

Research article

Open Access

Androgen regulation of the androgen receptor coregulators

Alfonso Urbanucci, Kati K Waltering, Hanna E Suikki, Merja A Helenius and Tapio Visakorpi*

Address: Institute of Medical Technology, University of Tampere and Tampere University Hospital, FI-33014 University of Tampere, Tampere, Finland

Email: Alfonso Urbanucci - alfonso.urbanucci@uta.fi; Kati K Waltering - kati.waltering@uta.fi; Hanna E Suikki - hanna.suikki@uta.fi; Merja A Helenius - merja.helenius@uta.fi; Tapio Visakorpi* - tapio.visakorpi@uta.fi

* Corresponding author

Published: 1 August 2008

Received: 14 December 2007

BMC Cancer 2008, 8:219 doi:10.1186/1471-2407-8-219

Accepted: 1 August 2008

This article is available from: <http://www.biomedcentral.com/1471-2407/8/219>

© 2008 Urbanucci et al; licensee BioMed Central Ltd.

This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/2.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Abstract

Background: The critical role of the androgen receptor (AR) in the development of prostate cancer is well recognized. The transcriptional activity of AR is partly regulated by coregulatory proteins. It has been suggested that these coregulators could also be important in the progression of prostate cancer. The aim of this study was to identify coregulators whose expression is regulated by either the androgens and/or by the expression level of AR.

Methods: We used empty vector and AR cDNA-transfected LNCaP cells (LNCaP-pcDNA3.1, and LNCaP-ARhi, respectively), and grew them for 4 and 24 hours in the presence of dihydrotestosterone (DHT) at various concentrations. The expression of 25 AR coregulators (*SRC1*, *TIF2*, *PIAS1*, *PIASx*, *ARIP4*, *BRCA1*, *β-catenin*, *AIB3*, *AIB1*, *CBP*, *STAT1*, *NCoR1*, *AES*, *cyclin D1*, *p300*, *ARA24*, *LSD1*, *BAG1L*, *gelsolin*, *prohibitin*, *JMJD2C*, *JMJD1A*, *MAK*, *PAK6* and *MAGE11*) was then measured by using real-time quantitative RT-PCR (Q-RT-PCR).

Results: Five of the coregulators (*AIB1*, *CBP*, *MAK*, *BRCA1* and *β-catenin*) showed more than 2-fold induction and 5 others (*cyclin D1*, *gelsolin*, *prohibitin*, *JMJD1A*, and *JMJD2C*) less than 2-fold induction. Overexpression of AR did not affect the expression of the coregulators alone. However, overexpression of AR enhanced the DHT-stimulated expression of *MAK*, *BRCA1*, *AIB1* and *CBP* and reduced the level of expression of *β-catenin*, *cyclinD1* and *gelsolin*.

Conclusion: In conclusion, we identified 5 coactivators whose expression was induced by androgens suggesting that they could potentiate AR signaling. Overexpression of AR seems to sensitize cells for low levels of androgens.

Background

Prostate cancer is the most and second most common male malignancy in the USA, and Europe, respectively [1,2]. The androgen-dependence of the growth of prostate cancer has been known for a long time [3]. The importance of the androgens was recently, once again, demonstrated in a large randomized study. In the study the use

of finasteride, an inhibitor of 5 α -reductase, which converts testosterone into more potent 5 α -dihydrotestosterone, was used for prevention of prostate cancer [4]. Almost 25% reduction in the prevalence of prostate cancer was observed in the treatment compared to a placebo arm. Due to the androgen dependence, the golden standard treatment for advanced prostate cancer has been

androgen withdrawal (i.e. castration) for the last half century [5]. The treatment is palliative and although most patients respond to it, the disease will eventually progress [6]. Such tumors, emerging during the androgen withdrawal, are called hormone-refractory ones.

The molecular mechanisms by which prostate cancer cells become resistant to endocrine therapy remain incompletely known. However, the key role of androgens and androgen receptor (AR), not just in early development but also in the progression of prostate cancer has now been demonstrated [7]. The recent finding on genetic rearrangement leading to formation of *TMPRSS2:ERG* fusion gene provides a model for molecular mechanisms how androgens act in promoting the early development of prostate cancer. Due to the rearrangement, the putative oncogene *ERG* becomes androgen regulated [8,9]. It has now also become apparent that the emergence of hormone-refractory prostate cancer is due to reactivation of AR-mediated signaling. It has been experimentally shown that overexpression on AR is required and it is sufficient to transform the growth of prostate cancer cells from androgen-dependence to -independence [10]. Furthermore, it has been shown that hormone-refractory tumors overexpress AR, and one-third of them contains amplification of the *AR* gene [11]. Mutations in the coding region of *AR* have also been found in antiandrogen treated prostate cancers [12,13]. And at least some of the mutations alter the sensitivity of the receptor to other steroid hormones or anti-androgens, such as 17 β -estradiol (E2) or hydroxyflutamide (HF) [14,15].

The transactivation of AR involves several coregulatory proteins [16,17]. Large number of coregulatory proteins have already been identified [18-20]. It has been suggested that changes in the expression of the coregulators may be involved in the development and progression of prostate cancer [18,21,22]. Since androgens and AR are known to be important in the prostate cancer tumorigenesis, it is possible that they also regulate the expression of the coregulators.

In order to identify AR coregulators whose expression is regulated by androgens, we measured the expression of 20 putative coactivators: *SRC1* (alias *NCOA1* nuclear receptor coactivator 1), *TIF2* (alias *NCOA2* nuclear receptor coactivator 2), *PIAS1* (protein inhibitor of activated *STAT*, 1), *PIASx* (alias *PIAS2* protein inhibitor of activated *STAT*, 2), *ARIP4* (alias *RAD54L2* *RAD54*-like 2 (*S. cerevisiae*)), *BRCA1* (breast cancer 1, early onset), *β -catenin* (alias *CTNNB1* catenin (cadherin-associated protein), beta 1, 88 kDa), *AIB3* (alias *NCOA6* nuclear receptor coactivator 6), *AIB1* (alias *NCOA3* nuclear receptor coactivator 3), *CBP* (alias *CREBBP* *CREB* binding protein (Rubinstein-Taybi syndrome)), *STAT1* (signal transducer and activator of

