Ornithine Decarboxylase Activity and Its Gene Expression Are Increased in Benign Hyperplastic Prostate

Xianxi Liu,[1]* Lei Wang,[2] Yuqin Lin,[2] Qingshan Teng,[2] Chunhua Zhao,[2] Haiyan Hu,[1] and Weiling Chi[1]

[1]*Laboratory of Molecular Biology, Shandong Medical University, Jinan, Shandong, People's Republic of China*
[2]*Department of Biochemistry, Shandong Medical University, Jinan, Shandong, People's Republic of China*

BACKGROUND. Ornithine decarboxylase (ODC) is the first key enzyme in the polyamine biosynthesis pathway. Polyamine is believed to participate in cellular proliferation and differentiation. To study the relationship between ODC and the pathogenesis of benign prostatic hyperplasia (BPH), the polyamine levels, ODC activities, and expression of ODC mRNA in benign hyperplastic and normal human prostates were assayed.
METHODS. Polyamine contents and ODC activities in tissue extracts were determined by reverse-phase high-performance liquid chromatography and spectrophotometric procedures, respectively. The ODC mRNA levels were assayed by Northern blot analysis.
RESULTS. The contents of putrescine, spermidine, and spermine in BPH tissues were 2.2, 3.4, and 6.0 times higher than those in normal tissues, respectively; the ODC activity of BPH tissue was about 3.2 times higher than in normal tissue; the expression level of ODC mRNA in the BPH tissues was greater than that of normal tissues.
CONCLUSIONS. The findings imply that 1) the increased ODC activity and polyamine content in prostatic tissue may correlate with the pathogenesis of BPH, and 2) the high level of ODC activity is induced by the overexpression of ODC mRNA. *Prostate 43:83–87, 2000.*

KEY WORDS: **ornithine decarboxylase; polyamine; gene expression; prostate; benign prostatic hyperplasia**

INTRODUCTION

The etiology of benign prostate hyperplasia (BPH) has been the subject of intense evaluation and speculation. It is widely accepted that androgens, growth factors, and epithelial-stromal cell interactions are associated with the development of BPH [1–4]. To maintain cellular content and functional activity, their biochemical and molecular mechanisms, normal or pathological, remain unknown. Ornithine decarboxylase (ODC), a rate-limiting enzyme of polyamine biosynthesis, catalyzes the synthesis of putrescine from ornithine. Putrescine is metabolized into spermidine and spermine by the addition of one or two polyamine groups, respectively [5]. Putrescine, spermidine, and spermine (termed polyamines) can stimulate the proliferation of cells [5]. ODC is a well-established indicator of tumor promotion [6], and its activity plays a critical role in cell transformation [7]. ODC activity is strikingly stimulated in response to various anabolic stimuli, such as hormones and growth factors in its appropriate target tissues [5,8]. Crozat et al. [9] reported that the ODC mRNA level and ODC activity were increased by androgens in the prostates of 3-day-

Grant sponsor: National Natural Science Foundation of China (No. 39470762).

*Correspondence to: Xianxi Liu, Laboratory of Molecular Biology, Shandong Medical University, Jinan, Shandong 250012, People's Republic of China. E-mail: mlwang@jn-public.sd.cninfo.net

Received 28 December 1998; Accepted 14 December 1999

old castrated mice and rats. In our previous studies, we reported that elevated 5α-reductase activity and accumulated dihydrotestosterone (DHT) content in benign hyperplastic tissue were possible causative factors in the pathogenesis of BPH, and we reported that human prostatic growth factor (hPGF) did increase tritiated thymindine incorporation into cultured fibroblasts [4,10]. In our present work we compared the polyamine content, ODC activity, and ODC mRNA level in BPH and normal human prostate. The data indicate that ODC activity, polyamine content, and ODC mRNA levels in BPH prostatic tissues are much higher than in normal prostatic tissues.

MATERIALS AND METHODS

Tissue Sampling

The fresh surgical specimens included 21 benign prostatic hyperplasias and 8 normal prostates from donors (20–40 years old) for kidney transplantation. Immediately after resection, the prostatic tissues were cleaned with cold PBS. All tissue specimens were frozen in liquid nitrogen and kept at −80°C until used. The histological examination of all specimens was made routinely. All BPH prostatic specimens were scored from S_{18}–S_{30} by I-PSS (International Prostate Symptom Score) criteria, and no cancer was detected in the normal specimens.

Preparation of Tissue Homogenate Supernatants

Prostatic tissue was weighed and homogenized in 10 volumes (v/w) of an extraction buffer consisting of 50 mmol/l Na_2HPO_4/NaH_2PO_4 (pH 7.2), 0.1 mmol/l 5-pyridoxal phosphate (Sigma Chemical Co., St. Louis, MO), 5 mmol/l DTT, and 1 mmol/l EDTA. After centrifugation for 20 min at 25,000*g*, the supernatants were collected for subsequent assays.

