

# Atlas of Dihydrotestosterone Actions on the Transcriptome of Prostate In Vivo

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**BACKGROUND.** Using serial analysis of gene expression (SAGE), we studied the transcriptomic changes in vivo by dihydrotestosterone (DHT) treatment in mice to better understand androgen effects in the prostate.

**METHODS.** Approximately 872,000 SAGE tags were isolated from intact and castrated (GDX) mice with and without DHT injection.

**RESULTS.** GDX significantly altered 431 transcripts, including 110 transcripts restored by DHT, and 146 potentially new transcripts. Totally, 187 transcripts were significantly affected by DHT treatment, of which 124 were induced and 63 were repressed. Interestingly and consistent with the prostate's secretory role, DHT up-regulated the expression of many genes involved in various steps of protein metabolism such as synthesis, folding, and secretion. GDX modulated the expression of genes which induce cell apoptosis and inhibit cell proliferation through polyamine biosynthesis, retinoid X receptor actions as well as several signaling pathways and some related factors. These results clarify DHT effects on prostate transcriptome in the areas of protein metabolism, cell proliferation and apoptosis. In addition, we detected gene expression changes in metabolic pathways, cytoskeleton, immunity and endoplasmic reticulum stress. Furthermore, knockdown of *S*-adenosylmethionine decarboxylase 1 in LNCaP cells confirmed the importance of androgen-regulated genes (ARGs) in prostate cancer cell growth.

**CONCLUSION.** Our data support the idea that ARGs are essential for the normal development of the prostate and can also be responsible for the pathogenesis of the prostate cancer.

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**KEY WORDS:** prostate; dihydrotestosterone; serial analysis of gene expression; transcriptome; androgen-regulated genes

## INTRODUCTION

Androgens are steroid hormones which induce the differentiation and maturation of male reproductive organs and the development of male secondary sex characteristics. Testosterone is the major androgen in circulation and dihydrotestosterone (DHT) is the primary nuclear androgen as well as the most potent androgen. These androgens exert their biologic functions on target tissues and cells mostly by transcriptional regulation of androgen-regulated genes (ARGs) through androgen receptor (AR) [1]. Thus, to understand the androgen action in the target tissues, it is necessary to identify ARGs.

Prostate is a highly androgen-dependent glandular tissue. The androgens play an essential role in prostate

development, growth and the pathogenesis of prostate diseases such as benign prostatic hyperplasia and adenocarcinoma [2,3]. Thus, investigating ARGs in prostate can help to extend our knowledge on prostate physiology and suggest new approaches for the prevention, early molecular diagnosis and treatment of prostate cancer.

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There are several previous studies on androgen targeted genes in the prostate cancer [4–7]. Gene expression profiling using serial analysis of gene expression (SAGE) have been carried out in some studies investigating genes differentially expressed in prostate cancer tissue [8] or genes regulated by androgen in prostate cancer cell lines in vitro [4–6]. These ARGs are unlikely to account for all the effects of androgens, especially in the early stage of prostatic disease, since androgens are responsible not only for initial prostate cancer growth but also for normal prostate development. Moreover, the change in the initial factor responsible for the development of prostate cancer may be blunted after the occurrence of cancer. Consequently, a more complete identification of ARGs in the prostate is necessary.

An in vivo model would maintain tissue properties stably during experimental treatment and allows hormonal intervention. Moreover, such a model would reflect tissue–tissue and/or tissue–organ interaction through circulating factors such as hormones and peptides, which are modulated by the hormonal intervention.

SAGE is a powerful method to analyze a large number of transcripts in a given tissue without any prior selection basis. With the advent of SAGE strategy, the expression of ARGs including known and novel genes in prostate tissue can be identified. Thus, SAGE offers a unique opportunity to understand the androgen action. The current study has investigated the transcriptomic changes induced by castration (GDX) and DHT on the normal mouse prostate tissue in vivo, using SAGE method. Here, we have identified 187 previously characterized transcripts and 89 novel transcripts which have rapidly responded to androgen within 24 hr in vivo. We have focused on gene regulation occurring within 24 hr since genes responding to DHT within this time period would be enriched in direct androgen targets before cell growth and tumorigenesis. The present findings confirm and extend previous studies and offer novel insights into the molecular mechanisms by which androgens affect the prostate in vivo such as DHT-induced protein synthesis/secretion, lipid/energy metabolism, endoplasmic reticulum stress, polyamine biosynthesis, cell proliferation and differentiation.

## MATERIALS AND METHODS

### Prostate Tissues Preparation

Prostate tissues were obtained from 51 male C57BL6 mice 10- to 12-week old for the intact group, and from 14 mice per group for GDX and GDX plus DHT treatment. The animals were purchased from Charles

River, Inc. (Québec, Canada), and had free access to Lab Rodent Diet No. 5002 (Ren's Feed and Suppliers, Ontario, Canada) and water. GDX was performed 7 days prior to organ collection for GDX and all DHT groups. DHT (0.1 mg/mouse) was injected 1 hr (DHT1hr), 3 hr (DHT3hr), 6 hr (DHT6hr), and 24 hr (DHT24hr) prior to sacrifice in the DHT groups. The control group (GDX) received vehicle solution (0.4% w/v Methocel A15LV Premium/5% ethanol) 24 hr prior to sacrifice. All mice were handled in a facility approved by the Canadian Council on Animal Care in accordance with the Guide for Care and Use of Experimental Animals. The entire prostate tissues of all mice from the same group were pooled together to eliminate inter-individual variations and to extract sufficient amount of mRNA for the analysis. The tissues were stored at  $-80^{\circ}\text{C}$  until RNA extraction.

### Transcriptome Analysis

The SAGE method was performed as previously described [9–12]. Polyadenylated RNA was extracted with mRNA Mini kit (Qiagen, Mississauga, Canada), annealed with the biotin-5'-T<sub>18</sub>-3' primer and converted to cDNA using the cDNA synthesis kit (Invitrogen, Carlsbad, CA). The resulting cDNA was digested with *Nla*III (anchoring enzyme) and the 3' restriction fragments were isolated with streptavidin-coated magnetic beads (Dynal Biotech, Oslo, Norway) and separated into two populations. Each population was ligated to one of the two annealed linker pairs and extensively washed to remove unligated linkers. The tag including the most 3' *Nla*III restriction site (CATG) of each transcript was released by digestion with *Bsm*FI (tagging enzyme). The blunting kit from Takara Co. (Kyoto, Japan) was used for the blunting and ligation of the two tag populations. The resulting ligation products containing the ditags were amplified by PCR with an initial denaturation step of 1 min at  $95^{\circ}\text{C}$  followed by 22 cycles of 20 sec at  $94^{\circ}\text{C}$ , 20 sec at  $60^{\circ}\text{C}$  and 2 sec at  $72^{\circ}\text{C}$  using 27 bp primers [12]. The PCR products were then digested with *Nla*III and the band containing the ditags was extracted from 12% acrylamide gel. The purified ditags were self-ligated to form concatemers. The concatemers ranging from 500 to 1,800 bp were isolated by agarose gel. The resulting DNA fragments were ligated into the *Sph*I site of pUC19 and cloned into UltraMAX DH5 $\alpha$ FT *E. coli* (Invitrogen). White colonies were screened by PCR to select long inserts for automated sequencing.

Sequence files were analyzed using the SAGEana program, a modification of SAGEparser [13]. Tags corresponding to linker sequences were discarded and duplicate concatemers were counted only once. To identify the transcripts, the sequences of 15 bp SAGE

tags (*Nla*III site CATG plus adjacent 11 bp tags) were matched with public databases. The classification of transcripts was mainly based on the genome directory [14] and gene ontology (<http://www.geneontology.org/>). The SAGE tags which did not match any sequence in the public databases were classified as potential novel transcripts.

#### Validation by Quantitative Real-Time PCR (Q-RT-PCR)

Pooled RNA samples of nine mice in each experimental group (GDX, DHT1hr, DHT3hr, DHT6hr, and DHT24hr), except for intact group, from an independent experiment were used for the Q-RT-PCR. First-strand cDNA was synthesized using 5 µg of isolated RNA in a reaction containing 200 U of Superscript III Rnase H-RT (Invitrogen), 300 ng of oligo-dT<sub>18</sub>, 500 mM deoxynucleotides triphosphate, 5 mM dithiothreitol and 34 U of human RNase inhibitor (Amersham Pharmacia, Piscataway, NJ) in a final volume of 50 µl. The reaction was performed at 50°C for 2 hr and then treated with RNase A for 30 min at 37°C. The resulting products were purified with Qiaquick PCR purification kits (Qiagen). The cDNA corresponding to 20 ng of total RNA was used to perform fluorescent-based real-time PCR quantification using the LightCycler real-time PCR apparatus (Roche, Inc., Nutley, NJ). Reagents were obtained from the same company and were used as described by the manufacturer. The conditions for PCR reactions were: denaturation at 95°C for 10 sec, annealing at 56–66°C for 5 sec and elongation at 72°C for 7–13 sec. The reaction was then heated for 3 sec at 2°C lower than the melting temperature of the DNA fragment. Reading of the fluorescence signal was taken at the end of the heating to avoid non-specific signal. A melting curve was performed to assess non-specific signal. Oligoprimer pairs that allow the amplification of approximately 200 bp were designed by GeneTools software (Biotools, Inc., Edmonton, AB) and their specificity was verified by blast in GenBank database. ARGs, GenBank accession numbers and regions used for the primer pairs were the following: heat shock 70 kDa protein 5 (HSPA5), AJ002387, 1418–1440; serine (or cysteine) proteinase inhibitor clade E member 2 (SERPINE2), X70296, 944–1202; nucleobindin 2 (NUCB2), AJ222586, 399–545; regulator of G-protein signaling 2 (RGS2), NM\_009061, 1565–1818; S-adenosylmethionine decarboxylase 1 (AMD1), D12780, 592–845; and Ia-associated invariant chain (Ii), NM\_010545, 400–647. Data calculation and normalization were performed using second derivative and double correction method using the housekeeping gene hypoxanthine guanine phosphoribosyl transferase 1 [15]. The expression levels of mRNA are

expressed as number of copies/µg total RNA using a standard curve of crossing point (Cp) versus logarithm of the quantity. The standard curve was established using known cDNA amounts of 0, 10<sup>2</sup>, 10<sup>3</sup>, 10<sup>4</sup>, 10<sup>5</sup>, and 10<sup>6</sup> copies of hypoxanthine guanine phosphoribosyl transferase 1 and a LightCycler 3.5 program provided by the manufacturer (Roche, Inc.). The Q-RT-PCR was performed in duplicate.

