

Androgen-modulated *p21* and *p53* gene expression in human non-transformed epithelial prostatic cells in primary cultures

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Received May 4, 2012; Accepted July 13, 2012

DOI: 10.3892/ijmm.2012.1082

Abstract. The prostate gland is under androgen control. The aim of the present study was to evaluate the expression of two genes that are regulators of the cell cycle, the *p53* and *p21* genes, in human non-transformed epithelial prostatic cells (HNTEPs) treated with different concentrations of hormones. Samples of prostate tissue were obtained from 10 patients between 60 and 77 years of age. HNTEP cells were grown in basal medium and treated with dihydrotestosterone (DHT) in different conditions for 4 h. A low concentration of DHT resulted in a significant increase in cell growth; this effect was eradicated by addition of the antiandrogen hydroxyflutamide. Furthermore, the low concentration of DHT induced lower mRNA levels in the *p53* and *p21* genes in HNTEP cells. In turn, high DHT concentrations induced a significant increase in the expression of the *p53* and *p21* genes. The present data suggest that the *p53* and *p21* genes play a role in the control of responsiveness and androgen dose-dependent cell proliferation in HNTEP cells. Further studies are required to assess the intracellular signaling pathway regulated by *p53* and *p21* under the influence of androgens and its implications for the pathophysiology of prostate diseases.

Introduction

Androgens are the main regulators of prostate growth and differentiation. The proliferation mechanisms activated by androgens involve transcription factors that operate together to maintain the balance between inhibition and cell prostate proliferation (1-3). The androgen receptor (AR) is expressed in normal prostate epithelial cells, in almost all primary prostate cancer cells and in most refractory prostate cancer cells (4). Androgen concentrations and actions are determinant for prostate enlargement and are dependent on AR activity (5,6). A cell line derived from a mouse xenograft

prostate carcinoma (PCa), CWR22R3, maintains a high level of AR expression. These cells were infected with retroviruses encoding AR-specific short hairpin RNA (shRNA), and AR downregulation was demonstrated by anti-AR immunoblotting; also, the cells expressing AR shRNA showed reduced cell density (6). Finally, blocking AR activity is a therapeutic approach for benign prostate hyperplasia (BPH) and PCa (7,8).

While the stimulatory effect of androgens on *in vivo* growth and development is well recognized in several animal models and in human prostate, the *in vitro* characterization of these effects is a complex issue. There have been reports on the effects of androgens on the growth of normal epithelial prostate cells (9), on stromal cells of BPH tissue (3) and on AR expression (7,8,10,11). By contrast, other studies were not able to show any effect of androgens, at different concentrations, on the cell proliferation of normal, hyperplastic or tumoral prostate cells (12-14). The fact that such studies were carried out on immortalized or transformed cells might be related to the variation in the results.

Studies using the LNCaP and MOP androgen-dependent prostate epithelial cancer cell lines have demonstrated a biphasic effect of androgens on proliferation, in which lower androgen concentrations have a maximum effect on cell proliferation (1,15-17). In addition, androgen shutoff genes (AS1, AS2 and AS3) have been demonstrated in LNCaP cells, and their gene expression was reported to be induced by higher concentrations of DHT (10^{-9} M) in the culture medium (18). We have also recently shown a stimulatory effect of androgens at low concentrations on cell proliferation of human non-transformed epithelial prostatic cells (HNTEP cells). This effect was eradicated by the addition of hydroxyflutamide (OH-Flutamide), an AR inhibitor (19). Moreover, this biphasic effect on HNTEP cell proliferation was associated with changes in mRNA levels of *c-myc* (19), *c-fos* and *c-jun* (20).

