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Gene Expression in Prostate Cancer Cells Treated With the Dual 5 Alpha-Reductase Inhibitor Dutasteride

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ABSTRACT: We sought preclinical data on the cellular and molecular effects of dutasteride in androgen-responsive, human prostate cancer (PCa) cells to better understand the mechanisms of action of 5 alpha-reductase inhibition in these cells. We used the human prostate cancer cell line LNCaP, which exhibits most features of PCa cells including androgen responsiveness. Our findings show that dutasteride kills PCa cells in vitro; it dramatically reduced viability and proliferation and disrupted genes and cellular pathways involved in metabolic, cell cycle, and apoptotic responses besides those expected in androgen-signaling pathways. Microchip gene array expression analysis revealed activation of genes in the FasL/tumor necrosis factor alpha (TNF-α) apoptotic and cell-survival pathways, correlating with the growth and survival effects in the LNCaP cells. Real-time polymerase chain reaction confirmed expression level changes seen by microarray analysis of candidate genes such as PLA2G2A, CDK8, CASP7, MDK, and NKX3.1. Collectively, our findings delineate the cellular and molecular effects of dutasteride in androgen-responsive PCa cells in vitro and may lead to its better therapeutic and chemopreventive use in PCa.

Key words: LNCaP, gene-expression profiling, REDUCE trial, apoptosis.

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Present treatment options for clinically localized human prostrate cancer (PCa) range from watchful waiting to radical prostatectomy, while androgen-deprivation therapy remains the main treatment option for advanced PCa. Projections for US males in 2004 suggest 230 110 new cases and 29 900 deaths from PCa (Jemal et al, 2004). Androgen deprivation of advanced PCa results in a median overall survival of only 23–37 months from initiation (Klotz, 2000). Better therapeutic and prevention options are urgently needed.

The prostate gland requires androgens for development and growth (Cunha, 1985). The natural ligands for the androgen receptor (AR) are testosterone and dihydrotestosterone (DHT). The majority of testosterone (95%) is produced by the testes, with the rest (5%) being produced by the adrenal glands (Partin and Rodriguez, 2002). Testosterone diffuses from the capillary bed in the prostatic stroma, across the basement membrane, and into the prostate basal epithelial cells. The basal cells express both 5α-reductase (5α-R) isoenzymes, 5α-R1 and 5α-R2, that convert testosterone to the more potent DHT steroid. DHT binds the AR with up to 10 times greater affinity than testosterone and activates gene transcription of androgen-regulated genes and cellular proliferation (Grossmann et al, 2001).

5α-R enzymatic activity converts 90% of testosterone to DHT in the prostate, and inhibition of this activity drastically reduces the amount of the more potent ligand available to the AR. 5α-R2 is the predominant isoenzyme in the human prostate, being expressed in both epithelial and stromal cells. Lesser amounts of 5α-R1 are also present in both types of prostate cells (Habib et al, 1998). Mutations in codon 49 of the gene encoding 5α-R2 (SRD5A2) have been shown to be associated with high-risk populations and are more prevalent in PCa than normal tissue (Ross et al, 1998; Jaffe et al, 2000). Additionally, these mutations are correlated with high enzymatic activity (Makridakis et al, 1997, 2000). Recently, it has been confirmed that a polymorphism in the SRD5A2 gene (specifically the V89L variant) may influence the risk of developing prostate cancer in men diagnosed at a younger age or with more aggressive disease (Cicek et al, 2004).

The Prostate Cancer Prevention Trial (PCPT), a 7-year chemoprevention trial with 18 882 men taking the drug finasteride, was the first successful demonstration of PCa prevention using finasteride, an inhibitor of 5α-R2 (18.4% of those receiving finasteride developed PCa compared with 24.8% on placebo; Thompson et al, 2003). A surprising finding from the PCPT involved an association between those taking finasteride and a greater incidence of higher Gleason grade tumors than those on placebo

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(Reynolds, 2003; Thompson et al, 2003). It remains unclear what led to the finasteride-associated higher grade cancers. One factor to be considered is the increase in bioavailable intraprostatic testosterone that occurs with finasteride treatment (Uygur et al, 1998). This unexpected finding supports the need to better delineate the cellular and molecular basis at work in prostate cancer cells during this type of antiandrogen therapy.

Dutasteride, a dual inhibitor of 5α-R1 and 5α-R2, has been approved for use in men with benign prostatic hyperplasia (BPH). Dutasteride suppresses serum DHT more effectively than finasteride (Bartsch et al, 2002). However, the clinical benefits of inhibiting both isoenzymes remain to be defined. The Reduction by Dutasteride of Prostate Cancer Events trial (REDUCE)[1] has been initiated and will involve 8000 men taking dutasteride for 5 years. The purpose of the study is to evaluate the safety and effectiveness of dutasteride in reducing the risk of prostate cancer. It is anticipated that inhibiting both 5α-R isoenzymes will result in a better clinical outcome. In addition to its use in the prevention of PCa, dutasteride could potentially be used in the early treatment of PCa because of its ability to reduce DHT levels in the prostate. However, results of clinical trials using dutasteride for treatment of BPH indicated that treatment with this drug can also result in increased levels of intraprostatic testosterone (Foley and Kirby, 2003).

The molecular effects of dutasteride on androgen-responsive PCa cells are unknown. Given the importance of mechanistic insights in the rational design and targeting of important biomolecules and their cellular pathways, here we present preclinical studies of dutasteride effects on the growth and proliferation of the androgen-responsive PCa cell line LNCaP. Time and dose-response treatment of LNCaP cells with dutasteride revealed a strong inhibition of cell viability and proliferation at doses comparable to those used in experimental animal models in vivo. Microarray gene-expression analysis under these conditions identified important genes and cellular pathways involved in metabolism, cell cycle, and apoptotic pathways, which are disrupted by dutasteride, in addition to androgen-signaling pathways. Real-time polymerase chain reaction confirmed expression level changes seen by microarray analysis of candidate genes such as PLA2G2A, CDK8, MDK, and NKX3.1. In addition, dutasteride affected several genes involved in the FasL/TNF-α apoptotic pathway and cell-survival pathways correlating with the viability and proliferation effects seen in LNCaP cells. Collectively, our findings delineate the cellular and molecular effects of dutasteride in androgen-responsive PCa cells in vitro. These findings pave the way for understanding the molecular basis of its effects in vivo and may lead to better use in the chemoprevention trials and possible treatment of PCa.

[1]Information on the REDUCE trial can be found at www.reducestudy.com/agi.

Materials and Methods

Cell Culture

LNCaP cells were obtained from ATCC (Manassas, Va) and used at passages 23–29. Cells were maintained in RPMI 1640 (Gibco/InVitrogen, Grand Island, NY) containing 9% fetal bovine serum (Biosource International, Camarillo, Calif), 100 U/ml penicillin, 100 μg/mL streptomycin, and 0.25 μg/mL fungizone (all Gibco/InVitrogen) in 5% CO_2 at 37°C. Concentration of testosterone in the media was determined by enzyme-linked immunosorbent assay (ELISA) to be less than or equal to 1 pmol. Cells were seeded in multiwell plates and left for 48 hours before treatment. Dutasteride was obtained from GlaxoSmithKline (Research Triangle Park, NC) and freshly prepared in dimethylsulfoxide (DMSO) at the time of use for each treatment. Media were changed every 24 hours, and cells were maintained in dark conditions during treatment.

Viability and Proliferation Assays

Cells were seeded in 96-well plates at 3×10^3 cells per well. After 48 hours media was changed to contain DMSO (vehicle) or concentrations ranging from 1 to 100 μM dutasteride for the indicated time periods. Viability was assayed using the CellTiter 96 Aqueous nonradioactive cell proliferation assay (Promega, Madison, Wis). Proliferation was assayed using the Cell Proliferation ELISA (colorimetric), BrdU incorporation assay (Roche, Indianapolis, Ind), both following the manufacturer's instructions.

