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Characterisation of steroid receptor expression in the human prostate carcinoma cell line 22RV1 and quantification of androgen effects on mRNA regulation of prostate-specific genes

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Abstract

In this study, the effect of natural androgens on the expression of androgen-regulated genes in the human prostate carcinoma cell line 22RV1 was characterised. To clarify the usefulness of the cells for in vitro studies concerning activation of androgen responsive genes by various steroidal compounds steroid receptor expression patterns had to be characterised intensively. Expression of androgen receptor (AR), estrogen receptor α (ERα) and β (ERβ), progestin receptor (PR) and glucocorticoid receptor α and β was investigated by the means of RT-PCR, immunocytochemistry, ligand binding or Western blot. 22RV1 cells were proved to express androgen receptor and less glucocorticoid receptor β on mRNA level. The confirmed mutation of the androgen receptor at codon H874 slightly apart from the steroid binding pocket seemed not to cause alteration of natural steroid hormone binding. mRNA expression of all progestin and estrogen receptor isoforms as well as glucocorticoid receptor α could not be detected. To study functional relevance of above-mentioned findings nine androgen-regulated genes were chosen to characterise the cell line and to determine androgenic effects using highly sensitive real-time RT-PCR. Addition of the three natural steroids dihydrotestosterone (DHT), testosterone, and 19-nortestosterone significantly influenced mRNA expression profiles. All compounds under study showed clear time-dependent and androgen-specific effects on transcriptional level. The results demonstrate that the cultivated human prostate carcinoma epithelial cells have a hormonal sensitivity correlated with the presence of specific receptors and can, therefore, serve as a selective model to study hormone action.

Keywords: 22RV1 cells; Androgen-regulated genes; Gene expression; Prostate-specific genes

1. Introduction

Gonadal steroid hormones do not only exert effects in organs related to reproductive function [1,2] or muscularity [3], but also in tissues that were not regarded as classical targets so far. The effects of steroid hormones are mediated through interaction with specific intracellular receptors, which are members of the nuclear receptor family [4,5]. Numerous tissues were shown to express mRNA transcripts for both estrogen receptors, subtypes estrogen receptor α (ERα) and β (ERβ) [6–9], androgen receptor (AR) [10–12] and progestin receptor (PR) [3,13]. However simultaneous occurrence of steroid receptors can be seen, e.g. in bovine skeletal muscle [14] or in bovine gastrointestinal tract [15,16]. Adams et al. [17] reported for the first time, the existence of ERβ on the protein level in the morphologically normal developing human fetal prostate. Androgen receptor gene expression in the monkey and dog uterus, that are typical organs for estrogen and progestin receptor expression, was observed by Adesanya-Famuyiwa et al. [18] and Sauerwein et al. [19]. Furthermore, organ specific cell culture systems showed expression of other steroid receptors at low concentrations, e.g. the human breast cancer cell line ZR-751 [20] and human breast cancer cells MFM-223 [21].

A cell line expressing only androgen receptor will be advantageous to study selective hormonal activity. The cell line 22RV1 was established from a human prostatic carci-

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noma xenograft (CWR22R) that was serially propagated in mice after castration-induced regression and relapse of the parental, androgen-dependent CWR22 xenograft [22]. In the present study steroid receptor expression was characterised in above-mentioned cells more detailed than currently known. Additionally, the role of AR-regulated genes (ARG) in androgen-independent growth of 22RV1 cells was investigated.

The androgen receptor is a member of the nuclear receptor superfamily [23,24] that functions as a ligand-inducible transcription factor mediating expression of target genes in response to receptor-specific ligands. Alteration of these regulation processes by environmental endocrine disruptors or modified physiological androgen production can cause incomplete masculinisation of the fetus and possibly impaired male fertility in adults [25,26]. Endocrine disruptors are exogenous substances or mixtures that alter functions of the endocrine system and cause adverse health effect in an intact organism, or its progeny, or (sub)populations [59].

Our aim was to establish a system showing a gene expression profile selectively regulated via androgen receptor—a system almost devoid of other steroid receptors unlike available biological tissues and most cell lines. Hence, the cell line 22RV1 was screened for steroid hormone receptors at the mRNA or protein level. Additionally, the AR was sequenced at the ligand pocket region and relative binding of natural steroid hormones was estimated. For expression profiling we identified nine androgen-regulated genes (AR, PSA, PSM, Drg1, TMPRSS2, PMEPA1, NKX3.1, 5α-reductase, cycline d1): AR is part of the steroid receptor family. Prostate-specific antigen (PSA) is a member of the human kallikrein gene family, and is well known as a prostate-specific tumour marker [27]. Prostate-specific membrane antigen (PSM), a transmembrane glycoprotein is almost entirely prostate specific, as is PSA. They may enable further delineation of the unique functions and behaviour of the prostate [28]. Differentiation-related gene (Drg1) is a gene showing to be diminished in colon, breast and prostate tumours [29]. Serine protease (TMPRSS2) plays a role in prostate carcinogenesis [30]. PMEPA1 gene, along with other highly androgen-induced prostate-specific genes, has potential to serve as an androgen signalling read-out biomarker in prostate tissue [31]. NKX3.1 is a candidate gene for opposing processes of androgen-driven differentiation of prostatic tissue and loss of differentiation during the progression of prostate cancer [32]. The enzyme steroid 5α-reductase catalyses the conversion of testosterone into the more powerful androgen, dihydrotestosterone [33]. Expression pattern of some genes had been assayed in an earlier study [34].