Measurement of Polyamine Content

Polyamine contents in prostatic tissue extracts were determined by reverse-phase high-performance liquid chromatography (HPLC) following precolumn derivatization with dansyl chloride [11]. The apparatus consisted of a HP1090M liquid chromatography and a HP1046A fluorescence detector. The separation was performed on a μ-Bondapak C_{18}. Excitation and emission wavelengths were 370 nm and 506 nm, respectively.

Assay of ODC Activity

Enzyme activity in tissue extracts was determined by spectrophotometric procedures [12]. One unit of enzyme activity was defined as 1 nmol putrescine produced in a 30-min incubation at 37°C. The protein concentration was measured according to the method of Lowry et al. [13].

Extraction of Total RNA

Total cellular RNA was extracted from the specimens according to previously described methods [14]. In brief, each specimen was homogenized in guanidinium isothiocyanate, and total RNA was extracted by a single-step method of RNA isolation. The concentration of RNA was measured at wavelengths of 260 nm and 280 nm, using a U-2000 spectrophotometer (Hitachi, Ltd., Tokyo, Japan).

Northern Blot Analysis

Northern blot analysis was carried out according to Sambrook et al. [15]. In brief, 30 μg of total RNA were fractionated by 1% agarose gel containing 0.66 mol/l formaldehyde, stained with ethidium bromide to assess integrity of RNA and detect differences in gel loading, transferred to a nylon membrane (Boehringer-Mannheim Co., Mannheim, Germany), and fixed at 120°C for 30 min. The blot was then prehybridized for 24 hr (at 42°C) in 50% formamide, 5 × SSC, 4 × Denhardt's solution, 0.1% sodium dodecyl sulfate (SDS), 0.1% sodium pyrophosphate, and 10 μg/ml denatured salmon sperm DNA. The membranes were hybridized in the same solution with a ^{32}P-labeled probe. Subsequently, the membranes were first washed with a high salt solution (2 × SSC, 0.1% SDS) for 30 min at 65°C and then with a low salt solution (0.2 × SSC, 0.1% SDS) for 30 min at 65°C. The membranes were subjected to autoradiography for 2 days at −70°C with an intensifying screen. The probe was pOD48 (kindly donated by Dr. Philip Coffino, University of California at San Francisco) labeled with ^{32}P-CTP by random primer translation after amplification, and linearized with *Eco*RI restriction enzyme. The autoradiographies were scanned with a dual-wavelength thin-layer chromatographic scanner (CS-930, Shimadzu Co., Kyoto, Japan) to quantify the levels of ODC mRNA.

Statistical Analysis

All numerical data were expressed as mean ± S, and statistical significance was assessed by Student's *t*-test. Results from liner recession analysis were considered statistically significant at $P < 0.05$.

TABLE I. Content of Polyamines in Homogenates from BPH and Normal Human Prostates ($\bar{x} \pm S$)†

		Putrescine		Spermidine		Spermine	
Group	n	nmol/g wet tissue	nmol/mg Pr.	nmol/g wet tissue	nmol/mg Pr.	nmol/g wet tissue	nmol/mg Pr.
Normal	8	24.14 ± 10.26	0.320 ± 0.172	4.72 ± 1.47	0.063 ± 0.026	32.98 ± 15.55	0.411 ± 0.183
BPH	21	52.86 ± 21.01*	0.489 ± 0.218	15.84 ± 4.64**	0.150 ± 0.051**	199.34 ± 67.20**	1.880 ± 0.650**

†Pr., protein.
*$P < 0.01$, compared with normal.
**$P < 0.001$, compared with normal.

RESULTS

Polyamine Content

The putrescine, spermidine, and spermine contents in human benign hyperplastic prostates were significantly higher than in normal prostates, as shown in Table I and Figure 1. The increase in spermine was the most significant.

ODC Activity

ODC activities in both BPH and normal human prostates are shown in Table II. The activity of ODC in the BPH prostate was much higher than in normal tissue.

Northern Blot Analysis

In Northern blot analysis, BPH tissues exhibited a higher expression of ODC mRNA in comparison with normal prostates (Fig. 2). The transcription levels were quantified by densitometric scanning analysis, and showed that the levels of ODC mRNA in BPH prostates were about 6–20-fold higher than in normal prostates.

