CHARACTERISATION OF GENE EXPRESSION PATTERNS IN 22RV-1 CELLS FOR DETERMINATION OF ENVIRONMENTAL ANDROGENIC/ANTIANDROGENIC COMPOUNDS

A.Hartel, A.Didier, M.W.Pfaffl and Heinrich H. D. Meyer
Institut für Physiologie, Technische Universität München
Weihenstephaner Berg 3
D-85350 Freising-Weihenstephan, Germany
Hartel@wzw.tum.de

oduction

ration of androgen receptor function due to endocrine disrupting compounds in the ronment may be responsible for impaired function and male development in lifestock. ed on human prostate carcinoma 22RV-1 cells, a cell culture expression system was blished to test androgenic/antiandrogenic function. Androgen dependent expression erns of six different marker genes (PSA, PSM, AR, NKX3.1, TMPRSS2, PMEPA1) were sured by highly sensitive Real-Time RT-PCR after treatment with three different pounds (fentinacetate, difenoconazole, tetramethrin) using DHT and R1881 as control stances. Final goal of the work is a sensitive screening method for the determination of rential gene expression caused by androgenic/antiandrogenic substances. Due to rent effects on gene expression, samples of unknown constitution may be characterised further physiochemical analysis by means of GC-MS or HPLC may by facilitated.

erials and Methods

ulture conditions

22RV1 cell line was obtained form DSMZ, Braunschweig (ACC 438) and cultured routinely in 40% RPMI um (Gibco), 40% Dulbecco´s MEM suplemented with 20% heat-inactivated FBS (Gibco) plus 100 units/l illin and 100 mg/l streptomycin.

reatment

were seeded in medium containing 20% charcoal-stripped FBS for 72h before treatment with steroids and cides and allowed to form a confluent monolayer. Synthetic androgen R1881 (1nM) was added and cells were sted at 0h, 6h, 24h after stimulation for RNA extraction. Same procedure was carried out with other ligands: oconazole (100nM), fentinacetate (100nM) tetramethrin (50nM) and DHT M). Control cultures were continuously grown in steroid-depleted untreated medium during the same time ds.

Extraktion and Real-Time RT-PCR quantification

RNA was isolated using peqGOLD TriFast™ according to the manufactures instructions. Synthesis of first d cDNA was performed with MMLV-RT (Promega) and random hexamer primers. Quantification of genes of st was carried out in LightCycler® (Roche Diagnostic, Germany) using LightCycler® DNA Master SYBR® n I technology. Fluorescence data reports were computed directly with LightCycler software 3.3 (Roche nostics, Germany). Data analysis was performed using Roche LC relative quantification software; Pfaffl, M.W.). Primers for all six androgen regulated genes (Table 1) were designed with HUSAR-software (DKFZ, elberg) and sythesised by MWG Biotech (Germany).

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ta were statistically processed in SigmaPlot® 2000 (SPSS Inc, Chcago, USA) and SigmaStat® 2.0 (Jandel oration, San Rafael, USA).

e 1. Primer characteristics and product lenght.

	PSA	PSM	AR	NKX3.1	TMPRSS2	PMEPA1
Forward imer (bp)	29-46	1195-1214	252-272	568-586	1104-1123	895-918
Reverse imer (bp)	170-187	1345-1364	558-578	740-761	1235-1254	1017-1040
Product ngth (bp)	159	170	326	261	151	146
Melting emp. (°C)	90.80	83.37	86.33	88.49	89.20	89.20

Results

1. Using Real-Time RT-PCR 22RV1 cells showed expression of androgen receptor only. Neither mRNA of estrogen receptor (ER) α and β nor mRNA of progestin receptor (PR) could be detected.