2. Materials and methods

2.1. Reagents

Dihydrotestosterone (DHT, 5α-androstan-17β-ol-3-one) was received from Sigma–Aldrich (Germany). Testosterone (4-androsten-17β-ol-3-one) and 19-nortestosterone (NT, 17β-hydroxy-4-estren-3-one) were obtained from Sigma. Charcoal (Norit A), and dextran (research grade, MG 65,000–73,000) were purchased from Serva (Germany). All steroids were dissolved in ethanol p.a. (final ethanol concentration was less than 0.1%).

2.2. Cell culture

22RV1 cells were obtained from Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ GmbH, Germany, ACC 438) and routinely cultured in 40% RPMI 1640 medium, 40% Dulbecco's MEM supplemented with 20% heat-inactivated fetal bovine serum (FBS) plus 100,000 units/l penicillin and 100 mg/l streptomycin (Sigma, Germany). Steroids were removed from FBS as described by Darbre et al. [35]. All media were obtained from Gibco-BRL (USA). Cells were split 1:6 every 7 days and cultures were maintained at 37 °C in a humidified atmosphere of 5% CO_2.

2.3. RNA preparation, RT and real-time PCR

Total RNA was isolated from 22RV1 cells using peqGold TriFast™ (peqLab Biotechnologie GmbH, Germany) with the guanidinium–isothiocyanate method [36] according to the manufacturer's instruction. Synthesis of first strand cDNA was performed by using 1000 ng total RNA and 200 U MMLV-reverse transcriptase (Promega, USA) according to the manufacturer's protocol. RT reaction was carried out in 40 μl reaction volume in a gradient cycler (Tgradient, Biometra®, Germany). All measurements of nucleic acid concentration were performed at 260 nm (OD_{260}) in a spectrophotometer (BioPhotometer®, Eppendorf, Germany).

Expression of steroid receptors was investigated using listed primers (Table 1). Subsequently, PCR products were subjected to gel-electrophoresis in 1.5% agarose gels containing 0.5 μg/ml ethidium bromide. Quantification of genes of interest was carried out in a LightCycler® (Roche Diagnostic, Germany) using LightCycler® DNA Master SYBR® Green I technology [37]. Master mixes for each PCR run were prepared as follows: 6.4 μl water, 1.2 μl $MgCl_2$ (4 mM), 0.2 μl forward primer (20 pmol), 0.2 μl reverse primer (20 pmol) and 1 μl Fast Start DNA Master SYBR® Green I mix (Roche Diagnostics, Germany). Master mix (9 μl) were combined with 1 μl (25 ng) reverse transcribed total RNA. Each sample was subjected to 40 PCR cycles (15 s at 95 °C for denaturation, 10 s at corresponding annealing temperature, 20 s at 72 °C for elongation and 3 s at 75–83 °C for a single fluorescence measurement). Primers for all nine androgen-regulated genes were designed using HUSAR-software (DKFZ, Heidelberg) and synthesised by MWG Biotech (Germany). We performed "second derivate maximum method" to determinate the crossing point using LightCycler Software 3.5 (Roche Molecular Biochemicals). Crossing point is defined as the point at which the fluorescence rises appreciably above the background fluorescence [37]. In the "second derivate

Table 1
Selected androgen-regulated genes and steroid receptors in human prostate carcinoma cell line

Identity	Accession no.	Upstream primer	Downstream primer	Size (bp)
PR	M15716	GAGAGCTCATCAAGGCAATTG	CACCATCCCTGCCAATATCTTG	227
ERα	NM000125	AGGGAAGCTCCTATTTGCTCC	CGGTGGATGTGGTCCTTCTCT	234
ERβ	NM001437	GCTTCGTGGAGCTCAGCCTG	AGGATCATGGCCTTGACACAGA	262
GRα	M10901	GAA GAG GGA GAT GGA GAC TG	TGT TGG AAT GAG AAG GGT GG	119
GRβ	AA814178	TTA CCA CAA CTC ACC CCTAC	CAG AGC AAA TGC CAT AAGAAAC	252
AR	M63505	TTGATTTTTCAGCCCATCCACTGGA	CCTGGTTTTCAATGAGTACCGCATTG	217
Ubiqutin	NM174133	AGTCGACCCTGCACCTGGT	GCTCCACCTCCAGGGTGA	194
PSA	AF113132	TGTCCGTGACGTGGATTG	GGTTCCACTGCTCCTCTGAG	159
5α red	M32313	CCTAAATACGCTGAAATGGAGG	CACACTTGGCAAGACATAGCC	131
TMPRSS2β	AF270487	AAGCTGCAGAAGCCTCTGAC	CAGCGTTCAGCACTTCTGAG	151
PSM	M99487	TCAGCACCACCAGATAGCAG	GGTTCCACTGCTCCTCTGAG	170
PMEPA1	AF224278	GGCAGAACACTCCGCGCTTCTTAG	CAAGCTCTCTTAGCTTGTGCATTC	146
NKX3.1	U80669	GCCAAGAACCTCAAGCTCAC	TCATAATGGTTGTCACCTGAGC	261
Drg1	AF078103	TACGGCTGATGACCTCATTG	GGTGATGGGCAGAGATGG	149
Cycline D1	NM053056	CACGTCCAGGTTCAACCC	TGCCTCAAAGTCCTGCTTG	195

GenBank accession numbers indicate gene sequences primers were designed from.

maximum method", a second derivate maximum within the exponential phase of the amplification curve is linearly related to a starting concentration of template cDNA molecules [37]. The PCR-product length was verified by gel electrophoresis as a single band at the expected length (data not shown). The specificity of the bans was conformed by the melting curve analysis of LightCycler software 3.5.