#### Cell Culture and Small Interfering RNA (siRNA) Transfection

Androgen-sensitive human prostate cancer (LNCaP) cells were purchased from the American Type Culture Collection (ATCC, Manassas, VA). They were maintained in phenol red-free RPMI-1640 medium (Fisher Scientific, Ottawa, Canada) supplemented with 10% (v/v) fetal bovine serum (FBS) (Wisent, Inc., St-Bruno, Canada) and antibiotics (100 U/ml penicillin and 100 µg/ml streptomycin) (Sigma-Aldrich Canada Ltd, Oakville, Canada) at 37°C under 95% air-5% CO<sub>2</sub> humidified atmosphere.

LNCaP cells were seeded at 3 × 10<sup>5</sup> cells/well on poly-L-lysine (Sigma-Aldrich Canada Ltd) treated 6-well cell culture plates with phenol red-free RPMI-1640 medium supplemented plus 10% FBS and antibiotics, and were allowed to adhere for 48 hr. The cells were transiently transfected using Lipofectamine<sup>TM</sup> 2000 (Invitrogen) in Opti-MEM<sup>®</sup> I Reduced Serum Medium (Invitrogen), according to the manufacturer's instruction. Briefly, 80 nM negative control, positive control (AR) or target (AMD1) Stelth<sup>TM</sup> RNAi oligo (Invitrogen) was transiently transfected into LNCaP cells for 24 hr, and then the medium was replaced with fresh steroid-reduced medium (phenol red-free RPMI-1640 medium supplemented plus 5% charcoal-stripped FBS (Wisent, Inc.) and antibiotics) with 10 pM R1881 for 72 hr. Mock treatment was performed with Lipofectamine<sup>TM</sup> 2000 alone. The GenBank accession numbers (positions) used for the sense sequences siRNA oligos were NM\_000044.2 (3008–3032) for AR and NM\_001634.3 (657–681) for AMD1.

Cell proliferation assay was performed 96 hr after the transfection by measuring DNA contents with diaminobenzoic acid (DABA) (Sigma-Aldrich Canada Ltd). In brief, medium was removed and remaining cells were fixed with 500 µl methanol per well. The 450 µl of filtered DABA solution (90 ml of 4N HCl (Fisher Scientific), 20 g of DABA and 10 g of carbon (Fisher Scientific)) was added in each well, and incubated for 60 min at 60°C. After placing the plate on ice, 3.75 ml of 1 N HCl was added in each well. The absorbance at 400 nm (excitation) and 508 nm (emission) was recorded with plate reader (Fluorolite 100, Opti-Ressources, Inc., Charny, Canada). The DNA

contents were calculated using DNA standards (MP Biochemical, Montreal, Canada). The values were triplicate measurements on three unique samples, and expressed with a ratio to the mock transfection.

Cell cycle analysis was also performed at 96 hr after the transfection with flow cytometry by staining cells with propidium iodide (PI) (Sigma-Aldrich Canada Ltd). In detail, medium containing cells were transferred to 5 ml tube and centrifuged for 5 min at 2,000 rpm. The cells were washed twice with 2 ml of phosphate buffer solution (PBS) (Invitrogen), in which 0.8 ml was used for following cell cycle analysis and cells in 1.2 ml were collected, frozen with liquid N<sub>2</sub> and stored at -80°C for Q\_RT-PCR analysis. The sample for cell cycle analysis was centrifuged for 5 min at 2,000 rpm and cells were re-suspended in 0.3 ml PBS. The 0.7 ml ice-cold ethanol (Fisher Scientific) was added and stored at -20°C for overnight. After washing cells with 3 ml PBS, cells were re-suspended in 250 µl of PI staining solution (5 ml of 0.1% Triton X-100 in PBS (Sigma-Aldrich Canada Ltd), 250 µl of 1 mg/ml PI and 2 mg DNase-free RNase A (Sigma-Aldrich Canada Ltd)), and incubated at 37°C for 30 min. The tube was placed on ice until analysis with EPICS XL set at 488 nm and System II Software (Beckman Coulter Canada, Inc., Mississauga, Canada). The values were expressed with a ratio to the mock transfection from pooled data of three independent experiments in triplicates for control as well as two independent experiments in triplicates for AR and AMD1.

Knockdown of the target genes and interferon response at 96 hr after the transfection was evaluated by the Q\_RT-PCR. The GenBank accession numbers (regions) used for the primer pairs were NM\_000044 (3152–3401) for AR, NM\_001634 (1946–2171) for AMD1, NM\_001547 (1645–1914) for interferon-induced protein with tetratricopeptide repeats 2 (IFIT2) and NM\_001549 (1261–1489) for interferon-induced protein with tetratricopeptide repeats 3 (IFIT3). The values were triplicate measurements on three unique samples, and expressed with a ratio to the mock transfection.

### Statistical Analysis

For the SAGE results, the comparative count display (CCD) test was used to identify the transcripts significantly differentially expressed ( $P \leq 0.05$ ) between the groups with more than a twofold change [16]. The data were normalized to 100,000 tags in order to facilitate visual comparison in the tables.

For the siRNA experiments, the one-way ANOVA test with the Fisher PLSD post hoc test or *t*-test was used to determine statistical significance between groups ( $P \leq 0.05$ ).

## RESULTS

Six SAGE libraries (Intact, GDX, GDX + DHT1hr, 3hr, 6hr, and 24hr) were generated to characterize the effect of GDX and DHT on prostate transcriptome. Approximately 150,000 tags were sequenced in each group, which corresponded to about 50,000 distinct tag species. A total of 872,587 tags were sequenced, which corresponded to 168,219 tag species. In total, GDX altered 431 transcripts, including 110 transcripts which were restored to control level by DHT, and 146 potentially new transcripts. Moreover, DHT altered 187 transcripts, of which 124 were induced and 63 were repressed. These transcripts are involved in a variety of functions such as protein/RNA expression, energy metabolism, cell proliferation, cell structure/motility, cell signaling and cell defense. Tables I–IX present the transcripts differentially expressed ( $P < 0.05$ ) between groups accordingly to their functions.

A major effect of DHT in the prostate is the induction of protein synthesis and secretory activity (Table I). DHT has up-regulated 25 transcripts involved in protein synthesis, folding and secretion activity, such as ribosomal protein S2/5/6/7 (RPS2/5/6/7), HSPA5, SERPINE2 and seminal vesicle secretion 3/5/6 (SVS3/5/6). In addition, GDX has up-regulated six transcripts related to RNA synthesis and splicing process whereas DHT has down-regulated half of them (DDX5 and PABP1). GDX also increased the expression of several transcription factors whereas DHT modulated four transcripts (Table II). The majority of the transcripts involved in lipid biosynthesis, as well as energy metabolism were up-regulated by DHT (Table III). DHT also induced transcripts involved in polyamine biosynthesis such as AMD1 and methionine adenosyltransferase II alpha (MAT2A) (Table IV). In addition, DHT increased the expression level of four transcripts involved in cell structure (Table V). Eight signal transducers including NUCB2 and RGS2 were modulated by GDX and the effects were reversed by DHT (Table VI). Moreover, DHT induced genes in cell defense, such as interleukin 25 (IL-25) and complement component 1 r subcomponent (C1R), while repressing the expression level of Ii (Table VII). Other transcripts such as lumican (LUM) and haptoglobin (HP) were also down-regulated by DHT, whereas some transcripts such as the expressed sequence tag (EST) similar to tumor differentially expressed 1 were up-regulated by DHT (Table VIII). Furthermore, 89 DHT responsive tags did not match any known mRNA from public databases, of which 54 were also concordantly regulated by GDX (Table IX).

Six characterized ARGs found by the SAGE were further validated by Q\_RT-PCR. The results of Q\_RT-PCR on both the previously known (HSPA5,

