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RESEARCH ARTICLE

5α-androstane-3α,17β-diol selectively activates the canonical PI3K/AKT pathway: a bioinformatics-based evidence for androgen-activated cytoplasmic signaling

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Abstract 5α-Androstane-3α,17β-diol (3α-diol) is reduced from the potent androgen, 5α-dihydrotestosterone (5α-DHT), by reductive 3α-hydroxysteroid dehydrogenases (3α-HSDs) in the prostate. 3α-diol is recognized as a weak androgen with low affinity toward the androgen receptor (AR), but can be oxidized back to 5α-DHT. However, 3α-diol may have potent effects by activating cytoplasmic signaling pathways, stimulating AR-independent prostate cell growth, and, more importantly, providing a key signal for androgen-independent prostate cancer progression. A cancer-specific, cDNA-based membrane array was used to determine 3α-diol-activated pathways in regulating prostate cancer cell survival and/or proliferation. Several canonical pathways appeared to be affected by 3α-diol-regulated responses in LNCaP cells; among them are apoptosis signaling, PI3K/AKT signaling, and death receptor signaling pathways. Biological analysis confirmed that 3α-diol stimulates AKT activation; and the AKT pathway can be activated independent of the classical AR signaling. These observations sustained our previous observations that 3α-diol continues to support prostate cell survival and proliferation regardless the status of the AR. We provided the first systems biology approach to demonstrate that 3α-diol-activated cytoplasmic signaling pathways are important components of androgen-activated biological functions in human prostate cells. Based on the observations that levels of reductive 3α-HSD expression are significantly elevated in localized and advanced prostate cancer, 3α-diol may, therefore, play a critical role for the transition from androgen-dependent to androgen-independent prostate cancer in the presence of androgen deprivation.

Keywords Androgen receptor · Cell proliferation · Gene expression · Microarray · PI3K/AKT pathway · Prostate cancer

Abbreviations

3α-diol	5α-Androstane-3α, 17α-diol
3α-HSD	3α-Hydroxysteroid dehydrogenase
5α-DHT	5α-Dihydrotestosterone
AR	Androgen receptor
ARG	Androgen responsive gene
BNT	Below noise threshold
FBS	Fetal bovine serum
GEO	Gene expression omnibus

Introduction

Androgens are responsible for physiological differentiation, growth, and maintenance as well as pathological development of androgen target tissues including the prostate. In mammals, the principal androgens are testosterone and its active metabolite 5α-dihydrotestosterone (5α-DHT) (McPhaul and Young 2001). Both testosterone

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and 5α-DHT bind and activate the androgen receptor (AR): 5α-DHT has a high affinity toward the AR with K_d (dissociation constant) = 10^{-10} M (Keenan et al. 1984), whereas the K_d of testosterone is approximately 1/3 of 5α-DHT (Grover and Odell 1975). After androgen binding, the ligand occupied AR translocates to the nucleus and leads to transcriptional activation of androgen responsive genes (ARGs) (Heinlein and Chang 2004).

In the prostate, 5α-DHT can be reduced to 5α-androstane-3α, 17β-diol (3α-diol) through the action of 3α-hydroxysteroid dehydrogenases (3α-HSDs). Two 3α-HSD isozymes, type 2 3α-HSD (AKR1C3) and type 3 3α-HSD (AKR1C2), are abundantly expressed in human prostate (Penning et al. 2000). Both 3α-HSDs possess dominant 5α-DHT reduction activity toward 3α-diol formation in vitro and in human prostate cells (Lin et al. 1997; Rizner et al. 2003). In contrast to 5α-DHT, the cellular responses to 3α-diol remain largely unknown. 3α-Diol has a low binding affinity to the AR ($K_d = 10^{-6}$ M) (Penning 1997), is generally considered a weak androgen and, as of yet, has no defined hormonal action. Therefore, it is believed that 3α-diol must be first converted to 5α-DHT by oxidative 3α-HSDs before exerting an androgenic action (Jacobi et al. 1978; Bauman et al. 2006).

However, the above assumption could not explain 3α-diol's action in a variety of androgen target tissues. 3α-Diol is more potent than 5α-DHT in the induction of prostatic hyperplasia in the castrated dog model (Walsh and Wilson 1976), and is involved in prostate formation in marsupial animals (Shaw et al. 2000). 3α-Diol has also been shown to be a critical hormone in virilizing the rat urogenital tract (Schultz and Wilson 1974), in the maintenance and regeneration of the prostate gland and seminal vesicles in a hypophysectomized rat model (Ahmad et al. 1978), and in parturition in a mouse model (Mahendroo et al. 1999). In addition, Wu et al. showed that elevated serum levels of 3α-diol metabolite in African-American and white men compared to Asian-American men, and suggested a correlation between 3α-diol metabolite and the increased prostate cancer risk seen in the first two groups (Wu et al. 2001). These reports suggest that 3α-diol may be an important hormone with its own functions through as yet undefined pathways.