2.4. Statistics

Statistical analysis of group differences was done by pair wise fixes reallocation randomisation test, which is implemented in the REST-XL software [38,39]. Differences in expression between control and treated samples were assessed in group means for statistical significance by randomisation tests. The mathematical mode was based on the PCR efficiency and the group mean crossing point difference between the treated versus the control group. The corresponding real-time PCR efficiency (E) in the exponential phase was calculated using the equation $E = 10^{[-1/\text{slope}]}$, applied to a dilution series ranging from 0.20 pg to 50 ng DNA in triplicate [23].

All data were statistically processed in SigmaPlot® 2000 (SPSS Inc., USA) and SigmaStat® 2.0 (Jandel Corporation, USA).

2.5. Radio receptor assay, relative binding affinities (RBA) and Western blot analysis

The protein concentration was evaluated with the BCA technique [40]. The AR assay and RBA estimation were performed as described previously [41].

For protein extraction approximately 10^8 cells were homogenised in lysis buffer (PBA) containing protease inhibitor. Protein samples (4.4 μg per lane) were separated on a 4–12% Bis–Tris Gel (NuPage, Invitrogen, CA, USA) in MOPS running buffer and transferred onto nitrocellulose membranes. Membranes were blocked with 1% skim milk powder in TBS-Tween (0.05 M Tris, 0.15 M NaCl, pH 7.4, 0.1% Tween 20) over night followed by incubation with a monoclonal antibody against PR (Clone 10A9, Coulter Immunotech, Marseille, France), ERα (2–185, Santa Cruz Biotechnology, CA, USA) or ERβ (PA1-311 Affinity BioReagents Inc., Golden, CA) in TBS-Tween, 1% dried skim milk powder, for 75 min at room temperature. Antibody-dilutions used were 1:200 (ERα), 1:500 (ERβ) and 1:50 (PR), respectively. After three times washing in TBS-Tween, 1% skim milk powder, the membranes were incubated with anti-mouse (ERα, PR) or anti-rabbit (ERβ) horseradish peroxidase-conjugated IgG secondary antibody (DAKO, Hamburg, Germany) at a dilution of 1:2000 in TBS-Tween, 1% skim milk for 45 min at room temperature. After washing in TBS-Tween and TBS alone the membrane was incubated with enhanced chemiluminescence reagent detection solution (Amersham, Buckinghamshire, UK) for 3 min in the dark. An X-ray film was exposed to the membrane to visualise protein expression. As a positive control recombinant human ERα or PR protein (Sigma, Munich, Germany) was used at a concentration of 30 ng per lane.

2.6. Immunocytochemistry of steroid receptors

Localisation for PR was performed as described previously [42]. The 22RV1 cells were cultured on microscope slides with a silicone insert. The slides were fixed for 5 min in 4% formaldehyde. Endogenous peroxidase activity was blocked by incubation in 1% hydrogen peroxidase for 30 min at room temperature. Non-specific binding was blocked by goat serum (1:10 dilution in PBS-buffer) for 30 min at room temperature. A primary antibody was added to each well and the slides incubated in a humidified chamber at 4 °C over night. A monoclonal mouse anti-PR antibody (MA1-410 Affinity BioReagents Inc., Golden, CA) was used; it detects both PR-A 94 kDa and PR-B 120 kDa protein isoforms by

staining at a concentration of 3 μg/μl. The ERα mouse monoclonal antibody from clone 1D5 (Coulter Immunotech Diagnostics, Germany) was applied at a dilution of 1:100. A rabbit polyclonal antibody (PA1-311 Affinity BioReagents Inc., Golden, CA) raised against the N-terminal region of ERβ (amino acids 54–71 of rat ERβ sequence, EMBL accession number AJ002603: AEPQKSPWCEARSLEH) was incubated as described by Rosenfeld et al. [43]. For control sections either primary or secondary antibody were omitted and replaced by buffer. Sections were incubated with DAB (3,3′diaminobenzidine hydrochloride from Sigma, Germany) alone to exclude residues of endogenous peroxidase activity. A lack of staining of controls showed the antigen specificity. After the incubation of PR or ERα, horseradish peroxidase-labelled goat anti-mouse IgG (DAKO, Hamburg, Germany) diluted at 1:200 was applied at room temperature for 1 h. Incubation of ERβ was followed by undiluted HRP-labelled goat secondary antibody detection systems against rabbit IgG (DAKO En-Vision^{TM}+, Hamburg, Germany) on equal terms. Peroxidase was visualised by applying 0.01% DAB and 0.01% hydrogen peroxide in PBS-buffer for 10 min in darkness at room temperature.

2.7. Functional expression studies and AR sequencing

Cells were seeded in medium containing 20% charcoal-stripped FBS for 72 h before treatment with steroids and allowed to form a 60–70% confluent monolayer. DHT, testosterone or 19-nortestosterone (10^{-7}, 10^{-8}, 10^{-9} M) was added and cells were harvested 0, 6, 24, 48 and 72 h after stimulation for RNA extraction. Control cultures were continuously grown in steroid-depleted untreated medium for the same time intervals.