Molecular mechanisms regulating the cell cycle are key processes in the proliferation and differentiation of a target tissue. These include both apoptosis and cell progression through the cell cycle. Apoptosis is a genetically regulated process, which requires the expression and action of gene products and coregulators in order to occur (21). Progression through the G1-phase cell cycle is associated with cyclins and cyclin-dependent kinase (CDK) complexes. This effect depends on several factors including the levels of CDK inhibitors (CDKIs) (22). The *p21* gene is a CDKI member of the CIP/KIP

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Key words: androgen, *p53*, *p21*, prostate, proliferation

family that includes *p27* and *p57*. *p21* prevents DNA synthesis and leads to growth arrest (23,24). The expression of the *p21* gene in prostate cells can be activated through growth factors (25), androgens (26,27) and *p53*, a suppressor gene (28).

p53 is a tumor-suppressor gene that regulates the expression of genes involved in cell cycle inhibition, apoptosis and genomic stability. The *p53* gene is the most commonly mutated or deleted gene in human cancer (28). Higher levels of mutated *p53* were found in prostate intraepithelial neoplasia (PIN), further implicating *p53* mutation or loss as an early event in prostate tumorigenesis (29). A recent study showed a decrease in *p53* mRNA levels after 8 h of treatment with dihydrotestosterone in a prostate cell line (LNCaP) compared with an untreated group. They also found 4 potential AREs in the *p53* gene: 1 in the 5' flanking region, 1 in the first exon and 2 sites in the first intron (30).

Although *p21* and *p53* appear to be associated with abnormal growth of prostate epithelial cells under the influence of androgens (26,27,30,31), their involvement in the biphasic effect of androgens on prostate cell proliferation has yet to be clearly defined. Therefore, the aim of the present study was to determine the effect of androgen at different concentrations on *p21* and *p53* gene expression in HNTEP cells.

Materials and methods

Cell culture. Samples of prostate tissue were obtained from retropubic prostatectomies of 10 patients between 60 and 77 years of age, diagnosed with BPH. Patients with malignant tumors were excluded. The local Ethics Committee approved the study protocol. Informed consent was obtained from all subjects.

HNTEP cells were cultured as previously described (32). Briefly, after removal of blood clots, prostate tissue was washed with Hank's balanced salt solution (HBSS; Gibco-BRL, Grand Island, NY, USA) plus kanamycin (0.5 mg/ml) (Sigma Chemical Co., St. Louis, MO, USA), and then finely minced into 2-3 mm pieces. Tissue fragments were treated with collagenase type IA (7.5 mg/g of tissue) (Sigma Chemical Co.) in HBSS. Enzymatic digestion proceeded for 3 h at 37°C with gentle shaking. The enzymatic reaction was interrupted by the addition of warm 199 culture medium (Gibco-BRL) plus kanamycin and 10% fetal bovine serum (FBS) (Gibco-BRL).

Epithelial cells were separated by differential filtration. Cell suspensions were distributed into 35-mm tissue-culture dishes (Corning Glassworks, NY, USA), 1×10^5 cells/dish, or into 24-well tissue-culture plates (NUNC™, Denmark), 2×10^4 cells/ml/plate, and maintained at 37°C in a humidified atmosphere of 95% air/5% CO₂ (NuAire, Inc., Plymouth, MN, USA). In addition, since it is difficult to observe the stimulatory effect of androgen on cell growth *in vitro*, a culture medium that was free of growth factors (such as insulin or EGF) other than those present in FBS was used. The basal medium consisted of medium 199 containing kanamycin (0.5 mg/ml) enriched with 5% charcoal-stripped FBS (cFBS). Cultures were kept in the same medium for the first 2 days, and then the medium was changed every 2 days.