transcription 1, 91 kDa), *p300* (alias *EP300* E1A binding protein p300), *ARA24* (alias *RAN*, member *RAS* oncogene family), *LSD1* (lysine-specific demethylase 1, alias AOF2 amine oxidase (flavin containing) domain 2), *BAG1L* (*BCL2*-associated athanogene, isoform 1L), *gelsolin* (*GSN* (amyloidosis, Finnish type)), *JMJD2C* (alias *JHDM3C/GASC1* jumonji domain containing 2C), *JMJD1A* (alias *JHDM2A* jumonji domain containing 1A), *MAK* (male germ cell-associated kinase), *MAGE11* (alias *MAGEA11* melanoma antigen family A, 11) and 5 putative corepressors: *NCoR1* (nuclear receptor co-repressor 1), *AES* (amino-terminal enhancer of split), *cyclin D1* (alias *CCND1*), *prohibitin (PHB)* and *PAK6* (*p21(CDKN1A)*-activated kinase 6) by using quantitative reverse transcription-PCR (Q-RT-PCR) in AR positive prostate cancer cell line LNCaP treated with different concentrations of DHT. To mimic the common overexpression of AR in hormone-refractory prostate cancer, we also stable transfected AR to LNCaP cell line. The LNCaP-ARhi cells express about 10-folds more AR mRNA and 3 to 4-folds more AR protein than that of the control (empty vector-transfected cell line: LNCaP-pcDNA3.1). The cell model was used to study whether the level of AR has an effect on the mRNA expression of the coregulators.

Methods

Cell culture protocols and transfections

pcDNA3.1(+) empty expression vector (Invitrogen Inc., Carlsbad, CA, USA) and pcDNA3.1(+) inserted with AR coding region were stable transfected into LNCaP (ATCC, Manassas, VA, USA) with Lipofectamine-Plus transfection reagent (Invitrogen Inc.). Transfected clones were selected with 400 μ g/ml geneticin (G418) for 2 weeks. The mRNA level of AR was examined from purified clones using Northern blotting and Q-RT-PCR with Light-Cycler (Roche Inc., Mannheim, Germany). The protein level of AR was analyzed using Western blotting with anti-AR antibody (441, NeoMarkers-Lab Vision Corporation, Fremont, CA, USA). LNCaP-pcDNA3.1 (transfected with empty pcDNA3.1-vector as a control) and LNCaP-ARhi (transfected with AR cloned into pcDNA3.1+ vector) cell lines were cultured according to the ATCC protocol with the addition of Geneticin 200 μ g/ml (Invitrogen Inc.). LNCaP-ARhi has about 10 times higher mRNA level of AR and 3 to 4 times higher protein level of AR than that of the LNCaP-pcDNA3.1 (Figure 1).

LNCaP-pcDNA3.1 and LNCaP-ARhi cells in the end of the exponential growing phase were divided in 1:4 ratio plates in RPMI1640 phenol free medium with 5% charcoal/dextran-treated (CCS) FBS (Hyclone Inc., South Logan, UT, USA), 1% glutamine (Invitrogen Inc.) and 1% penicillin-streptomycin (BioWhittaker Inc., Walkersville, MD, USA) for 4 days. The medium was then changed to phenol free RPMI1640 including 10% CCS-FBS (Hyclone

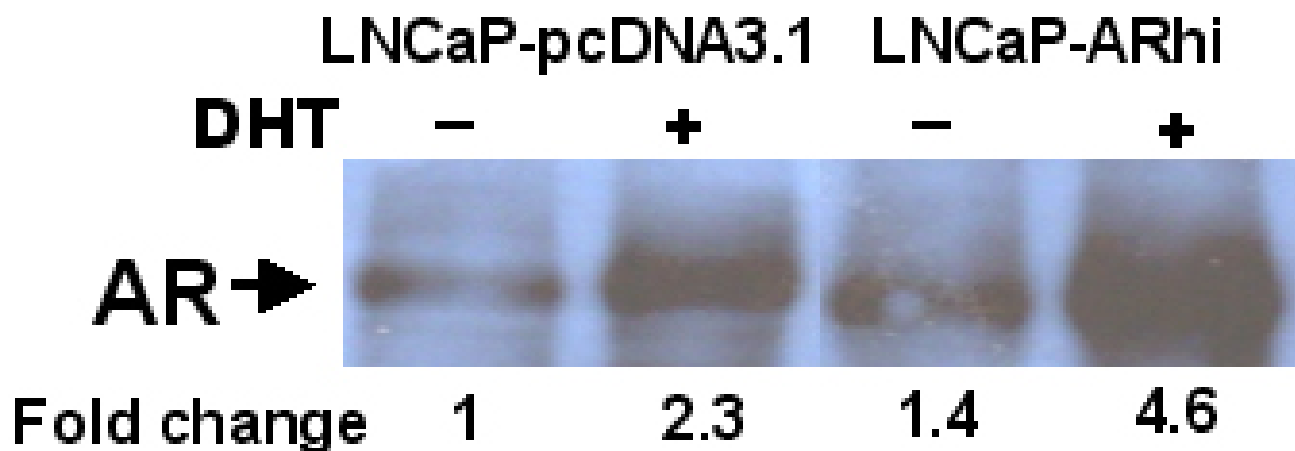


Figure 1
Western blot analysis of AR in empty vector transfected (LNCaP-pcDNA3.1) and AR-cDNA transfected (LNCaP-ARhi) LNCaP cells. Cells were grown either in the absence or presence of DHT. Nuclear fraction of LNCaP-ARhi shows clearly higher AR expression than the control LNCaP-pcDNA3.1. The quantification of the bands by ImageJ is given below the bands.

Inc.), 1% Glutamine (Invitrogen Inc.), 1% Pest (Invitrogen Inc.) and 0, 0.1, 1.0, 10 or 100 nM DHT. The cells were harvested into TRIZOL reagent (Life Technologies Inc., Gaithersburg, MD, USA) after 4 h and 24 h, followed by total RNA isolation according to the manufactures protocol.

RT-PCR

The cDNA was synthesized with AMV Reverse Transcriptase and oligo(dT)12-18 primer according to the manufacturer's protocol (Finnzymes, Espoo Finland). The standards were prepared mixing total RNA from untreated LNCaP cells and universal RNA (Clontech Laboratories, Inc., Mountain View, CA, USA) in a ratio of 1:5. After first strand cDNA synthesis, serial dilutions corresponding to 1000, 200, 40, 8, 1.6, 0.32, 0.064 µg of RNA pool were prepared and stored in aliquots. The PCR reactions were performed in LightCycler apparatus (Roche Inc.) using an LC Fast Start DNA SYBR Green I Kit (Roche Diagnostics, Mannheim, Germany). The final volume of each reaction was 20 µl containing 2 µl of cDNA sample (or standard), 4 mM MgCl₂ (except for PAK6 (5 mM) and JMJD1A (3 mM)), 0.5 µM each primer, and 1× ready-to-use SYBR Green I reaction mix including Taq DNA polymerase, reaction buffer and a deoxynucleotide triphosphate mix. The cycling conditions were designed according to the manufacturer's guidelines which are given in additional file 1: primers' table. The primers were designed for amplifying regions of the mRNAs derived from different exons to avoid amplification of genomic DNA. The melting curve analysis with the LightCycler together with 1.5% agarose gel electrophoresis of the products were used to

ensure that right size product without significant background was amplified in the reaction. The expression levels of the coregulators were normalized to the expression levels of TATA-box binding protein (TBP) as previously described [23].