The CWR22 mutant AR exon H was amplified using two-step PCR method as described [44,45]. The amplified fragments were commercially sequenced to specify the gained PCR product (Medogenomix, Germany).

3. Results

3.1. Receptor mRNA identification, primer and real-time RT-PCR product specificity

In humane prostate epithelial cell line 22RV1 mRNA of AR und GRβ were detected by real-time RT-PCR. Neither mRNA of estrogen receptor α and β (ERα and ERβ) nor mRNA of three isoforms of progestin receptor (PR) and glucocorticoid receptor α could be found. Additionally, the intensity of androgen receptor expression was significantly higher by the factor five than expression of GRβ on mRNA level. The effect of hydrocortisone via the GRβ on the expression of the investigated genes was tested, but no significant effect was observed (data not shown). Specificity of the desired products in 22RV1 cell line total RNA were documented by high resolution gel electrophoresis (Fig. 1), and additionally by melting curve analysis (data not shown).

3.2. In vitro protein expression

Protein expression for ERα, ERβ and PR was tested in vitro with monoclonal anti-receptor antibodies by Western blot analysis. In vitro expression for AR protein was tested with radio receptor assay. Western blot revealed three isoforms of PR in the positive control of endometrium but was negative in 22RV1 cells (Fig. 2C). Observed bands correspond to 116, 92 and 65 kDa molecular weight, thus representing PR isoforms A, B and C [46]. The antibody against ERα detected a 60 kDa band in the positive control (endometrium) and was negative in the 22RV1 samples (Fig. 2A). The antibody against ERβ identified two bands at approximately 62 and 58 kDa in the control endometrium in contrast to cell line samples where no band was detectable (Fig. 2B). 22RV1 human prostate carcinoma cells contained active androgen receptor at a binding capacity of 9.8 fmol/mg protein.

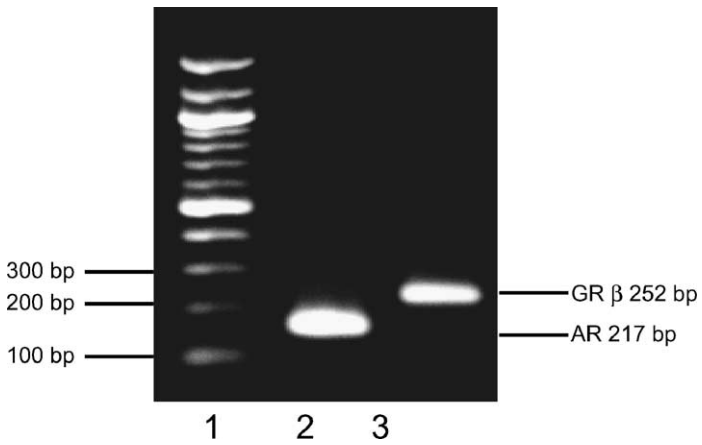

Fig. 1. Expression of AR and GRβ (lane 1: MassRle^{TM} DNA Ladder Mix; lane 2: AR RT-PCR product; lane 3: GRβ RT-PCR product) in 22RV1cell line.