Caspase Assays

Caspase 7 activity was assessed using a caspase-glo 3/7 kit (Promega). Cells were seeded at 3×10^3 per well in 96-well plates and treated for the indicated times with 0 to 50 μM dutasteride. Assays were performed following the manufacturer's instructions.

Microarray Gene-Expression Analysis

Total RNA was isolated from LNCaP cells after treatment with either vehicle alone (DMSO) or 10 μM dutasteride in DMSO for 48 hours using Trizol (InVitrogen, Carlsbad, Calif) followed by further cleaning with Rneasy kit (Qiagen, Valencia, Calif). Triplicate samples of each were quality checked using Agilent, labeled, and hybridized to U95Av2 microchip arrays following the manufacturer's instructions (Affymetrix, Santa Clara, Calif). The microarray data were normalized using cyclic loess normalization (Dudoit et al, 2002). Genes were identified as being differentially expressed between the untreated group and the dutasteride-treated group with a linear mixed model, similar to that proposed by Chu et al (Chu et al, 2002). The genes were ranked according to their P value (smallest to largest). An arbitrary decision was made to focus attention on the top 200 differentially expressed genes as measured by their P value.

Real-Time Polymerase Chain Reaction

Two-step real-time polymerase chain reaction was performed using cDNA prepared from RNA isolated as described above using first strand cDNA synthesis kit (Roche, Indianapolis, Ind) and SYBR Green polymerase chain reaction (PCR) Master Mix (Applied Biosystems, Foster City, Calif) on an ABI PRISM 7700 SDS following the manufacturer's instructions. The primers for SYBR green amplification were designed using Primer3 software (Rozen and Skaletsky, 2000), and both forward and reverse primers were used at a final concentration of 900 nM. PCR products (120–150 bp) were run on 1.2% agarose gels to check for nonspecific amplification. Relative quantitation was used to determine fold change in expression levels by the comparative C_T method using the formula $2^{-\Delta\Delta C_T}$, where C_T is the threshold cycle of amplification.

Results

Dutasteride Effects on Viability and Proliferation of LNCaP Cells

In LNCaP cells, 0.5–10 μM dutasteride inhibits conversion of ^{3}H-testosterone to ^{3}H-DHT by more than 99% (Lazier et al, 2004). To confirm the effects of dutasteride on LNCaP cells, a time and dose-response experiment was performed. LNCaP cells growing in optimal conditions (RPMI with 9% fetal bovine serum) were treated with varying concentrations of dutasteride (1–100 μM) for 24–96 hours, at which time both viability and proliferation were assayed. Viability and cell number were reduced even at 1 μM dutasteride after just 48 hours of treatment, and both endpoints continued decreasing in a dose-dependent manner (Figure 1A and B). Likewise, morphology was noticeably altered after 48 hours of treatment with 1 μM dutasteride and began to resemble cells undergoing androgen deprivation as described in detail (Murillo et al, 2001). Cell proliferation as determined by BrdU incorporation was reduced by approximately 50% after 48 hours of treatment with 10 μM dutasteride, confirming the inhibitory effects of dutasteride on the growth of LNCaP cells (Figure 1C). These experiments were routinely performed in media containing whole serum; results of experiments done in media containing charcoal-stripped serum paralleled those in whole serum, but with changes in morphology and viability occurring earlier (data not shown). Cell death was mainly apoptotic as judged by increased Annexin V staining, with some necrotic cells observed at 50–100 μM. Thus, growth, proliferation, and viability of LNCaP cells were strongly reduced by dutasteride treatment. These androgen-responsive PCa cells showed typical androgen-deprivation effects, similar to those observed by culturing LNCaP cells in androgen-depleted media, starting at 1 μM concentrations of dutasteride in whole serum for 48 hours. However, the effects of dutasteride treatment appear to be more detrimental to LNCaP cells in vitro than androgen deprivation alone.

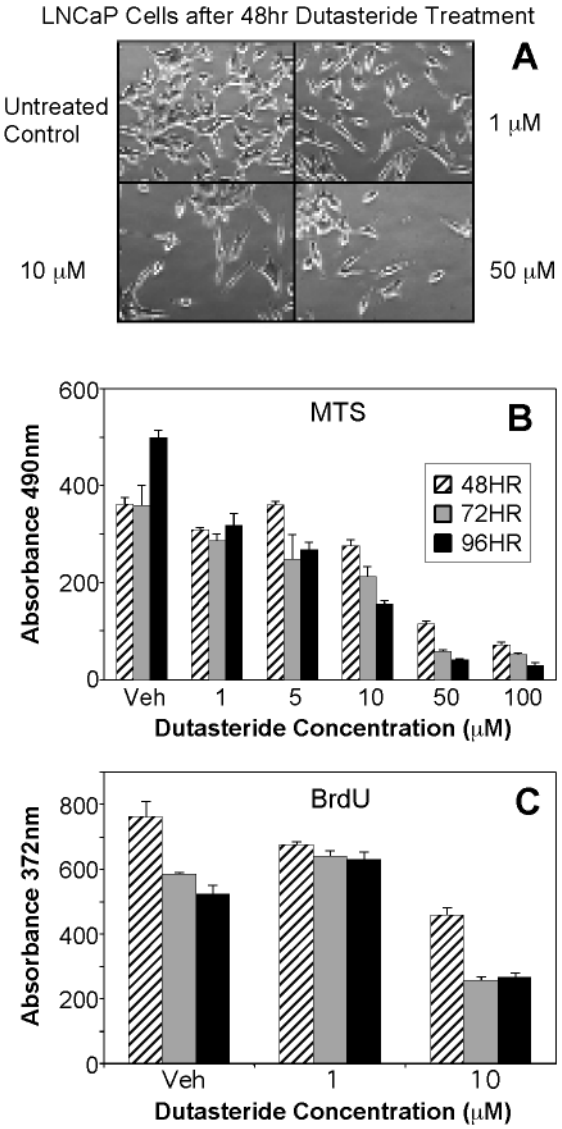
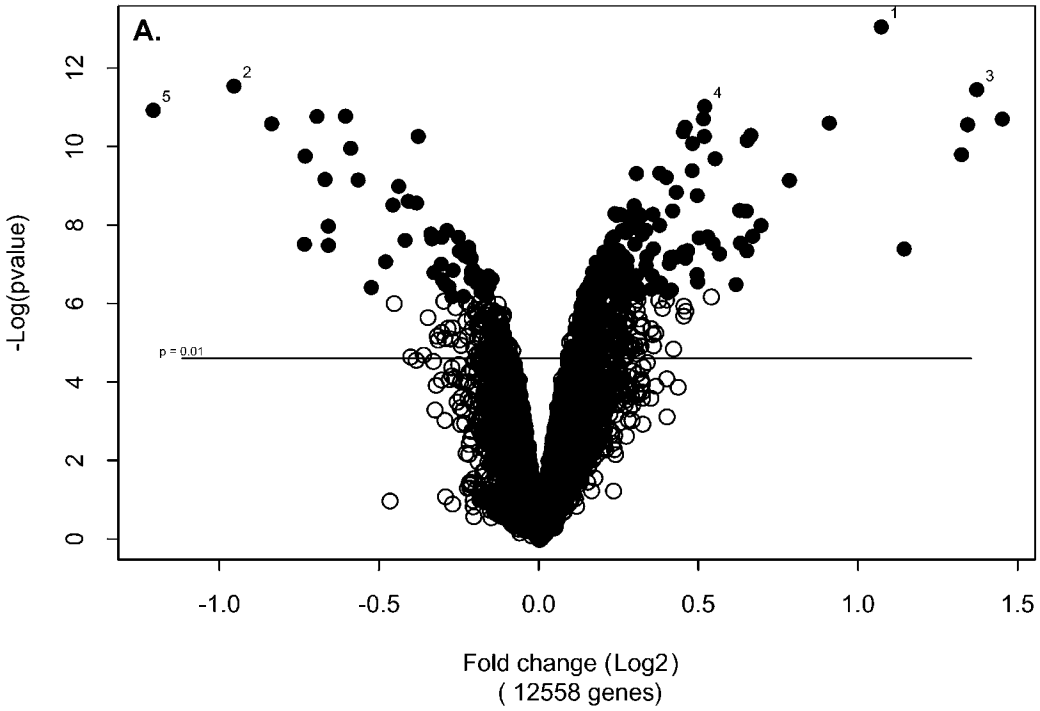