Western analysis

Cytoplasmatic and nuclear proteins were isolated from subconfluent cells of LNCaP-ARhi. Proteins were separated in 10% SDS-PAGE gel followed by transfer to a membrane (Immobilon-P, Millipore Corp., Billerica, MA) using BIORAD transblot® semi-dry transfer cell (Bio-Rad Laboratories, Inc). After blocking in PBS 0.1% Tween 5% BSA (or 5% non-fat dry milk), the membranes were incubated with primary antibody (AR: dilution 1: 200 Androgen Receptor Ab-1 (AR441) Neomarkers, Fremont, CA; β-catenin: dilution 1:2000 BD Transduction Laboratories, Inc.; MAK: dilution 1:500 MAK antibody (C-term), ABGENT San Diego, CA; CBP: dilution 1:1000, R&D Systems, Inc.) over night at +4 °C. After washes and incubation with secondary antibody (DAKO A/S, Denmark) the bound antibody was visualized on autogradiography film using Western Blotting Luminol Reagent (Santa Cruz, Inc.) according to the manufacturer's protocol. Pan-actin antibody (dilution 1:1000 pan AB-5, clone ACTN05, Neomarkers, Fremont, CA;) was used as a reference.

Statistical analysis

Grubb's test was used to detect the outlier values in the repetitive runs for each gene. One-way ANOVA (Parametric) test with Bonferroni post-test was used to evaluate the statistical significance of the expression level's changes.

Results

To identify androgen regulated coregulators, empty vector transfected LNCaP (LNCaP-pcDNA3.1) was grown in different concentrations of DHT. The total RNA was then collected at two time points (4 h and 24 h). To confirm the success of the DHT stimulation, the expression of prostate-specific antigen (PSA), known androgen regulated gene was first measured (Figure 2). PSA was strongly induced by DHT ($p < 0.0001$ at 4 h and 24 h).

Next, we screened expression of *SRC1*, *TIF2*, *PIAS1*, *PIASx*, *ARIP4*, *BRCA1*, *β-catenin*, *AIB3*, *AIB1*, *CBP*, *STAT1*, *NCoR1*, *AES*, *cyclin D1*, *p300*, *ARA24*, *LSD1* and *BAG1L* coregulators in triplicates (three independent Q-RT-PCRs' runs). Of the coregulators, *PIASx* ($p = 0.0601$ at 4 h and $p = 0.6488$ at 24 h), *BRCA1* ($p = 0.4172$ at 4 h and $p = 0.0377$ at 24 h), *β-catenin* ($p = 0.0272$ at 4 h and $p = 0.4158$ at 24 h), *AIB3* ($p = 0.0690$ at 4 h and $p = 0.1455$ at 24 h), *AIB1* ($p = 0.0401$ at 4 h and $p = 0.2541$ at 24 h), *CBP* ($p = 0.0184$ at 4 h and $p = 0.0588$ at 24 h), *cyclin D1* ($p = 0.2039$ at 4 h and $p = 0.4432$ at 24 h), *ARA24* ($p = 0.0220$ at 4 h and $p = 0.2502$ at 24 h) and *BAG1L* ($p = 0.0359$ at 4 h and $p = 0.3981$ at 24 h), showed dose dependent response to androgens, whereas *SRC1*, *TIF2*,

PIAS1, *ARIP4*, *STAT1*, *NCoR1*, *AES*, *p300* and *LSD1* did not.

PIASx, *BRCA1*, *β-catenin*, *AIB3*, *AIB1*, *CBP*, *cyclin D1*, *ARA24* and *BAG1L* were selected for additional Q-RT-PCR runs in triplicates. In addition, expression of *gelsolin*, *prohibitin*, *JMJD2C*, *JMJD1A*, *MAK*, *PAK6* and *MAGE11* was measured in triplicates. Figures 3 and 4 show the expression pattern of these 16 coregulators upon DHT stimulation. The genes that showed significant dose dependent expression were *AIB1*, *CBP*, *MAK*, *BRCA1*, *β-catenin*, *cyclin D1*, *gelsolin*, *prohibitin*, *JMJD1A*, and *JMJD2C*. Table 1 indicates the p-values of the sixteen genes. *Gelsolin* showed reduced and increased expression at 4 and 24 hour time points, respectively. Others exhibited an androgen-dependent increase in the expression of the mRNAs. However, only *AIB1*, *CBP*, *MAK*, *BRCA1*, and *β-catenin* showed more than 2-folds induction after stimulation with DHT.

In order to evaluate the effect of the level of AR expression on the expression of the coregulators, we utilized LNCaP-based overexpression model (LNCaP-ARhi). The PSA response in the LNCaP-ARhi cell line at 24 hours was significantly ($p < 0.0001$) stronger than in the empty vector transfected cell line (LNCaP-pcDNA3.1) (Figure 2). PSA

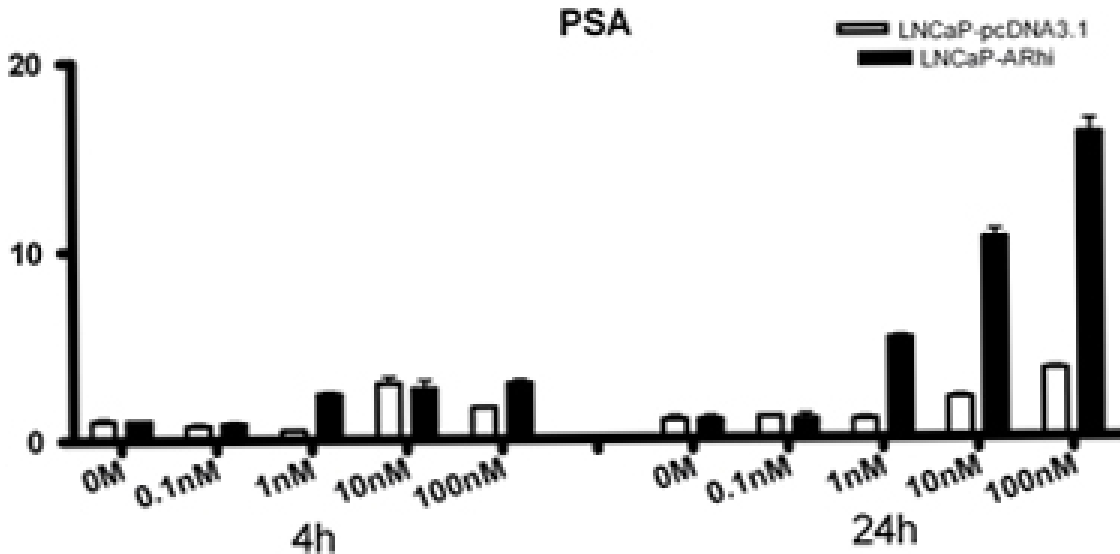


Figure 2 Expression of PSA in LNCaP-pcDNA3.1 and LNCaP-ARhi according to Q-RT-PCR. The cells were cultured in presence of DHT at different concentrations. After 4 and 24 hours, the cells were collected and expression of PSA and TBP mRNA was measured in triplicates by Q-RT-PCR. The bars and whiskers represent mean + S.E.M. of PSA/TBP values normalized against the 0 M of each time point.

