

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

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Abstract 5 α -Androstane-3 α ,17 β -diol (3 α -diol) is reduced from the potent androgen, 5 α -dihydrotestosterone (5 α -DHT), by reductive 3 α -hydroxysteroid dehydrogenases (3 α -HSDs) in the prostate. 3 α -diol is recognized as a weak androgen with low affinity toward the androgen receptor (AR), but can be oxidized back to 5 α -DHT. However, 3 α -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 3 α -diol-activated pathways in regulating prostate cancer cell survival and/or proliferation. Several canonical pathways appeared to be affected by 3 α -diol-regulated responses in LNCaP cells; among them are apoptosis signaling, PI3K/AKT signaling, and death receptor signaling pathways. Biological analysis confirmed that 3 α -diol stimulates AKT activation; and the AKT pathway can be activated independent of the classical

AR signaling. These observations sustained our previous observations that 3 α -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 3 α -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 3 α -HSD expression are significantly elevated in localized and advanced prostate cancer, 3 α -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

3 α -diol	5 α -Androstane-3 α , 17 α -diol
3 α -HSD	3 α -Hydroxysteroid dehydrogenase
5 α -DHT	5 α -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 5 α -dihydrotestosterone (5 α -DHT) (McPhaul and Young 2001). Both testosterone

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and 5 α -DHT bind and activate the androgen receptor (AR): 5 α -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 5 α -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, 5 α -DHT can be reduced to 5 α -androstan-3 α , 17 β -diol (3 α -diol) through the action of 3 α -hydroxysteroid dehydrogenases (3 α -HSDs). Two 3 α -HSD isozymes, type 2 3 α -HSD (AKR1C3) and type 3 3 α -HSD (AKR1C2), are abundantly expressed in human prostate (Penning et al. 2000). Both 3 α -HSDs possess dominant 5 α -DHT reduction activity toward 3 α -diol formation in vitro and in human prostate cells (Lin et al. 1997; Rizner et al. 2003). In contrast to 5 α -DHT, the cellular responses to 3 α -diol remain largely unknown. 3 α -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 3 α -diol must be first converted to 5 α -DHT by oxidative 3 α -HSDs before exerting an androgenic action (Jacobi et al. 1978; Bauman et al. 2006).

However, the above assumption could not explain 3 α -diol's action in a variety of androgen target tissues. 3 α -Diol is more potent than 5 α -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). 3 α -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 3 α -diol metabolite in African-American and white men compared to Asian-American men, and suggested a correlation between 3 α -diol metabolite and the increased prostate cancer risk seen in the first two groups (Wu et al. 2001). These reports suggest that 3 α -diol may be an important hormone with its own functions through as yet undefined pathways.

However, the mechanism of 3 α -diol-regulated cellular physiology remains largely unknown. Through gene expression profiling analysis, we identified that 3 α -diol and 5 α -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 3 α -diol-activated signaling pathways using bioinformatics approaches based on genes that are regulated by 3 α -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). 3 α -Diol and mouse anti- β -actin antibody were purchased from Sigma-Aldrich (St. Louis, MO). [α - 32 P]dATP (3,000 Ci/mmol, 10 μ Ci/ μ l) 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 3 α -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 μ g/ml streptomycin at 37°C and 5% CO₂. 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) 3 α -diol stimulation.

To determine the involvement of the AR in 3 α -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 μ g AR specific (pSiAR-EGFP) or control siRNA (pSi-Con-EGFP) plasmid in 100 μ l 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 3 α -diol stimulation.

cDNA-based membrane array hybridization and image acquisition

To identify transcription profiling in LNCaP cells following 3 α -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). 3 α -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 3 α -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 3 α -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 3 α -diol-regulated cellular responses. The responsive genes were not ranked by gene expression levels.

Western blot analysis

Total cellular proteins were harvested following 3 α -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 3 α -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 3 α -diol administration. Of these 825 genes, 416 genes were expressed below the noise levels but up- or down-regulated significantly following 3 α -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 3 α -diol stimulation. The list of 3 α -diol responsive genes is provided in supplemental Table 1.