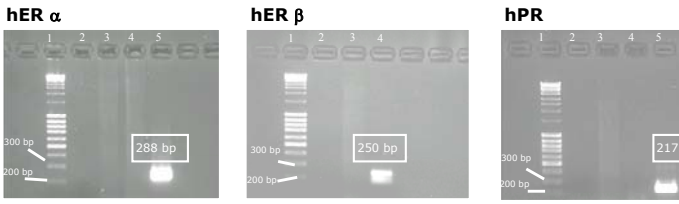


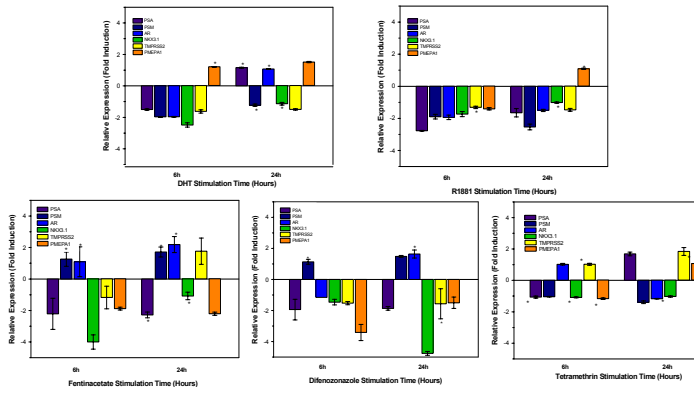
Figure 1. Expression of hER α (lane 1 MassRle™ DNA Ladder Mix; lanes 2-4 RNA extraced from 22RV1; standard – human cord blood), hER β (lane 1 MassRle™ DNA Ladder Mix; lanes 2,3 RNA extraced from 2 line 4 standard – human cord blood) and hPR (lane 1 MassRle™ DNA Ladder Mix; lanes 2-4 RNA extraced 22RV1; lanes 5,6 standard – human cord blood) receptor in 22RV1cell line.

2. Primer specifity, Real-Time PCR efficiency and intra- and interassay variation (Table 2) could be analy LightCycler® (Roche Diagnostic, Germany).

Table 2. Characterisation and validation of androgen regulated genes with Real-Time RT-PCR

	PSA	PSM	AR	NKX3.1	TMPRSS 2	PMEPA1	Ubic
PCR efficiency	2.07	2.13	1.99	1.90	1.98	1.92	1.
Quantification range	25-0.2ng	25-0.04ng	25-0.04ng	25-0.2ng	25-0.04ng	25-0.04ng	25-0
Test linearity Correlation	total RNA (r = 0.98)	total RNA (r = 0.99)	total RNA (r = 0.92)	total RNA (r = 1.00)	total RNA (r = 0.98)	total RNA (r = 1.00)	total (r =
Intraassay variation (n = 3)	0.3%	0.8%	1.6%	0.5%	1.0%	0.4%	0.
Interassay variation (n = 3)	2.6%	3.2%	4.9%	2.6%	42.3%	3.5%	1.

3. Gene expression showed different patterns depending on the compound under study



(*) indicates non significant differences between treatment groups (P<0.05)

Figure 2. Time- and substance dependent effects on marker gene expression (n = 6).

Discussion and Future Prospects

22RV1 cells are highly suitable for bio-response linked analysis of androgenic/antiandrogenic compounds.

Proving that described cell line does only express hAR, is important to ensure selective binding and gene activation via this receptor.

Due to potential expression of estrogen or progestin receptor, interpretation of gene expression pattern is not possible in a system including all types of receptors such as laboratory animals.

Stimulation of cells for 6h seemed to be sufficient to obtain substance-specific transcription patterns.

Main advantage in comparison to reporter-gene assays is that six genes can be analysed in parallel.

Using an endogenous gene expression system provides a model which is therfore closer to complex *in vivo* condition.

To simplify the test system for higher sample throughput, RNA isolation will be performed only once after stimulation of 22RV1 cells.

- **Expression patterns are substance–specific**
- **A broader spectrum of compounds will be analysed**
- **Additive/subtractive effects in mixed samples have to be determined**
- **System will be adapted for analysis of putative andorgenic ingredients in e.g. surface- or drinking water**