However, the mechanism of 3α-diol-regulated cellular physiology remains largely unknown. Through gene expression profiling analysis, we identified that 3α-diol and 5α-DHT regulate distinct gene expression patterns in androgen sensitive human prostate cancer LNCaP cells (Nunlist et al. 2004; Zimmerman et al. 2004). In the present study, we identified potential 3α-diol-activated signaling pathways using bioinformatics approaches based on genes that are regulated by 3α-diol in human prostate LNCaP cells.

Materials and methods

Reagents and chemicals

Human prostate cancer cell line LNCaP was obtained from ATCC (CRL-1740; Manassas, VA). 3α-Diol and mouse anti-β-actin antibody were purchased from Sigma-Aldrich (St. Louis, MO). [α-^{32}P]dATP (3,000 Ci/mmol, 10 μCi/μl) was obtained from Amersham Pharmacia (Piscataway, NJ). RPMI 1640 medium, OPTI-MEM, penicillin–streptomycin, fetal bovine serum (FBS), and Lipofectamine 2000 were purchased from Invitrogen (Grand Island, NY). Charcoal-dextran treated (CD) FBS with testosterone levels $<10^{-10}$ M was obtained from HyClone (Logan, UT). Atlas nylon Human Cancer 1.2 cDNA Expression Array was purchased from Clontech Laboratories (Palo Alto, CA). Rabbit anti-phospho AKT Ser(473) antibody was purchased from Cell Signaling Technology (Danvers, MA).

LNCaP cell culture, transfection, and 3α-diol treatment

LNCaP cells were maintained in the complete growth medium consisting of RPMI 1640 supplemented with 10% FBS, 5 units/ml penicillin, and 5 μg/ml streptomycin at 37°C and 5% CO_2. Cells were passaged every 3–5 days or whenever cells reached 70–80% confluence. To perform androgen stimulation, 1×10^6 LNCaP cells were first seeded in 60 mm tissue culture plates, subjected to serum deprivation by culturing the cells with OPTI-MEM supplemented with 1% CD FBS for 24 h followed by 10^{-11} M (for array analysis) or 10^{-8} M (for Western blot analysis) 3α-diol stimulation.

To determine the involvement of the AR in 3α-diol-regulated cytoplasmic signaling, AR expression was suppressed by transfecting LNCaP cells with a plasmid-based siRNA construct as we previously described (Yang et al. 2005). Briefly, 2×10^6 LNCaP cells were first mixed with 3 μg AR specific (pSiAR-EGFP) or control siRNA (pSi-Con-EGFP) plasmid in 100 μl of Nucleofector solution R, and transfection was accomplished using the Nucleofector transfection device (Amaxa Biosystems; Gaithersburg, MD). Cells were then returned to pre-warmed completed medium and incubated in a cell incubator for 24 h. Cells were then subjected to serum deprivation for another 24 h followed by 10^{-8} M 3α-diol stimulation.

cDNA-based membrane array hybridization and image acquisition

To identify transcription profiling in LNCaP cells following 3α-diol stimulation, cDNA-based membrane arrays were used. There were a total of 1,176 genes present in the Atlas Human Cancer 1.2 Expression Array. For a complete list of genes that were printed on these arrays, see

http://www.clontech.com/products/detail.asp?product_id=10454&tabno=2. Procedures for total RNA isolation and quantitation, radiolabeled probes preparation and purification, as well as membrane hybridization and washing were described previously (Nunlist et al. 2004; Zimmerman et al. 2004). Following the washing steps, array membranes were exposed to phosphor-imaging screens (Packard BioScience; Meriden, CT); and images were captured by a Cyclone storage phosphorimager system (Packard BioScience). Results from the phosphor-imaging system were presented as digital light units and interpreted using OptiQuant image analysis software (Packard BioScience).

Data normalization and target gene identification

To perform array analysis, the background of the array membranes was first determined by the average and standard deviation (SD) of all 276 background spots, consisting of spots between probes areas and spots on the rims indicated in the array membranes for each array. Total system noise around the true zero point was normally distributed with right-tail positively skewed. Points exceeding 3 SD above the mean were eliminated until the true normal distribution was successively observed; usually this procedure involved the removal of no more than 5–10 points. The mean of the remaining background measurements was subtracted from all expression measurements as reported by others (Dozmorov and Centola 2003). Genes whose expression levels exceeded 2 SD above the background levels were considered to be expressed genes. In contrast, genes whose expression levels did not exceed the 2 SD thresholds were marked as below noise threshold (BNT). 3α-Diol-regulated genes were identified if their expression levels changed from BNT to exceed the 2 SD thresholds (off-on genes) or the opposite (on-off genes) following 3α-diol stimulation. Full microarray data were deposited in Gene Expression Omnibus (GEO) and are accessible on the GEO web-site (GSE8860).

Network generation and pathway analysis

The collective responsive genes were used to identify underlying pathways regulated by 3α-diol in prostate cells. The responsive genes were analyzed as one set by Ingenuity Pathway Analysis (IPA; Ingenuity® Systems, Redwood City, CA, http://www.ingenuity.com), a web-based bioinformatics application, to identify canonical pathways over-represented in the data set. The identified pathways were then evaluated against the full expression data to identify each gene's potential participation in 3α-diol-regulated cellular responses. The responsive genes were not ranked by gene expression levels.

