

## Effect of epristeride on expression of TGF- $\beta$ receptor in rat prostatic epithelial cells *in vitro*

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**KEY WORDS** prostatic hyperplasia; epristeride; rats; transforming growth factor beta receptors; reverse transcriptase polymerase chain reaction; Western blotting

### ABSTRACT

**AIM:** To assess the effect of epristeride on the expression of transforming growth factor  $\beta$  type II receptor (T $\beta$ R II) in rat prostatic epithelial cells *in vitro*.

**METHODS:** RT-PCR and Western blot were used to quantitatively detect the mRNA and protein expressions of T $\beta$ R II in rat prostatic epithelial cells treated or untreated with epristeride. Immunocytochemical staining method was used to qualitatively analyze the expression of T $\beta$ R II protein. **RESULTS:** After treatment with epristeride 180 or 360 nmol/L, T $\beta$ R II mRNA expression levels were  $0.56 \pm 0.08$  and  $0.59 \pm 0.07$ , respectively, which were significantly up-regulated compared with control cells ( $0.38 \pm 0.04$ ,  $P < 0.05$ ); expression level of T $\beta$ R II protein were  $3163 \pm 920$  and  $6769 \pm 1941$ , respectively, which were also markedly up-regulated compared with control cells ( $536 \pm 240$ ,  $P < 0.05$ ). Immunostaining showed weak positive reaction in control cells, while strong staining of T $\beta$ R II was found in epristeride-treated cells. **CONCLUSION:** Epristeride may up-regulate the expression of T $\beta$ R II to induce apoptosis of prostatic epithelial cells.

### INTRODUCTION

Development of benign prostatic hyperplasia (BPH) appears to be dependent on the conversion of testosterone to dihydrotestosterone (DHT), which is enzymatically

mediated by a steroid 5 $\alpha$ -reductase<sup>[1]</sup>. Epristeride (SK and F105657) is a novel uncompetitive 5 $\alpha$ -reductase inhibitor, which can reduce the prostate growth by inhibiting the conversion of testosterone to dihydrotestosterone<sup>[2]</sup>. Although epristeride is shown to decrease prostate size and intraprostatic DHT level<sup>[3]</sup>, its molecular mechanisms underlying growth inhibition induced by epristeride are incompletely described.

Our previous study demonstrated that epristeride induced prostatic atrophy histologically in experimental models and reduced the number of prostatic epithelial cells via an apoptotic mechanism<sup>[3]</sup>. The transforming growth factor beta (TGF- $\beta$ ) family is composed of a series of isoforms, which regulates very distinct cellular functions, including proliferation and differentiation<sup>[4]</sup>. TGF- $\beta$  has been detected in normal and abnormal human prostate<sup>[5]</sup>. TGF- $\beta$ 1 seems to be a potent inhibitor of prostatic epithelial cell proliferation and has been shown to mediate apoptosis of epithelial cells *in vitro*<sup>[6]</sup>. Signaling by TGF- $\beta$  is dependent on binding to cell surface receptors, three of which designated as T $\beta$ R I, II, and III have been cloned. In a proposed model, TGF- $\beta$  binds to the type II receptor, and then the type I receptor is recruited and phosphorylated by the type II receptor. It has also been demonstrated that castration-induced involution in the rat prostate was accompanied by an increase in TGF- $\beta$  expression, and administration of TGF- $\beta$ 1 can induce programmed cell death in the rat prostate<sup>[7]</sup>. These results have established an important role of TGF- $\beta$  in programmed cell death in the rat prostate.

To investigate whether epristeride-induced atrophic changes in BPH were mediated by TGF- $\beta$ , A semi-quantitative RT-PCR was established to assess the expression of T $\beta$ R II mRNA in cultured primary prostatic epithelial cells. Protein level was detected quantitatively by Western blot and immunocytochemistry.

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## MATERIALS AND METHODS

**Drug and reagents** Epristeride was provided by Yangzhou Pharmaceutical Factory, China. All primary antibodies used in the experiment were purchased from Santa Cruz Biotech Inc (California, CA, USA).