Table 1: p-values of the One-way ANOVA (Parametric) test

| Gene | LNCaP-pcDNA3.1 | | LNCaP-ARhi | |
|-------------------|----------------|----------|------------|----------|
| | 4 h | 24 h | 4 h | 24 h |
| <i>AIB1</i> | 0.0019 | < 0.0001 | 0.0023 | < 0.0001 |
| <i>CBP</i> | 0.0478 | 0.0103 | 0.0035 | 0.0205 |
| <i>MAK</i> | 0.1385 | 0.0014 | 0.0090 | 0.1625 |
| <i>BRCA1</i> | 0.0007 | < 0.0001 | 0.0022 | < 0.0001 |
| <i>β-catenin</i> | 0.0336 | < 0.0001 | < 0.0001 | < 0.0001 |
| <i>Cyclin D1</i> | 0.0094 | 0.0094 | 0.6382 | 0.4697 |
| <i>Gelsolin</i> | 0.0016 | < 0.0001 | 0.0029 | 0.4211 |
| <i>Prohibitin</i> | 0.0291 | 0.0084 | 0.0114 | 0.0053 |
| <i>JMJD1A</i> | 0.0276 | 0.0009 | < 0.0001 | 0.4592 |
| <i>JMJD2C</i> | 0.0588 | 0.0064 | 0.0259 | 0.6450 |
| <i>BAG-1L</i> | 0.0029 | 0.1467 | 0.0244 | 0.0150 |
| <i>PIASx</i> | 0.9371 | 0.8551 | 0.8224 | 0.7616 |
| <i>PAK6</i> | 0.1075 | 0.4876 | 0.3359 | 0.6410 |
| <i>MAGE11</i> | 0.7256 | 0.5952 | 0.6438 | 0.6014 |
| <i>AIB3</i> | < 0.0001 | 0.0135 | < 0.0001 | 0.3818 |
| <i>ARA24</i> | 0.0016 | 0.0276 | 0.0007 | 0.0013 |

p-values of the 16 genes were measured in triplicate in the second round analysis.

expression was stimulated already in 1 nM concentration in the LNCaP-ARhi, whereas the stimulation was evident in 10 nM in the LNCaP-pcDNA3.1.

None of the coregulators showed significant alterations in the expression in 0 M DHT between the LNCaP-pcDNA3.1 and LNCaP-ARhi. However, induction of expression in lower concentrations of DHT in LNCaP-ARhi compared to LNCaP-pcDNA3.1 was found in *MAK*, *CBP*, *AIB1*, and *BRCA1*. In contrast, the androgen regulation of *β-catenin*, *cyclin D1* and *gelsolin*, which was apparent in LNCaP-pcDNA3.1 at 24 h was less strong in LNCaP-ARhi (Figures 3 and 4).

Western blot analyses of *β-catenin*, *CBP* and *MAK* were in concordance with the Q-RT-PCR results (see additional file 2: WB analysis).

Discussion

We systematically studied the androgen regulation of the expression of twenty-five AR coregulators. 5 (20%) coregulators showed statistically significant over 2-fold induction by DHT. These were *AIB1*, *CBP*, *MAK*, *BRCA1* and *β-catenin*. In addition, expression of five (20%) other coregulators (*cyclin D1*, *gelsolin*, *prohibitin*, *JMJD1A*, and *JMJD2C*) was significantly, but less than 2-fold, induced by androgens.

AIB1 (Amplified in Breast Cancer 1) encodes for a well characterized protein with histone acetyltransferase activity. It is thought to be a nuclear receptor coactivator that interacts with various nuclear hormone receptors enhanc-

ing their transcriptional activity in a hormone dependent fashion. It is a member of the p160/steroid receptor coactivator (SRC) family and recruits p300/CBP-associated factor, with histone acetylation activity, and CBP (CREB binding protein) as a part of the transcription multisubunit coactivation complex [19,24]. *AIB1* has been found to be amplified in about 10% of breast cancers [25]. Previous studies have indicated that estrogens might have a suppressive role in respect to the expression of *AIB1* [26,27]. Here we found that at 4 hours time point *AIB1* is nearly 2-fold induced by any concentration of DHT while at 24 hours time point a 4-fold induction was observed. The highest induction was at 1 nM DHT while at 10 nM the expression was declined. We used web-based TESS (Transcription Element Search Software) program and TRANSFAC database [28] to search for canonical binding sites for AR in the 2 kb upstream the transcription start site (TSS) of *AIB1*. The program identified several glucocorticoid receptor (GR) responsive elements (GREs) and two putative androgen responsive elements (AREs) at -527 and at -1291 bp from the TSS.

Similarly to *AIB1*, *CBP* was nearly 2-folds induced at 4 hours time point by any concentration of DHT while at 24 hours time point a 4-folds induction can be observed, although such induction can be seen also in 10 nM DHT concentrated media. In contrast with our results, Comuzzi et al. have suggested that *CBP* expression is down-regulated after treating LNCaP cells with synthetic androgen methyltrienolone (R1881) for 48 hours at concentrations of 0.01 nM and 1 nM [29].

MAK (Male Germ Cell-Associated Kinase) is a recently identified AR coactivator [30] that initially has been found to be strongly androgen-regulated in mice kidney [31]. In addition, it has previously been demonstrated that *MAK* is transcriptionally induced by DHT in LNCaP cells showing 9-fold induction by 10 nM DHT at 24 hours [32]. This is confirmed by our finding of 6.5-fold induction at 24 hours time point by 10 nM DHT. *MAK* has been found to be associated with AR and being corecruited in the transcriptional complex enhancing AR activity. Furthermore the modulation of its expression strongly alters the vitality of the cells affecting AR pathway mediated signaling [30]. Jia et al. have shown that *MAK* promoter is a direct target of AR and identified putative ARE sites within the promoter of *MAK* (about -3500 bp from the TSS), and that AR is recruited to the *MAK* promoter after DHT stimulation according to chromatin immunoprecipitation (ChIP) analysis [33]. Altogether the data indicates that the expression of *MAK* is regulated by androgens and since it functions as an AR co-activator, it forms a putative feedback loop augmenting the effects of androgens.