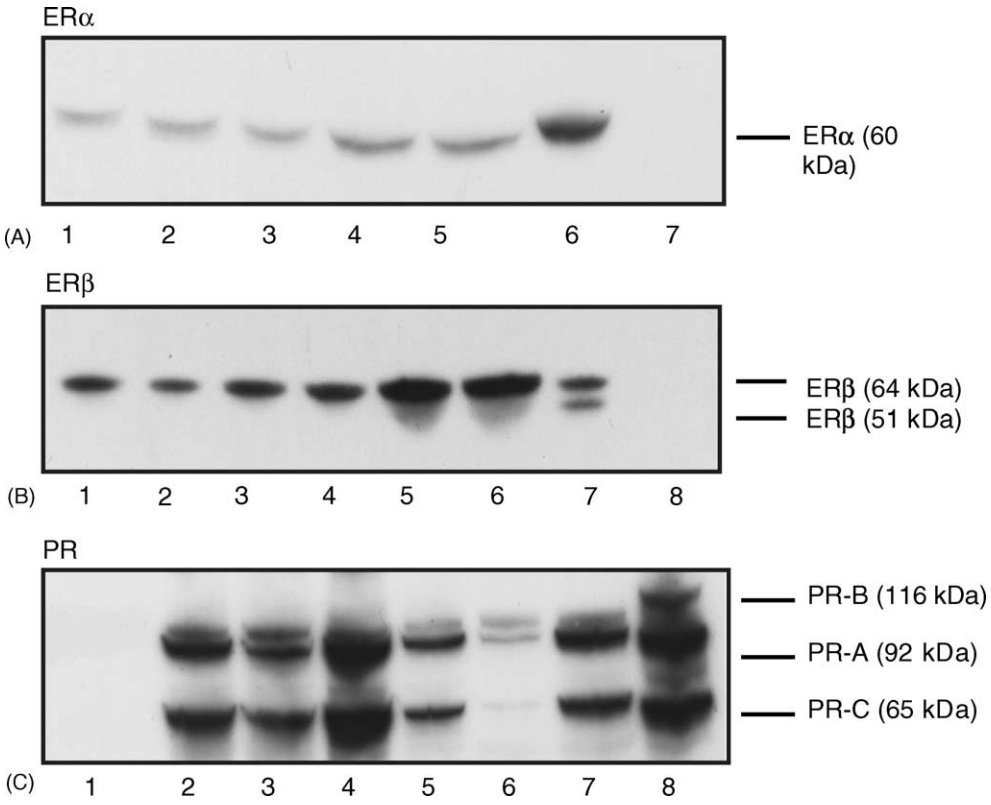

Fig. 2. Western blot analysis of ERα, ERβ and PR of 22RV1 cells. (A) Lanes 1–5: endometrium; lane 6: ERα protein; lane 7: 22RV1 cell line. (B) Lanes 1–6: endometrium; lane 7: ERβ protein; lane 8: 22RV1 cell line. (C) Lane 1: 22RV1 cell line; lanes 2–7: oviduct; lane 8: PR protein.

The order of relative binding affinities to the natural steroids was DHT > testosterone > estradiol > progesterone. The highest RBA to the AR showed the natural ligands DHT (100%) and testosterone (35%), in comparison the hormones estradiol and progesterone had low affinities of 7.4 and 3.6%, respectively (RBA for recombinant human wild type AR: DHT 100%, testosterone 31.3%, estradiol 4.9% and progesterone 3.8% [47]).

Automated sequencing of androgen receptor exon H DNA from 22RV1 cells detected a C to T mutation at position 4046, numbered according to Lubahn et al. [44], that changed codon 874 from CAT coding for histidine to TAT coding for tyrosine. In the region analysed only mutation H874 was found. No similar indication of an AR gene mutation was detected in exons A through G.

3.3. *In vitro ERα, ERβ and progestin receptor protein localisation*

The PR protein was also not detectable by immunocytochemical methods using monoclonal antibody MA1-410. An intensive staining of nuclei was found in oviduct epithelial cells before and after Mayer Haemalaun counterstain (Fig. 3). The immunocytochemical evaluation of ERα, ERβ with monoclonal antibodies revealed a negative reaction in 22RV1 cells (Fig. 4).

3.4. *Steroid treatment*

Results of mRNA profiles upon androgen stimulation during a 72 h time course are depicted in Figs. 5–7. As gene expression was similar for all three concentrations applied in this study, results were taken together and depicted as average value of concentrations 10^{-7}, 10^{-8}, 10^{-9} M. DHT and testosterone showed similar expression pattern and will therefore be discussed together. The majority of androgen-responsive genes were clearly up-regulated up to 48 h of treatment. Thereafter especially TMPRS2, NKX3.1 and PSM were significantly down-regulated. In contrast to DHT that poses an up-regulatory effect on six genes during the overall period of time, this up-regulation weakens upon testosterone application within 24 h.

For 19-nortestosterone a bi-phased effect could be observed. Up-regulation of all genes under study that was found after 6 h turned into down-regulation of six target genes within the 24 h incubation period. From this time onward the majority of target genes proved to be up-regulated again. Down-regulatory effects after 48 and 72 h of 19-nortestosterone exposure were not as strong as those seen after DHT and testosterone treatment. For almost all androgen-responsive genes detected in the screening, the changes in gene expression were detectable within 6–24 h, suggesting that they are primary effects of androgens. The

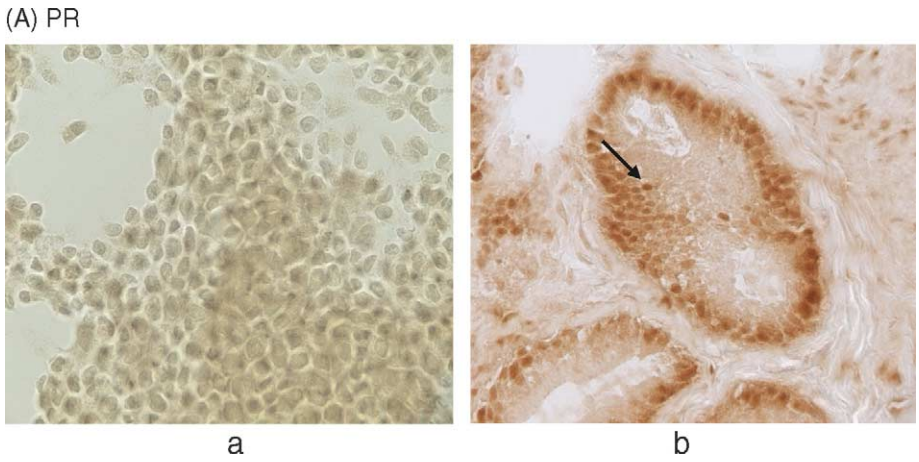

Fig. 3. Immunocytochemical localisation of PR. (a) Nuclei of 22RV1—negative; (b) oviduct epithelial cells—positive control (both 40× magnification).

secondary effect was observed after 24 h. Upon stimulation with 19-nortestosteron a "shock-reaction" was observed after 6 h—all genes were up-regulated. After 48 h the expression pattern stabilised, and all genes showed characteristic expression effects. The minimal AR expression was observed after 24 h, afterwards, expression strongly increased.

PSA, a prostate-specific tumour marker gene showed, as expected, up-regulation with all substances during the whole time of the experiment. After stimulation with DHT and testosterone up-regulation of 5α-reductase gene was observed. Upon treatment with 19-nortestosterone up-regulation of 5α-reductase is shifted to 48 h after stimulation.