Figure 1. Effects of dutasteride treatment on LNCaP cells' morphology, viability, and proliferation. (**A**) Cells were treated for 48 hours with increasing concentrations of dutasteride and light microscopy photographs obtained at a 20× magnification. Cell viability was determined by MTS assay (**B**) and proliferation by BrdU incorporation (**C**) after treatment at the indicated times and dutasteride concentrations. Results represent experiments performed in triplicate.

Cellular and Genetic Pathways Affected by Dutasteride in LNCaP Cells

RNA was isolated from vehicle-treated and 10 μM dutasteride-treated cells at 48 hours and used to prepare probes for hybridizing Affymetrix U95Av2 microchip gene arrays. RNA from this time point was chosen based on the fact that after 48 hours of treatment with 10 μM dutasteride, cell proliferation was reduced by approximately 50%; therefore, pathways being affected by the



B. Top 25 Transcripts

rank	genbank	symbol	p-value
1	S39329	KLK2	0.00000
2	M25915	CLU	0.00001
3	X07730	KLK3	0.00001
4	U07919	ALDH1A3	0.00002
5	U91618	NTS	0.00002
6	M22430	PLA2G2A	0.00002
7	M17733	SC5DL	0.00002
8	AF099989	SPAK	0.00002
9	Antigen, P	NO.SIFseq	0.00002
10	AL034374	HELO1	0.00002
11	U08854	UGT2B15	0.00003
12	X07730	KLK3	0.00003
13	AA131149	S100P	0.00003
14	AB020637	KIAA0830	0.00003
15	Antigen, P	NO.SIFseq	0.00003
16	U80669	NKX3A	0.00003
17	J03779	MME	0.00003
18	X80692	MAPK6	0.00004
19	Oncogene T	NO.SIFseq	0.00004
20	U29091	SELENBP1	0.00005
21	X05332	KLK3	0.00005
22	U16799	ATP1B1	0.00006
23	AL079298	MCCC2	0.00006
24	AI635895	DSIPI	0.00008
25	AA255502	H4FG	0.00009

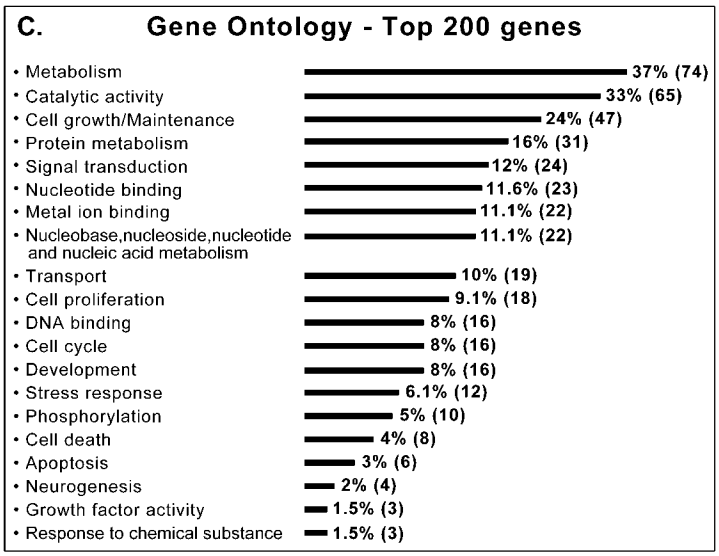


Figure 2. Microarray gene-expression analysis. (**A**) Volcano plot of gene expression in treated and untreated LNCaP cells. The X-axis position represents the ''fold-change'' plotted on a log base 2 scale. The Y-axis position shows the *P* value of the significance of expression difference plotted on a negative log base 10 scale. The filled circles represent the top 200 genes that were selected for further analysis. The top 5 of these are identified 1–5. All cells were vehicle treated or dutasteride treated (10 μM) for 48 hours. Results represent experiments performed in triplicate. (**B**) List of top 25 transcripts affected by dutasteride treatment of LNCaP cells. (**C**) Ontological sorting of top 200 transcripts ($P < .001$) affected by dutasteride treatment (10 μM at 48 hours).

drug were likely to be fully engaged. For the purpose of this communication, we focused our analysis on the top 200 differentially regulated gene transcripts ($P \leq .001$) and used the DAVID gene ontology annotation tool to group genes by function (Figure 2A through C) (Chu et al, 2002; Dudoit et al, 2002; Dennis et al, 2003). Subsequent ontological analysis revealed metabolism and catalytic activity gene pathways as the predominant divergences between treated and nontreated cells (Figure 2C). These were followed by cell growth/maintenance and pro-

A

Androgen signaling genes

- **AR** *androgen receptor*
- **PSA** *prostate specific antigen*
- **NKX3A** *homeobox protein NKX3.1*
- **UGT2B15** *UDP glucuronosyl transferase*

Metabolism genes

- **PLA2G2A** *phospholipase A2*
- **CDK8** *cell division protein kinase 8*

Apoptosis-related genes

- **Caspase 7**
- **Caspase 8**
- **BNIP3** *BCL2 E1B protein-interacting 3*
- **BIRC1** *baculoviral IAP repeat-containing protein*

Neurogenesis genes

- **MDK** *neurite growth-promoting factor*

C

GENE	ACC	FOLD CHANGE
AR	M23263	**2.01**
PSA	X07730	**0.27**
NKX3A	U80669	**0.22**
UGT2B15	U08854	**3.09**
PLA2G2A	M22430	**7.47**
CDK8	X85753	**3.36**
CASPASE 7	U67319	**2.85**
CASPASE 8	X98172	**4.86**
BNIP3	AF002697	**4.71**
BIRC1	U80017	**1.77**
MDK	M94250	**2.17**

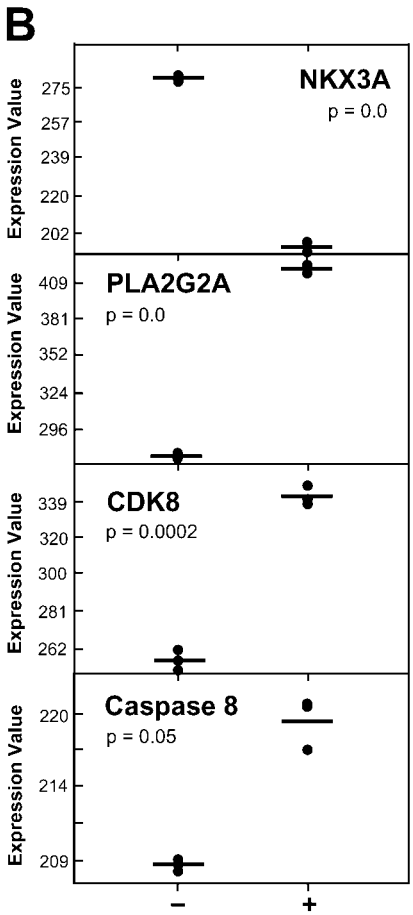


Figure 3. Real-time polymerase chain reaction (RTPCR) analysis of selected, differentially expressed genes in dutasteride-treated and control LNCaP cells. (**A**) Of the 17 selected genes from the microarray findings, the 11 differentially expressed as confirmed by RTPCR are shown under their ontological group. (**B**) Dot plots of 4 of these genes illustrating microchip array expression values in vehicle-treated (−) and dutasteride-treated (+) cells. Arrays were probed in 3 separate experiments with each dot representing 1 experiment. (**C**) Genes, accession numbers, and their respective expression levels displayed as fold change of treated compared with untreated cells as determined by RTPCR.

tein metabolism, which together with the first 2 groups are consistent with the major anabolic effects of androgens in responsive cells. Only genes involved in signal transduction functions were more numerous than other cell and nucleic acid metabolism. Apparently less numerous, but critically important, were gene groups involving stress, phosphorylation, and cell death, which we further analyzed given the cell stress and death effects of dutasteride on LNCaP cells.