Cell proliferation. MTT colorimetric assay was used to study cell proliferation. The MTT assay is based on the reduction

of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; Sigma Chemical Co.) to a blue formazan product by mitochondrial dehydrogenase found in viable cells (33). Epithelial cells (2×10^4 cells/ml) were allowed to adhere overnight in 24-well tissue-culture plates in basal medium. On the following day, designated Day 0, the basal medium was removed and the experimental medium was applied. The experimental medium consisted of basal medium and treatments with DHT (10^{-8} , 10^{-10} and 10^{-13} M) alone or combined with hydroxyflutamide (OH-FLU) at 10^{-6} M or ethanol vehicle (control medium). Cells were cultured under routine conditions, and the medium was changed on Day 2. On Day 6, 50 μ l of 5 mg/ml MTT in PBS was added to each well. After 4 h of incubation at 37°C, the medium was removed, 100 μ l of DMSO was added, and optical density was measured at 540 nm in an ELISA reader (Benchmark Microplate Reader). The data represent the mean of 3-6 wells of each treatment. Experiments were repeated at least three times, using samples from different patients.

Extraction of RNA and synthesis of cDNA. Cells were grown in basal medium deprived of serum for 4 h, and then treated with DHT or ethanol vehicle in different conditions for 4 h. Subsequently, the mRNA was extracted. The extraction of RNA and the synthesis of cDNA were carried out as previously described (19). Prostatic cells in culture were washed twice with PBS and homogenized in phenol-guanidine isothiocyanate (TRIzol; Invitrogen, Carlsbad, CA, USA). Total-RNA was extracted with chloroform and precipitated with isopropanol by 12,000 x g centrifugation at 4°C. The RNA pellet was washed twice with 75% ethanol, resuspended in diethylpyrocarbonate-treated water, and quantified by light absorbance at 260 nm. First-strand cDNA was synthesized from 1 μ g total-RNA, using the SuperScript Preamplification System (Invitrogen). After denaturing the template RNA and primers at 65°C for 5 min, reverse transcriptase was added in the presence of 20 mM Tris-HCl (pH 8.4) plus 50 mM KCl, 2.5 mM MgCl₂, 0.5 mM dNTP mix and 10 mM dithiothreitol, and incubated at 42°C for 50 min. The mixture was heated at 70°C to interrupt the reaction, and then incubated with *E. coli* RNase for 20 min at 37°C to destroy the untranscribed RNA.

Real-time PCR conditions. Amplification and detection were performed with the MiniOpticon Real-Time PCR detection system (Bio-Rad Life Science Research, Hercules, CA, USA). Duplicate samples were used. The PCR mixture contained 1.25 μ l SYBR-Green, 2 ng cDNA at 1:50 dilution, 3 mM MgCl₂, 20 mM Tris-HCl pH 8.4 plus 50 mM KCl, 0.2 mM dNTP mix, 1 unit TaqDNA polymerase and 0.4 μ M of each primer in a 25- μ l final volume. The reaction conditions were 94°C for 2 min for hot-start, and 39 cycles of 94°C for 50 sec, 59°C for 30 sec and 72°C for 40 sec for the *p53* gene; and 94°C for 50 sec, 57°C for 30 sec and 72°C for 40 sec for the *p21* gene. The sequences of primers employed were: *p53* gene, sense, 5'-CTGAGGTTGGCTCTGACTGTACCA-3' and antisense, 5'-CTCATTACAGCTCTCGGAACATCTC-3' (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi); *p21* gene, sense, 5'-CTCAG7AGGAGGCGCCATG 3' and antisense, 5'-GGGCGGATTAGGGCTTCC-3' (25). For normalization of the expression levels, the expression of β 2-microglobulin

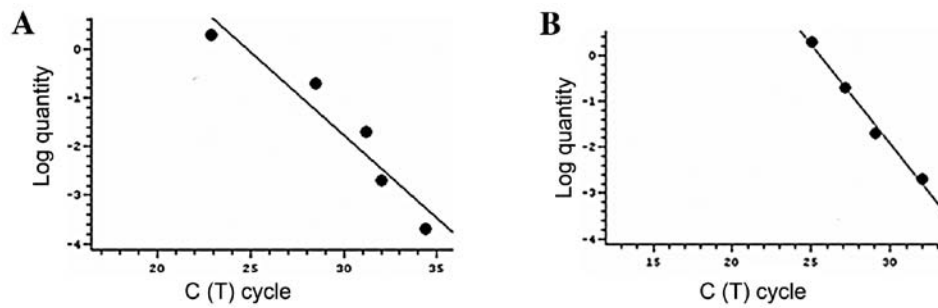


Figure 1. Standard curves of real-time PCR for the (A) *p53* and (B) *p21* genes performed on a dilution series of cDNA standard. Real-time PCR assay was analyzed in the linear phase and a fit linear function of the log of relative fluorescence vs. cycle number with a typical R^2 value >0.9 .