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

Signaling pathways, most affected by 3 α -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 overrepresented in overlapping canonical pathways. In the analysis of 3 α -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, 3 α -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 3 α -diol treatment	After 3 α -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

Table 1 continued

	Accession number	Before 3 α -diol treatment	After 3 α -diol treatment
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

3 α -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 3 α -diol administration. 3 α -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 3 α -diol treatment (Fig. 3). Analysis of the cell cycle genes showed that the majority of cell cycle regulators remained constant before and after 3 α -diol administration indicating cell cycle progression remained constant even in the presence of 3 α -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 3 α -diol, the activation of death receptor signaling apparently was countered by the activation of the PI3K/AKT survival pathway. In addition, 3 α -diol-treated LNCaP cells might evade from cell death through an NF- κ B-independent pathway, since I κ B

Fig. 1 3 α -Diol-activated canonical pathways in human prostate cancer LNCaP cells. Several canonical pathways were statistically overrepresented in differentially expressed genes following 3 α -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

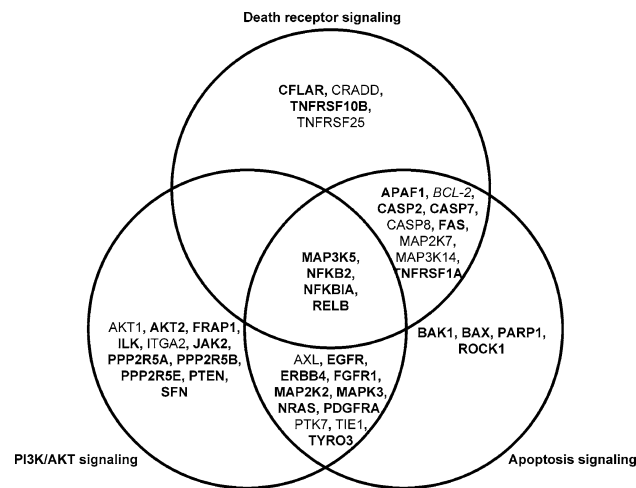
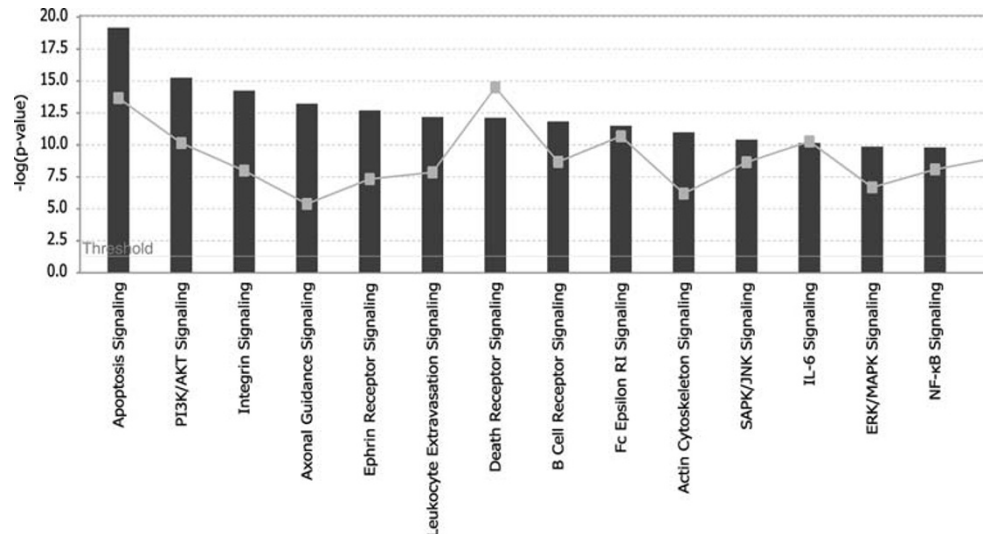


Fig. 2 Venn diagram of overlapping genes in three overrepresented canonical pathways. Genes that are shared between and among apoptosis signaling, PI3K/AKT signaling, and death receptor signaling pathways are shown. Bold, normal, and italic characters represent genes that are up-regulated, down-regulated,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