DISCUSSION

It is widely accepted that androgen is associated with the development of BPH [1,2,4]. The mechanism of androgen action remains unclarified. However, there is increasing evidence that stromal-epithelial interactions play an essential role in the development and growth of the prostate, and in the pathogenesis of BPH [16]. The classic experiments of Cunha et al. [17] support the concept that a multitude of epithelial features are regulated by androgen indirectly through androgen-dependent mediators of stromal origin. Tenniswood [18] proposed the existence of prostate-derived growth factors that modulate the replicative and transcriptional processes of the prostate. Furthermore, several reports about growth factors in human

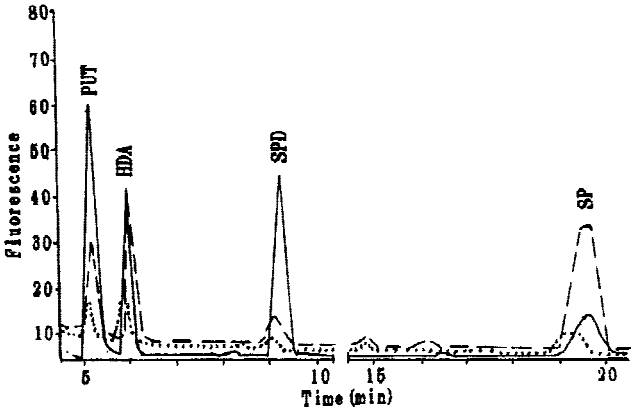

Fig. 1. Chromatogram of polyamines. Solid line, standard of polyamines; dotted line, polyamines from normal tissue; dashed line, polyamines from BPH tissue; PUT, putrescine; SPD, spermidine; SP, spermine; HD, intrinsic standard. The contents of putrescine, spermidine, and spermine in BPH tissues were 2.2, 3.4, and 6.0 times higher than in normal tissues, respectively.

TABLE II. Activity of ODC in Homogenates From BPH and Normal Human Prostate ($\bar{x} \pm S$)

		ODC activity	
Group	n	U/mg protein	U/g wet tissue
Normal	8	2.25 ± 0.59	168.05 ± 31.30
BPH	21	5.39 ± 1.24	536.27 ± 98.25
Significance		$P < 0.001$	$P < 0.01$

prostatic tissues have appeared [19,20]. Growth factors are a potent mediator of cellular proliferation, differentiation, and cell death [21]. They are involved in the action mechanism of androgen. But the biochemical and molecular mechanisms of androgen action remain unknown.

In the present study, we determined that ODC activity and its production of polyamines are contained in BPH and normal human prostates. The results indicate that ODC activity and polyamine content are

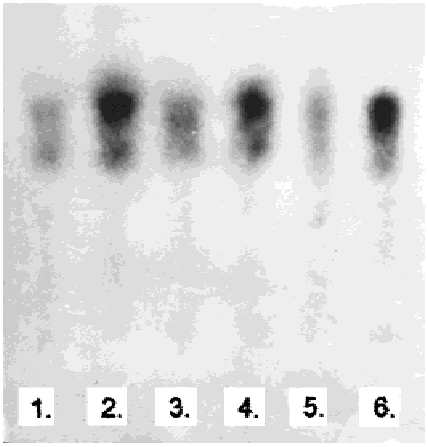

Fig. 2. Northern blot analysis of ODC mRNA isolated from normal prostates (**lanes 1, 3, 5**) and BPH prostates (**lanes 2, 4, 6**). Densitometric scanning analysis (450 nm) showed the absorbing values as 56,678, 540,918, 80,024, 397,450, 27,309, and 184,745, respectively.

much higher than in a normal prostate. These results indicate that the increasing ODC activity and polyamine content in prostatic tissues are associated with the pathogenesis of BPH. ODC and polyamines can promote DNA replication, RNA transcription, and protein biosynthesis, so that ODC and polyamines can stimulate cell growth and differentiation [8]. ODC is an androgen-dependent enzyme. Its activity is regulated by androgen, in that it occurs not only in several androgen-regulated tissues, but also in target tissues exhibiting both hyperplastic and hypertrophic responses to male sex steroids, such as rat prostate, seminal vesicles, and mouse kidney [9]. Previous studies in this and other laboratories indicated that the DHT concentration in BPH prostates is increased. These results suggest that ODC is probably the mediator of androgen-induced growth in the human prostate.

Previous studies indicated that ODC activity is regulated by androgen at multiple levels, including the rate of ODC gene transcription, stabilization of ODC mRNA species, rate of enzyme protein synthesis, and prolongation of the half-life of enzyme protein [21–23]. In the present study we demonstrated that the ODC mRNA concentration in BPH prostates was much higher than in normal human prostates (6–20-fold in Northern hybridization). This result indicated that the increase of ODC activity in BPH prostate was probably due to the increase of ODC gene expression. Regulation of ODC mRNA gene expression remains unclarified. Crozat et al. [9] reported that ODC gene expression was increased by androgen in mice and rat prostates. Eisenberg and Janne [24] reported that there was an ARE-like (Androgen Response Element) DNA motif starting at about –910 nt from the cap site in the murine ODC promoter, which was capable of binding to recombinant androgen receptor protein in vitro. These results suggest that ODC gene expression in the human prostate may be regulated by androgen. Altogether, we think that androgen stimulates the growth of the prostate partially through regulation of ODC mRNA expression. However, further studies are necessary to confirm this suspicion.