**TABLE I. Differentially Expressed Transcripts Involved in Protein Expression**

Tags	I	GDx	GDx + DHT					Abbr.	Description (UniGene; GenBank)
			1hr	3hr	6hr	24hr			
<b>Protein synthesis</b>									
AGGCAGACAGT	187*	33	72	72	116*	87	EEF1A1	Eukaryotic translation elongation factor 1 alpha 1 (196614; BC018223)	
GACCCCAAAA	3*	30	30	19	12	18	EEF2	EST eukaryotic translation elongation factor 2 (27818; BY666406)	
AATATCGAAA	10*	1	2	3	3	4	EIF3S6	EST eukaryotic translation initiation factor 3, subunit 6 (880; AK088269)	
GCTTTGGTGA	16	8	14	17	21	31*	EIF5A	Eukaryotic translation initiation factor 5A (196607; BC024899)	
CTGAAACATCTC	94*	26	43	53	80*	66	RPLP0	Acidic ribosomal phosphoprotein PO (5286; BC011106)	
CAAGGTGACAG	53	22	42	35	51	64*	RPS2	Ribosomal protein S2 (1129; BC002186)	
CCTTTGAGATC	33	15	32	29	49*	34	RPS5	Ribosomal protein S5 (5291; AK012481)	
GCCAGATGCCG	43	15	20	25	48*	35	RPS6	Ribosomal protein S6 (1139; BC010604)	
GCGAGAAGCCG	4	0	12*	6	12*	9*	RPS7	EST ribosomal protein S7 (5281; C80084)	
AATTTCAAAAC	56*	184	116	145	142	111	RPS17	Ribosomal protein S17 (42767; BC002044)	
GACGGTTGTGC	1*	11	11	6	5	3	RPL26	EST ribosomal protein L26 (3229; AV304482)	
CCAGAACAGAC	55*	153	135	155	137	110	RPL30	Ribosomal protein L30 (3487; BC002060)	
AGGAAAGCCGC	89*	334	281	257	198	215	RPL36	Ribosomal protein L36 (11376; BC021595)	
GGATTTGGCTT	148*	402	409	381	330	325	RPLP2	Ribosomal protein, large P2 (14245; BC012413)	
<b>Chaperones and protein folding</b>									
TCATCTTTAAC	30*	0	2	1	8	10*	CALR	Calreticulin (1971; BC003453)	
CACATACACGGG	6	0	9*	7	10*	11*	FKBP2	FK506 binding protein 2 (4234; BC031824)	
CAGGAGGAGTT	28*	3	1	5	5	8	GRP58	Glucose regulated protein, 58 kDa (709; BC033439)	
TCAGCAACTGC	13*	1	1	4	8	6	HSPA5	EST heat shock 70 kDa protein 5 (glucose-regulated protein) (918; BB050254)	
CTCTGGGGTCA	20	7	6	10	10	38*	HSPA5	EST heat shock 70 kDa protein 5 (glucose-regulated protein) (918; BY421170)	
TGGTGTAGGAA	141	76	78	82	108	228*	HSPA5	Heat shock 70 kDa protein 5 (glucose-regulated protein) (918; AK004578)	
GTGAGCCCAT	37*	8	19	20	27	17	HSP90	EST heat shock protein 1, beta (2180; BQ925902)	
GAGCGTTTGG	49	21	28	28	50	64*	PPIA	Peptidylprolyl isomerase A (5246; AK012491)	
CGGCCACTGGA	48*	11	10	24	20	41*	PDIR	Protein disulfide isomerase-related protein (182959; BC006865)	
TCACAGACACG	21*	0	1	2	3	6	TRA1	Tumor rejection antigen gp96 (4526; AK088148)	
<b>Protein trafficking and transport</b>									
AGACATTTTAA	6*	69	42	58	41	30	ANX4	Annexin A4 (619; U95371)	
ATTCTCTGGAT	11*	1	0	4	4	7	ATP2A2	ATPase, Ca+++ transporting, cardiac muscle, slow twitch 2 (42255; AJ131821)	
TTTPTTGGTGTG	4*	23	13	16	17	12	ATP6V1G1	ATPase, H+ transporting, V1 subunit G isoform 1 (29868; BC003429)	
TGAACACAATG	0*	12	6	1	2	2	MSCP	EST mitochondrial solute carrier protein (36710; BB067030)	
CTATGGCTTCT	5*	28	14	11	12	14	RAB11A	EST RAB11a, member RAS oncogene family (1387; NM_017382)	
CGGGCACCTAT	10*	0	2	1	8*	7	SSR4	Signal sequence receptor, delta (831; BC008172)	
TTTACACGTGTG	20*	1	1	7	9	9	SLC12A2	Solute carrier family 12, member 2 (4168; U13174)	
CATFGCTGTGA	1	9	3	5	3	0*	SLC39A1	Solute carrier family 39 (iron-regulated transporter), member 1 (28756; BC003438)	
GCAATGGCCCTC	1*	12	8	8	7	12	SLC9A3R1	Solute carrier family 9 (sodium/hydrogen exchanger), isoform 3 regulator 1 (27842; U74079)	
<b>Protein modification/targeting</b>									
CCTGCAAGGAG	3*	19	15	13	13	17	COPG1	Coatomer protein complex, subunit gamma 1 (26422; BC024686)	
TCCTCTCTCTG	9*	0	1	2	3	3	COPA	Coatomer protein complex subunit alpha (30041; BC024070)	
TATAATCCACA	10*	0	1	2	1	1	MAN1A	EST mannosidase 1, alpha (117294; BC063961)	
TTGCAAAAAGC	41*	4	4	8	7	17	MAN1A	Mannosidase 1, alpha (117294; BC015265)	
GGATCTCTGGGT	3*	19	12	14	19	13	TKT	Transketolase (154387; AK012794)	

(Continued)

**TABLE I. (Continued)**

Tags	I	GDx	GDx + DHT				Abbr.	Description (UniGene; GenBank)
			1hr	3hr	6hr	24hr		
Protein turnover								
TTTTATTCTCC	3*	24	14	19	13	10	CAPN12	Calpain 12 (81144; BC028751)
CTCAGCCTGG	31*	115	88	101	62	93	CTSD	Cathepsin D (231395; X52886)
TACTGGGAGCT	4*	21	11	15	12	16	CTSH	Cathepsin H (2277; NM_007801)
ATAGCCCCAAA	1*	20	17	10	5	3*	CTSS	Cathepsin S (3619; BC002125)
CCTTGCTCAAT	87*	219	186	263	164	148	CST3	Cystatin C (4263; BC002072)
TTGATCCCCAT	12*	1	1	1	6	4	LAMP1	Lysosomal membrane glycoprotein 1 (16716; AY069968)
TTCAGAGAATA	0*	26	21	13	20	17	MMP15	EST matrix metalloproteinase 15 (7283; BF384352)
Secretion								
CCCCCAAAAA	4*	79	56	19*	22*	23*	LCN2	EST lipocalin 2 (9537; BC020275)
ATGCAGTGGCG	414*	8	11	10	35*	179*	SPINK3	Serine protease inhibitor, Kazal type 3 (272; AK007841)
TGTTCACTAAA	49	29	35	45	60	81*	SERPINE2	Serine (or cysteine) proteinase inhibitor, clade E, member 2 (3093; AK045954)
AACCAACGATC	380*	128	191	208	138	169	SVP2	EST seminal vesicle protein, secretion 2 (143501; BB123697)
ACCAGACACG	193*	4	6	10	9	21*	SVP2	EST seminal vesicle protein, secretion 2 (143501; BB872622)
AGAGAAGATGG	3	0	0	0	1	9*	SVS3	EST seminal vesicle secretion 3 (118769; BY374235)
ACCACCTCACA	24*	0	2	0	0	9*	SVS3	Seminal vesicle secretion 3 (118769; AF242220)
CCCTGCCACTG	13*	0	0	1	0	12*	SVS3	Seminal vesicle secretion 3 (118769; AK035235)
GAAAC TTGAAT	93*	32	49	21	14	58	SVS5	EST seminal vesicle secretion 5 (140154; BY373663)
GGGCC TAGAAA	2,319*	315	437	299	240	809*	SVS6	Seminal vesicle secretion 5 (140154; X57139)
TCC TGAGGATG	42*	5	28*	7	8	25*	SVS7	Seminal vesicle secretion 6 (3787; NM_013679)
ATGGCCTGAGA	514*	140	328*	353*	490*	371*	SVP7	Seminal vesicle protein, secretion 7 (99349; AFI34204)
CCCTCCCCTCC	35	17	33	33	60*	35	SVP7	EST seminal vesicle protein, secretion 7 (99349; BB118614)
GGCTCTGTTCT	261*	82	124	70	59	210*	SVP29	EST seminal vesicle protein 29 (1286; BB107209)

I, intact; GDx, gonadectomy; DHT, dihydrotestosterone.

\*Significantly different ( $P < 0.05$ ) from GDx.

<sup>a</sup>Genes previously reported to be regulated by androgens.

**TABLE II. Differentially Expressed Transcripts Involved in RNA Expression**

Tags	I	GDx	GDx + DHT				Abbr.	Description (UniGene; GenBank)
			1hr	3hr	6hr	24hr		
Transcription factors								
ACACCAAAAA	1*	12	5	7	5	2	AEBP1	AE binding protein 1 (4665; X80478)
TGATGTTCCAC	3*	17	11	22	10	6	BAZ2A	EST bromodomain adjacent to zinc finger domain, 2A (200834; BF018079)
TAAAAAGTTCTC	1*	18	9	9	12	13	ELF1	E74-like factor 1 (24876; U19617)
GGATATGTGGT	6*	30	22	16	19	11	EGR1	early growth response 1 (181959; M22326)
TGGAAAAGTGAA	3*	37	16	14	10*	7*	FOS	FBJ osteosarcoma oncogene (5043; BC029814)
GAGCGTGTGC	10*	1	2	1	2	2	GATA2	GATA binding protein 2 (1391; AB000096)
TTTTTGTAAAA	4*	21	7	4*	8	4*	HDAC7A	Histone deacetylase 7A (258313; AK030863)
TTAAAAGTATTG	8*	0	0	0	2	2	NKX3A	NK-3 transcription factor, locus 1 (Drosophila) (3520; U88542)
TTCCATTTAAA	3*	20	9	10	12	13	CID	Nuclear DNA binding protein (2485; NM_020558)
TAGTTGAAAAA	2*	15	6	6	8	4	NR4A1	Nuclear receptor subfamily 4, group A, member 1 (119; BC004770)
TGCTTGAACAG	4	1	4	3	6	12*	NFIB	EST nuclear factor I/B (4025; AW743765)
GAACAATGGAG	3	0	1	4	8*	7	RPO2TC1	RNA polymerase II transcriptional coactivator (41746; BC010967)
GATCCTAAGCC	1*	12	14	15	8	7	STAT6	EST signal transducer and activator of transcription 6 (121721; BC029318)
ATGTAATAAAG	1*	15	10	13	6	6	TGIF	TG interacting factor (8155; BC012700)
RNA synthesis—RNA processing								
GCCTTCCAATA	50*	146	109	116	97	99	DDX5	DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 5 (19101; BC009142)
CATTGCCCTTCA	13*	70	28	42	23*	25*	DDX5	EST DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 5 (19101; AV267859)
ACACATTTATTT	13*	72	33	35	26	22*	PABP1	EST poly(A) binding protein, nuclear 1 (7723; BB100395)
AATAAAGTTGT	24*	129	67	42*	57	42*	PABP1	EST poly(A) binding protein, nuclear 1 (7723; BC072940)
GGCAGCACAAA	3*	24	21	15	12	20	HNRPL	EST heterogeneous nuclear ribonucleoprotein L (9043; BB399813)
CAGTTGGGTTT	5*	22	9	12	10	10	HNRPA1	Heterogeneous nuclear ribonucleoprotein A1 (27927; AK007802)

I, intact; GDx, gonadectomy; DHT, dihydrotestosterone.

\*Significantly different ( $P < 0.05$ ) from GDx.

<sup>a</sup>Genes previously reported to be regulated by androgens.

