+	−	±
CaP4	+ +	−	+	−
CaP5	ND	ND	−	+
CaP6	−	−	−	−
Number of positive/total CaP cases	2/3	1/3	1/4	2/4

[a] ND: not determined.

4. Discussion

By using RT-PCR and in situ hybridisation we have found that the mRNAs of both 5α-R isoenzymes are expressed in normal, hyperplastic and malignant prostate tissue. 5α-R1 mRNA was preferentially expressed by the epithelial cells, whereas 5α-R2 mRNA was expressed by both epithelial and stromal cells.

Evidence pointing to the existence of 5α-R1 in epithelial cells was provided by our report showing, using a biochemical approach, that 5α-R1 is the isoenzyme expressed in the human prostate cell line DU145 [17]. Since this cell line is derived from a brain metastasis of an epithelial prostate cancer [23], our result implied the presence of the isoenzyme in malignant epithelium. This observation has been further confirmed and extended to another cancer cell line HPC-36 M [24].

Using polyclonal antibodies against 5α-R2 in immunohistochemical analyses of normal human prostate tissue, it has been found a strong signal in secretory epithelium (especially in basal cells), a weak staining of the stroma and no 5α-R2 mRNA in muscle [25]. Our data on 5α-R2 mRNA location are in accordance with these findings.

Although we tested at least six serial sections of each tissue specimen with sense and antisense riboprobes, one BPH and one prostate cancer sample proved to be negative for both 5α-R mRNAs. We do not yet know whether these different labelling patterns represent regional variation within a single prostate specimen or between prostates from different individuals. This could be a reason explaining the differences between our results and those recently reported by Bruchovsky et al. [16].

Quantification of the expression levels of the mRNAs indicated that both 5α-R mRNAs expression is increased in BPH tissue in comparison with normal prostate. It has been shown very recently that mRNA expression of a variety of growth factors is identical in BPH tissue and the transitional zone of normal prostate (S. Gil Diez de Medina, F. Radvanyi, D. Chopin, personal communication).

The increase observed in 5α-R isoenzymes expression could reflect one of the steps that lead to prostate cells hypertrophying. Conversely in prostate cancer tissue, 5α-Rl mRNA expression was also increased, but not 5α-R2, suggesting that 5α-R1 isoform could be involved in the neoplastic transformation of the tissue [24].

Much of the work on prostate 5α-R has been directed towards its role in the etiology of BPH and prostate cancer. In mice, mesenchyma is the inducing element in prostate development during embryogenesis and puberty [26]. In man also, the stroma appears to play the initiating role in the development of adenomatous nodules and to be the major constituent of hyperplastic tissues. However, why the prostate should have two 5α-reductase isoenzymes still remains an enigma.

References

[1] S. Andersson, D.W. Russell, Structural and biochemical properties of cloned and expressed human and rat steroid 5α-reductases, Proc. Natl. Acad. Sci. USA 87 (1990) 3640–3644.

[2] S. Andersson, D.M. Berman, E.P. Jenkins, D.W. Russell, Deletion of steroid 5α-reductase 2 gene in male pseudohermaphroditism, Nature 354 (1991) 159–161.

[3] E.P. Jenkins, S. Andersson, J. Imperato-McGinley, J.D. Wilson, D.W. Russell, Genetic and pharmacological evidence for more than one human steroid 5α-reductase, J. Clin. Invest. 89 (1992) 293–300.

[4] A.E. Thigpen, D.L. Davis, A. Milatovich, B. Mendonca, J. Imperato-McGinley, J.E. Griffin, U. Francke, J.D. Wilson, D.W. Russell, Molecular genetics of steroid 5α-reductase 2 deficiency, J. Clin. Invest. 90 (1992) 799–809.

[5] F. Labrie, Y. Sugimoto, V. Luu-The, J. Simard, Y. Lachance, D. Bachvarov, G. Leblanc, F. Durocher, N. Paquet, Structure of human type Il 5(α-reductase gene, Endocrinology 131 (1992) 1571–1573.

[6] C. Iehlé, S. Délos, O. Filhol, P.M. Martin, Baculovirus-directed expression of human prostatic steroid 5α-reductase 1 in an active form, J. Steroid Biochem. Mol. Biol. 46 (1993) 177–182.

[7] C. Iehlé, S. Délos, O. Guirou, R. Tate, J-P. Raynaud, P.M. Martin, Human prostatic steroid 5α-reductase isoforms—a comparative study of selective inhibitors, J. Steroid Biochem. Mol. Biol. 54 (1995) 273–279.

[8] E.P. Jenkins, C.L. Hsieh, A. Mllatovich, K. Normington, D.M. Berman, U. Francke, D.W. Russell, Characterization and chromosomal mapping of a human steroid 5α-reductase gene and pseudogene and mapping of the mouse homologue, Genomics 11 (1991) 1102–1112.

[9] V. Luu-The, Y. Sugimoto, L. Puy, Y. Labrie, Solache I. Lopez, M. Singh, F. Labrie, Characterization, expression and immunohistochemical localisation of 5α-reductase in human skin, J. Invest. Dermatol. 102 (1994) 221–226.