We chose genes from 3 of these ontological groups plus several genes known to be involved in androgen signaling and confirmed the array results using real-time PCR (RTPCR; Figure 3A through C). Of the 17 genes chosen, 11 generated RTPCR profiles consistent with the array data (Figure 3C). Four genes showed no differences in expression levels between untreated and treated samples, and 2 were expressed at extremely low or undetectable levels (data not shown).

We next sought to identify the apoptotic and survival pathways that were affected in dutasteride-treated cells and the cell death genes activated. Two components of the FasL/TNF-α apoptotic signaling pathway, caspase 7 and caspase 8, were found to be up-regulated in cells treated with dutasteride. To further determine the functional significance of the gene-expression changes of caspase 7 and correlations with cell death seen, we used a DEVD cleavage assay to detect enzymatic activity of caspase 7. The enzymatic activity of caspase 7 increased in a dose-dependent manner at 48 hours for the treated cells (Figure 4C), providing functional significance and further confirming that this pathway is being activated by dutasteride treatment in LNCaP cells.

Discussion

In the prostate, dutasteride effectively blocks both 5α-R1 and -R2 isoenzymes, thus greatly reducing the amount of DHT available to bind the AR and direct proliferation (Bartsch et al, 2002). It may be potentially advantageous

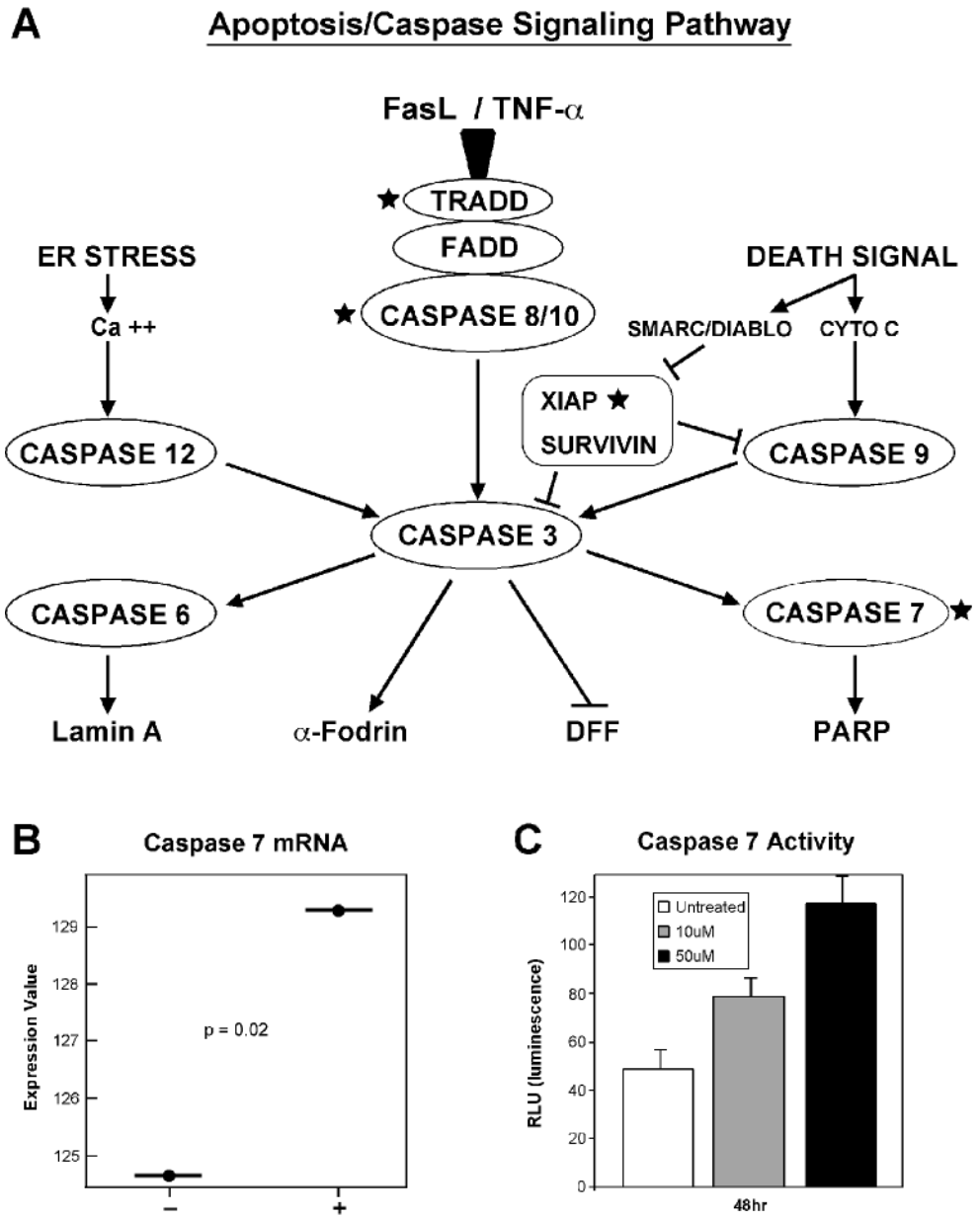


Figure 4. Schematic diagram of the FasL/TNF-α cell death and survival signaling pathway components. (**A**) Star marks genes affected by dutasteride treatment of LNCaP cells as determined by gene-expression changes seen in microchip array hybridizations and confirmed by RTPCR. (**B**) Expression values of caspase 7 mRNA in control-treated (−) or dutasteride-treated (+) cells. Results are from an experiment performed in triplicate. (**C**) Caspase 7 activity as determined by DEVD-cleavage activity in LNCaP cells untreated (vehicle) and the indicated dutasteride treatment doses for 48 hours. Results are from experiments performed in triplicate.

to effect this dual inhibition for preventing or treating PCa. However, as the PCPT findings suggested, as yet unknown mechanisms may come into play during steroid 5α-R inhibition (Reynolds, 2003; Thompson et al, 2003). The REDUCE trial has been initiated, further supporting the logic of better delineating the cellular and molecular effects of such dual inhibition on androgen-responsive PCa cells. Understanding such mechanisms may aid in better use of dutasteride as a chemopreventive and treatment drug for PCa.

As in the PCPT, the present findings revealed a more complex picture than expected. However, overall, dutasteride treatment of LNCaP PCa cells results in cells that phenotypically resemble LNCaP cells undergoing androgen deprivation in vitro (Murillo et al, 2001). In those studies, LNCaP cells underwent apoptosis and neuroendocrine differentiation. If the androgen-deprivation conditions are maintained chronically, there is an eventual rise of androgen-independent LNCaP cell sublines (Murillo et al, 2001). In the present studies, dutasteride in-

duces these changes, including induction of neurogenesis genes such as MDK.