### Rat ventral prostatic epithelial cell culture

Primary culture of rat prostatic epithelial cells was carried out as described previously<sup>[8]</sup>. Cells were cultured in 2 mL of a medium consisting of RPMI-1640 (GIBCO) supplemented with 10 % bovine calf serum, glutamine 2 mmol/L, benzylpenicillin 100 kU/L, streptomycin 100 mg/L, epidermal growth factor (EGF) 10  $\mu$ g/L, cholera toxin 10  $\mu$ g/L, and transferrin 5 mg/L. Cultures were incubated in a humidified atmosphere of 5 % carbon dioxide and 95 % air at 37 °C. On the 12th day, cells were harvested and the following tests were carried out.

**Reverse transcription polymerase chain reaction (RT-PCR)** Cultured prostatic cells were plated at a density of  $1 \times 10^5$  cells in 25-cm<sup>2</sup> culture flasks containing serum-free RPMI-1640 for 24 h. Then cells were treated with two different concentrations of epristeride (180 and 360 nmol/L) for 72 h, untreated cells were used as control. Cells were washed and further incubated for 24 h in drug- and serum-free culture medium. All the treated and control groups were harvested with trypsin/edetic acid (0.05 %/0.01  $\mu$ mol  $\cdot$  L<sup>-1</sup>), centrifuged at 3500  $\times$  g for 5 min. The supernatants were discarded and cells were washed twice with PBS (in mmol/L: Na<sub>2</sub>HPO<sub>4</sub> 8.1, NaH<sub>2</sub>PO<sub>4</sub> 1.9, and NaCl 0.145; pH 7.4). Total RNA from the various fresh cell pellets was immediately extracted by TRIzol (GIBCO). PCR primers were as following: T $\beta$ R II, 5'-GGTCAT-TATCCAAGTGACGG-3' (sense), 5'-CAGCTTGCC-TTGTAGACCT-3' (antisense), amplicon 303 bp;  $\beta$ -actin, 5'-CCTCTATGCCAACACAGTGC-3' (sense), 5'-GTACTCCTGCTTGCTGATCC-3' (antisense), amplicon 211 bp. Oligonucleotide primers specific to T $\beta$ R II mRNA were designed from known genomic and cDNA information<sup>[9]</sup>. Synthesis of cDNA was carried out with reverse transcriptase (M-MLV, GIBCO) from 1  $\mu$ g of total RNA. Reverse-transcribed single strand cDNA 2  $\mu$ L was subjected to 25 cycles of PCR in 50  $\mu$ L of buffer [Tris-Cl 10 mmol/L (pH 8.3), KCl 50 mmol/L, MgCl<sub>2</sub> 1.5 mmol/L, dNTPs 50  $\mu$ mol/L, Thermus aquaticus polymerase 2.5 U (Sigma), and 0.2  $\mu$ mol/L of specific primers for T $\beta$ R II and  $\beta$ -actin].

Experiment was performed using known positive

control for T $\beta$ R II (rat kidney) to determine the optimal number of cycles of PCR to which cDNA products should be co-amplified,  $\beta$ -actin was amplified as an internal standard on all of the samples simultaneously for quantitation. Each cycle consisted of denaturation at 94 °C for 1 min, annealing at 55 °C for 1 min, and extension at 72 °C for 5 min. PCR products were separated by electrophoresis on 3 % agarose gel. Relative ethidium bromide-stained band intensities were assessed by densitometry (Molecular Dynamics, ImageQuant Software, Sunyvale, CA). Each RT-PCR was repeated thrice using different preparation of RNA.