DHT and testosterone prevented mRNA induction of PSM after 48 and 72 h. Maximal down regulation was observed after 72 h (1.85-fold). High expression of cycline d1 (1.49-fold) was observed after 6 h stimulation with testosterone.

4. Discussion

The human prostate carcinoma cell line 22RV1 has been established 5 years ago [22] from the CWR22 xenograft. Nevertheless, detailed information on steroid receptor expression of this cell line was not available from current literature. Herein we show an overview on steroid receptor expression on both mRNA and protein level. The fact that neither ERα, ERβ nor PR and GRα are expressed on mRNA and protein level indicates that interferences of compounds under study with respective receptor mediated gene activation pathways can be excluded. The human prostate carcinoma cell line produces human androgen receptor almost devoid of any other steroid hormone receptor—in contrast, receptor preparation of animal or human tissues in principle contain all receptors. Interaction with GRβ during treatment with androgenic ligands cannot be excluded completely, because this receptor is present on mRNA level. Anyhow, activation with hydrocortisone did not affect expression of the genes investigated here and transcripts of AR are the predominate ones showing five-fold higher expression levels.

One potential limitation in using a CWR22 derived model might be the mutation at amino acid 874 from histidine to tyrosine [48]. Sequencing of AR exon H from 22RV1 cells confirmed the mutation at codon 874 close to the steroid binding pocket. Anyhow, relative binding affinity of natural steroids to AR from 22RV1 cells was very similar to wild type AR what agrees with an observation in CRW22 xenografts showing in vivo preservation of steroid specificity [49]. The induction of transcriptional activity in both mutant and wild type AR with higher concentrations natural steroid concentrations seems likely.

Taking into account this information 22RV1 cells seem to be highly suitable for our scientific approach. For the first time, we could prove that 22RV1 cells can be used for studies on endocrine disrupting potential of various compounds by selective expression analysis of androgen receptor regulated genes. For estrogen-responsive genes such a system is already available [50]. It is another major advantage of our system that cell growth is independent from the presence of androgens in the culture medium. Growth over 72 h in androgen depleted control medium does have no negative effect on cell viability and transcriptional potential.

Second important point in our study was to test the functionality of our cell culture based expression system in the presence of naturally occurring androgens. This question was covered by time and concentration dependent stimulation of 22RV1 cells with three endogenous androgens. An in vitro system like ours contrasts the approach of reporter gene-assays. These test systems mainly focus on the expression of a single gene being under control of a respective steroid promoter region. In contrast our cell culture allows analysis of various androgen-responsive genes that are relevant under in vivo conditions.

22RV1 cells react to androgens with specific alteration of gene expression pattern in comparison to untreated control cells. Higher effects of DHT on transcription reflect that it is a more potent ligand for AR compared to testosterone. Interestingly, 5α-reductase is more consistently expressed in DHT than in testosterone treated cells. 19-Nortestosterone that is