Western blot analysis

Total cellular proteins were harvested following 3α-diol stimulation with RIPA buffer supplemented with proteinase inhibitors as we described (Yang et al. 2005). Protein concentration was determined by bicinchoninic acid (BCA) protein assay kit (Pierce; Rockford, IL). Total proteins separation and transfer were also performed as previously described procedures (Yang et al. 2005). Phospho-AKT and β-actin expression was detected by incubating protein membranes with appropriate primary antibodies against these molecules followed by peroxidase-conjugated secondary antibodies incubation. Immunoreactive proteins were detected using the enhanced chemiluminescent (ECL) reagent (Pierce).

Results

Identification of 3α-diol responsive genes in human prostate cancer LNCaP cells

Of the total of 1,176 genes on the array, 825 genes were identified to be expressed following 3α-diol administration. Of these 825 genes, 416 genes were expressed below the noise levels but up- or down-regulated significantly following 3α-diol stimulation: 305 genes were significantly up-regulated, whereas another 111 genes were down-regulated to below the noise threshold level. The remaining 409 genes remained on before and after 3α-diol stimulation. The list of 3α-diol responsive genes is provided in supplemental Table 1.

Identification 3α-diol-regulated signaling pathways using canonical pathway analysis

Signaling pathways, most affected by 3α-diol, included apoptosis signaling, PI3K/AKT signaling, and death receptor (Fig. 1). The PI3K/AKT signaling pathway appeared to have the highest statistical significance among all identified pathways, whereas the death receptor signaling had the highest ratio of genes overrepresented in the pathway.

With accumulating knowledge about cellular processes, the majority of the identified genes are participants in multiple canonical pathways. Therefore, it was not surprising that genes identified in the dataset are over-represented in overlapping canonical pathways. In the analysis of 3α-diol responsive genes, multiple genes were shown to be shared by more than one pathways as shown in the Venn diagram (Fig. 2). For examples, 3α-diol-regulated, MAP3K5, NFKB2, NFKB1A, and RELB were commonly shared among apoptosis signaling, PI3K/AKT signaling, and death receptor signaling pathways.

Table 1 Genes overrepresented in Cell Cycle, PI3K/AKT, apoptosis and death receptor signaling canonical pathways. Expression level are shown as Log10 values

	Accession number	Before 3α-diol treatment	After 3α-diol treatment
Cell cycle controlling genes			
Cyclin D3	M92287	3.22	BNT
CDK2	M68520	3.02	3.60
CDC25A	M81933	2.20	3.43
TP53	M14694	3.30	3.70
Cyclin D1	X59798	3.54	3.93
E2F-1	M96577	3.34	3.84
RB1	M15400	3.50	4.18
CDK4	M14505	3.37	4.50
CDC2	X05360	3.31	4.65
Cyclin B1	M25753	3.69	5.02
Cyclin D2	D13639	BNT	BNT
CDC25C	M34065	BNT	3.35
APC	M74088	BNT	3.44
PI3K/AKT signaling			
AXL	M76125	2.42	BNT
ITGA2	M28249	2.20	BNT
PTK7	U33635	3.88	BNT
TIE1	X60957	1.47	BNT
AKT1	M63167	3.17	BNT
AKT2	M77198	BNT	3.16
FRAP1	L34075	BNT	1.63
ILK	U40282	BNT	3.66
PPP2R5E	L76703	BNT	3.01
JAK2	AF005216	BNT	3.58
RELB	M83221	BNT	3.90
MAP3K5	D84476	BNT	3.67
NFKB2	X61498	BNT	3.57
SFN	AF029082	BNT	3.48
NFKBIA	M69043	BNT	3.86
FGFR1	M37722	BNT	3.34
MAPK3	X60188	BNT	3.58
EGFR	X00588	BNT	3.73
PPP2R5B	L42374	BNT	2.62
PPP2R5A	L42373	BNT	4.22
TYRO3	D17517	BNT	3.81
ERBB4	L07868	BNT	2.65
PDGFRA	M21574	BNT	3.27
PTEN	U92436	BNT	4.08
MAP2K2	L11285	BNT	3.61
NRAS	X02751	BNT	4.20
Apoptosis			
MAP3K14	Y10256	3.72	BNT
MAP2K7	AF022805	2.91	BNT
CASP8	U60520	2.83	BNT
CASP7	U37448	BNT	2.68
ROCK1	U43195	BNT	3.28
PARP1	M18112	BNT	3.81
TNFRSF1A	M33294	BNT	3.14
BAK1	U23765	BNT	3.38
FAS	Z70519	BNT	3.14
BAX	L22474	BNT	3.45
APAF1	AF013263	BNT	2.96
CASP2	U13021	BNT	4.07
Death receptor signaling			
TNFRSF25	Y09392	3.67	BNT
CRADD	U84388	3.34	BNT
CFLAR	AF010127	BNT	2.39
TNFRSF10B	AF016268	BNT	1.92

3α-Diol augmented PI3K/AKT signaling for prostate cell growth

Major players in the identified pathways are shown in Table 1 along with their expression levels. The complete mapping of the identified genes in canonical pathways for PI3K/AKT, apoptosis, and cell death are shown in Fig. 3 and Supplemental Figs. 1S and 2S. The PI3K/AKT signaling pathway is involved in a variety of biological functions; among them are cell cycle progression, cell death, and cell growth. We showed that 26 genes identified from the Cancer cDNA array are involved in the PI3K/AKT signaling pathway. The majority of these genes were up-regulated following 3α-diol administration. 3α-Diol-stimulated prostate cell growth may be mediated through activated AKT to cRAF-MEK1/2-ERK1/2 pathway.