**Western blot** Total protein was obtained from cultured prostate epithelial cells. Rat prostate epithelial cells  $1 \times 10^5$  were cultured in the presence or absence of epristeride (180 or 360 nmol/L) in serum-free medium for 72 h, then harvested and lysed with 100  $\mu$ L ice-cold lysis buffer (Tris-Cl 20 mmol/L, pH 7.4, NaCl 50 mmol/L, edetic acid 5 mmol/L, NaF 50 mmol/L, sodium pyrophosphate 30 mmol/L, NaVO<sub>4</sub> 100  $\mu$ mol/L, PMSF 1 mmol/L, aprotinin 10 mg/L, leupeptin 10 mg/L, and 1 % of Triton X-100) at 4 °C for 30 min, and ultracentrifuged at 100 000  $\times$  g, 4 °C for 45 min. The protein in the supernatant was mixed with sample buffer [Tris-Cl 50 mmol/L, pH 6.8, DTT 10 mmol/L, 2 % SDS, 0.1 % bromophenol blue, 10 % glycerol], fractionated by 7.5 % SDS-polyacrylamide gel, and electrotransferred onto a nitrocellulose membrane. The membrane was probed consecutively with anti-T $\beta$ R II rabbit polyclonal antibody (dilution 1 : 500) and horseradish peroxidase-conjugated anti-rabbit IgG. The blots were visualized by enhanced chemiluminescence (ECL, Amersham Pharmacia Biotech). The densities of the immunoblots were quantitated with an image analyzer.

**Immunocytochemical assay of T $\beta$ R I protein expression** After a 72 h treatment with epristeride, cells ( $1.0 \times 10^4$ /well) were collected and smeared on the slides precoated with 0.01 % poly-L-lysine (Sigma). The T $\beta$ R II immunoreactivity was detected by the ABC (avidin-biotin-peroxidase complex) method. The primary antibody used was anti-T $\beta$ R II rabbit polyclonal antibody (Santa Cruz Biotech Inc) (1 : 150) and secondary antibody used was a biotinylated-antibody goat anti-rabbit IgG (Huamei Biotech, China) (1 : 200). The experiment was performed at least three times and representative photos were taken by Olympus System (Japan).

**Statistical analysis** Statistical analysis was per-



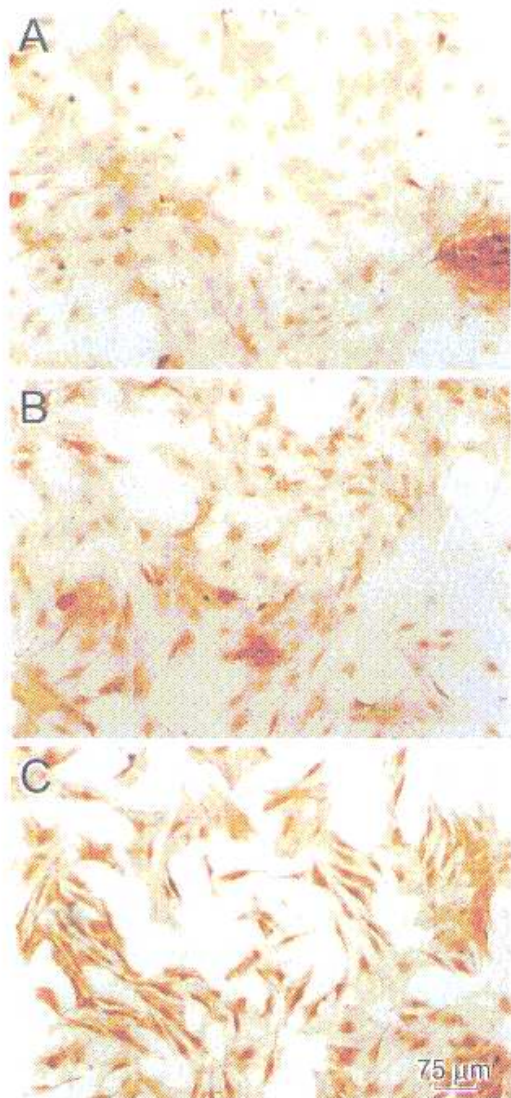


Fig 3. Immunocytochemical detection of T $\beta$ RII expression in prostatic epithelial cells. (A) untreated; (B) treated with episteride 180 nmol/L; (C) episteride 360 nmol/L.  $\times 133$ .

have been reported<sup>[3]</sup>. However, molecular mechanisms associated with its growth-inhibitory effect are not clear. Concentrations of episteride used in this experiment were determined according to our preliminary experiment results, which showed that these two concentrations were equal to the intraprostatic concentration of episteride when SD rats were treated with episteride by gavage at minimal effective doses 3 and 6 mg/kg.