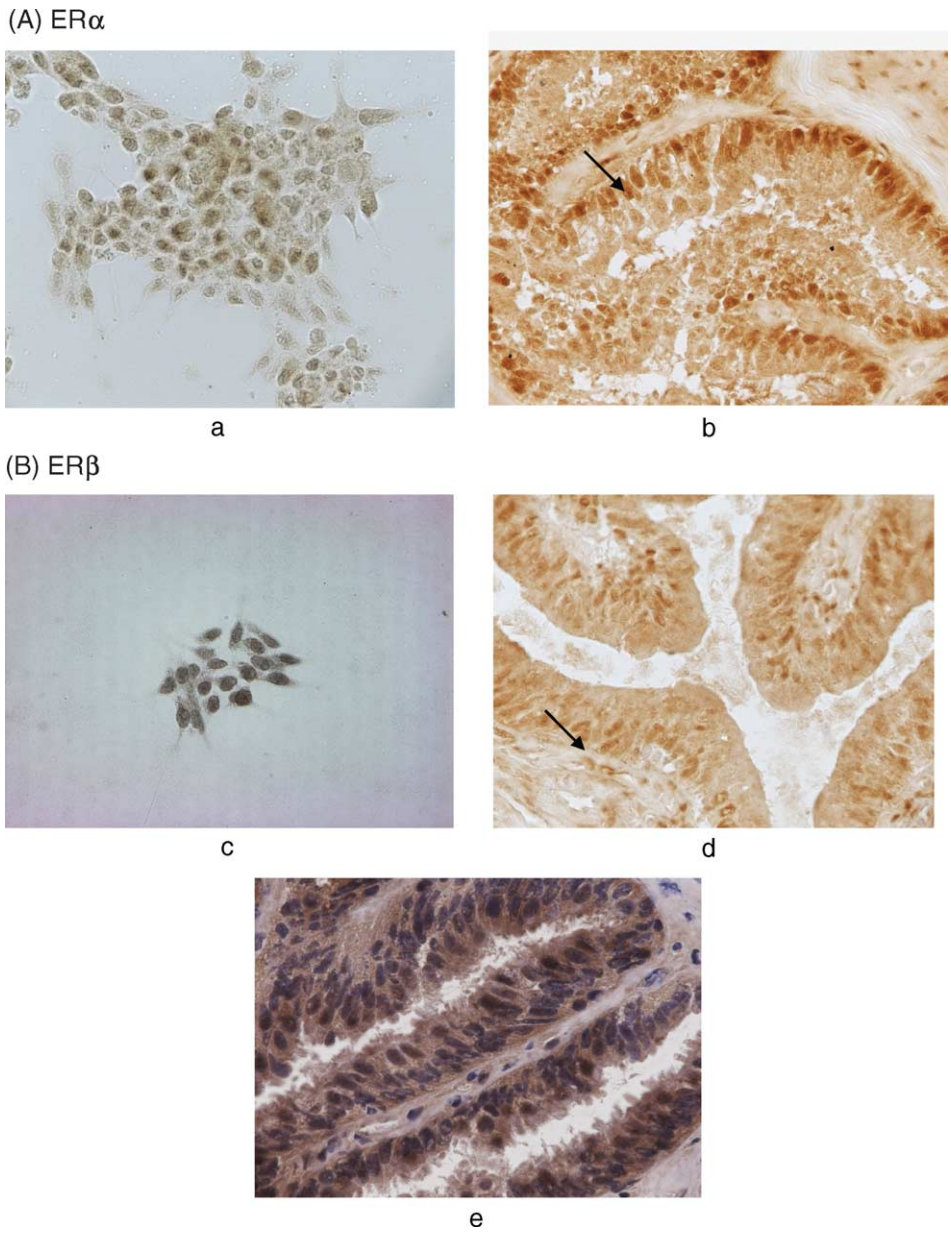

Fig. 4. Immunocytochemical localisation of ERα and ERβ. A(a): ERα in the nuclei of 22RV1—negative; A(b): oviduct epithelial cells—positive control. B(c): ERβ in the nuclei of 22RV1 cells—negative; B(d): oviduct epithelial cells—positive control; B(e): oviduct epithelial cells after Mayer Haemalaun counterstain (40× magnification).

structurally more different exhibits a modified gene expression pattern with bi-phased up-regulation of target genes. 19-Nortestosterone is known to be more resistant to biotransformation in vivo than DHT or testosterone. This resistance may explain strong up-regulation after 48 and 72 h. Nevertheless, it remains unclear why transcriptional activity is mostly down-regulated after 24 h. The fact that AR expression increased only after 24 h could be explained by the low AR concentration in 22RV1 cells (10 fmol/mg protein) and a longer activation period of AR. Expression of PSA mRNA is androgen-regulated [27] and after treatment with 19-nortestosterone up-regulation of PSA genes was observed only after 48 h. Due to the fact that 19-nortestosterone is less active compared to the other androgens, a longer regulation period is probably needed.

The non-identical effects of androgens on androgen-regulated genes (ARG) mRNA turnover in this cell line suggest that different mechanisms are involved in maintaining ARG mRNA expression. Presumably, the regulation of ARG in prostate carcinoma cell line is as complex as in intact prostate tissues [51]. This complexity necessitates future detailed studies on the molecular basis of androgenic regulation of ARG in prostate carcinoma cell lines.

Lately much attention has been turned to the potential adverse effects of endocrine disrupters to human health [52–57] or to the physiologically delicate hormonal balance e.g. by

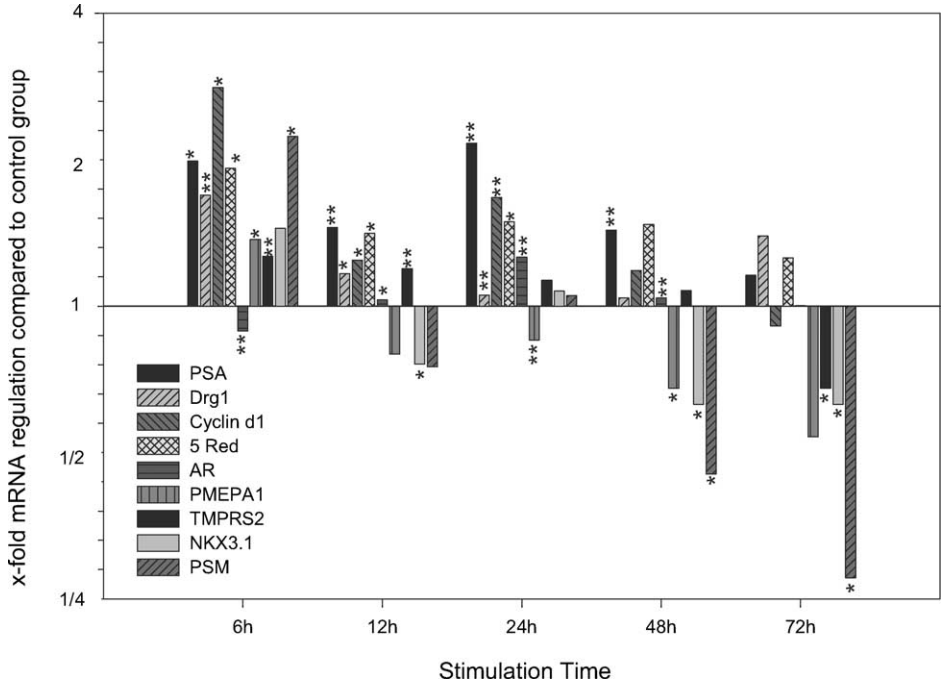

Fig. 5. Time response to testosterone (calculated mean values at 10^{-7}, 10^{-8}, 10^{-9} M testosterone) induction of mRNA expression level in 22RV1 cells in comparison with untreated control (each $n = 9$). Expression changes were shown as *x*-fold up- or down-regulation (mean ± S.D.). $^{*}P < 0.05$, $^{**}P < 0.01$ indicate significant differences between treatment groups.