Other branches of this pathway, such as cell cycle progression, cell death, cell survival, seemed to be unaffected, since genes involved these biological activities remained unchanged following 3α-diol treatment (Fig. 3). Analysis of the cell cycle genes showed that the majority of cell cycle regulators remained constant before and after 3α-diol administration indicating cell cycle progression remained constant even in the presence of 3α-diol. Up-regulation of some members of the apoptosis and death receptor signaling was observed; however, key blocks such as non-expression of BCL-2 and BAD apparently terminated transmission of cell death signals. Although the death receptor signaling pathway was up-regulated in response to 3α-diol, the activation of death receptor signaling apparently was countered by the activation of the PI3K/AKT survival pathway. In addition, 3α-diol-treated LNCaP cells might evade from cell death through an NF-κB-independent pathway, since IκB

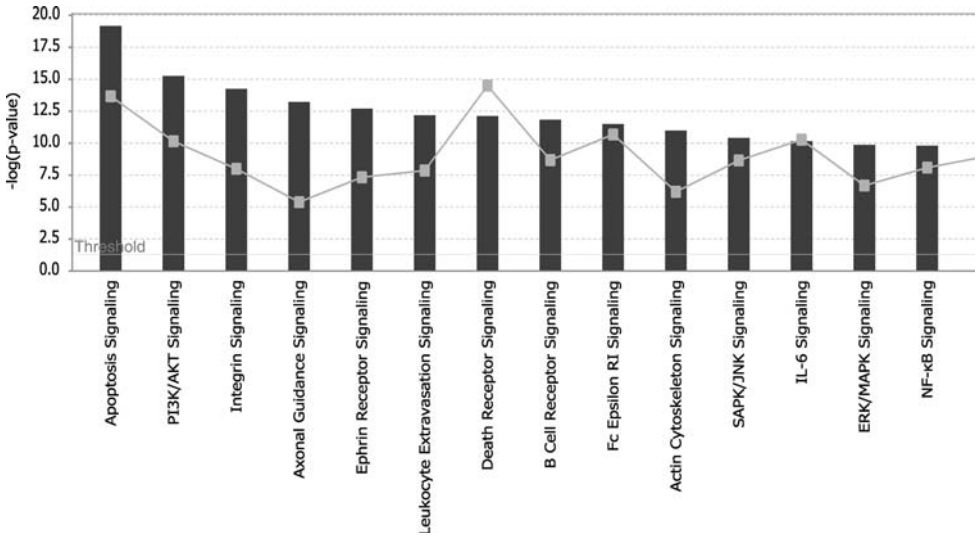

Fig. 1 3α-Diol-activated canonical pathways in human prostate cancer LNCaP cells. Several canonical pathways were statistically overrepresented in differentially expressed genes following 3α-diol exposure. Threshold *P*-value was set to $P < 0.05$ which is shown as -log(*P*-value). The gray line is presented as ratios of number of genes in a given pathway identified from the array analysis divided by total number of genes that make up that pathway

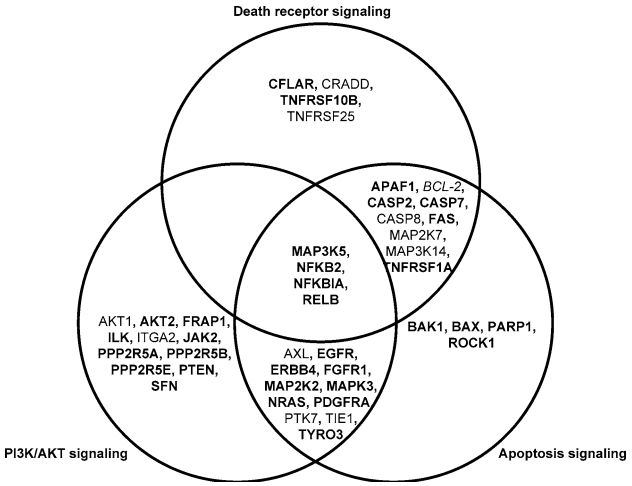

Fig. 2 Venn diagram of overlapping genes in three overrepresented canonical pathways. Genes that are shared between and among apoptosis signaling, PI3K/AKT signaling, and death receptor signaling pathways are shown. Bold, normal, and italic characters represent genes that are up-regulated, down-regulated, and BNT, respectively, from the array analysis

expression was up-regulated to prevent NF-κB from functioning in 3α-diol-treated cells.