**TABLE III. Differentially Expressed Transcripts Involved in Metabolism**

Tags	I	GDX	GDX + DHT					Abbr.	Description (UniGene; GenBank)
			1hr	3hr	6hr	24hr			
<b>Amino acid</b>									
GAAACTCTACT	50*	4	9	13	17	12	CDO1	Cysteine dioxygenase 1, cytosolic (29996; BC013638)	
AAGCTGCCCTC	10*	0	0	0	1	2	OAT	Omithine aminotransferase (13694; BC008119)	
<b>Lipid</b>									
GAAAAGTTGGCC	18*	67	81	87	60	46	APOD	Apolipoprotein D (2082; X82648)	
ATATTTTATA	1*	13	6	12	7	5	OXCT	EST 3-oxoacid CoA transferase (13445; AK010029)	
TCAAACTTGGT	4*	25	16	22	8	11	FABP5	Fatty acid binding protein 5, epidermal (741; BC002008)	
AGCCAAGAGAG	2	1	10*	3	3	8	FADS2	EST fatty acid desaturase 2 (38901; AK083282)	
CAGGACTCCGT	21	11	28	24	36	56*	SCD2	Stearoyl-Coenzyme A desaturase 2 (193096; BC040384)	
CTGCCCCCCGA	2*	24	1*	1*	1*	0*	SAA3	Serum amyloid A 3 (14277; X03479)	
GCCCAGACCTG	6*	36	35	35	12	12	SDHI	Sorbitol dehydrogenase 1 (104920; AK015059)	
<b>Sugar/glycolysis</b>									
GCCACTCTACT	24*	4	3	7	9	21*	AKR1B3	Aldo-keto reductase family 1, member B3 (aldose reductase) (451; BC021655)	
TAGTATGGTAA	127*	4	13	10	12	24*	AKR1B7	Aldo-keto reductase family 1, member B7 (14460; AK002705)	
GACATTTAAAG	1*	14	8	8	9	9	GBA	Acid beta glucosidase (5031; BC006663)	
CAGTGTGTTC	8*	0	0	1	0	1	BPGM	2,3-bisphosphoglycerate mutase (197824; AK043412)	
GACCTCATTC	9*	0	3	6	4	3	ENO3	Enolase 3, beta muscle (29994; BC013460)	
<b>Energy/TCA cycle</b>									
TTTCGTCTTTT	22*	0	1	2	13*	4	ATP6	ATP synthase F0 subunit 6; ATP6 (NC_001569; Pos:8126)	
AACCTAATAA	20*	2	3	4	4	4	ATP6	ATP synthase F0 subunit 6; ATP6 (NC_001569; Pos:8129)	
TTGATGTATCT	27*	1	0	1	1	1	ATP8	ATP synthase F0 subunit 8; ATP8 (NC_001569; Pos:7788)	
ATTTATCACAA	8*	0	1	2	1	0	ATP8	ATP synthase F0 subunit 8; ATP8 (NC_001569; Pos:7791)	
GTCCAAGAACA	1*	17	8	8	5	5	ATP51	EST ATP synthase, H+ transporting, mitochondrial F0 complex, subunit g (14663; AV113345)	
GCTGCCCTCCA	169*	37	76	65	66	121*	COX1	Cytochrome c oxidase subunit 1; (NC_001569; Pos:6816)	
GAAATTTATTA	14*	0	1	2	0	2	COX2	Cytochrome c oxidase subunit 2; (NC_001569; Pos:7082)	
TGTTGGTACGA	18*	3	8	5	19*	16	COX3	Cytochrome c oxidase subunit 3; (NC_001569; Pos:8934)	
CTCGGCTTCA	10*	108	97	97	60	60	COX3	Cytochrome c oxidase subunit 3; (NC_001569; Pos:9322)	
AGGACAAATAT	115*	2	3	4	15*	5	CYTB	Cytochrome b, CYTB; MITG.13 (NC_001569; Pos:14542)	
AGGAGGACTTA	57*	5	6	7	6	4	MTND2	NADH dehydrogenase subunit 2; (NC_001569; Pos:4413)	
GTAGTGGAGT	30*	7	11	21	25	22	MTND3	NADH dehydrogenase subunit 3; (NC_001569; Pos:9682)	
ATGACTGATAG	122*	5	5	10	29*	22	MTND4	NADH dehydrogenase subunit 4; (NC_001569; Pos:11233)	
ACTACCATCAG	16*	1	1	2	3	5	MTND5	NADH dehydrogenase subunit 5; (NC_001569; Pos:12434)	
ATGATGTGAAT	10*	0	0	0	0	1	MTND5	NADH dehydrogenase subunit 5; (NC_001569; Pos:12847)	

I, intact; GDX, gonadectomy; DHT, dihydrotestosterone.  
 \*Significantly different ( $P < 0.05$ ) from GDX.  
<sup>a</sup>Genes previously reported to be regulated by androgens.

**TABLE IV. Differentially Expressed Transcripts Involved in Cell Proliferation and Differentiation**

Tags	I	GDX	GDX + DHT					Abbr.	Description (UniGene; GenBank)
			1hr	3hr	6hr	24hr			
Cell cycle									
TACTATGAGAT	9*	0	1	2	3	4	AES	Amino-terminal enhancer of split (2626; X73361)	
CTTCTTTCTGA	15*	49	47	39	37	34	GAS6	Growth arrest specific 6 (3982; BC005444)	
Polyamine related									
AAACGAAAGTT	41*	8	5	10	19	30*	MATZA	Methionine adenosyltransferase II, alpha (29815; BC003451)	
TTTGGTGGGACT	19*	0	1	4	13*	9*	AMD1	5-adenosylmethionine decarboxylase 1 (7880; BC011110)	
GCAGCGCCCTCC	4,727*	2,041	2,218	2,516	2,429	3,651	SBP	Spermine binding protein (46428; AK009406)	

I, intact; GDX, gonadectomy; DHT, dihydrotestosterone.

\*Significantly different ( $P < 0.05$ ) from GDX.

<sup>a</sup>Genes previously reported to be regulated by androgens.

SERPINE2, RGS2, and AMD1) and unknown (NUCB2 and Ii) ARGs showed concordant results with the SAGE data (Fig. 1).

In order to explore the role of ARGs in human prostate cancer cell growth, we have knockdowned AMD1 by 81% using siRNA in the LNCaP cells (Table X). As seen after AR siRNA knockdown, AMD1 siRNA decreased DNA content whereas it increased the proportion of cells in apoptosis. Moreover, interferon responses were the same as the negative control siRNA oligo.

## DISCUSSION

### Protein Expression

Consistent with the physiological effects of androgens on prostatic secretory activity [17], many transcripts involved in protein synthesis, folding and secretion were up-regulated by DHT. Importantly, many ARGs involved in protein secretion were increased by DHT, such as SVS 3/5/6/7, which contributes to maintain the integrity and transport of spermatozoa [18], as well as serine protease inhibitor kazal type 3 and SERPINE2 (also known as PN-I) which have been implied in androgen enhanced prostate secretion [19,20]. These increases explain well the specialized androgen-induced secretion of prostate. On the other hand, DHT suppressed mRNA level of EST lipocalin 2, also called 24p3 and Ngai, an innate immunity related gene which enhances spermatozoa motility by elevating intracellular pH, cAMP accumulation and protein tyrosine phosphorylation [21]. In addition, GDX increased several transcripts with roles in protein trafficking and transport, including annexin A4 and EST RAB11a member RAS oncogene family which are involved in membrane remodeling, vesicle fusion and trafficking, respectively [22,23].

Several ARGs involved in the initial steps of polypeptide synthesis such as eukaryotic translation factors and ribosomal proteins were also up-regulated by DHT. These results support the current view that DHT induces anabolic metabolism in prostate through up-regulation of these genes. Eukaryotic translation elongation factor 1 alpha 1 (EEF1A1), eukaryotic translation elongation factor 2 (EEF2) and eukaryotic translation initiation factor 5A (EIF5A) are all involved in regulation of proliferation [24–26] and/or transformation of eukaryotic cells [25,26]. Eukaryotic translation initiation factor 3 subunit 6 (EIF3S6), also known as Int6, can disrupt cell cycle control and/or promote tumorigenesis [27]. DHT up-regulated EEF1A1 and EIF5A whereas GDX decreased EEF1A1 and EIF3S6. In addition, DHT increased several ribosomal protein S except for RPS17. Thus, DHT may increase protein

**TABLE V. Differentially Expressed Transcripts Involved in Cell Structure/Motility**

Tags	I	GDx	GDx + DHT					Abbr.	Description (UniGene; GenBank)
			1hr	3hr	6hr	24hr			
<b>General</b>									
TCTCCAGGCCGA	43*	1,463	1,335	1,238	1,052	889	a	CLU	Clusterin (196344; AF248057)
C CAAAGTCTTT	3*	20	14	16	15	17		LMNA	Lamin A (3438; X14170)
Cytoskeletal cellular shape and motility									
AACTGCTTCAA	8*	32	23	27	23	22	a	ARPC1B	Actin related protein 2/3 complex, subunit 1B (30010; AF162768)
TATATATATTT	16*	0	1	0	3	4		ELMO2	EST engulfment and cell motility 2, ced-12 homolog ( <i>C. elegans</i> ) (35064; BB503829)
CTCCTGGACAC	41	14	31	36	46*	27	† <sup>a</sup>	GSN	Gelsolin (21109; NM_146120)
GACATCAAGTC	23*	72	72	56	42	55		KRT1-19	Keratin complex 1, acidic, gene 19 (1012; BC034561)
CCTACAGTTGA	21*	2	4	8	9	6		MYLF	Myosin light chain, alkali, fast skeletal muscle (1000; AK003182)
AGAGAAGAGTG	10*	1	3	3	2	2		MYH1	Myosin, heavy polypeptide 1, skeletal muscle, adult (42156; AJ293626)
GAGCAGACCGT	22*	1	4	9	15*	4	↑	MYH4	Myosin, heavy polypeptide 4, skeletal muscle (35531; AJ278733)
AAAGTCATTGA	84*	26	29	22	35	32	a	TPM1	Tropomyosin 1, alpha (121878; AK077713)
CAC TGACCTCC	17	4	13	21*	18	4	† <sup>a</sup>	TPM2	Tropomyosin 2, beta (646; AK003186)
TGACAGAAGAG	31*	6	11	9	17	7		TNNC	Troponin C, fast skeletal (1716; BC024390)
GAGGCCCGGAA	31*	8	13	16	18	11		TNNI2	Troponin I, skeletal, fast 2 (39469; BC028515)
ACTGTCCGGGC	16*	2	1	13*	10	9	↑	TNNI3	Troponin T3, skeletal, fast (14546; BC003747)
Extracellular matrix and collagen biosynthesis									
AGAAATGAGATC	13*	0	1	4	6	3		DCN	Decorin (1987; X53929)
AAGCGCTCCCT	12*	0	2	2	1	4		COL4A6	EST procollagen, type IV, alpha 6 (155586; BC023678)
CCTGGTGAAG	45*	0	0	0	2	0		MUC10	Mucin 10, submandibular gland salivary mucin (200411; BC042902)

I, intact; GDx, gonadectomy; DHT, dihydrotestosterone.

\*Significantly different ( $P < 0.05$ ) from GDx.

<sup>a</sup>Genes previously reported to be regulated by androgens.