Dutasteride effectively inhibits both viability and proliferation of LNCaP prostate cancer cells within 48 hours of treatment with 1–10 μM, consistent with DHT's importance in these cells' growth and survival. However, these cells are in whole media containing less than or equal to 1 pmol testosterone, so observed effects cannot totally be explained by inhibition of conversion of testosterone to DHT. Our array data have revealed up-regulation of 2 genes, UGT2B15 and UGT2B17, in LNCaP cells treated with dutasteride (Figures 2B and 3A and C). These UDP-glucuronosyltransferases specifically recognize DHT and its metabolites, androstane-3α, 17β-dial, and androsterone, leading to inactivation and subsequent secretion by the cells of the respective inactive derivatives (Turgeon et al, 2001). It has been suggested that these enzymes have an important role in inactivating androgens in steroid target tissues (Turgeon et al, 2003). Increased expression of these genes with dutasteride treatment is potentially another mode of action for this drug in prostate cells. In addition to blocking conversion of testosterone to DHT, dutasteride can further reduce the amount of androgen available to prostate cells by up-regulating enzymes that degrade DHT or any of its metabolites that may be present in the cells. A recent report examining the effects of dutasteride on the growth and proliferation of LNCaP cells has noted that after treatment with exogenous DHT in combination with dutasteride, cell proliferation is still dramatically decreased (Lazier et al, 2004). This is consistent with our findings that the effects dutasteride is having on LNCaP cells are not solely related to inhibition of testosterone to DHT conversion.

We first focused on genes involved in the androgen-signaling pathway since it has been shown that inhibition of 5α-R in LNCaP cells affects androgen-regulated genes (Zhu et al, 2003). RTPCR findings confirmed the microchip gene-expression data, showing up-regulation of AR mRNA and down-regulation of NKX3.1 and PSA (KLK3) after 48 hours of dutasteride treatment. Previous studies have demonstrated that PSA gene expression in LNCaP cells is mediated via conversion of testosterone to DHT (Zhu et al, 2003); thus, blocking this conversion with dutasteride is affecting this and possibly other AR-regulated genes. These findings suggest that dutasteride stimulates LNCaP cells to rapidly respond to the decreased DHT levels and AR action by up-regulating AR transcription. Under androgen-deprived conditions the AR can bind other ligands or function in a ligand-independent manner to promote growth and proliferation; therefore, expression levels of the AR can be critical to cell survival under such conditions (Culig et al, 2003). Additionally, LNCaP cells contain a mutation in the ligand-binding domain of the AR, leading to speculation that 5α-R inhibitors may be working through this mutation in these cells. However, Long et al tested several novel androgen-synthesis and/or 5α-R and inhibitors, along with finasteride, for their effects on cell growth and their ability to bind AR, specifically LNCaP mutant AR vs wild type AR in transfected PC-3 cells. They reported that while finasteride's growth inhibitory properties are specific for the LNCaP AR, the other dual inhibitors they tested interacted equally with both receptors. In binding assays, finasteride competed to a small degree with synthetic androgen R1881 equally well for both mutated and wild type AR (Long et al, 2000).

There is evidence that in LNCaP cells treated with finasteride, down-regulation of PSA is a result of the inhibition of the complex formation between nuclear proteins and the steroid receptor-binding consensus (SRBC) site in the PSA promoter (Wang et al, 1997), although in those experiments finasteride was used at higher concentrations, 25–100 μM, than the dutasteride concentration we have used (1–10 μM). In our studies, in agreement with those of Zhu et al (Zhu et al, 2003), both 5α-R1 and 5α-R2 can be RTPCR amplified in LNCaP cells (data not shown). It is unclear whether it matters which isoenzyme is inhibited in LNCaP cells; however, dutasteride inhibits both and thus diminishes the potential of any testosterone conversion to DHT. Nevertheless, it would be important to further define additional dutasteride interactions, if any, with other cellular proteins.

More importantly, the DNA microarray gene-expression analysis of LNCaP cells under these conditions revealed genes involved in apoptotic, metabolic, and cell cycle pathways, which are in addition to the expected androgen-signaling pathway. Specifically, several genes in the FasL/TNF-α apoptotic pathway were found to be up-regulated with dutasteride treatment (Figure 4A), pointing to a possible engagement of this cell death pathway by dutasteride in PCa cells. Moreover, several genes involved in cell survival or resistance to apoptosis, such as BIRC1 (baculoviral IAP repeat-containing 1), showed increased levels of expression.

Studies have shown that caspase 8 activation is necessary for TNF-α–related apoptosis inducing ligand (TRAIL)-mediated apoptosis in LNCaP cells (Rokhlin et al, 2002). Although we did not formally test this possibility, it is likely that this is one of the pathways by which apoptosis is occurring in LNCaP cells treated with dutasteride. In support of this possibility were our findings showing several key players of this pathway being affected by dutasteride. For example, mRNA levels of TRADD, caspase 7, caspase 8, and BIRC1 were increased, as was caspase-dependent, DEVD-cleavage activity. Strictly speaking, the latter activity can represent caspase 3 and 7 enzymatic activity; however, caspase 3 is considered to act upstream of caspase 7 and enzymatic

separation of the two is not possible (Thornberry et al, 1997). Regardless of caspase 3 contributions to our assayed DEVD-activity, both caspases are downstream effectors of cell death (Nunez et al, 1998). Of note, prior studies of LNCaP cells undergoing apoptosis have also shown induction and activation of caspase 7 (Marcelli et al, 1998; Marcelli et al, 1999). Little change in message levels was seen for FasL or TNF-α; however, their exquisite regulation is primarily posttranscriptional and their engagement does not necessitate new mRNA (Beyaert et al, 2002; Schultz and Harrington, 2003).

The phospholipase A_2 gene (PLA2G2A) was found to be one of the most highly up-regulated genes in dutasteride-treated cells. Activation of phospholipases such as PLA2 results in accumulation of arachidonic acid (AA) (Seilhamer et al, 1989). Accumulation of AA and inhibition of AA metabolism, leading to increased apoptosis, has been implicated as a chemopreventive mechanism for anti-inflammatory drugs (Kelloff, 2000). Activation of PLA2, resulting in increased apoptosis, is possibly another mode of action for dutasteride-induced cell death and potential chemopreventive action in prostate cells. However, more studies are needed to explore this hypothesis in PCa cells.

Another gene found to be up-regulated in dutasteride-treated cells was CDK8, a gene involved in the regulation of transcription. CDK8 has been shown to regulate transcription by targeting the CDK7/cyclinH subunits of TFllH and providing a link between mediator complexes and basal transcription (Di Pietro et al, 1999; Akoulitchev et al, 2000). It is possible that under the dutasteride-treatment conditions, the dramatic switch in the LNCaP cell's transcription program may be aided by induction of such genes as CDK8.

The genes we have described illustrate the variety of cellular responses taking place in LNCaP cells treated with dutasteride. Our studies were performed in a human PCa cell line in vitro; however, LNCaP cells have been shown to exhibit most of the characteristics of human, androgen-responsive, PCa. Although the doses we have used in our in vitro studies correspond to those that have been used in vivo in animal studies (both rat and dog), they are significantly higher than levels achieved in human clinical trials; the highest concentration reported for dutasteride in prostate tissue was 457 ng/ml (approximately 1 μM) after treatment with 5 mg per day (Roger Rittmaster, personal communication). We are currently examining the effects of dutasteride on LNCaP cells at the levels being used in the REDUCE trial (0.5 mg dose per day) and have observed some of the same genes being regulated at the RNA level as early as 24 hours after treatment (preliminary data not shown); hence, we believe that these in vitro data represent a valid starting point for assessing dutasteride's effects on PCa cells. The in vivo findings of the REDUCE trial could further aid our understanding of dutasteride effects in prostate cells. Collectively, our findings delineate the cellular and molecular effects of dutasteride in androgen-responsive PCa cells in vitro. Further analysis of those changes that are important with regard to cell death vs cell survival will result in a better understanding and potential use of dutasteride in the prevention or treatment of prostate cancer.