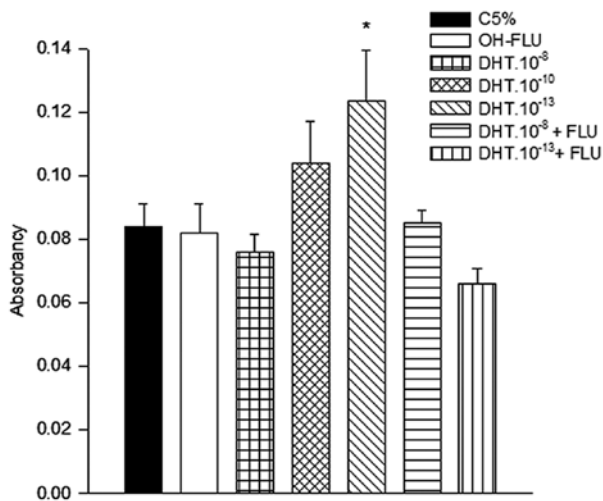


Figure 2. Effect of dihydrotestosterone (DHT) on primary culture of HNTEP cells. Cells were grown in medium 199 with 5% charcoal-stripped fetal bovine serum supplemented with or without antiandrogen hydroxyflutamide (OH-FLU) or with DHT. 10^{-13} , 10^{-10} or 10^{-8} M alone or with OH-FLU. Cell proliferation was studied by MTT colorimetric assay on Day 6. Each bar represents the mean \pm SEM of 6 separate experiments performed in 9-19 wells each. For statistical analysis, ANOVA followed by Duncan's test was used. * $P < 0.05$ vs. control group (C), OH-FLU, 10^{-8} and DHT. 10^{-13} + FLU.

(sense, 5'-ATCCAGCGTACTCCAAAGATTCAG-3' and antisense, 5'-AAATTGAAAGTTAAGTATGCACGC-3' (34) was used as a housekeeping gene.

Standard curves and efficiency. All samples were automatically processed for melting-curve analysis of amplified cDNA. The melting temperature (T_m) is specific to each amplicon. The melting temperature for *p53* was 89°C , for *p21* 93°C and for β 2-microglobulin 83°C . Standard curves were constructed by plotting the C_T (cycle threshold) values of the real-time PCR performed on a dilution series of cDNA standard. The real-time PCR assay was analyzed in the linear phase, and a linear function was fitted of the log of relative fluorescence vs. cycle number with a typical R^2 value >0.9 (Fig. 1). The results of the gene expression corrected by the housekeeping gene were expressed as the proportional change (1-, 2- or 3-fold) in relation to the control group.

Western blotting. Protein samples of HNTEP cells in culture were obtained with TRIzol reagent (Invitrogen) following the