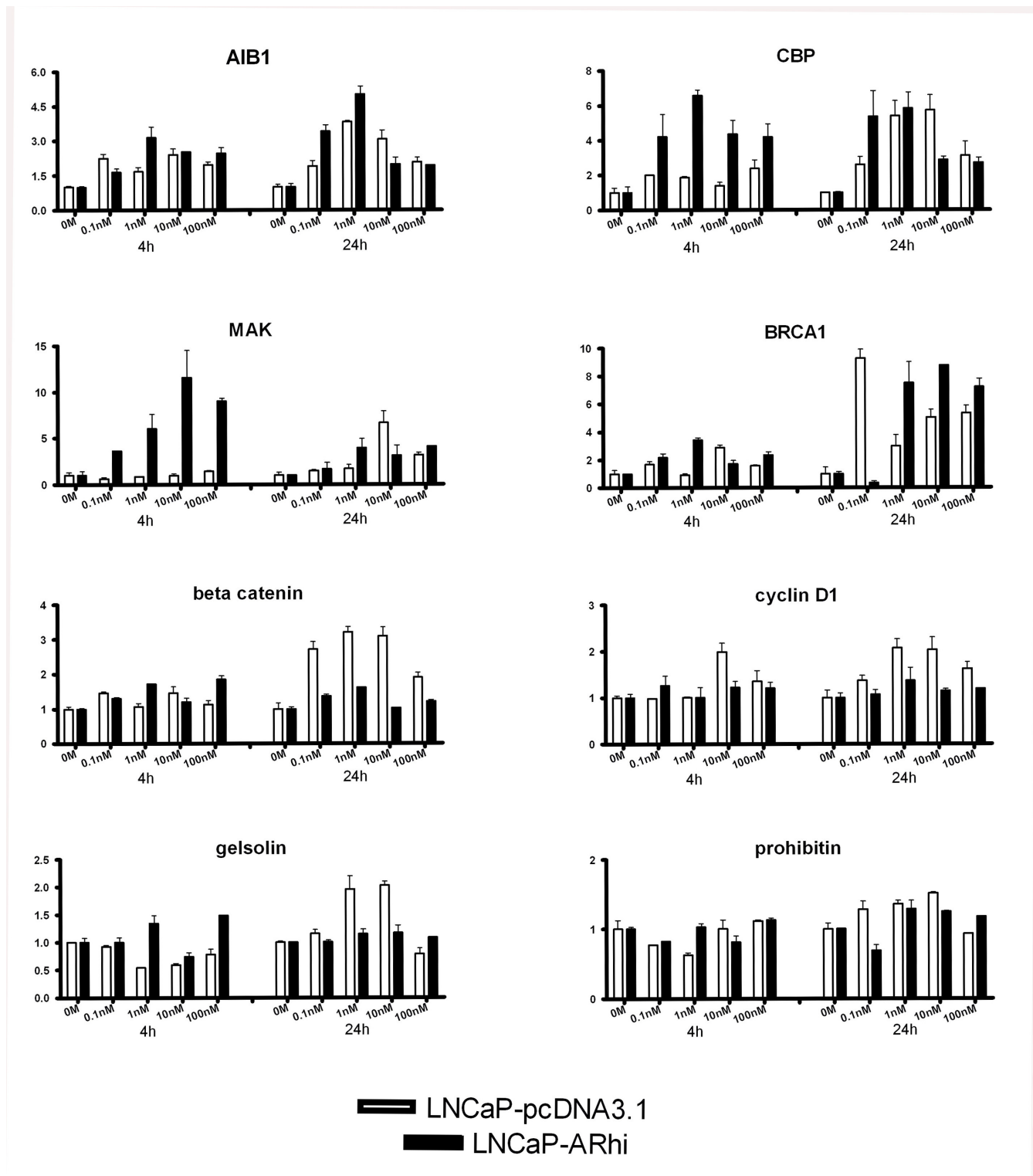


Figure 3
 The expression of *AIB1*, *CBP*, *MAK*, *BRCA1*, β -*catenin*, *cyclin D1*, *gelsolin* and *prohibitin* in LNCaP-pcDNA3.1 and LNCaP-ARhi at 4 and 24 hours according to Q-RT-PCR. The measurements were done in triplicates. The bars and whiskers represent mean + S.E.M. of each gene against the TBP, normalized against the 0 M of each time point. p-values are given in Table I.