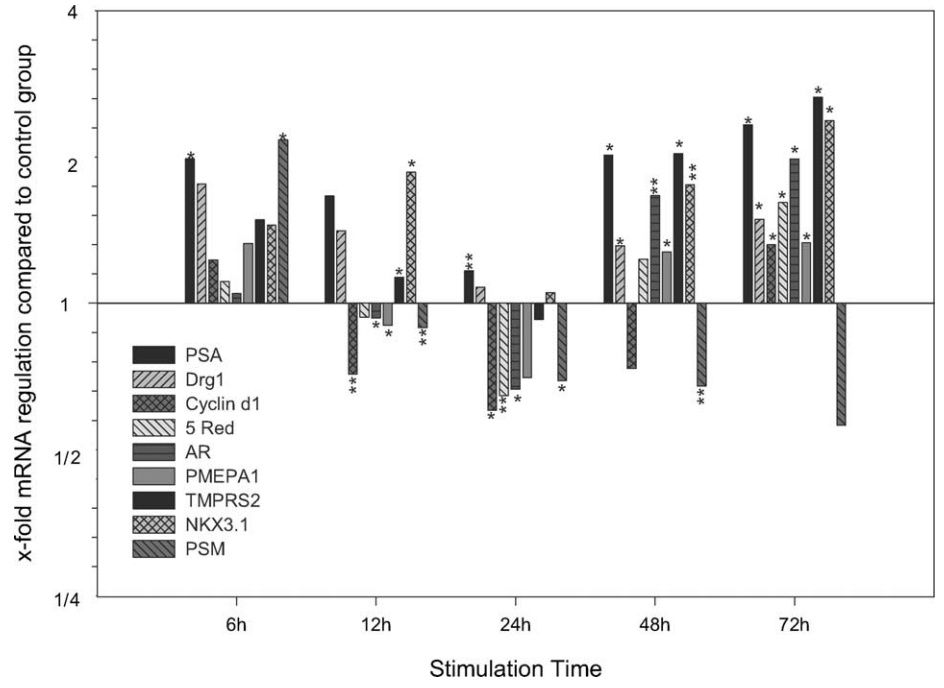

Fig. 6. Time response to 19-nortestosterone (calculated mean values at 10^{-7}, 10^{-8}, 10^{-9} M 19-nortestosterone induction of mRNA expression level in 22RV1 cells in comparison with untreated control (each $n = 9$). Expression changes were shown as *x*-fold up- or down-regulation (mean ± S.D.). $^{*}P < 0.05$, $^{**}P < 0.01$ indicate significant differences between treatment groups.

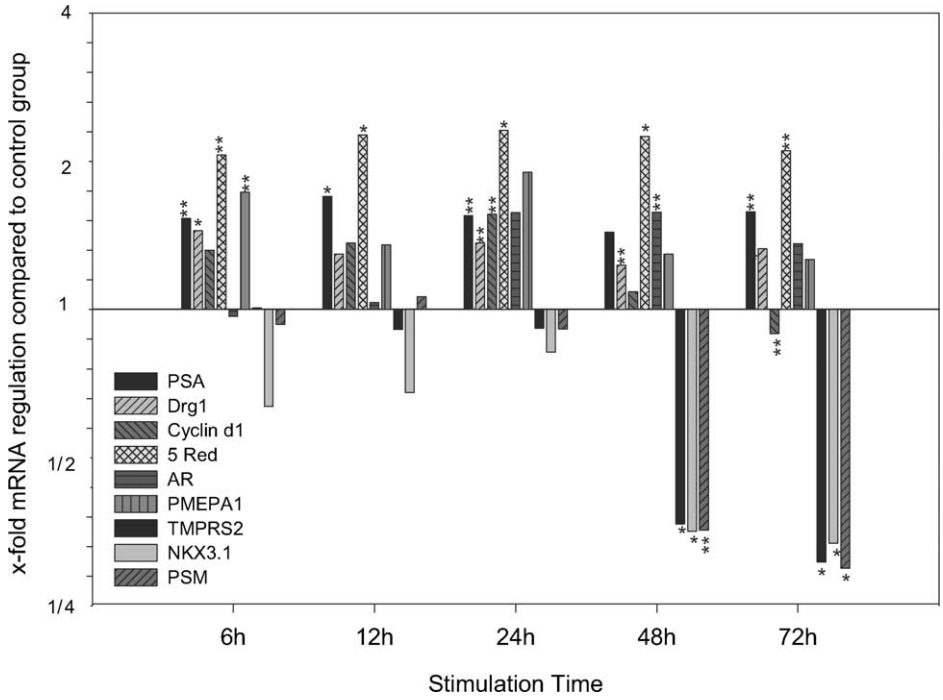

Fig. 7. Time response to DHT (calculated mean values at 10^{-7}, 10^{-8}, 10^{-9} M DHT) induction of mRNA expression level in 22RV1 cells in comparison with untreated control (each $n = 9$). Expression changes were shown as x-fold up- or down-regulation (mean ± S.D.). $^{*}P < 0.05$, $^{**}P < 0.01$ indicate significant differences between treatment groups.

mimicking the activity of hormones or by suppressing the normal hormone action [58–60]. Gene expression in the described cell line may provide a selective tool to understand androgenic side activities more specifically.

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