manufacturer's protocol. The protein concentration was determined by the Bradford method (35). Sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis was carried out using a miniprotein system (Bio-Rad Life Science Research) with broad-range molecular weight standards. Total protein of $6.5 \mu\text{g}$ was loaded onto each lane with a loading buffer containing 0.375 M Tris (pH 6.8), 50% glycerol, 12% SDS, 0.5 M dithiothreitol and 0.002% bromophenol blue. Samples were heated at 100°C for 3 min prior to gel loading. Following electrophoresis, proteins were transferred to nitrocellulose membranes (Schleicher and Schuell) using an electrophoresis transfer system (mini Trans-Blot Electrophoretic Transfer Cell) at 110 V for 1-2 h. The membranes were then washed with TTBS (20 mM Tris-HCl, pH 7.5; 150 mM NaCl; 0.05% Tween-20, pH 7.4) and 8% non-fat dry milk for 90 min. The membranes were incubated overnight at 4°C with the primary antibody (monoclonal anti-p21 antibody and monoclonal anti-p53) (Upstate Biotechnology) diluted in TTBS. After washing, the membranes were incubated for 2 h at room temperature with secondary antibody (rabbit anti-mouse IgG conjugate 1:3,000 (Bio-Rad Life Science Research), washed with TBS (20 mM Tris-HCl; 150 mM NaCl, pH 7.5), and developed with the chemoluminescence ECL Western Blotting system (Amersham) followed by apposition of the membranes to autoradiographic film (Kodak X-Omat). The optical density (OD) of the bands was analyzed using an image-processing system (Image Master VDS; Pharmacia Biotech, Uppsala, Sweden). Ponceau S staining was used as a protein loading control for p21 and β -tubulin (1:10,000) for p53.

Statistical analysis. Data are reported as means and standard error of the mean (SEM). Differences between groups were assessed by analysis of variance, followed by Duncan's test. All analyses were performed using the Statistical Package for Social Sciences (SPSS, Chicago, IL, USA). Data were considered to indicate statistically significant differences at $P < 0.05$.

Results

The effect of DHT on the growth of HNTEP cells was examined on Day 6 of culture. Data were compared to those of cells treated with control medium. Cells treated with DHT at a concentration of 10^{-13} M showed a higher proliferation rate ($P < 0.05$) compared to cells from the other groups: DHT. 10^{-8} M, control medium, OH-FLU or DHT. 10^{-13} M combined with OH-FLU (Fig. 2).

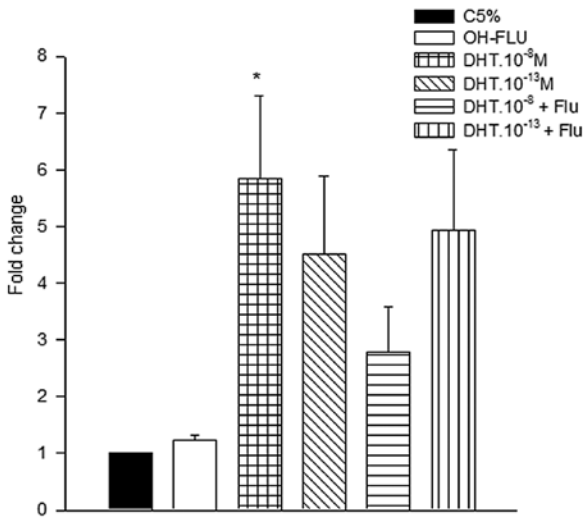


Figure 3. *p53* mRNA quantitative results by real-time PCR. The graphic represents the fluorescence change in the relative expression of *p53* in HNTep cells treated with hydroxiflutamide (OH-FLU) at 10⁻⁶ M or dihydrotestosterone at 10⁻⁸ and 10⁻¹³ M isolated or combined with OH-FLU. Comparisons between groups were analyzed by ANOVA followed by Duncan's test. *P<0.05 vs. control group, OH-FLU.10⁻⁶, DHT.10⁻¹³ and DHT.10⁻⁸ + OH-FLU.