Apoptosis has been proposed as a mechanism to ex-

plain involution in androgen-deprived prostates. In rats, castration triggers apoptosis in epithelial and stromal cells throughout the ventral lobe<sup>[10,11]</sup>. The TGF- $\beta$  signaling system plays an established role in apoptosis in different transformed and non-transformed epithelial cells<sup>[12]</sup> and also in the stroma<sup>[13]</sup>. TGF- $\beta$  is a potent inhibitor of prostatic epithelial cells and it induces epithelial apoptosis<sup>[14,15]</sup>. TGF- $\beta$  receptors are predominantly expressed in prostatic epithelial cells. Undoubtedly, the TGF- $\beta$  system is regulated by androgens. Castration increases the levels of TGF- $\beta$  mRNA, which return to normal after androgen replacement. TGF- $\beta$  receptors are also up-regulated by castration. The findings that the T $\beta$ RII is under negative androgen control is also consistent with the proposed role of TGF- $\beta$  as a growth inhibiting factor in normal prostate<sup>[7]</sup>. It had been also reported that T $\beta$ RI and T $\beta$ RII were up-regulated and localized in the glands undergoing involution after treated with finasteride. The up-regulation of these receptors could be one of the earliest changes observed in the responsive secretory cell as a result of inhibitory signal<sup>[16]</sup>. Our observation of a marked increase both in T $\beta$ RII mRNA level and in protein level in epithelial cells treated with episteride 360 nmol/L is a further confirmation of a mediation of episteride action by the TGF- $\beta$  pathway.

In summary, our data demonstrated that episteride could up-regulate T $\beta$ RII mRNA and protein expression and episteride-induced apoptosis might be correlated with up-regulation of T $\beta$ RII. These results provide further understanding of the mechanism of episteride against BPH.

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finasteride-treated human hyperplastic prostates correlate with an up-regulation of TGF- $\beta$  receptor expression. *Prostate* 1998; 37: 84-90.

### 爱普列特对体外大鼠前列腺上皮细胞 TGF- $\beta$ 受体表达的影响

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**关键词** 前列腺增生; 爱普列特; 大鼠; 转化生长因子- $\beta$  受体; 逆转录聚合酶链反应; 蛋白质印迹

**目的:** 研究爱普列特对体外培养前列腺上皮细胞转化生长因子 $\beta$ 受体II(T $\beta$ RII)表达的影响。 **方法:** 逆转录PCR法及Western blot定量检测体外培养前列腺上皮细胞T $\beta$ RII mRNA及蛋白表达, 免疫组织化学染色定性分析爱普列特对前列腺上皮细胞T $\beta$ RII表达的影响。 **结果:** T $\beta$ RII mRNA表达水平分别 $0.56 \pm 0.08$ 和 $0.59 \pm 0.07$ , 与对照组细胞( $0.38 \pm 0.04$ ,  $P < 0.05$ )相比明显上调; T $\beta$ RII蛋白的表达水平分别为 $3163 \pm 920$ 和 $6769 \pm 1941$ , 与对照组细胞( $536 \pm 240$ ,  $P < 0.05$ )相比明显上调; 免疫组化结果表明未经爱普列特处理的上皮细胞T $\beta$ RII表达为弱阳性, 经爱普列特处理后的上皮细胞T $\beta$ RII表达明显增强。 **结论:** 爱普列特诱导前列腺上皮细胞凋亡可能与上调T $\beta$ RII表达有关。

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