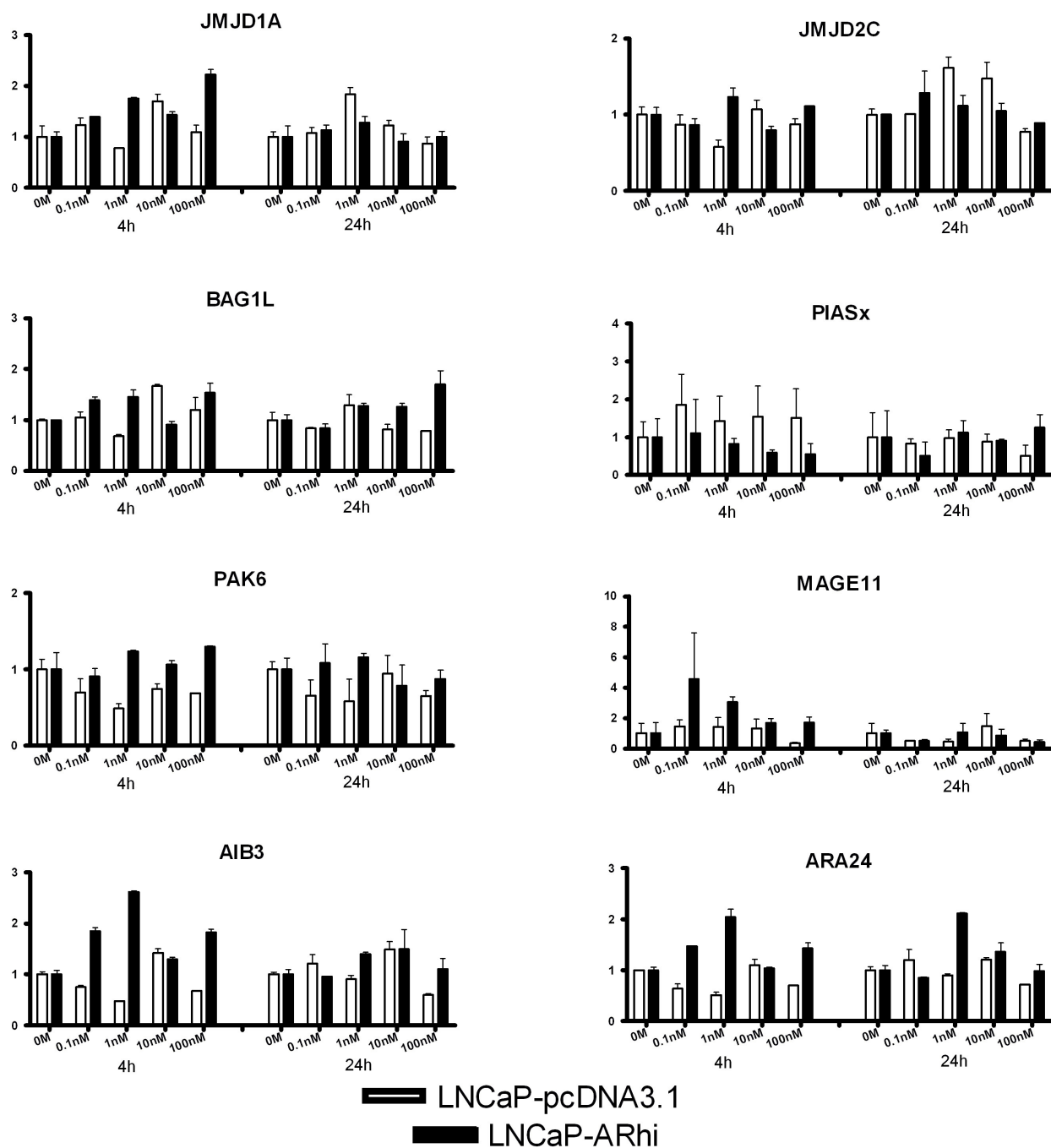


Figure 4
 The expression of *JMJD1A*, *JMJD2C*, *BAG1L*, *PIASx*, *PAK6*, *MAGE11*, *AIB3*, and *ARA24* in LNCaP-pcDNA3.1 and LNCaP-ARhi at 4 and 24 hours according to Q-RT-PCR. The measurements were done in triplicates. The bars and whiskers represent mean + S.E.M. of each gene against the TBP, normalized against the 0 M of each time point. p-values are given in Table I.

BRCA1 (breast cancer 1) is a well characterized gene in breast cancer since mutations in this gene are responsible of at least 40% of inherited breast cancers and more than 80% of inherited ovarian cancers [34,35]. *BRCA1* is also an AR coactivator that has been demonstrated to enhance AR transactivation in prostate and breast cancer cell lines with a synergistic effect with other p160 coactivators [36]. Our data shows an androgen-mediated induction of the expression of *BRCA1*. It has previously been demonstrated that *BRCA1* is an estrogen induced gene in breast and ovarian cancer cells [37,38].

β -catenin is an adherens junction protein with altered expression in various tumor types. Its fundamental role as a multifunctional oncoprotein has been established [39]. The gene encodes for a protein that interacts with AR in close proximity with p160 coactivator family and it is thought to be important for the formation of the AR transcription complex [40,41]. There is some evidence that AR could also have an important role as a scaffolding protein in β -catenin translocation from the cytoplasm into the nucleus [42-44]. β -catenin has been shown to augment AR transcriptional function in a ligand-dependent fashion [43,45]. Here we show that β -catenin is induced up to 2.5-folds at 24 hours time point, already at a concentration of 0.1 nM of DHT. The induction reaches then the 3-folds and declines at 2-folds at 100 nM showing a significant androgen regulation.

In addition to the above mentioned coregulators, *cyclin D1*, *gelsolin*, *prohibitin*, *JMJD1A* and *JMJD2C* showed a significant dose dependent effect in their expression level mainly at 24 hours time point. However, the induction was very mild, less than 2-fold, suggesting either only weak androgen effect or secondary mechanisms for the induction.

It has been demonstrated that AR is commonly overexpressed in hormone-refractory prostate cancer [11,46,47]. In addition, it has been shown that the overexpression is capable to transform androgen dependent growth of prostate cancer cell to independence [9]. To study the effect of AR overexpression, we stable transfected AR to LNCaP cell line leading to 3 to 4-fold increased expression of AR. The growth of LNCaP-ARhi was stimulated on lower concentrations of androgens than the growth of LNCaP-pcDNA3.1 (unpublished data). None of the coregulators were up- or downregulated by the level of AR alone since there was no significant difference in the expression of the coregulators at 0 M of DHT. Instead, the overexpression of AR seems to sensitize cells to androgens. This was evident e.g. for *PSA*, which was significantly upregulated more and in lower concentrations of DHT in LNCaP-ARhi than in LNCaP-pcDNA3.1 cells. Similarly, the expression of *MAK*, *CBP*, and *BRCA1* was increased more in LNCaP-

ARhi than in LNCaP-pcDNA3.1, and, at least, a 10-fold sensitization was observed. For *MAK* and *CBP* this was apparent in 4 h time point, whereas for *BRCA1* at 24 h time point. Interestingly, the increased expression of AR did not affect all androgen-regulated coregulators. *β -catenin*, *cyclin D1* and *gelsolin*, which showed androgen-regulation in LNCaP-pcDNA3.1 at 24 hours were not regulated by androgens in LNCaP-ARhi. The data suggest that the AR overexpression has distinct effects on different target genes. It should be recognized that during the androgen withdrawal, there are still adrenal androgens left. For example, the DHT concentration is only partially depleted [48]. Thus, the genes that are regulated in reduced DHT concentrations due to AR overexpression might be important in the progression of prostate cancer during the hormonal therapy.

The effects of androgens on gene expression were measured at two time points, 4 h and 24 h. The 4 h time points represent a time interval where direct effects of androgens administration should be seen, although the effect can be mild. On the other hand, also secondary mechanisms can already be activated at 24 hours time point. All coregulators (*AIB1*, *CBP*, *MAK*, *BRCA1*, β -catenin) that were induced more than 2-fold by DHT, showed the induction already at 4 time point in one or both cells lines (LNCaP-pcDNA3.1 and LNCaP-ARhi) suggesting direct regulation. Whereas, coregulators with lower-level of induction showed the effect variably in 4 and 24 hour points, which may also suggest secondary mechanisms of androgen regulation.

Conclusion

In conclusion, by systematic measurement of 25 AR coregulators, we identified 5 coregulators whose expression seems to be significantly androgen-regulated indicating that they could be a part of positive feedback mechanisms potentiating the AR signaling. Overexpression of AR seems to sensitize cells in terms of androgen regulation but the effect is gene-specific since not all androgen-regulated genes were up-regulated in AR overexpressing model.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

AU: acquired the data through PCR, prepared the manuscript, analyzed and interpreted the data. KW: made the cell-culture work. HM: supported the PCR work. HS: established the AR overexpression cell lines. TV: designed the study, helped preparing the manuscript and analyzing and interpreting the data. All authors read and approved the final manuscript.