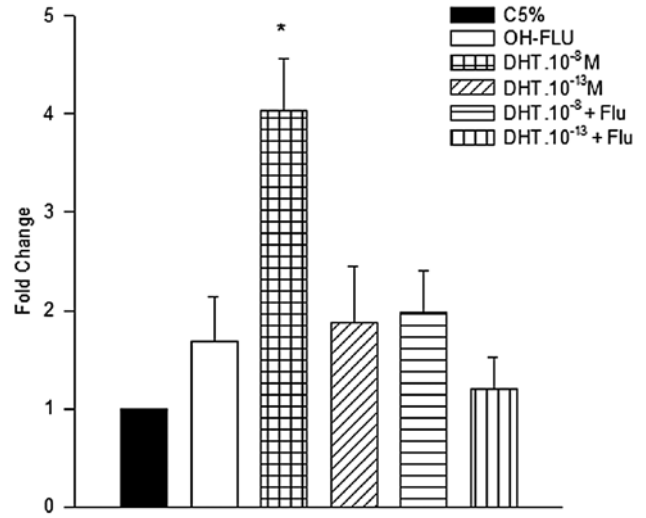


Figure 5. *p21* mRNA quantitative results by real-time PCR. The graphic represents the fluorescence change in relative expression of *p21* in HNTep cells treated with hydroxiflutamide (OH-FLU) at 10⁻⁶ M or dihydrotestosterone at 10⁻⁸ and 10⁻¹³ M isolated or combined with OH-FLU. Comparisons between means for different groups were analyzed by ANOVA followed by Duncan's test. *P<0.05 vs. the others groups.

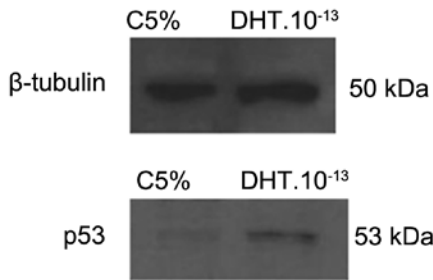


Figure 4. Autoradiogram of p53 protein levels of HNTep cells treated with DHT.10⁻¹³ M for 4 h. Samples were separated in a 12% SDS-PAGE, transferred to a nitrocellulose membrane and incubated with anti-AR antibody. beta-tubulin staining was used as protein loading control.

A quantitative analysis of mRNA levels of the *p53* and *p21* genes in HNTep cells was performed after 4 h with different treatment conditions.

The effect of DHT treatment on *p53* and *p21* mRNA levels in HNTep cells was estimated by quantitative analysis. The *p53* mRNA levels were 6-fold higher in HNTep cells treated with DHT.10⁻⁸ M compared to the control group. The *p53* mRNA level of this group was also higher compared with the OH-FLU.10⁻⁶, DHT.10⁻¹³ and DHT.10⁻⁸ + OH-FLU groups (Fig. 3). To confirm the presence of *p53* in HNTep cells, we evaluated the protein levels of p53 treated for 4 h with DHT at 10⁻¹³ M. Western blot analysis showed an immunoreactivity of p53 corresponding to a 53 kDa protein in HNTep cells (Fig. 4).

The data presented in Fig. 5 show that HNTep cells treated with DHT.10⁻⁸ M expressed 4-fold higher *p21* mRNA levels than the control group. This level of expression was also significantly higher compared with the other groups. The protein level of *p21* (21 kDa) was analyzed after 4 h with androgen treatment. The data shown in Fig. 6 suggest an increase in the protein level of *p21* in the group treated with DHT.10⁻⁸ M compared to the other groups.

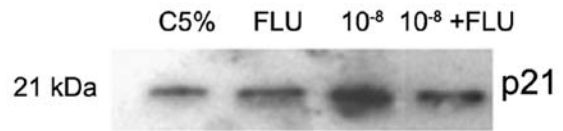


Figure 6. Representative western blot analyses of the *p21* gene in HNTep cells with different treatments for 4 h. Ponceau S staining was used as protein loading control.

Discussion

The results of the present study showed that low concentrations of androgens exerted a positive effect on cell proliferation in HNTep cells, and high concentrations maintained proliferation similar to that of the control. There are several experimental models for the study of prostate growth (1,7,12,36) and each of these models responds differently to stimulation. Therefore, these models may not be appropriate for investigating the effects of androgens on growth and differentiation of prostate epithelial cells. The epithelial prostate cells (HNTep) were established as an *in vitro* model to study the androgen dependence of human prostate, and have previously been characterized both functionally and morphologically (32).