**TABLE VI. Differentially Expressed Transcripts Involved in Cell Signaling**

Tags	I	GDx	GDx + DHT					Abbr.	Description (UniGene; GenBank)
			1hr	3hr	6hr	24hr			
Signal transducer									
GTGTTTAAAG	6*	26	22	17	17	15	BZRP	Benzodiazepine receptor, peripheral (1508; BC002055)	
CTCTGAATACT	10*	1	3	1	3	9	CAI	Calcium binding protein, intestinal (2442; J05186)	
ACAAACTTAGG	18*	3	6	8	12	11	CAM1	Calmodulin 1 (34246; M19381)	
AGCAGGTTTTC	1*	17	12	7	7	10	CAM2	EST calmodulin 2 (18041; AV168251)	
TCATCTTTAAC	30*	0	2	1	8	10*	CALR	Calreticulin (1971; BC003453)	
GCAAAAGCAAA	1*	13	7	10	4	9	ARPP19	Cyclic AMP phosphoprotein (30728; NM_021548)	
TTTCTCATTTGT	0	1	13*	1	0	1	PTPRK	ESTs, A48066 protein-tyrosine-phosphatase, receptor type kappa precursor (250836; AV174021)	
ATTTGAAAATAA	8*	46	27	19	27	20	GNAI2	Guanine nucleotide binding protein, alpha inhibiting 2 (196464; BC037130)	
TCTTGAAATTA	1*	11	2	1*	1*	0*	GBP2	Guanylate nucleotide binding protein 2 (24038; BC032882)	
CGCTGTACAGA	1*	13	12	10	8	2	IGFBP3	Insulin-like growth factor binding protein 3 (29254; AK077477)	
GCAACCTCCCA	10*	1	0	1	3	2	IGFBP5	Insulin-like growth factor binding protein 5 (578; L12447)	
TTTTAAATAAAA	0*	9	3	1	3	0*	IGTP	Interferon gamma induced GTPase (858; AK088315)	
ACAAAGGTTAA	9	4	22*	14	19*	12	LMO4	LIM only 4 (29187; BC010278)	
AGCCAAACAAA	4*	22	21	16	16	12	MAP3-K12	Mitogen activated protein kinase kinase 12 (4358; AK081623)	
AGTGAGATTGA	23*	1	6	4	8	22*	NUCB2	Nucleobindin 2 (9901; BC010459)	
ACTGATTGCAG	3*	18	13	18	13	7	PLAA	Phospholipase A2, activating protein (22724; AK075934)	
AATAAAAGTGG	5*	39	23	16	12*	17	PLCB3	Phospholipase C, beta 3 (6888; BC035928)	
CCAGCCTGTGA	3	14	6	4	2*	6	RGL2	EST ral guanine nucleotide dissociation stimulator-like 2 (43777; AW55065)	
TAGATTTGGGG	4*	21	6	16	4*	17	ARAF	raf-related oncogene (220946; BC004757)	
TTACCACATAG	10*	1	0	6	15*	4	RGS2	Regulator of G-protein signaling 2 (28262; BC023001)	
TCTAAACAATA	10*	0	1	0	6	1	RCN2	Reticulocalbin 2 (1782; AK077486)	
GGGATCTTCAA	30*	3	3	10	13	21*	FRAG1	Similar to FGF receptor activating protein 1 (258004; BC004794)	

I, intact; GDx, gonadectomy; DHT, dihydrotestosterone.  
 \*Significantly different ( $P < 0.05$ ) from GDx.  
<sup>a</sup>Genes previously reported to be regulated by androgens.

**TABLE VII. Differentially Expressed Transcripts Involved in Cell/Organism Defense**

Tags	I	GDx	GDx + DHT						Abbr.	Description (UniGene; GenBank)
			1hr	3hr	6hr	24hr	3hr	6hr		
Homeostasis—general										
TAAAAATTGTAG	18*	2	0	4	8	3	3	CKMT2	EST creatine kinase, mitochondrial 2 (20240; AK009042)	
CAAAAGATTAAA	4*	21	10	12	14	5	5	CAR3	EST carbonic anhydrase 3 (300; AV012596)	
AGTGCCTCTGCT	15*	1	5	8	6	7	7	GSTM2	Glutathione S-transferase, mu 2 (14601; BC037068)	
CAGCTTCGAAT	0*	12	11	12	8	7	7	GSTM2	Glutathione S-transferase, theta 2 (24118; BC012707)	
AAATFAAAAACA	3*	25	18	16	10	8	8	MGST1	Microsomal glutathione S-transferase 1 (14796; BC009155)	
CAGCCTGAAAA	8	19	12	10	3*	20	20	SEPR	EST selenoprotein R (28212; BY703886)	
TPTCCAGGTGT	18*	76	51	60	51	30	30	SEPW1	Selenoprotein W, muscle 1 (42829; NM_009156)	
CCCTGAGGGGT	3*	24	9	16	5*	7	7	TRF	Transferrin (37214; BC012313)	
Homeostasis—DNA repair										
TAATAAAAAATT	8*	32	15	10	19	11	11	HMGNI	High mobility group nucleosomal binding domain 1 (2756; X53476)	
Immunity										
CATCTGAAAAA	9*	97	44	70	48	38	38	ADN	EST adipisin (4407; AW215391)	
TPTTCAAAAAA	153*	45	28	31	66	49	49	B2M	Beta-2 microglobulin (163; AK019389)	
CTCTGACTTAC	24*	81	81	92	66	81	81	BSG	Basigin (726; BC010270)	
CACTGGCCTTC	1*	12	12	11	8	14	14	BSG	EST basigin (726; AV212250)	
TGGGGCCTCTC	3*	21	12	13	15	9	9	C3	Complement component 3 (19131; K02782)	
CCAGAGATGGC	28*	1	0	1	3	12*	12*	CUR	Complement component 1, r subcomponent (24276; BC004637)	
TACACCCCTCA	13*	0	1	0	1	2	2	CEA-CAM10	CEA-related cell adhesion molecule 10 (30300; BC003346)	
CCAGAGGCAGT	13*	1	1	7	5	4	4	CEACAM2	CEA-related cell adhesion molecule 2 (155711; BC024320)	
TTATGGAATTG	48*	187	119	104	100	148	148	CD24A	CD24a antigen (6417; M58661)	
GAGTGGATTCT	13*	48	53	67	39	42	42	CD63	Cd63 antigen (4426; BC012212)	
GCGCCCTTCCC	3*	21	32	20	20	13	13	CCL21	Chemokine (C-C motif) ligand 21 (leucine) (196097; BC025974)	
GAGGATCTGT	12*	1	1	4	4	6	6	DEFB1	EST defensin beta 1 (5341; BY088280)	
GTATGTGGGAA	2*	15	6	5	6	6	6	SAMHD1	EST SAM domain and HD domain, 1 (196457; BF148012)	
GTTCAAGTGAC	18*	174	120	147	87	53*	53*	Ii	Ia-associated invariant chain (258212; BC003476)	
TATCCTGAATG	3*	26	23	16	17	21	21	LY6A	Lymphocyte antigen 6 complex, locus A (8180; BC002070)	
GAGGACTGCCA	39*	151	165	187	167	186	186	LY6E	Lymphocyte antigen 6 complex, locus E (788; BC019113)	
TGTCAGTCTGT	1*	14	8	6	9	6	6	LYZ5	Lysozyme (45436; BC002069)	
TGCGGGGGTGG	9	3	10	4	9	22*	22*	IL-25	Interleukin 25 (29925; AY038184)	
CTGAGTTTGAG	3*	22	9	10	6	9	9	NKTR	Natural killer tumor recognition sequence (23964; L04289)	

I, intact; GDx, gonadectomy; DHT, dihydrotestosterone.

\*Significantly different ( $P < 0.05$ ) from GDx.

<sup>a</sup>Genes previously reported to be regulated by androgens.

**TABLE VIII. Differentially Expressed Transcripts Involved in Other Functions**

Tags	I	GDX	GDX + DHT					Abbr.	Description (UniGene; GenBank)
			1hr	3hr	6hr	24hr	24hr		
GGCCAAAGTGGC	4*	28	21	18	14	19	APIS1	Adaptor protein complex AP-1, sigma 1 (833; M62418)	
TGACAAAACCGT	99*	0	2	1	5	7	MSMB	Beta-microseminoprotein (2540; U89840)	
AAGGTTGACTC	3*	24	10	18	15	13	CLK4	CDC like kinase 4 (9488; BC012675)	
GAAAATGAGAA	1*	337	1717*	266	52*	20*	COP56	EST COP9 (constitutive photomorphogenic) homolog, subunit 6 (Arabidopsis thaliana) (3981; BQ255747)	
AAAAAGTACCA	342	313	402	616	971*	635	EDV	Endogenous sequence related to the Duplan murine retrovirus (218091; AK002956)	
CTAATAAAGCC	115*	430	319	390	314	280	FAU	Finkel-Biskis-Reilly murine sarcoma virus (FBR-MuSV) ubiquitously expressed (fox derived) (4890; AFI47745)	
AGAGTGTTACT	1	8	2	3	0*	1	FATP	ESTs, Highly similar to FATP_MOUSE Long-chain fatty acid transport protein (FATP) [M.musculus] (38165; AI156620)	
GAAGAGGGGGA	2*	19	3*	7	3*	3*	HP	Haptoglobin (26730; M96827)	
CCCAGTGTGTG	47*	0	0	0	1	9*	N/A	Hypothetical protein 9530003A11 (207324; AK035222)	
CTTACCAATAT	10*	0	1	7	6	4	ND1	Influenza virus NS1A binding protein, Nd1 (33764; BC004092)	
TTATGATTAAT	1*	20	15	10	10	4*	LUM	Lumican (18888; BC005550)	
GGGAGCGAAAA	14*	2	6	10	8	12	ID2	Inhibitor of DNA binding 2 (1466; BC006921)	
TTAAAA CGTGC	1	8	2	4	0*	6	MOR-F4L2	EST mortality factor 4 like 2 (27218; BB089351)	
AACTGGGATTA	8	1	0*	0	1	1		EST RIKEN clone E330022E08 product (171547; BY565559)	
CACCTTGGTGC	3*	74	49	54	19*	25*		Mouse 19.5 mRNA (4992; AK081478)	
GGAGGTAGACC	614	99*	72	98	82	282*	TGM4	RIKEN clone 9530008N10 product (221277; AK035279)	
TTCTAATCGGT	1925*	793	613	810	791	968	PBSN	Probasin (8034; AF005204)	
ATTTTACCTT	1	10	6	5	4	1*		RIKEN cDNA 0610030H11 gene (181488; BC038913)	
ATGGCATCAGG	3	10	1*	9	5	7	SGT	Small glutamine-rich tetrapeptide repeat (TPR)-containing (30068; BC003836)	
CCCAGGCTTGG	1*	12	8	5	5	4	SRC	Rous sarcoma oncogene (22845; BC039953)	
AAGGAGTTACA	4*	22	17	11	11	11	SUI1-RS1	EST suppressor of initiator codon mutations, related sequence 1 (13886; AV166443)	
GTGCATTTGTA	1*	12	10	7	7	3	TGFBI	Transforming growth factor, beta induced (14455; AK084431)	
AACTACAGCTG	8	1	2	1	10*	8	TDE1	EST tumor differentially expressed 1 (4962; BM223255)	

I, intact; GDX, gonadectomy; DHT, dihydrotestosterone.