ACKNOWLEDGMENTS

Many thanks for the kindly donation of pOD48 from Dr. Philip Coffino, University of California at San Francisco, CA. We also appreciated the help from Dr. Fu Shanji, Affiliated Hospital of Shandong Medical University, China, in the work of measurement.

REFERENCES

1. Lee C, Kozlowski JM, Grayhack JT. Etiology of benign prostatic hyperplasia. Urol Clin North Am 1995;22:237–246.
2. Griffiths K, Eaton CL, Harper ME, Peeling B, Davies P. Steroid hormones and the pathogenesis of benign prostatic hyperplasia. Eur Urol [Suppl] 1991;20:68–77.
3. Bosch RJLH. Pathogenesis of benign prostatic hyperplasia. Eur Urol [Suppl] 1991;20:27–30.
4. Luo D, Lin Y, Liu X, Qin Z, Zhao C, Zhang Y, Yu Z. Effect of prostatic growth factor, basic fibroblast growth factor, epidermal growth factor, and steroids on the proliferation of human fetal prostatic fibroblast. Prostate 1996;28:352–358.
5. Pegg AE. Recent advances in the biochemistry of polyamines in eukaryotes. Biochem J 1986;234:249–262.
6. O'Brien TG, Simsiman RC, Boutwel RK. Induction of the polyamine biosynthetic enzymes in mouse epidermis and their specificity for tumor promotion. Cancer Res 1975;35:2426–2433.
7. Auvinen M, Dasinen A, Andeersson LC, Holtte E. Ornithine decarboxylase activity is critical for cell transformation. Nature 1992;360:355–358.
8. Pegg AE, McCann PP. Polyamine metabolism and function. Am J Physiol 1982;243:212–221.
9. Crozat A, Palvimo J, Julkunen M, Janne OA. Comparison of androgen regulation of ornithine decarboxylase and s-adenosylmethione decarboxylase gene expression in rodent kidney and accessory sex organs. Endocrinology 1992;130:1131–1144.
10. Lin Y, Cui X, Luo D, Yao Z, Liu X. The 5α-reductase activity and DHT content in normal and hyperplastic human prostate. Natl Med J China 1991;71:263–266.
11. Bowlin TL, Mckown BJ, Sunkara PS. Ornithine decarboxylase induction and polyamine biosynthesis are required for the growth of interleukin-2 and interleukin-3 dependent cell lines. Cell Immunol 1986;98:341–350.
12. Ngo TT, Brillhart KL, Dowis RH. Spectrophotometric assay for ornithine decarboxylase. Anal Biochem 1987;160:290–294.
13. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the folinphenol reagent. J Biol Chem 1951;193:265–275.
14. Chomczynski P, Sacchi N. Single step method of RNA isolation by acid guanidinum thiocyanate phenol chloroform extraction. Anal Chem 1987;162:156–159.

15. Sambrook KJ, Fritsch EF, Maniatis T. Molecular cloning, second edition. 1989. p 7.35–7.55.
16. Coffey DS, Pienta KJ. New concepts in studying the control of normal and cancer growth of prostate. Prog Clin Biol Res 1987; 239:1–73.
17. Cunha GR, Donjacour AA, Cooke PS, Mu S, Bigsby RM, Higgin SJ, Sugimura Y. The endocrinology and developmental biology of the prostate. Endocr Rev 1987;8:338–362.
18. Tenniswood M. Epithelial-stromal interactions in the control of gene expression in the prostate. Prostate 1986;9:375–385.
19. Nishi N, Matuo Y, Kunitami K, Talenaka I, Usami M, Kotako T, Wada F. Comparative analysis of growth factors in normal and pathologic human prostate. Prostate 1988;13:39–48.
20. Sherwood ER, Fong CJ, Lee C, Kozlowski TM. Basic fibroblast growth factor: a potential mediator of stromal growth in the human prostate. Endocrinology 1992;130:2955–2963.
21. Pegg AE. Polyamine metabolism and its importance in neoplastic growth and as a target for chemotherapy. Cancer Res 1988; 48:759–774.
22. Heby O, Persson L. Molecular genetics of polyamines synthesis in eukaryotic cells. Trends Biochem Sci 1990;15:153–158.
23. Wallon VM. Regulation of ornithine decarboxylase during cell growth, changes in the stability and transcription of the mRNA, and in the turnover of the protein. Mol Cell Biochem 1995;146: 39–44.
24. Eisenberg LM, Janne OA. Nucleotide sequence of the 5′-flanking region of the murine ornithine decarboxylase gene. Nucleic Acids Res 1989;17:2359–2360.