In our study, the effect of the low concentration of androgen was blocked by adding the anti-androgen OH-FLU to the culture medium. In a parallel study, we assessed the AR gene expression in HNTep cells using the same mRNA employed for the *p21* and *p53* analysis in these data. In this case, the AR gene expression did not change with different treatments of DHT (data not shown). These data confirmed our previous studies on cell proliferation (19) and supported the theory that the mitogenic effect of the low dose of DHT in HNTep cells is regulated by its own receptor, the AR. There are few studies regarding the effects of androgens in non-transformed cell models. A study by Shao *et al* (17) confirmed a biphasic effect

of androgens on prostate cancer cells using the prostate cancer cell line LNCaP. They showed that a concentration higher than 1 nM of a synthetic androgen, R1881, inhibited cell growth, whereas concentrations of 0.1 nM and below stimulated proliferation. This biphasic effect of androgens on cancer cells was previously demonstrated by other groups (1,15,16), but the effect of androgen on the growth of the non-transformed cells remains uncertain. The actions of androgen are controversial and complex, since after binding to androgen, AR is able to recruit general transcription factors to its target gene promoters. It has become clear that the transcriptional activity of AR is regulated by coregulators, including both coactivators and corepressors, by various mechanisms (37).

The present study also showed that the expression of the *p53* and *p21* genes differs according to the androgen concentrations added to the culture medium of HNTEP cells. Thus, cells incubated at low DHT concentrations exhibited lower *p53* and *p21* mRNA levels than those treated with DHT at 10^{-8} M. The inhibition of these tumor suppressor genes suggested that under the influence of low concentrations of androgens, HNTEP cells are induced to progress into the cell cycle.

Androgens can stimulate proliferation and differentiation and suppress apoptosis in the prostate gland (21). Defects in apoptotic signaling pathways are common in cancer cells. Inhibition of apoptosis is important for tumor initiation, since apoptosis is involved in the process of eliminating cells with different anomalies that lead to malignant transformation. There are many apoptosis-modulating proteins, such as Akt and *p53* (38,39). Mutations of the *p53* gene are associated with tumors and proliferative disturbance of prostate tissue (29). In this study, we worked with the wild-type form of *p53*, demonstrating an increase of *p53* mRNA levels with the higher dose of DHT after 4 h of treatment. We showed that the anti-androgen hydroxyflutamide blocked this effect, indicating that the *p53* gene is a target of androgen modulation in HNTEP cells. In the present study we also assessed the protein expression of *p53* in HNTEP cells to determine the presence of functional protein. The results regarding protein levels of *p53* are controversial. Bruckheimer *et al* (40), reported an increase in the protein levels of *p53* and *Bax* after treatment with 1 nM of DHT in a hormone-sensitive LNCaP-FGC cell line. Moreover, some studies have shown a decrease in protein levels of the *p53* gene in LNCaP after 24 h of treatment with DHT over a range of 10^{-11} to 10^{-7} M (41). These results could be due to post-transcriptional adjustments which result in *p53* stabilization. Rokhlin *et al* (30), using the same cell line (LNCaP), also reported a decrease in the levels of *p53* mRNA after 3 days of treatment with 10^{-11} and 10^{-9} M of DHT, and an important role in TNF- α mediated apoptosis in LNCaP cells (39). There are androgen-responsive elements in the *p53* gene, which may explain the regulation of this gene expression by androgens (30). There is little information on androgen modulation of *p53* expression in non-transformed epithelial prostate cells. Khan *et al* (42) reported a higher expression of the *p53* gene in the prostate gland of E6-AP (E6-associated protein)-null mice, suggesting that E6-AP deletion attenuates the growth and development of the prostate gland by interfering with AR function as well as by stimulating *p53*-mediated apoptosis.