\*Significantly different ( $P < 0.05$ ) from GDX.

<sup>a</sup>Genes previously reported to be regulated by androgens.

**TABLE IX. Potential Novel Transcripts Differentially Expressed**

Tags	GDX + DHT					GDX + DHT						
	I	GDX	1hr	3hr	6hr	24hr	I	GDX	1hr	3hr	6hr	24hr
GAAAAAGAGAA	0	7	27*	5	1	0	↑	107	527*	76	12*	6*
GAAAAAAGAGAA	0	4	21*	1	1	0	↑	25	8	9	8	4*
GAATATGGCGA	0	3	22*	6	1	0	↑	20	11	10	5	4*
GAAAAATGAGAC	0	3	24*	1	0	0	↑	13	4	1*	3	2
ACGACTTGAAA	0	3	20*	2	0	0	↑	19	6	5	3*	7
TTTTTCATGT	0	3	17*	2	0	0	↑	21	22	14	4*	9
GAAAAAGGAGAA	0	2	15*	2	0	0	↑	10	6	4	3	1*
AAAAATGACGA	0	2	13*	1	0	0	↑	104	114	73	46	31*
TCCTAAAGTGT	0	1	15*	1	1	0	↑	82	56	46	33	22*
ATTTCCAGTTT	0	1	10*	1	2	0	↑	41	8	14	7*	6*
AAATATGGCAA	0	1	10*	1	0	0	↑	29	11	13	8*	12
GAGAAATGAGAA	0	1	12*	1	1	1	↑	38	22	11*	6*	9*
CTGTTCCGGCC	0	1	3	2	10*	3	↑	56	25	22	13*	14*
GAAAAATGATGA	1	3	17*	5	2	1	↑	140	61	45	21*	23*
TGGTTTTTGTGTC	1	3	25*	7	1	3	↑	36	18	20	6*	10*
ATTTTCACTTT	1	1	21*	1	1	1	↑	28	11	7	3*	10
GAAAAATGTGAA	1	1	18*	3	1	0	↑	32	15	14	9*	12
CAGTAGCTGAA	1	0	1	3	9*	1	↑	0	0	2	11*	9*
GGAGATCTTTC	2	3	21*	2	0	0	↑	63	40	29	13*	30
GAAGGTCCAGC	3	4	4	5	26*	9	↑	1	5	7	14*	11*
AGTGTGGGTAC	5	0	1	7	15*	7	↑	2	3	9	9	33*
TTGGCCGGCGCT	6	1	8	13*	9	7	↑	1	3	1	4	13*
CTCCACTATGT	6	4	22*	5	3	4	↑	2	2	7	17*	8
TAGAGACTGCC	6	8	31*	3	3	4	↑	1	3	4	17*	15
GCACTTAGAAA	6	1	3	2	12*	7	↑	0	2	1	2	9*
GTTACAGACCT	8	5	15	24*	48*	27*	↑	6	4	8	21	28*
GGACCTGTTA	8	3	12	24*	24*	16*	↑	1	4	3	3	13*
GGAGAAGGGGG	8	2	8	11	21*	13	↑	1	3	8	3	12*
GCGGTGAATCA	8	1	4	5	4	12*	↑	6	8	10	14	27*
CTCCACTATGT	12	5	22*	2	3	2	↑	1	2	4	12*	22*
TGGGCCCCGGAG	13	5	4	13	46*	22	↑	4	8	4	12	21*
AAAAAGTACCT	13	9	7	25	48*	14	↑	1	1	0	1	20*
ATCTCGAAAAC	48	29	38	51	84*	43	↑	6	3	5	11	30*
ATCATAGTCTT	1	8	7	4	5	0*	↓	2	3	7	39*	41*
GTGACCCACGGG	49	110	51	51	30*	52	↓	1	4	4	5	19*
AAAAATGAGAA	0*	15	62*	9	1*	1*	↓	37	42	55	61	176*
AAATATGGCGA	0*	10	36*	9	1*	1*	↓	15	16	36	43	150*

C AAAACATACT	0*	19	12	11	13	2*	↓	AAAAGGCTTTC	171*	1	7	19*	14*	↑
G AAAACGAGAA	0*	10	37*	4	3	0*	↓	AATGCTATGCT	274*	4	24*	34*	45*	↑
G AAAATGATAA	0*	15	134*	9	4	1*	↓	GCAAAATCCTTT	361*	11	44*	40*	62*	↑
G AATATGGCAA	0*	13	84*	11	1*	1*	↓	GTACCTGTGAG	532*	24	78*	138*	480*	↑
T CCACAGTGG	0*	15	79*	13	1*	1*	↓	GATGAAGGTAT	634*	29	65	61	108*	↑
G CCGTTCTTAG	0*	26	17	10	4*	7	↓	GCTCCTGAGGG	724*	46	171*	258*	264*	↑
G GAATAACGCC	0*	9	6	4	0*	2	↓	TGCCAACTGAT	1,318*	303	373	417	655*	↑
G GATTGGCTG	0*	8	1	4	0*	0*	↓			260				↑

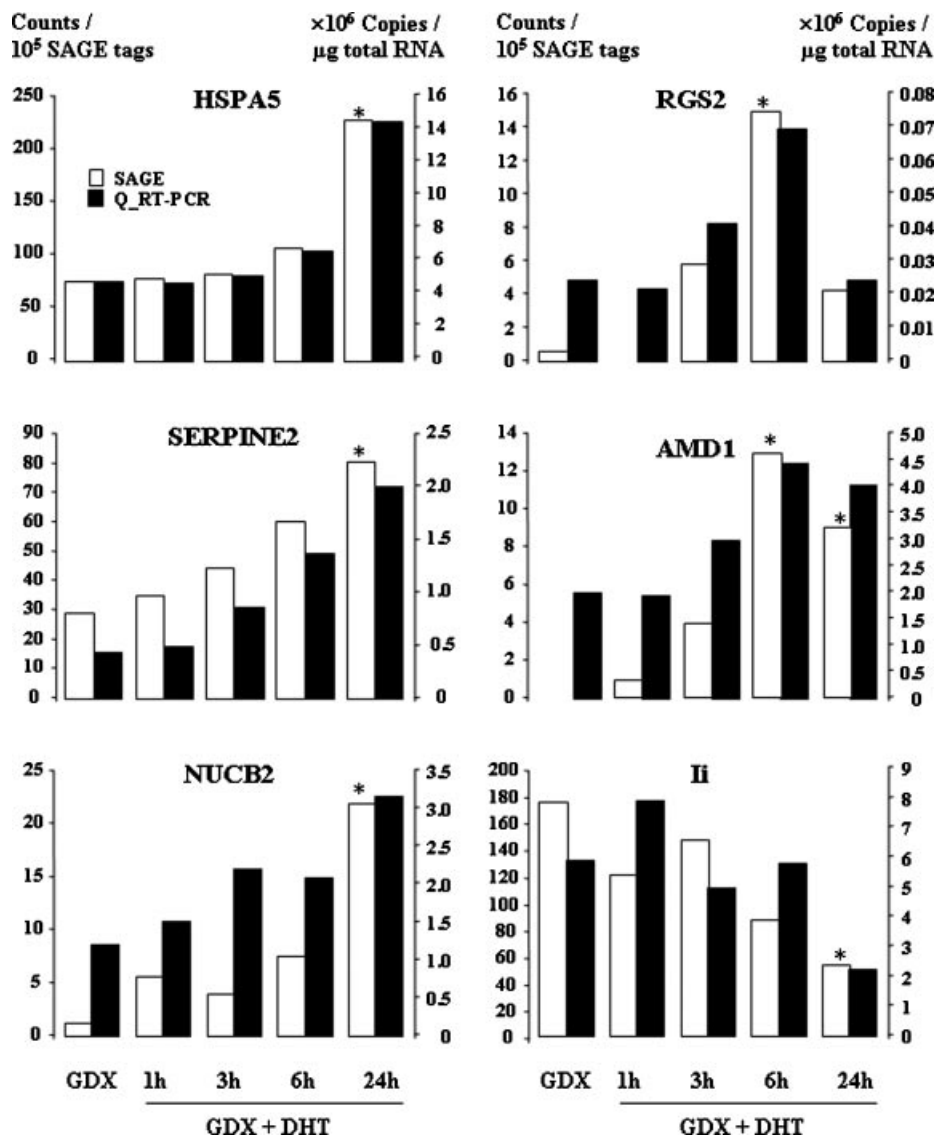
L, intact; GDx, gonadectomy; DHT, dihydrotestosterone.

\*Significantly different ( $P < 0.05$ ) from GDx. Ninety-four no match tags regulated by GDx.

synthesis and translation processing by up-regulating these transcripts, some of which may also play positive role in cell proliferation and transformation. On the other hand, GDx up-regulated several ribosomal protein L. Since it is not clear why RNAs coding for ribosomal proteins L have been increased by GDx, further studies are needed to investigate the role of up-regulation of these ribosomal proteins after GDx.

Transcripts involved in protein folding and chaperones such as FK506 binding protein (FKBP) 2, peptidylprolyl isomerase A (PPIA), HSPA5, calreticulin (CALR) and protein disulfide isomerase-related protein (PDIR) were increased by DHT. The up-regulation of HSPA5 could be a linchpin on DHT actions in endoplasmic reticulum (ER). HSPA5 and CALR are known ER-resident chaperones, which may fold secretion proteins after these polypeptide products are transported into the ER. In ER, HSPA5 retains the activating transcription factor 6 (ATF6) by inhibiting its Golgi localization signals. The dissociation of HSPA5 during ER stress allow ATF6 to be transported to the Golgi, which activates the transcription of ER molecular chaperones [28]. Moreover, HSPA5 is homologous to Kar2p which is required for protein biogenesis in the yeast ER [29]. Furthermore, mitochondrial HSP70 family members are reported to be essential mediators of mitochondrial biogenesis [30]. Indeed, mitochondrial transcripts such as ATP synthase F0 subunit 6 (ATP6), cytochrome *c* oxidase subunit 1 and 3 (COX1 and 3), cytochrome *b* (CYTB) and NADH dehydrogenase subunit 4 (MTND4) were increased by DHT in the current study. On the other hand, GDx significantly decreased the expression of EST heat shock protein 1 beta (HSP90), another chaperone protein. HSP90 has been reported to bind and activate the AR, as well as to interact with FKBP5 in the function of mature heterocomplexes [4]. In the current study, DHT increased the transcript encoding FKBP2, in agreement with another report [31]. FKBP5 have peptidylprolyl isomerase (PPIase) activity and the transcription of PPIA was also up-regulated by DHT in the current study. Taken together, DHT may induce protein folding and maturation through up-regulating gene expression of HSPA5, CALR, FKBP2, and PPIA.