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References

Akoulitchev S, Chuikov S, Reinberg D. TFIIH is negatively regulated by cdk8-containing mediator complexes. *Nature.* 2000;407:102–106.

Bartsch G, Rittmaster RS, Klocker H. Dihydrotestosterone and the concept of 5 alpha-reductase inhibition in human benign prostatic hyperplasia. *World J Urol.* 2002;19:413–425.

Beyaert R, Van Loo G, Heyninck K, Vandenabeele P. Signaling to gene activation and cell death by tumor necrosis factor receptors and Fas. *Int Rev Cytol.* 2002;214:225–272.

Chu T-M, Weir B, Wolfinger R. A systematic statistical linear modeling approach to oligonucleotide array experiments. *Mathematical Biosci.* 2002;176:35–51.

Cicek MS, Conti DV, Curran A, Neville PJ, Paris PL, Casey G, Witte JS. Association of prostate cancer risk and aggressiveness to androgen pathway genes: SRD5A2, CYP17, AND the AR. *Prostate.* 2004;59: 69–76, 2004.

Culig Z, Klocker H, Bartsch G, Steiner H, Hobisch A. Androgen receptors in prostate cancer. *J Urol.* 2003;170:1363–1369.

Cunha GR. Mesenchymal-epithelial interactions during androgen-induced development of the prostate. *Prog Clin Biol Res.* 1985;171:15–24.

Dennis G Jr, Sherman BT, Hosack DA, Yang J, Gao W, Lane HC, Lempicki RA. DAVID: Database for Annotation, Visualization, and Integrated Discovery. *Genome Biol.* 2003;4:P3.

Di Pietro C, Rapisarda A, Bonaiuto C, Lizzio MN, Engel H, Amico V, Scalia M, Amato A, Grzeschik KH, Sichel G, Purrello M. Genomics of the human genes encoding four TAFII subunits of TFIID, the three subunits of TFIIA, as well as CDK8 and SURB7. *Somat Cell Mol Genet.* 1999;25:185–189.

Dudoit S, Yang YH, Callow MJ, Speed TP. Statistical method for identifying genes with differential expression in replicated cDNA microarray experiments. *Stat Sin.* 2002;12:111–139.

Foley CL, Kirby RS. 5 alpha-reductase inhibitors: what's new? *Cur Opin Urol.* 2003;13:31–37.

Grossmann ME, Huang H, Tindall DJ. Androgen receptor signaling in androgen-refractory prostate cancer. *J Natl Cancer Inst.* 2001;93: 1687–1697.

Habib FK, Ross M, Bayne CW, Grigor K, Buck AC, Bollina P, Chapman K. The localisation and expression of 5 alpha-reductase types I and II mRNAs in human hyperplastic prostate and in prostate primary cultures. *J Endocrinol.* 1998;156:509–517.

Jaffe JM, Malkowicz SB, Walker AH, MacBride S, Peschel R, Tomaszewski J, Van Arsdalen K, Wein AJ, Rebbeck TR. Association of SRD5A2 genotype and pathological characteristics of prostate tumors. *Cancer Res.* 2000;60:1626–1630.

Jemal A, Tiwari RC, Murray T, Ghafoor A, Samuels A, Ward E, Feuer

EJ, Thun MJ. Cancer statistics, 2004. *CA Cancer J Clin.* 2004;54:8–29.

Kelloff, GJ. Perspectives on cancer chemoprevention research and drug development. *Adv Cancer Res.* 2000;78:199–334.

Klotz L. Hormone therapy for patients with prostate carcinoma. *Cancer.* 2000;88:3009–3014.

Lazier CB, Thomas LN, Douglas RC, Vessey JP, Rittmaster RS. Dutasteride, the dual 5 alpha-reductase inhibitor, inhibits androgen action and promotes cell death in the LNCaP prostate cancer cell line. *Prostate.* 2004;58:130–144.

Long BJ, Grigoryev DN, Nnane IP, Liu Y, Ling Y-Z, Brodie AM. Antiandrogenic effects of novel androgen synthesis inhibitors on hormone-dependent prostate cancer. *Cancer Res.* 2000;60:6630–6640.

Makridakis N, Ross RK, Pike MC, Chang L, Stanczyk FZ, Kolonel LN, Shi CY, Yu MC, Henderson BE, Reichardt JK. A prevalent missense substitution that modulates activity of prostatic steroid 5 alpha-reductase. *Cancer Res.* 1997;57:1020–1022.

Makridakis NM, di Salle E, Reichardt JK. Biochemical and pharmacogenetic dissection of human steroid 5 alpha-reductase type II. *Pharmacogenetics.* 2000;10:407–413.

Marcelli M, Cunningham GR, Haidacher SJ, Padayatty SJ, Sturgis L, Kagan C, Denner L. Caspase-7 is activated during lovastatin-induced apoptosis of the prostate cancer cell line LNCaP. *Cancer Res.* 1998; 58:76–83.

Marcelli M, Cunningham GR, Walkup M, He Z, Sturgis L, Kagan C, Mannucci R, Nicoletti I, Teng B, Denner L. Signaling pathway activated during apoptosis of the prostate cancer cell line LNCaP: overexpression of caspase-7 as a new gene therapy strategy for prostate cancer. *Cancer Res.* 1999;59:382–390.

Murillo H, Huang H, Schmidt LJ, Smith DI, Tindall DJ. Role of PI3K signaling in survival and progression of LNCaP prostate cancer cells to the androgen refractory state. *Endocrinology.* 2001;142:4795–4805.

Nunez G, Benedict MA, Hu Y, Inohara N. Caspases: the proteases of the apoptotic pathway. *Oncogene.* 1998;17:3237–3245.

Partin A, Rodriguez R. The molecular biology, endocrinology, and physiology of the prostate and seminal vesicles. In: Walsh PC, ed. *Campbell's Urology.* 8th ed. Philadelphia, Pa: Saunders; 2002:1237–1296.

Reynolds T. Prostate cancer prevention trial yields positive results, but with a few cautions. *J Natl Cancer Inst.* 2003;95:1030–1031.

Rokhlin OW, Guseva NV, Tagiyev AF, Glover RA, Cohen MB. Caspase-8 activation is necessary but not sufficient for tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-mediated apoptosis in the prostatic carcinoma cell line LNCaP. *Prostate.* 2002;52:1–11.

Ross RK, Pike MC, Coetzee GA, Reichardt JK, Yu MC, Feigelson H, Stanczyk FZ, Kolonel LN, Henderson BE. Androgen metabolism and prostate cancer: establishing a model of genetic susceptibility. *Cancer Res.* 1998;58:4497–4504.

Rozen S, Skaletsky H. Primer3 on the WWW for general users and for biologist programmers. *Methods Mol Biol.* 2000;132:365–386.

Schultz DR, Harrington WJ Jr. Apoptosis: programmed cell death at a molecular level. *Semin Arthritis Rheum.* 2003;32:345–369.

Seilhamer JJ. Pruzanski W, Vadas P, Plant S, Miller JA, Kloss J, Johnson LK. Cloning and recombinant expression of phospholipase A2 present in rheumatoid arthritic synovial fluid. *J Biol Chem.* 1989;264:5335–5338.

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.

Thornberry NA, Rano TA, Peterson EP, Rasper DM, Timkey T, Garcia-Calvo M, Houtzager VM, Nordstrom PA, Roy S, Vaillancourt JP, Chapman KT, Nicholson DW. A combinatorial approach defines specificities of members of the caspase family and granzyme B. Functional relationships established for key mediators of apoptosis. *J Biol Chem.* 1997;272:17907–17911.