Previous studies have reported *p53* expression in prostate tumors and immortalized cell lines (43,44). The *p53* gene

may influence the expression of other genes; the present study analyzed the expression of the *p21* gene, which has been reported to be a target of *p53* action (23,28,42,45).

The *p21* gene induces apoptosis mechanisms and blocks the progression into the cell cycle (24,45). Although the role of the *p21* gene in prostate diseases is not well established, the existence of an androgen-responsive element in its promoter region suggests that this gene may be modulated by the androgen receptor (46). Evidence from the LNCaP and HPr-1 cell lines suggests that *p21* is involved in the mechanism of androgen action at different doses (26,27,31,47). Our results showed a decrease in *p21* gene expression in HNTEP cells treated with low androgen concentrations, and an increase at high doses. These data are consistent with the results of others who reported an increase in *p21* mRNA levels in LNCaP treated with a high dose (10^{-8} M) of synthetic androgen (48). Moreover, a human cell line PC-3 transfected with AR, when treated with 10 nM of DHT, showed upregulation of *p21* levels, and the knockdown of AR expression also resulted in downregulation of *p21* protein levels (49). A recent study by semiquantitative RT-PCR analysis showed a high expression of the *p21* gene in DU145-Id4 cells, a lineage of DU145 cells transfected with Id4 (inhibitor of differentiation 4, a member of the Id gene family) and PrEC cells (an immortalized lineage of normal human prostatic epithelial cells) as compared to parental DU145 cells. In this study a highly significant increase (over 15-fold by real-time PCR) in *p53* gene expression was found in the DU145-Id4 cell line compared to DU145 cells (50). To the best of our knowledge, this is the first report demonstrating the regulation of suppressor genes *p53* and *p21* by androgen in human non-transformed epithelial prostatic cells in primary culture (HNTEP).

We also analyzed the protein levels of *p21*. Protein expression tended to increase when HNTEP cells were treated with high concentrations of DHT (10^{-8} M). The increase in the *p21* protein levels by high doses of androgens has been demonstrated in other cell-culture models using different prostate-cell lines (26,27,48). The results of the proliferation rates of the HNTEP cells submitted to androgen treatment could be interpreted as indirect evidence favoring the hypothesis of involvement of these genes in the regulation of the cell cycle, associated with different doses of androgens. Taken together, these data may indicate that under the influence of androgens the expression of both *p53* and *p21* genes could be differentially changed in order to allow modulation in cell proliferation.

Benign prostatic hyperplasia (BPH) is the most prevalent disease of the prostate. The incidence of BPH coincides with decreasing circulating testosterone levels in senescence (51) and is associated with an imbalance between the rate of cell proliferation and cell death. The association between low concentrations of androgens and prostate cell proliferation in our *in vitro* system of HNTEP culture cells could be seen as similar to the endocrine events occurring in male senescence and prostate growth. Thus, changes in the expression of the *p21* and *p53* genes might be part of the mechanisms by which low androgen concentrations are related to BPH. In this context, studies on suppressor genes continue to be performed, in order to find molecular markers for states of uncontrolled growth in target tissues for androgen action (31,43,52-54). In conclusion, the present data showed that HNTEP cell proliferation could

be induced by low androgen concentrations, and was related to lower *p53* and *p21* gene expression at these concentrations. Further studies are required to assess the intracellular signaling pathway regulated by *p53* and *p21* under the influence of androgen stimulation in human prostate epithelial cells and its implications for the pathophysiology of benign and neoplastic transformation of the human adult prostate.

Acknowledgements

The authors thank the Graduate Research Group (GPPG) at Clinical Hospital de Clínicas de Porto Alegre for the editorial support provided, and the Urology Services at Hospital de Clínicas de Porto Alegre, Hospital São Lucas/PUCRS and Hospital Ernesto Dorneles for the support in obtaining prostatic tissue. This study was supported in part by Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul (FAPERGS) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

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