Genes involved in protein turnover including the D/H/S members of cathepsin (CTS) family and EST matrix metalloproteinase 15 (MMP15) were significantly increased by GDx. Cathepsins function in the lysosomal degradation of proteins regulating cell growth and tissue homeostasis. Recently, CTSD, also known as CD, has been reported to mediate p53-dependent apoptosis by a transcriptional mechanism [32]. Thus, the current data suggest its potential role in GDx-induced apoptosis. Moreover, CTSD secreted by



**Fig. 1.** Validation of ARGs by Q-RT-PCR. \*Significantly different from GDX. Six characterized ARGs found by the SAGE were further validated by Q-RT-PCR. The results of Q-RT-PCR on both the previously known (HSPA5, SERPINE2, RGS2, and AMD1) and unknown (NUCB2 and Ii) ARGs showed concordant results with the SAGE data.

**TABLE X.** Effects of AR and AMD1 siRNA on LNCaP Cell Growth and Interferon Response

	Control	AR	AMD1
Target mRNA expression	—	0.24 ± 0.02*	0.15 ± 0.04*
Cell cycle <sup>a</sup>			
Apoptosis	2.09 ± 0.90	4.00 ± 1.51*	6.73 ± 1.73*
S-phase	0.61 ± 0.12	0.32 ± 0.17*	0.55 ± 0.11
DNA contents	1.04 ± 0.26	0.76 ± 0.09*	0.43 ± 0.08*
IFIT2 mRNA expression	0.19 ± 0.08	0.17 ± 0.05	0.09 ± 0.03
IFIT3 mRNA expression	0.11 ± 0.04	0.06 ± 0.02	0.06 ± 0.01

Values are mean ± SD.

\*Significantly different ( $P < 0.05$ ) from control siRNA.

<sup>a</sup>Pooled values of three independent experiments in triplicates for control as well as two independent experiments in triplicates for AR and AMD1.

prostate carcinoma cells contributes to the prevention of tumor growth and angiogenesis-dependent metastases through production of angiostatic peptides [32]. CTSS is the principal lysosomal cysteine protease, and the degradation of Ii by CTSS is responsible for major histocompatibility complex (MHC) class II maturation and presentation in vivo [33]. Both CTSS and Ii were increased by GDX and decreased by DHT. On the other hand, GDX increased the mRNA level encoding EST MMP15, a member of endopeptidases family which degrades the extracellular matrix. Several studies have pointed out that MMP family genes are associated with tumorigenesis [34,35]. The present results suggest that GDX may induce protein degradation through the expression of these genes. It is also worth noting that GDX increased the mRNA expression of basigin (BSG, also called CD147 and extracellular MMP inducer: EMMPRIN), which induces MMP synthesis in cancer cells [35].

Interestingly, we have found a temporal order of the changes in these many synthetic and secretory transcripts. The expression of genes involved in ribosomal proteins synthesis began to increase within 6 hr, whereas genes active in protein folding and secretion were induced later.

### RNA Expression

GDX increased the expressions of several transcripts involved in RNA synthesis and splicing process whereas DHT restored half of them, such as DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 5 (DDX5) and EST poly(A) binding protein, nuclear 1. DDX5, which has a putative RNA helicase for pre-mRNA processing and alternative splicing, has been thought to act as a transcriptional co-activator. However, DDX5 (p68) has recently been shown to have a repressor activity and to interact with histone deacetylase I (HDAC1) and DDX17 (p72) [36]. DDX5 could act as co-activator and/or co-repressor differently depending on the context of the promoter and the transcriptional complex in which it exist [36]. Thus, DHT might activate the transcription in the prostate by suppressing the expression of DDX5. Two other family members, DDX15 and 16, are two other transcriptional co-activators induced by DHT in prostate cancer cell [4,6]. GDX has increased the expression of most transcription factors, 10 out of 14, whereas DHT modulated four, of which half were induced. Indeed, five of these transcription factors including early growth response 1 (EGR1), FBJ osteosarcoma oncogene (FOS), GATA binding protein 2 (GATA2), NK-3 transcription factor locus 1 (NKX3A) and nuclear receptor subfamily 4 group A member 1 (NR4A1) are known ARGs. GDX has induced transcription repressors such

as AE binding protein 1 (AEBP1), EST bromodomain adjacent to zinc finger domain 2A (BAZ2A), histone deacetylase 7A (HDAC7A), nuclear DNA binding protein (C1D), TG interacting factor (TGIF) whereas DHT induced activators EST nuclear factor I/B (NFIB) and RNA polymerase II transcriptional coactivator (PRO2TC1). DHT and GDX might activate and/or repress transcription in the prostate through regulation of these transcripts. Additionally, in consistence with the GDX-induced apoptosis, the transcription factors EGR1, FOS, HDAC7A and NR4A1, which are responsible for prostate apoptosis [31,37–39] were all increased by GDX, whereas DHT has repressed FOS and HDAC7A.

### Metabolism

The de novo unsaturation of fatty acid is regulated by individual desaturase enzymes that introduce double bonds between defined carbons of the fatty acyl chain. In the present study, DHT has induced gene expression of EST fatty acid desaturase 2 (FADS2) and stearoyl-Coenzyme A desaturase 2 (SCD2), which introduce double bond in the  $\Delta^6$  and  $\Delta^9$  position of fatty acids, respectively. The first double bond introduced into a saturated fatty acid is nearly always in the  $\Delta^9$  position. The principal product of SCD, an iron-containing enzyme that catalyze a rate-limiting step in the synthesis of unsaturated fatty acids, is oleic acid which has anticancer effect on breast cancer cells [40]. In human prostate cancer cells, androgen induced de novo fatty acid synthesis through fatty acid synthase (FAS) [41], and RNA interference-mediated down-regulation of FAS suppresses triglycerides and phospholipids synthesizes and induces apoptosis, which results in inhibition of LNCaP cell growth [42]. These data suggest that the androgen differentially modulates de novo saturate/unsaturated fatty acids synthesis in normal prostate tissue and prostate cancer cells.

GDX also decreased gene expressions of two aldo-keto reductase family (AKR) members, AKR1B3 and 7, while DHT replacement increased them within 24 hr. AKR1B7 (also known as mouse vas deferens protein, MVDP), the major secretory component of the mouse vas deferens, encodes an enzyme responsible for detoxification of isocaproaldehyde (a byproduct of side-chain cleavage of cholesterol in the first step of steroidogenesis) and 4-hydroxynonenal (a lipid peroxidation product). AKR1B7 is regulated by ACTH in adrenals, FSH in ovaries, and LH/hCG in testicular leydig cells [43], whereas its strict androgen-dependence is only mouse-specific in the vas deferens [44]. Mouse-specific 77-bp sequence in the promoter is probably an androgen-responsive region containing a

single ARE, which is likely to explain the mouse-specific androgen responsiveness of AKR1B7 *in vivo* [44]. The current study has shown, for the first time, the induction of mRNA expression of the AKR1B7 gene by DHT in mice prostate. Interestingly, Martinez et al. [43] have suggested the role of AKR1B7 in the protection of spermatozoa against peroxidative damage in the vas deferens. Several transcripts involved in mitochondrial oxidative phosphorylation such as ATP6, COX1/3, CYTB and MTND4 were repressed by GDX and increased by DHT. The elevated levels of metabolism in lipid, sugar and mitochondrial oxidative phosphorylation may reflect the mitogenic or secretory stimulus of androgen by supplying their necessary energy source.

### Immunity

DHT inhibits and even suppresses immune functions [45]. Consistently, GDX increased the expression levels of most of these immunity-enhanced genes in this group (13 out of 18) and DHT decreased it. On the other hand, DHT increased IL-25 and complement component 1 r subcomponent (C1R). IL-25 is a pro-inflammatory cytokine which induces gene expression of IL-4 and IL-13 in Th2 T cells [46]. C1R is the first component of the classical pathway of the complement system. GDX suppressed its expression level as well as those of beta-2 microglobulin (B2M), EST defensin beta 1 (DEFB1), CEA-related cell adhesion molecule (CEACAM) 2 and 10. B2M is the beta-chain of MHC class I molecules, and DEFB1 protects the urinary tract against infections [47]. Interestingly, CEACAM10 has been shown to be predominantly expressed in seminal vesicles and decreased after castration, consistently with the current results in the prostate [48].

### Cell Structure and Motility

The prostate consists of collagen, smooth muscle and striated fast and slow twitch myofibers [49]. We have found many differentially expressed transcripts encoding for components of extracellular matrix, cytoskeleton and their modifying enzymes. For example, GDX decreased three extracellular matrix components, decorin (DCN), EST procollagen type IV alpha 6 and mucin 10 submandibular gland salivary mucin, while inducing cytoskeleton genes, keratin complex 1 acidic gene 19 and actin related protein 2/3 complex subunit 1 B. These two latter genes are involved in actin polymerization and membrane protrusion. Interestingly, the genes of myofiber components down-regulated by GDX were all of fast type, including troponin C fast skeletal, troponin I skeletal fast 2, troponin T3 skeletal fast (TNNT3) and myosin light chain alkali fast skeletal muscle. In addition, DHT has

increased gene expression of myosin heavy polypeptide 4 skeletal muscle (MYH4), tropomyosin 2 beta (TPM2), TNNT3 and gelsolin (GSN), suggesting that these genes are involved in prostate cell growth and remodeling.

### Cell Proliferation

In prostate, androgen has anabolic effect on protein expression, lipid metabolism, cytoskeleton as well as cell proliferation. DHT has induced the expression of MAT2A and AMD1, which promote polyamine biosynthesis. MAT2A catalyses the biosynthesis of S-adenosylmethionine and AMD1 converts putrescine to metabolically active spermidine and spermine. Polyamine biosynthesis contributes for spermatogenesis as well as tumorigenesis and organ hypertrophy. Steady supply of polyamines is a prerequisite for cell proliferation [50]. GDX down-regulated spermine binding protein (SBP), a known androgen dependent gene [51,52].

GDX has induced the expression of interferon gamma induced GTPase (IGTP) and DHT has suppressed it. IGTP mediates cell survival through activation of PI3K/AKT and inhibition of glycogen synthase kinase-3 (GSK-3) activity [53]. In the prostate cancer cell, GSK3-beta activity is essential for androgen-stimulated gene expression and cell proliferation [53]. In the present study, DHT repressed IGTP. Therefore, DHT may induce cell proliferation via suppression of IGTP, which can result in activation of GSK3-beta.