3α-diol elevated AR-independent AKT activation

To determine the involvement of the AR in 3α-diol-activated AKT expression and phosphorylation, AR expression was suppressed in LNCaP cells by a plasmid-based siRNA technique through transient transfection. Transfection efficiency was monitored by the enhanced green fluorescence protein (EGFP) expression driven by cytomegolovirus (CMV) promoter encoded in the same plasmid construct in transfected cells using flow cytometry. Using the Amaxa Nucleofector transfection system, greater than 60% transfection efficiencies were achieved in all experiments. Without cell sorting to enrich transfected LNCaP cells, transfection experiments with the AR specific siRNA plasmid had an average of 90% suppression in AR expression at 48 h following transfection as determined by Western blot analysis. In AR-silenced LNCaP cells, levels of total AKT expression were elevated between 1 and 8 h following 3α-diol stimulation (Fig. 4). 3α-Diol was also capable of stimulating AKT phosphorylation at Ser(473) in LNCaP cells in an AR-independent manner, and levels of AKT phosphorylation were more prominent in AR-silenced LNCaP cells as compared to parental or mock transfected LNCaP cells (Fig. 4).

Discussion

We and others have demonstrated that 3α-diol can be an active androgen with its own androgenic action (Nunlist et al. 2004; Agapova et al. 2006). More importantly, 3α-diol may play a significant role in promoting the development of androgen-independent prostate cancer through AR-independent mechanisms. This analysis was aimed at both identifying genes whose expression was altered and key pathways whose signaling was switched on or off following 3α-diol exposure in AR-positive human prostate cancer LNCaP cells. This report, therefore, would not only spotlight gene expression changes but also understand molecular processes that remain active or inactive in 3α-diol-stimulated prostate cells. We used 10^{-11} M 3α-diol to identify genes and pathways that are regulated by this androgen, since this concentration of 3α-diol stimulated elevated cell proliferation without significantly *trans*-activating AR in LNCaP cells (Nunlist et al. 2004).

To emphasize cancer progression, the cDNA membrane array used in this analysis focused on the central pathways

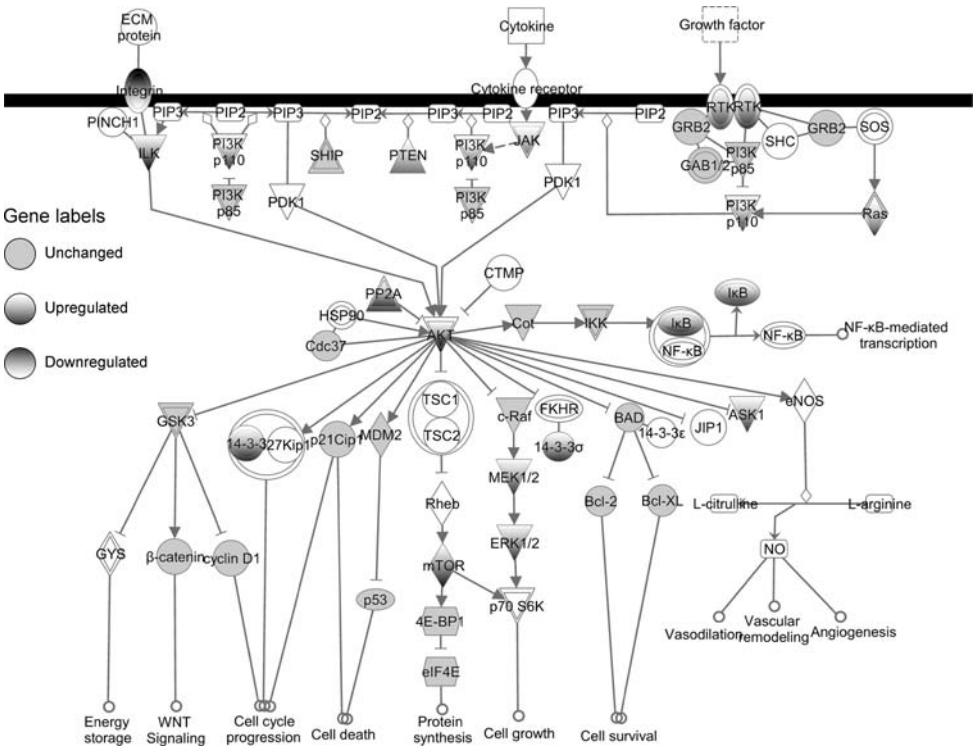

Fig. 3 PI3K/AKT signaling pathway activated by 3α-diol. Networks of PI3K/AKT signaling pathway are shown. Genes that are up- or down-regulated by 3α-diol are marked by gradient fill, whereas genes that are not regulated by 3α-diol are marked as gray color. Genes that are not colored indicate the absence of these genes in the membrane array