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

3 α -diol elevated AR-independent AKT activation

To determine the involvement of the AR in 3 α -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 cytomegalovirus (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 3 α -diol stimulation (Fig. 4). 3 α -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 3 α -diol can be an active androgen with its own androgenic action (Nunlist et al. 2004; Agapova et al. 2006). More importantly, 3 α -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 3 α -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 3 α -diol-stimulated prostate cells. We used 10⁻¹¹ M 3 α -diol to identify genes and pathways that are regulated by this androgen, since this concentration of 3 α -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

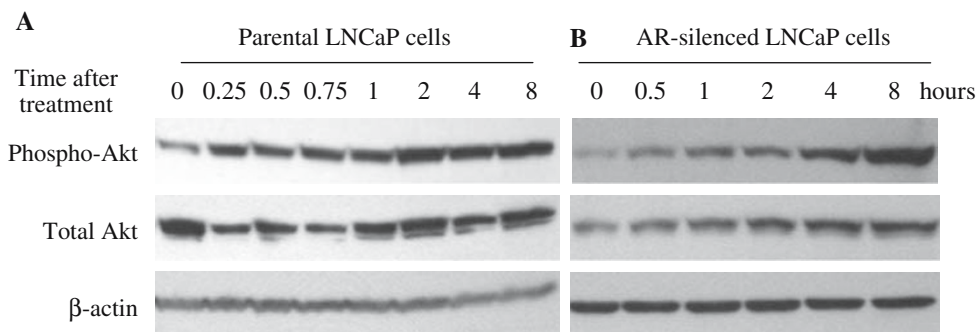
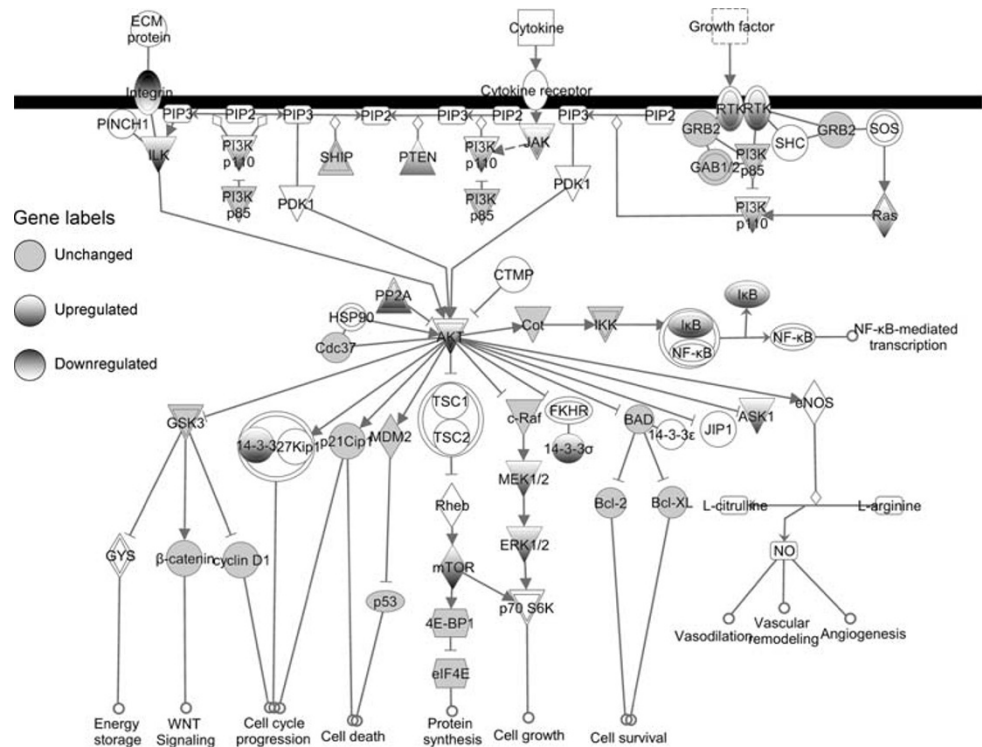


Fig. 4 Western blot analysis of 3α -diol-regulated AKT expression and phosphorylation in LNCaP cells. (a) Temporal regulation of AKT expression and phosphorylation between 15 min and 8 h in LNCaP cells treated with 10^{-8} M 3α -diol. (b) Temporal regulation of AKT expression and phosphorylation in AR-silenced 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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