[10] P.S. Rennie, N. Bruchovsky, M.G. McLoughlin, F.H. Batzhold, E.E. Dustan-Adams, Kinetic analysis of 5α-reductase isozymes in benign prostatic hyperplasia, J. Steroid Biochem. 19 (1983) 169–173.

[11] R.W. Hudson, Comparison of nuclear 5α-reductase activities in the stromal and epithelial fractions of human prostatic tissue, J. Steroid Biochem. 26 (1987) 349–353.

[12] J.M. Le Goff, P.M. Martin, T. Ojasoo, J-P. Raynaud, Nonmichaelian behaviour of 5α-reductase in human prostate, J. Steroid Biochem. 33 (1989) 155–163.

[13] K.S. Hirsch, C.D. Jones, J.E. Audia, S. Andersson, L. McQuaid, N.B. Stamm, B.L. Neubauer, P. Pennington, R.E. Toomey, D.W. Russell, LY191704: a selective, nonsteroidal inhibitor of human steroid 5α-reductase type 1, Proc. Natl. Acad. Sci. USA 90 (1993) 5277–5281.

[14] A.E. Thigpen, R.I. Silver, J.M. Gulleyardo, M.L. Casey, J.D. McConnell, D.W. Russell, Tissue distribution and ontogeny of steroid 5α-reductase isozyme expression, J. Clinic. Invest. 92 (1993) 903–910.

[15] P. Bonnet, E. Reiter, M. Bruyninx, B. Sente, D. Dombrowicz, J. De Leval, J. Closset, G. Hennen, Benign prostatic hyperplasia and normal prostate aging: differences in types 1 and Il 5α-reductase and steroid hormone receptor messenger ribonucleic acid (mRNA) levels, but not in insulin-like growth factor mRNA levels, J. Clin. Endocrinol. Metab. 77 (1993) 1203–1208.

[16] N. Bruchovsky, M.D. Sadar, K. Akakura, S.L. Goldenberg, K. Matsuoka, P.S. Rennie, Characterisation of 5α-reductase gene expression in stroma and epithelium of human prostate, J. Steroid Biochem. Mol. Biol. 59 (1996) 397–404.

[17] S. Délos, C. Iehlé, P.M. Martin, J-P. Raynaud, Inhibition of the activity of 'basic' 5α-reductase (type 1) detected in DU145 cells and produced in insect cells, J. Steroid Biochem. Mol. Biol. 48 (1994) 347–352.

[18] M. Kaefer, J.E. Audia, N. Bruchovsky, R.L. Goode, K.C. Hsiao, I.Y. Leibovitch, J.H. Krushinski, C. Lee, C.P. Steidle, D.M. Sutkowski, B.L. Neubauer, Characterisation of type 1 5α-reductase activity in DU 145 human prostate adenocarcinoma cells, J. Steroid Biochem. Mol. Biol. 58 (1996) 195–205.

[19] L'.H Ouafik, V. May, D.W. Saffen, B.A. Eipper, Thyroid hormone regulation of peptidylglycine α-amidating monooxygenase expression in anterior pituitary gland, Mol. Endocrinol. 10 (1990) 1497–1505.

[20] J.M. Chirgwin, A.E. Przybyla, R.J. MacDonald, W.J. Rutter, Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease, Biochemistry 18 (1979) 5294–5299.

[21] M.G. Peterson, N. Tanese, B.F. Pugh, R. Tjian, Functional domains and upstream activation properties of cloned human TATA binding protein, Science 248 (1990) 1625–1630.

[22] S. Gil de Diez Medina, D. Chopin, A. El Marjou, A. Delouvée, W.J. Larochelle, A. Hoznek, C. Abbou, S.A. Aaronson, J.P. Thiery, F. Radvanyi, Decreased expression of keratinocyte growth factor receptor in a subset of human transitional cell bladder carcinomes, Oncogene 14 (1997) 323–330.

[23] K.R. Stone, D.D. Mickey, H. Wunderli, G. Mickey, D.F. Paulson, Isolation of human prostate carcinoma cell line (DU145), Int. J. Cancer 21 (1978) 274–281.

[24] C.M. Smith, S.A. Ballard, N. Worman, R. Buettner, J.R.W. Masters, 5α-Reductase expression by prostate cancer cell lines and benign prostatic hyperplasia in vitro, J. Clin. Endocrino. Metab. 81 (1996) 1361–1366.

[25] W. Eicheler, P. Tuohimaa, P. Vilja, K. Adermann, W.G. Forssmann, G. Aumüller, Immunocytochemical localisation of human 5α-reductase 2 with polyclonal antibodies in androgen target and non-target human tissues, J. Histochem. Cytochem. 42 (1994) 667–675.

[26] G.R. Cuhna, A.A. Donjacour, P.S. Cooke, S. Mee, R.M. Bigsby, S.J. Higgins, Y. Sugimura, The endocrinology and developmental biology of the prostate, Endocrinol. Rev. 8 (1987) 338–360.