Turgeon D, Carrier JS, Chouinard S, Belanger A. Glucuronidation activity of the UGT2B17 enzyme toward xenobiotics. *Drug Metab Dispos.* 2003;31:670–676.

Turgeon D, Carrier JS, Levesque E, Hum DW, Belanger A. Relative enzymatic activity, protein stability, and tissue distribution of human steroid-metabolizing UGT2B subfamily members. *Endocrinology.* 2001;142:778–787.

Uygur MC, Arik AI, Altug U, Erol D. Effects of the 5 alpha-reductase inhibitor finasteride on serum levels of gonadal, adrenal, and hypophyseal hormones and its clinical significance: a perspective clinical study. *Steroids.* 1998;63:208–213.

Wang LG, Liu XM, Kreis W, Budman DR. Down-regulation of prostate-specific antigen expression by finasteride through inhibition of complex formation between androgen receptor and steroid receptor-binding consensus in the promoter of the PSA gene in LNCaP cells. *Cancer Res.* 1997;57:714–719.

Zhu YS, Cai LQ, You X, Cordero JJ, Huang Y, Imperato-McGinley J. Androgen-induced prostate-specific antigen gene expression is mediated via dihydrotestosterone in LNCaP cells. *J Androl.* 2003;24:681–687

Appendix 1.