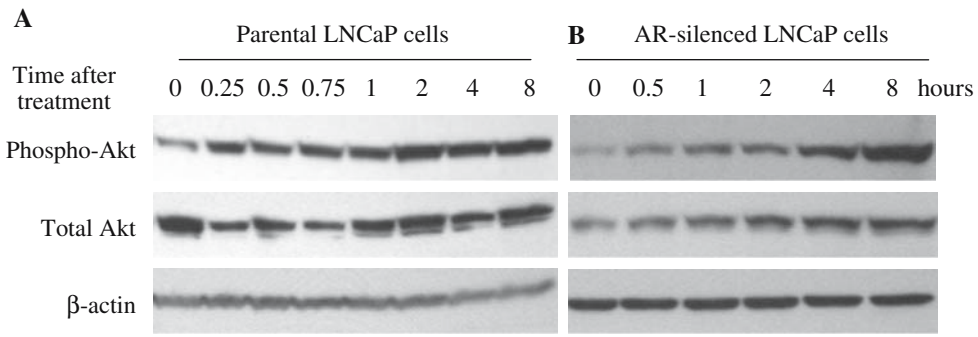

Fig. 4 Western blot analysis of 3α-diol-regulated AKT expression and phosphorylation in LNCaP cells. (**a**) Temporal regulation of AKT expression and phosphorylation between 15 min and 8 h in LNCaP cells treated with 10^{-8} M 3α-diol. (**b**) Temporal regulation of AKT expression and phosphorylation in AR-silenced LNCaP cells. LNCaP cells were transiently transfected with the pSiAR-EGFP plasmid, and subjected to serum deprivation followed by 10^{-8} M 3α-diol stimulation. Levels of AKT expression and phosphorylation were determined between 30 min and 8 h following 3α-diol stimulation. Experiments were repeated twice and a representative result was presented

represented in cancer, including cell cycle, growth factor signaling, cell death, and survival. Following the analysis of a total of 1,176 well-annotated, cancer-related genes, we have reported the confirmation of the unique expression patterns of 3α-diol versus 5α-DHT responsive genes using quantitative RT-PCR (Zimmerman et al. 2004), and Western blot analysis (Yang et al. 2008). We continued to investigate biological pathways that are affected by 3α-diol responsive genes, and to study roles of these pathways in regulating prostate cancer cell survival and growth.

Canonical pathway analysis suggested that apoptosis signaling and cytoplasmic signaling (PI3K/AKT and ERK/MAPK) were linked to 3α-diol-regulated prostate cell physiology or pathology. These cytoplasmic signaling pathways might be directly activated by 3α-diol without going through the AR, since (1) 5α-DHT was not detected in LNCaP cells following 3α-diol stimulation as determined by radiochemical assay (Rizner et al. 2003) and LC/MS/MS (Yang et al. 2008), (2) AR *trans*-activation activity was not significantly elevated with low concentrations of 3α-diol in LNCaP cells (Nunlist et al. 2004), and (3) AKT activation was observed in 3α-diol-stimulated, AR-silenced LNCaP cells. For the first time, the cytoplasmic signaling pathways, apoptosis signaling, and death receptor signaling were

directly linked to 3α-diol-regulated biological effects in prostate cells. These findings underlie mechanisms of 3α-diol-stimulated prostate cancer cell growth and possibly androgen-independent prostate cancer progression.

We have demonstrated that LNCaP cells could not survive without functional AR (Yang et al. 2005); and supplementation of 5α-DHT did not protect AR-silenced LNCaP cells from undergoing apoptosis (Yang et al. 2008). In contrast, 3α-diol supported cell survival and proliferation in AR-silenced LNCaP cells and in AR negative human prostate cancer PC-3 cells (Yang et al. 2008). Based on these observations, we expected that genes associated with cell survival and/or apoptosis should appear as 3α-diol-regulated genes in the array analysis. Androgen removal did up-regulate pro-apoptotic genes; but inhibition of BCL-2 or BAD following 3α-diol administration might prolong prostate cell survival. In addition, the expression of cell cycle genes remained constant before and after 3α-diol stimulation suggesting that cells continue to undergo cell cycle progression in the presence of 3α-diol. Our bioinformatics analysis proposed potential signaling pathways that are responsible for 3α-diol-regulated androgenic effects such as suppression of apoptosis and promotion of cell growth to support prostate cancer cell survival and proliferation.

5α-DHT activates both genomic and non-genomic actions through AR *trans*-activation and cytoplasmic signaling, respectively. The interactions between the potent androgen and the AR have been studied in the most detail (Heinlein and Chang 2004). Although AR-dependent genomic action may ultimately turn out to be the most important for physiological development of androgen target tissues (Whitacre et al. 2002; Cheng et al. 2006), 5α-DHT-activated non-genomic action has been strongly implicated in pathological progression of androgen-independent prostate cancer (Sun et al. 2006; Cinar et al. 2007). In the mode of non-genomic action, it has been proposed that androgens may act as mediators of secondary transcription factors, as regulators of autocrine and paracrine mediators of gene expression, or as mediators in secretion of other hormones that regulate androgenic effects in androgen target tissues (Verhoeven and Swinnen 1999; Grillo et al. 2005). Some of these effects have been attributed to membrane AR (Papakonstanti et al. 2003; Grillo et al. 2005) or other plasma membrane bound receptors (Lieberherr and Grosse 1994; Estrada et al. 2003).