Additional material

Additional file 1

Additional Table 1 – primers' sequences and cycling conditions. primers' sequences and cycling conditions.

Click here for file

[<http://www.biomedcentral.com/content/supplementary/1471-2407-8-219-S1.pdf>]

Additional file 2

Additional Figure 1. Western blot analysis. Western analysis of β -catenin, CBP, and MAK in LNCaP-ARhi cells treated with different concentrations of DHT.

Click here for file

[<http://www.biomedcentral.com/content/supplementary/1471-2407-8-219-S2.pdf>]

Acknowledgements

The authors wish to acknowledge Ms. Mariitta Vakkuri for the technical assistance. This work was supported by Academy of Finland, Cancer Society of Finland, Reino Lahtikari Foundation, Sigrid Juselius Foundation, Finnish Cultural Foundation, the Medical Research Fund of Tampere University Hospital, and the European Union FP6, CANCURE Programme (Contract Number: MEST-CT-2005-020970).

References

- Cooperberg MR, Moul JW, Carroll PR: **The changing face of prostate cancer.** *J Clin Oncol* 2005, **23**:8146-8151.
- Boyle P, Ferlay J: **Cancer incidence and mortality in Europe, 2004.** *Ann Oncol* 2005, **16**:481-488.
- Isaacs JT: **Role of androgens in prostatic cancer.** *Vitam Horm* 1994, **49**:433-502.
- Thompson IM, Goodman PJ, Tangen CM, Lucia MS, Miller GJ, Ford LG, Lieber MM, Cespedes RD, Atkins JN, Lippman SM, Carlin SM, Ryan A, Szczepanek CM, Crowley JJ, Coltman CA Jr: **The influence of finasteride on the development of prostate cancer.** *N Engl J Med* 2003, **349**:215-224.
- Huggins C, Hodges CV: **Studies on prostatic cancer, I: the effect of castration, of estrogen and of androgen injection on serum phosphatases in metastatic carcinoma of the prostate 1941.** *J Urol* 2002, **168**(1):9-12.
- Gittes RF: **Carcinoma of the prostate.** *N Engl J Med* 1991, **324**:236-245.
- Linja MJ, Visakorpi T: **Alterations of androgen receptor in prostate cancer.** *J Steroid Biochem Mol Biol* 2004, **92**:255-264.
- Tomlins SA, Rhodes DR, Perner S, Dhanasekaran SM, Mehra R, Sun X-W, Varambally S, Cao X, Tchinda J, Kuefer R, Lee C, Montie JE, Shah RB, Pienta KJ, Rubin MA, Chinnaiyan AM: **Recurrent fusion of TMPRSS2 and ETS transcription factor genes in prostate cancer.** *Science* 2005, **310**:644-648.
- Perner S, Demichelis F, Beroukhir R, Schmidt FH, Mosquera J-M, Setlur S, Tchinda J, Tomlins SA, Hofer MD, Pienta KG, Kuefer R, Vessella R, Sun X-W, Meyerson M, Lee C, Sellers WR, Chinnaiyan AM, Rubin MA: **TMPRSS2:ERG fusion-associated deletions provide insight into the heterogeneity of prostate cancer.** *Cancer Res* 2006, **66**:8337-8341.
- Chen CD, Welsbie DS, Tran C, Baek SH, Chen R, Vessella R, Rosenfeld MG, Sawyers CL: **Molecular determinants of resistance to antiandrogen therapy.** *Nat Med* 2004, **10**:33-39.
- Linja MJ, Savinainen KJ, saramäki OR, Tammela TM, Vessella RL, Visakorpi T: **Amplification and overexpression of androgen receptor gene in hormone-refractory prostate cancer.** *Cancer Res* 2001, **61**:3550-3555.
- Taplin ME, Bubleby GJ, Ko YJ, Small EJ, Upton M, Rajeshkumar B, Balk SP: **Selection for androgen receptor mutations in prostate cancers treated with androgen antagonist.** *Cancer Res* 1999, **59**:2511-2515.
- Wallén MJ, Linja M, Kaartinen K, Schleutker J, Visakorpi T: **Androgen receptor gene mutations in hormone-refractory prostate cancer.** *J Pathol* 1999, **189**:559-563.
- Haapala K, Hyytinen ER, Roiha M, Laurila M, Rantala I, Helin HJ, Koivisto PA: **Androgen receptor alterations in prostate cancer relapsed during a combined androgen blockade by orchiectomy and bicalutamide.** *Lab Invest* 2001, **81**:1647-1651.
- Hara T, Miyazaki J-I, Araki H, Yamaoka M, Kanzaki N, Kusaka M, Miyamoto M: **Novel mutations of androgen receptor: a possible mechanism of bicalutamide withdrawal syndrome.** *Cancer Res* 2003, **63**:149-153.
- Lee DK, Chang C: **Molecular communication between androgen receptor and general transcription machinery.** *J Steroid Biochem Mol Biol* 2003, **84**:41-44.
- Rahman M, Miyamoto H, Chang C: **Androgen receptor coregulators in prostate cancer: mechanisms and clinical implications.** *Clin Cancer Res* 2004, **10**:2208-2219.
- Culig Z, Comuzzi B, Steiner H, Bartsch G, Hobisch A: **Expression and function of androgen receptor coactivators in prostate cancer.** *J Steroid Biochem* 2004, **92**:265-271.
- Chmelar R, Buchanan G, Need EF, Tilley W, Greenberg NM: **Androgen receptor coregulators and their involvement in the development and progression of prostate cancer.** *Int J Cancer* 2007, **120**(4):719-733.
- Burd CJ, Morey LM, Knudsen KE: **Androgen receptor corepressors and prostate cancer.** *Endocr Relat Cancer* 2006, **13**:979-994.
- Balk SP: **Androgen receptor as a target in androgen-independent prostate cancer.** *Urology* 2002, **60**:132-139.
- Fujimoto N, Mizokami A, Harada S, Matsumoto T: **Different expression of androgen receptor coactivators in human prostate.** *Urology* 2001, **58**:289-294.
- Linja MJ, Porkka KP, Kang Z, Savinainen KJ, Jänne OA, Tammela TLJ, Vessella RL, Palvimo JJ, Visakorpi T: **Expression of androgen receptor coregulators in prostate cancer.** *Clin Cancer Res* 2004, **10**:1032-1040.
- Heinlein CA, Chang C: **Androgen receptor (AR) coregulators: An overview.** *Endocr Rev* 2002, **23**:175-200.
- Anzick SL, Kononen J, Walker RL, Azorsa DO, Tanner MM, Guan X-Y, Sauter G, Kallioniemi O-P, Trent JM, Meltzer PS: **AIB1, a steroid receptor coactivator amplified in breast and ovarian cancer.** *Science* 1997, **277**:965-968.
- Lauritsen KJ, List H-J, Reiter R, Wellstein A, Riegel AT: **A role for TGF- β in estrogen and retinoid mediated regulation of the nuclear receptor coactivator AIB1 in MCF-7 breast cancer cells.** *Oncogene* 2002, **21**:7147-7155.
- Lonard DM, Tsai SY, O'Malley BW: **Selective estrogen receptor modulators 4-hydroxytamoxifen and raloxifene impact the stability and function of SRC-1 and SRC-3 coactivator proteins.** *Mol Cell Biol* 2004, **24**:14-24.
- TESS: **Transcription Element Search Software on the WWW** [<http://www.cbil.upenn.edu/tess>]
- Comuzzi B, Nemes C, Schmidt S, Jasarevic Z, Lodde M, Pycha A, Bartsch G, Offner F, Culig Z, Hobisch A: **The androgen receptor coactivator CBP is up-regulated following androgen withdrawal and is highly expressed in advanced prostate cancer.** *J Pathol* 2004, **204**:159-166.
- Ma A-H, Xia L, Desai SJ, Boucher DL, Guan Y, Shih H-M, Shi X-B, deVere White RW, Chen H-W, Tepper CG, Kung H-J: **Male germ cell-associated kinase, a male-specific kinase regulated by androgen, is a coactivator of androgen receptor in prostate cancer cells.** *Cancer Res* 2006, **66**:8439-8447.
- Snider LD, King D, Lingrel JB: **Androgen regulation of MAK mRNAs in mouse kidney.** *J Biol Chem* 1985, **260**:9884-9893.
- Xia L, Robinson D, Ma A-H, Chen H-C, Wu F, Qiu Y, Kung H-J: **Identification of human male germ cell-associated kinase, a kinase transcriptionally activated by androgen in prostate cancer cells.** *J Biol Chem* 2002, **277**:35422-35433.
- Jia L, Coetzee GA: **Androgen receptor-dependent PSA expression in androgen-independent prostate cancer cells does not involve androgen receptor occupancy of the PSA locus.** *Cancer Res* 2005, **65**:8003-8008.
- Rahman N, Stratton MR: **The genetics of breast cancer susceptibility.** *Annu Rev Genet* 1998, **32**:95-121.