Comparision of LNCaP and LNCaP + Dutasteride

rank	genbank	cytoband	symbol	affy id	unigene	locus link	Mean Diff (log 2 scale)	Ratio of Means (raw scale)	t-statistic	p-value
1	S39329	19q13.3-q1	KLK2	217_at	Hs.181350	3817	1.0700	2.0995	40.9582	0.00000
2	M25915	8p21-p12	CLU	36780_at	Hs.75106	1191	-0.9574	0.5150	-28.0543	0.00001
3	X07730	19q13.-q13	KLK3	1805_g_at	Hs.171995	354	1.3689	2.5828	27.4280	0.00001
4	U07919	15q26	ALDH1A3	36686_at	Hs.75746	220	0.5170	1.4310	24.6208	0.00002
5	U91618	12q21	NTS	33998_at	Hs.80962	4922	-1.2105	0.4321	-24.0182	0.00002
6	M22430	1p35	PLA2G2A	614_at	Hs.76422	5320	-0.6081	0.6561	-23.1513	0.00002
7	M17733	11q23.3	SC5DL	31557_at	Hs.288031	6309	-0.6980	0.6164	-23.0887	0.00002
8	AF099989	2q24.3	SPAK	40966_at	Hs.199263	27347	0.5130	1.4270	22.7440	0.00002
9	Antigen,	NO_.SIF	NO_.SIF	1514_g_at	NO_.SIF	NO_.SIF	1.4491	2.7304	22.7229	0.00002
10	AL034374	6p21.1-p12	HELO1	33821_at	Hs.250175	60481	0.9070	1.8751	22.1245	0.00002
11	U08854	4q13	UGT2B15	33068_f_at	Hs.150207	7366	-0.8395	0.5588	-22.0356	0.00003
12	X07730	19q13.-q13	KLK3	1804_at	Hs.171995	354	1.3404	2.5323	21.9195	0.00003
13	AA131149	4p16	S100P	34319_at	Hs.2962	6286	0.4553	1.3711	21.5612	0.00003
14	AB020637	11q21	KIAA0830	40455_at	Hs.167115	23052	0.4496	1.3657	20.9362	0.00003
15	Antigen,	NO_.SIF	NO_.SIF	1662_r_at	NO_.SIF	NO_.SIF	0.6613	1.5815	20.4647	0.00003
16	U80669	8p21	NKX3A	445_at	Hs.55999	4824	0.5160	1.4299	20.3249	0.00003
17	J03779	3q25.1-q25	MME	1389_at	Hs.1298	4311	-0.3804	0.7682	-20.3192	0.00003
18	X80692	15q21	MAPK6	36926_at	Hs.271980	5597	0.6500	1.5691	19.8265	0.00004
19	Oncogene	NO_.SIF	NO_.SIF	1842_at	NO_.SIF	NO_.SIF	0.4790	1.3938	19.4178	0.00004
20	U29091	1q21-q22	SELENBP1	37405_at	Hs.334841	8991	-0.5919	0.6634	-18.8226	0.00005
21	X05332	19q13.-q13	KLK3	40794_at	Hs.171995	354	1.3214	2.4991	18.0847	0.00005
22	U16799	1q22-q25	ATP1B1	37669_s_at	Hs.78629	481	-0.7346	0.6010	-17.9104	0.00006
23	AL079298	5q12-q13	MCCC2	36432_at	Hs.167531	64087	0.5496	1.4637	17.6091	0.00006
24	AI635895	Xp21.1-q25	DSIPI	36629_at	Hs.75450	1831	0.4777	1.3925	16.3077	0.00008
25	AA255502	6p21.3	H4FG	39969_at	Hs.46423	8364	0.3759	1.2977	16.0614	0.00009
26	U17163	7p22	ETV1	37055_at	Hs.89566	2115	0.3031	1.2338	16.0152	0.00009
27	AI557497	6	NCUBE1	39039_s_at	Hs.184325	51465	0.3968	1.3166	15.6154	0.00010
28	U06641	4q13	UGT2B15	33069_f_at	Hs.150207	7366	-0.6724	0.6275	-15.4057	0.00010
29	Tubulin,	NO_.SIF	NO_.SIF	330_s_at	NO_.SIF	NO_.SIF	-0.5688	0.6742	-15.3600	0.00010
30	Antigen,	NO_.SIF	NO_.SIF	1661_i_at	NO_.SIF	NO_.SIF	0.7825	1.7201	15.3215	0.00011
31	M22430	1p35	PLA2G2A	37017_at	Hs.76422	5320	-0.4416	0.7363	-14.7402	0.00012
32	AF002697	14q11.2-q1	BNIP3	38010_at	Hs.79428	664	0.4279	1.3453	14.1807	0.00014
33	AA522530	10pter-q26	FLJ20500	39827_at	Hs.111244	54541	0.4935	1.4079	13.8819	0.00016
34	X85753	13q12	CDK8	1189_at	Hs.25283	1024	-0.4112	0.7520	-13.3700	0.00018
35	AL049987	NULL	NULL	40552_s_at	Hs.166361	NULL	-0.3858	0.7654	-13.2215	0.00019
36	X01703	12q12-12q1	K-ALPHA-1	40567_at	Hs.334842	10376	-0.4598	0.7271	-13.0387	0.00020
37	U83660	13q32	ABCC4	1931_at	Hs.139336	10257	0.2959	1.2276	12.9903	0.00020
38	AF075587	13q22	KIAA0916	39777_at	Hs.151411	23077	0.6262	1.5435	12.6070	0.00023
39	AF060502	1p36.11-1p	PEX10	41281_s_at	Hs.247220	5192	0.4164	1.3346	12.5623	0.00023
40	U75329	21q22.3	TMPRSS2	34996_at	Hs.318545	7113	0.6471	1.5660	12.5502	0.00023
41	AL050162	7q31.2	TES	32134_at	Hs.165986	26136	0.2352	1.1771	12.3393	0.00025
42	AA760866	4q28.2-q31	NDUFC1	38485_at	Hs.84549	4717	0.3542	1.2783	12.2918	0.00025
43	AF024710	6p12	VEGF	1953_at	Hs.73793	7422	0.3118	1.2413	12.2845	0.00025
44	D89053	2q34-q35	FACL3	33880_at	Hs.268012	2181	0.2527	1.1914	12.2776	0.00025
45	AL050073	11q22	AASDHPPT	35761_at	Hs.64595	60496	0.2415	1.1822	12.2038	0.00026
46	AF045584	11p11.2-p1	POV1	33708_at	Hs.18910	8501	0.3167	1.2454	12.1388	0.00026
47	AI800499	6q21	AIM1	32112_s_at	Hs.161002	202	0.2768	1.2115	12.0540	0.00027
48	AI557240	2q12-q21	DBI	37692_at	Hs.78888	1622	0.2973	1.2289	11.7025	0.00030
49	X12433	15q26.1	HS1-2	41088_at	Hs.99364	11057	0.3759	1.2977	11.4351	0.00033
50	AL035304	1q24	DKFZP564B1	37000_at	Hs.76285	25874	0.6935	1.6171	11.4309	0.00033
51	U59209	4q13	UGT2B17	33672_f_at	Hs.183596	7367	-0.6619	0.6321	-11.3666	0.00034
52	U41060	18q12.1	LIV-1	1798_at	Hs.79136	25800	0.2981	1.2295	11.2550	0.00036
53	AA059408	21q11	STCH	38676_at	Hs.288799	6782	0.3325	1.2592	11.0723	0.00038
54	W28186	8q22.1	LC27	41188_at	Hs.296398	55353	-0.2905	0.8176	-11.0596	0.00038
55	U48807	8p12-p11	DUSP4	1788_s_at	Hs.2359	1846	0.2583	1.1961	11.0403	0.00038
56	AA442799	16p12.1	FLJ20274	40868_at	Hs.268371	55623	0.2698	1.2057	10.8917	0.00040
57	X52015	2q14.2	IL1RN	37603_at	Hs.81134	3557	-0.3410	0.7895	-10.8150	0.00041
58	AF060568	11q23.1	ZNF145	39681_at	Hs.37096	7704	0.3212	1.2494	10.7735	0.00042
59	AB006625	19q13.4	PEG3	39701_at	Hs.139033	5178	0.6673	1.5881	10.6476	0.00044
60	X16396	2p12	MTHFD2	40074_at	Hs.154672	10797	0.5262	1.4401	10.6107	0.00045
61	AA977580	2q34-q35	FACL3	33881_at	Hs.268012	2181	0.2328	1.1751	10.6016	0.00045
62	D79206	20q12	SDC4	35844_at	Hs.252189	6385	-0.2545	0.8383	-10.5822	0.00045
63	X83301	NULL	NULL	41643_at	Hs.352067	NULL	-0.3067	0.8085	-10.5793	0.00045
64	U25182	Xp22.13	PRDX4	38435_at	Hs.83383	10549	0.4994	1.4137	10.5321	0.00046
65	J05032	2p14-q21.3	DARS	32615_at	Hs.80758	1615	0.2271	1.1705	10.5110	0.00046
66	AL080184	2	LOC51141	35833_at	Hs.7089	51141	-0.3375	0.7914	-10.4627	0.00047
67	AF038182	5q21.1	LOC90355	33466_at	Hs.25925	90355	-0.4213	0.7468	-10.3657	0.00049
68	L25931	1q42.1	LBR	288_s_at	Hs.152931	3930	0.2228	1.1670	10.1777	0.00052
69	W27949	6q24	C6orf34	41454_at	Hs.111029	23593	0.6288	1.5462	10.1737	0.00053
70	AB000584	19p13.1-13	PLAB	1890_at	Hs.296638	9518	0.5428	1.4568	10.1400	0.00053
71	AA526812	1q42.12	FLJ10326	36647_at	Hs.262823	55699	0.2980	1.2294	10.1006	0.00054
72	AB018257	21q22.11	ZNF294	39005_s_at	Hs.288773	26046	-0.7370	0.6000	-10.0987	0.00054
73	X06956	2q36.1	TUBA1	36591_at	Hs.75318	7277	-0.6616	0.6322	-10.0242	0.00056
74	AF041210	5	TRIM28	34296_at	Hs.228059	10155	-0.2223	0.8572	-9.8945	0.00059
75	AW007731	7p13	H2AV	39092_at	Hs.301005	94239	0.3562	1.2800	9.8060	0.00061
76	AA658877	19q13.3-q1	KLK2	41721_at	Hs.181350	3817	1.1416	2.2062	9.7951	0.00061
77	U80034	13q12	MIPEP	36830_at	Hs.68583	4285	0.2473	1.1870	9.6900	0.00063
78	AB011004	1q23.1	UAP1	41242_at	Hs.21293	6675	0.6488	1.5679	9.6834	0.00064
79	AF070641	NULL	NULL	37156_at	Hs.10684	NULL	0.4635	1.3789	9.6834	0.00064
80	AF023676	11q13	TM7SF2	35628_at	Hs.31130	7108	-0.2518	0.8399	-9.6564	0.00064
81	M24069	12p13.1	CSDA	39839_at	Hs.198726	8531	0.4511	1.3671	9.6017	0.00066
82	U08316	Xp22.2-p22	RPS6KA3	865_at	Hs.173965	6197	0.2017	1.1501	9.5980	0.00066
83	U48437	19q13.1	APLP1	36148_at	Hs.74565	333	-0.2215	0.8577	-9.5921	0.00066
84	AL080118	14q32.13	DKFZP564F1	41437_at	Hs.275352	26175	0.2049	1.1526	9.5700	0.00067
85	X61123	12q22	BTG1	37294_at	Hs.77054	694	0.5635	1.4779	9.4797	0.00069
86	M83667	8p11.2-p11	CEBPD	1052_s_at	Hs.76722	1052	0.2220	1.1664	9.4713	0.00069
87	M69023	15	TSPAN-3	38612_at	Hs.100090	10099	-0.2345	0.8500	-9.3643	0.00072
88	X56807	18q12.1	DSC2	39302_at	Hs.239727	1824	0.2622	1.1993	9.3485	0.00073
89	AL049246	3q23	FLJ10618	34804_at	Hs.42484	55186	0.3356	1.2619	9.3085	0.00074
90	AF014402	5q11	PPAP2A	34797_at	Hs.41569	8611	0.4178	1.3359	9.2816	0.00075
91	V00572	Xq13	PGK1	37677_at	Hs.78771	5230	0.4102	1.3288	9.2532	0.00076
92	W26659	8	LOC51669	36975_at	Hs.279921	51669	0.4236	1.3413	9.2432	0.00076
93	X75346	1q32	MAPKAPK2	1439_s_at	Hs.75074	9261	-0.2214	0.8577	-9.2430	0.00076
94	D86322	4q28.3-q31	CLGN	40710_at	Hs.86368	1047	0.4566	1.3723	9.2185	0.00077
95	AL039458	NULL	LIG1	34800_at	Hs.4193	26018	0.2529	1.1916	9.2137	0.00077
96	U48705	6p21.3	DDR1	1007_s_at	Hs.75562	780	-0.2142	0.8620	-9.2084	0.00077
97	AI056696	5q14.3	CETN3	35232_f_at	Hs.29463	1070	0.2245	1.1684	9.1487	0.00079
98	U84388	12q21.33-q	CRADD	1211_s_at	Hs.155566	8738	0.2242	1.1681	9.1015	0.00081
99	AL050290	Xp22.1	SAT	34304_s_at	Hs.28491	6303	0.2739	1.2091	9.0922	0.00081
100	X01060	3q26.2-qte	TFRC	37324_at	Hs.77356	7037	-0.4829	0.7156	-9.0033	0.00084

* U95Av2 microchip array data for LNCaP cells treated with 10μM dutasteride for 48 hours. Top 100 gene transcripts sorted by *P* value are shown. Mean Diff represents LNCaP untreated control compared to dutasteride treated. For the full set of data representing 12 558 gene transcripts, contact Lucy Schmidt at schmidt.lucy@mayo.edu. The entire database can be found at the following web site: http://mayoresearch.mayo.edu/mayo/research/tindall_lab/.