However, our findings suggested that at least some of AR-independent androgenic effects ascribed to 5α-DHT may result from the accumulation of 3α-diol in prostate cells. In the prostate, 5α-DHT can be reduced to 3α-diol through the action of reductive 3α-HSDs. Between the two major 3α-HSD isozymes, AKR1C2 and AKR1C3, in human prostate (Lin et al. 1997; Penning et al. 2000), both isozymes catalyze 5α-DHT reduction activity toward the formation of 3α-diol (Lin et al. 1997; Rizner et al. 2003). Elevated steady state levels of these 3α-HSD transcripts have been demonstrated in primary cultures of human prostate cancer cells as compared to epithelial cells derived from normal prostate (Lin et al. 1997; Rizner et al. 2003). Furthermore, elevated AKR1C3 expression has been demonstrated in localized and advanced prostate cancer (Nakamura et al. 2005; Fung et al. 2006; Stanbrough et al. 2006). Androgen deprivation therapy is intended to suppress the accumulation of potent androgens in the cancerous prostate. In contrast, levels of the potent androgens such as testosterone and 5α-DHT remain relatively constant in the prostate before and after androgen deprivation therapy (Nishiyama et al. 2004; Titus et al. 2005). With the elevated reductive 3α-HSD expression and availability of their substrate, it is possible that 3α-diol is accumulated in cancerous prostate even in patients undergoing androgen deprivation therapy. Based on the bioinformatics analysis presented in this study, potential 3α-diol accumulation may ultimately responsible for the development of androgen-independent prostate cancer.

Summary

3α-Diol can be an active androgen with its own androgenic function. To support the concept that 3α-diol can be responsible for prostate cancer progression in the presence of androgen deprivation therapy, we presented a bioinformatics evidence that 3α-diol can directly alter the status of PI3K/AKT as well as other apoptosis and cell growth pathways to promote prostate cell survival and proliferation in a cell culture system. The relationship between 3α-diol accumulation and androgen-independent prostate cancer progression deserves further investigation.

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References

Agapova OA, Malone PE, Hernandez MR (2006) A neuroactive steroid 5α-androstane-3α,17β-diol regulates androgen receptor level in astrocytes. J Neurochem 98:355–363

Ahmad N, Warren DW, Haltmeyer GC (1978) The effects of 5α-reduced androgens on maintenance and regeneration of prostate glands and seminal vesicles in castrated and hypophysectomized rats. Anat Rec 192:543–554

Bauman DR, Steckelbroeck S, Williams MV, Peehl DM, Penning TM (2006) Identification of the major oxidative 3α-hydroxysteroid dehydrogenase in human prostate that converts 5α-androstane-3α,17β-diol to 5α-dihydrotestosterone: a potential therapeutic

target for androgen dependent disease. Mol Endocrinol 20: 444–458

Cheng H, Snoek R, Ghaidi F, Cox ME, Rennie PS (2006) Short hairpin RNA knockdown of the androgen receptor attenuates ligand-independent activation and delays tumor progression. Cancer Res 66:10613–10620

Cinar B, Mukhopadhyay NK, Meng G, Freeman MR (2007) Phosphoinositide 3-kinase-independent non-genomic signals transit from the androgen receptor to Akt1 in membrane raft microdomains. J Biol Chem 282:29584–29593

Dozmorov I, Centola M (2003) An associative analysis of gene expression array data. Bioinformatics 19:204–211

Estrada M, Espinosa A, Muller M, Jaimovich E (2003) Testosterone stimulates intracellular calcium release and mitogen-activated protein kinases via a G protein-coupled receptor in skeletal muscle cells. Endocrinol 144:3586–3597

Fung KM, Samara ENS, Wong C, Metwalli A, Krlin R, Bane B, Liu CZ, Yang JT, Pitha JT, Culkin DJ, Kropp BP, Penning TM, Lin HK (2006) Increased expression of type 2 3α-hydroxysteroid dehydrogenase/type 5 17β-hydroxysteroid dehydrogenase (AKR1C3) and its relationship with androgen receptor in prostate carcinoma. Endocr Relat Cancer 13:169–180

Grillo ML, Jacobus AP, Scalco R, Amaral F, Rodrigues DO, Loss ES, Wassermann GF (2005) Testosterone rapidly stimulates insulin release from isolated pancreatic islets through a non-genomic dependent mechanism. Horm Metab Res 37:662–665

Grover PK, Odell WD (1975) Correlation of in vivo and in vitro activities of some naturally occurring androgens using a radioreceptor assay for 5α-dihydrotestosterone with rat prostate cytosol receptor protein. J Steroid Biochem 6:1373–1379

Heinlein CA, Chang C (2004) Androgen receptor in prostate cancer. Endocr Rev 25:276–308

Jacobi GH, Moore RJ, Wilson JD (1978) Studies on the mechanism of 3α-androstanediol-induced growth of the dog prostate. Endocrinology 102:1748–1755

Keenan BS, Greger NG, Hedge AM, McNeel RL (1984) Cytosol androgen receptor (AR) in human skin fibroblasts: characterization of the binding reaction and differentiation from androgen binding molecules of lower affinity. Steroids 43:159–178

Lieberherr M, Grosse B (1994) Androgens increase intracellular calcium concentration and inositol 1,4,5-trisphosphate and diacylglycerol formation via a pertussis toxin-sensitive G-protein. J Biol Chem 269:7217–7223

Lin HK, Jez JM, Schlegel BP, Peehl DM, Pachter JA, Penning TM (1997) Expression and characterization of recombinant type 2 3α-hydroxysteroid dehydrogenase (HSD) from human prostate: demonstration of bifunctional 3α/17β-HSD activity and cellular distribution. Mol Endocrinol 11:1971–1984