35. Irminger-Finger I, Siegel BD, Leung W-C: **The functions of breast cancer susceptibility gene 1 (BRCA1) product and its associated proteins.** *Biol Chem* 1999, **380(2)**:117-128.
36. Park JJ, Irvine RA, Buchanan G, Koh SS, Park JM, Tilley WD, Stallcup MR, Press MF, Coetzee GA: **Breast cancer susceptibility gene 1 (BRCA1) is a coactivator of the androgen receptor.** *Cancer Res* 2000, **60**:5946-5949.
37. Gudas JM, Nguyen H, Li T, Cowan KH: **Hormone-dependent regulation of BRCA1 in human breast cancer cells.** *Cancer Res* 1995, **55**:4561-4565.
38. Romagnolo D, Annab LA, Thompson TE, Risinger JI, Terry LA, Barrett JC, Afshari CA: **Estrogen upregulation of BRCA1 expression with no effect on localization.** *Mol Carcinog* 1998, **22**:102-109.
39. Morin PJ: **Beta-catenin and cancer.** *Bioessays* 1999, **21**:1021-1030.
40. Song LN, Herrrell R, Byers S, Shah S, Wilson EM, Gelaman EP: **beta-catenin binds to the activation function 2 region of the androgen receptor and modulates the effects of the N-terminal domain and TIF2 on ligand-dependent transcription.** *Mol Cell Biol* 2003, **23**:1674-1687.
41. Song LN, Gelaman EP: **Interaction of beta-catenin and TIF2/GRIPI in transcriptional activation by the androgen receptor.** *J Biol Chem* 2005, **280**:37853-37867.
42. Pawloski JE, Ertel JR, Allen MP, Xu M, Butler C, Wilson EM, Wierman ME: **Liganded androgen receptor interaction with beta-catenin.** *J Biol Chem* 2002, **277**:20702-20710.
43. Yang F, Li X, Sharmu M, Sasaki CY, Longo DL, Lim B, Sun Z: **Linking beta-catenin to androgen-signaling pathway.** *J Biol Chem* 2002, **277**:11336-11344.
44. Mulholland DJ, Cheng H, Reid K, Rennie PS, Nelson CC: **The androgen receptor can promote beta-catenin nuclear translocation independently of adenomatous polyposis coli.** *J Biol Chem* 2002, **277**:17933-17943.
45. Truica CI, Byers S, Gelmann EP: **beta-catenin affects androgen receptor transcriptional activity and ligand specificity.** *Cancer Res* 2000, **60**:4709-4713.
46. Visakorpi T, Hyytinen E, Koivisto P, Tanner M, Keinänen R, Palmberg C, Palotie A, Tammela TM, Isola J, Kallioniemi O-P: **In vivo amplification of the androgen receptor gene and progression of human prostate cancer.** *Nat genet* 1995, **9**:401-406.
47. Latil A, Bieche I, Vidaud D, Lidereau R, Berthon P, Cussenot O, Vidaud M: **Evaluation of androgen, estrogen (ER alpha and ER beta), and progesterone receptor expression in human prostate cancer by real-time quantitative reverse transcription-polymerase chain reaction assays.** *Cancer Res* 2001, **61**:1919-1926.
48. Mostaghel EA, Page ST, Lin DW, Fazli L, Coleman IM, True LD, Knudsen B, Hess DL, Nelson CC, Matsumoto AM, Bremner WJ, Gleave ME, Nelson PS: **Intraprostatic androgens and androgen-regulated gene expression persist after testosterone suppression: therapeutic implications for castration-resistant prostate cancer.** *Cancer Res* 2007, **67**:5033-5041.

Pre-publication history

The pre-publication history for this paper can be accessed here:

<http://www.biomedcentral.com/1471-2407/8/219/prepub>

Publish with **BioMed Central** and every scientist can read your work free of charge

"BioMed Central will be the most significant development for disseminating the results of biomedical research in our lifetime."

Sir Paul Nurse, Cancer Research UK

Your research papers will be:

- available free of charge to the entire biomedical community
- peer reviewed and published immediately upon acceptance
- cited in PubMed and archived on PubMed Central
- yours — you keep the copyright

Submit your manuscript here:
http://www.biomedcentral.com/info/publishing_adv.asp