Mahendroo MS, Porter A, Russell DW, Word RA (1999) The parturition defect in steroid 5α-reductase type 1 knockout mice is due to impaired cervical ripening. Mol Endocrinol 13:981–992

McPhaul MJ, Young M (2001) Complexities of androgen action. J Am Acad Dermatol 45:S87–S94

Nakamura Y, Suzuki T, Nakabayashi M, Endoh M, Sakamoto K, Mikami Y, Moriya T, Ito A, Takahashi S, Yamada S, Arai Y, Sasano H (2005) In situ androgen producing enzymes in human prostate cancer. Endocr Relat Cancer 12:101–107

Nishiyama T, Hashimoto Y, Takahashi K (2004) The influence of androgen deprivation therapy on dihydrotestosterone levels in the prostatic tissue of patients with prostate cancer. Clin Cancer Res 10:7121–7126

Nunlist EH, Dozmorov I, Tang Y, Cowan R, Centola M, Lin HK (2004) Partitioning of 5α-dihydrotestosterone and 5α-androstane-3α, 17β-diol activated pathways for stimulating human prostate cancer LNCaP cell proliferation. J Steroid Biochem Mol Biol 91:157–170

Papakonstanti EA, Kampa M, Castanas E, Stournaras C (2003) A rapid, nongenomic, signaling pathway regulates the actin reorganization induced by activation of membrane testosterone receptors. Mol Endocrinol 17:870–881

Penning TM (1997) Moledular endocrinology of hydroxysteroid dehydrogenases. Endocr Rev 18:281–305

Penning TM, Burczynski ME, Jez JM, Hung CF, Lin HK, Ma H, Moore M, Palackal N, Ratnam K (2000) Human 3α-hydroxysteroid dehydrogenase isoforms (AKR1C1-AKR1C4) of the aldo-keto reductase superfamily: functional plasticity and tissue distribution reveals roles in the inactivation and formation of male and female sex hormones. Biochem J 351:67–77

Rizner TL, Lin HK, Peehl DM, Steckelbroeck S, Bauman DR, Penning TM (2003) Human type 3 3α-hydroxysteroid dehydrogenase (aldo-keto reductase 1C2) and androgen metabolism in prostate cells. Endocrinology 144:2922–2932

Schultz FM, Wilson JD (1974) Virilization of the Wolffian duct in the rat fetus by various androgens. Endocrinology 94:979–986

Shaw G, Renfree MB, Leihy MW, Shackleton CH, Roitman E, Wilson JD (2000) Prostate formation in a marsupial is mediated by the testicular androgen 5α-androstane-3α,17α-diol. Proc Natl Acad Sci USA 97:12256–12259

Stanbrough M, Bubley G, Ross K, Golub TR, Rubin MA, Penning TM, Febbo PG, Balk SP (2006) Increased expression of genes converting adrenal androgens to testosterone in androgen-independent prostate cancer. Cancer Res 66:2815–2825

Sun YH, Gao X, Tang YJ, Xu CL, Wang LH (2006) Androgens induce increases in intracellular calcium via a G protein-coupled receptor in LNCaP prostate cancer cells. J Androl 27: 671–678

Titus MA, Schell MJ, Lih FB, Tomer KB, Mohler JL (2005) Testosterone and dihydrotestosterone tissue levels in recurrent prostate cancer. Clin Cancer Res 11:4653–4657

Verhoeven G, Swinnen JV (1999) Indirect mechanisms and cascades of androgen action. Mol Cell Endocrinol 151:205–212

Walsh PC, Wilson JD (1976) The induction of prostatic hypertrophy in the dog with androstanediol. J Clin Invest 57:1093–1097

Whitacre DC, Chauhan S, Davis T, Gordon D, Cress AE, Miesfeld RL (2002) Androgen induction of in vitro prostate cell differentiation. Cell Growth Differ 13:1–11

Wu AH, Whittemore AS, Kolonel LN, Stanczyk FZ, John EM, Gallagher RP, West DW (2001) Lifestyle determinants of 5α-reductase metabolites in older African-American, white, and Asian-American men. Cancer Epidemiol Biomarkers Prev 10:533–538

Yang Q, Fung KM, Day WV, Kropp BP, Lin HK (2005) Androgen receptor signaling is required for androgen-sensitive human prostate cancer cell proliferation and survival. Cancer Cell Int 5:8

Yang Q, Titus M, Fung KM, Lin HK (2008) 5α-Androstane-3α,17β-diol supports human prostate cancer cell survival and proliferation through androgen receptor-independent signaling pathways: implication of androgen-independent prostate cancer progression. J Cell Biochem (in press)

Zimmerman RA, Dozmorov I, Nunlist EH, Tang Y, Li X, Cowan R, Centola M, Frank MB, Culkin DJ, Lin HK (2004) 5α-Androstane-3α,17β-diol activates pathway that resembles the epidermal growth factor responsive pathways in stimulating human prostate cancer LNCaP cell proliferation. Prostate Cancer Prostatic Dis 7:364–374