GDX increased the mRNA levels of serum amyloid A 3 (SAA3) and apolipoprotein D (APOD), which inhibit cell proliferation [54,55]. DHT has increased the gene expression of LIM only 4 (LMO4) which is highly expressed in proliferating cells [56] and similar to FGF receptor activating protein 1 (FRAG1), which activates the fibroblast growth factor receptor of rat osteosarcoma cells (FGFR2-ROS) [57]. Interestingly, several differentially expressed transcripts directly interact with AR as co-regulator, such as amino-terminal enhancer of split (AES) [58], CALR [59], gelsolin (GSN) [60], and TGIF [61]. GDX has decreased the mRNA levels of AES and CALR whereas DHT has induced CALR and GSN. After 12 hr castration, rapid loss of nuclear AR has been observed [62]. Thus, the decrease of AR modulators by GDX in the current results is consistent with this phenomenon. It is likely that GDX may not only reduce AR but also its co-regulators. Taken together, DHT may induce cell proliferation in prostate not only through the up-regulation of polyamine biosynthesis, but also through some factors such as IGTP, SAA3, APOD, LMO4, and FRAG1 as well as some AR co-regulators.

### Tumorigenesis

Molecular and cellular bases supporting the efficiency of selenium for the prevention of prostate carcinogenesis are emerging [63]. Selenium potentially prevents carcinogenesis through its effects on oxidative stress, DNA repair, inflammation, apoptosis, proliferation, carcinogen metabolism, testosterone production, angiogenesis, fat metabolism, and immune function. Selenium effects can be indirect (via the interaction with selenoproteins) and/or direct (via the interaction with selenium molecular targets such as p21, protein kinase C and AR) [63]. In the prostate, these effects are supported by data showing that selenium accumulates in human prostate, as well as selenium accumulates, DNA damage reduces and apoptosis increases in the prostates of older dogs [63]. In the current results, DHT repressed EST selenoprotein R (SEPR) and GDX induced selenoprotein W muscle 1 (SEPW1). These two selenium binding molecules could be involved in the preventive effects of selenium on prostate carcinogenesis. In addition, a recent study [64] has shown that HSPA5, which was also up-regulated by DHT in the present study, mediates the anticancer activity of selenium.

RGS2 can also cause androgen-independent activation of AR in prostate cancer cells. Its repression has been suggested to be an important step during prostate tumorigenesis and progression [65]. Moreover, as previously described above, eukaryotic translation factors EEF1A, EEF2, EIF5A, and EIF3S6 are also involved in tumorigenesis. DHT and GDX might also activate and/or repress tumorigenesis in the prostate through regulation of these transcripts.

### Cell Apoptosis

GDX has increased FOS expression and DHT has suppressed it within 6 hr. The nuclear phosphoprotein FOS forms heterodimeric complexes with members of the v-JUN avian sarcoma virus 17 oncogene homolog (JUN) family of proteins and contributes to the activator protein-1 (AP-1) transcription factor complex. Although a recent study suggested that FOS has no function in epithelia apoptosis in the mouse male accessory sex organs, including ventral prostate [66], direct experimental data in FOS-deficient mice have shown that FOS is essential for apoptosis and regression of the prostate following castration [37]. Thus, the current results support the view that FOS is important in the initiation of apoptosis under androgen modulation.

In addition to some known prostate apoptotic moleculars induced by GDX, such as FOS and clusterin (CLU) (also known as testosterone-repressed prostate messenger-2, TRPM-2) [67,68], the current results iden-

tified many novel ARGs in diverse signaling pathways that are apoptosis related including p53 pathway, retinoid X receptor (RXR) actions, calcium-dependent pathways and IGF signaling pathway. Since p53 inhibits prostate growth by inducing pro-apoptotic genes and cell cycle inhibitor, p53 can be expected to be regulated by DHT. However, p53 mRNA level was unaltered by DHT in this study. Similarly in both LNCaP cancer cell lines and rat ventral prostate, mRNA level of p53 did not change, whereas the protein expression and several p53-target genes were modulated by DHT [31]. Likewise, in this study two p53 target genes were induced by GDX, including EGR1 which encodes an anti-proliferative/pro-apoptotic protein that activate p53 expression [69], and the p53-activated gene, insulin-like growth factor binding protein 3 (IGFBP3) [70]. Thus, the current results support the view that AR may indirectly inhibit p53 function post-transcriptionally and/or through p53 target genes such as IGFBP3 and EGR1 [31].

Two transcription factors in RXR actions were additionally identified, NR4A1 (also called TR3, NGFI-B, and Nur77) and TGIF. RXR regulates the NR4A1-dependent apoptotic pathway by modulating its nuclear export and mitochondrial targeting through switching the RXR/NR4A1 heterodimerization interfaces [71]. Androgens rapidly induced NR4A1 in LNCaP prostatic cancer cells, whereas castration in rat ventral prostate induced the expression of NR4A1 [38]. Similarly, GDX induced NR4A1 in the current study. GDX increased TGIF, which interferes with the retinol binding protein 2-RXR element and prevents transcription by RXR. Thus, the current results suggest that GDX may modulate RXR actions by up-regulating NR4A1 and TGIF.

In the current study, HDAC7 was up-regulated by GDX and down-regulated by DHT. Similarly to some pro-apoptotic factors such as cytochrome *c* and second mitochondria-derived activator of caspase (Smac/DIABLO), HDAC7 translocates from mitochondria or nucleus to cytoplasm in response to apoptotic stimuli in several human cell lines including normal prostate epithelial cells, PC-3 and LNCaP [39]. Moreover, HDAC7, interacts directly with silencing mediator for retinoid and thyroid hormone receptors (SMRT) and HDAC5, and promotes SMRT-mediated transcriptional repression [72].

Several differentially expressed genes between GDX and DHT treated mice participate in the different signal transduction pathways, indicating that various signaling pathways might be activated simultaneously. They include serine/threonine protein kinase family members (mitogen activated protein kinase kinase kinase 12 and raf-related oncogene), calcium binding proteins (CALR, calcium binding protein intestinal (CAI),

calmodulin 1/2 (CAM 1/2), NUCB2 and reticuocalbin 2 (RCN2)), IGF signaling modulators (IGFBP3 and 5) and a group of small GTPases and their associated factors (IGTP, RGS2, ral guanine nucleotide dissociation stimulator like 2, guanine nucleotide binding protein alpha inhibiting 2, guanylate nucleotide binding protein 2 and phospholipase c beta 3). In particular, these results suggest important insights into the androgen modulation of calcium related pathways and IGF signaling.

Previous studies pointed to a potential role of intracellular calcium levels in the activation of castration-induced apoptosis in the prostate [73]. In addition, several studies demonstrated that GDX induced-apoptosis is suppressed by blocking the influx of  $\text{Ca}^{2+}$  or its release from intracellular store in normal rodent prostate [73,74]. Here, we identified some additional ARGs involved in this mechanism including NUCB2 and the ARG CALR [51,74]. The expression levels of these two calcium-binding proteins were decreased by GDX and increased by DHT. GDX also decreased other calcium binding proteins such as CAI, CAM1, and RCN2. Since GDX induced-apoptosis is regulated by intracellular calcium level, we have identified the genes related to calcium such as NUCB2, CALR, CAI, CAM1, and RCN2, as candidates which can regulate GDX induced-apoptosis.

As expected, GDX and DHT regulated the IGF signaling system, which plays an essential role in prostate growth. GDX suppressed the mRNA level of IGFBP5, while inducing those of IGFBP3 and transferrin (TRF). The latter is identified as a specific IGFBP3-binding protein [75]. Moreover, the expression of TRF was decreased 6 hr after DHT treatment. Since IGFBP3 was induced by p53 in apoptosis as previously described [70], the up-regulation of both TRF and IGFBP3 by GDX might be the molecular mechanism in GDX-induced apoptosis.

DHT decreased the mRNA level of solute carrier family 39 (iron-regulated transporter) member 1, SLC39A1 (also known as ZIP). As a major zinc uptake transporter, this gene may be involved in the unique function and capability of accumulating extremely high intracellular levels of zinc in prostate secretory epithelial cells. This zinc-accumulation can inhibit cell growth and induce mitochondrial apoptogenesis [76,77]. Thus, this ARG may play a role in androgen modulation of cell growth or apoptosis in prostate cells through its zinc accumulation action.

### Oxidative Stress

Recent studies reported that oxidative stress is one of the major factors associated with the progression of prostate cancer through the accumulation of DNA

damage. Notably, several oxidative stress related genes were differentially expressed in GDX and DHT treated mice, including selenoproteins (SEPR and SEPW1), CLU and some chaperones and folding molecules especially located in ER (HSPA5, CALR, PDIR and glucose regulated protein 58 kDa (GRP58)). Unbalance of ER homeostasis leads to accumulation of unfolded or misfolded proteins and puts stress to ER, which activates cell death-promoting pathways. DHT increased HSPA5, CALR and PDIR, whereas GDX decreased them, as well as GRP58 which is also part of the protein folding machinery in ER [78]. PDIR is another ER-stress inducible gene, which acts as a catalyst or protein folding in the lumen of ER. Thus, the current results show that DHT induce ER stress responsive genes in the prostate. Consistently, Travers et al. [79] also reported that many aspects of secretory pathways were affected by unfold protein response (UPR), including protein translocation, glycosylation, folding, degradation in ER as well as vesicle trafficking and transport between ER and Golgi to distal secretions. Activation of lipid biosynthesis and metabolism may also constitute a component of UPR. Indeed, many genes involved in such secretory pathways and lipid metabolism are identified in the current study. Furthermore, one of these ER stress response genes, PDIR, was induced by androgen in prostate cancer cells [80].

### Cellular Senescence

Unexpectedly, we found that some genes involved in cellular senescence processing, were differentially expressed after DHT administration. Genes up-regulated during senescence such as GSN, RPS6/7 were induced by DHT whereas genes down-regulated during senescence such as EGR1, cyclic AMP phosphoprotein (ARPP19) and RPS17 were induced by GDX. Cellular senescence, similarly to apoptosis, is thought to be a mechanism of tumor suppression [81]. The fact that DHT induced genes up-regulated in senescence suggests that DHT has a potential role to activate cellular senescence processing.

### Novel Transcripts

Eighty-nine potential novel transcripts were differentially expressed after DHT treatment. These tag species did not match any known cDNA sequences in public databases. Further characterization of these transcripts will help to unravel the signaling and metabolic pathways regulated by DHT in the prostate.

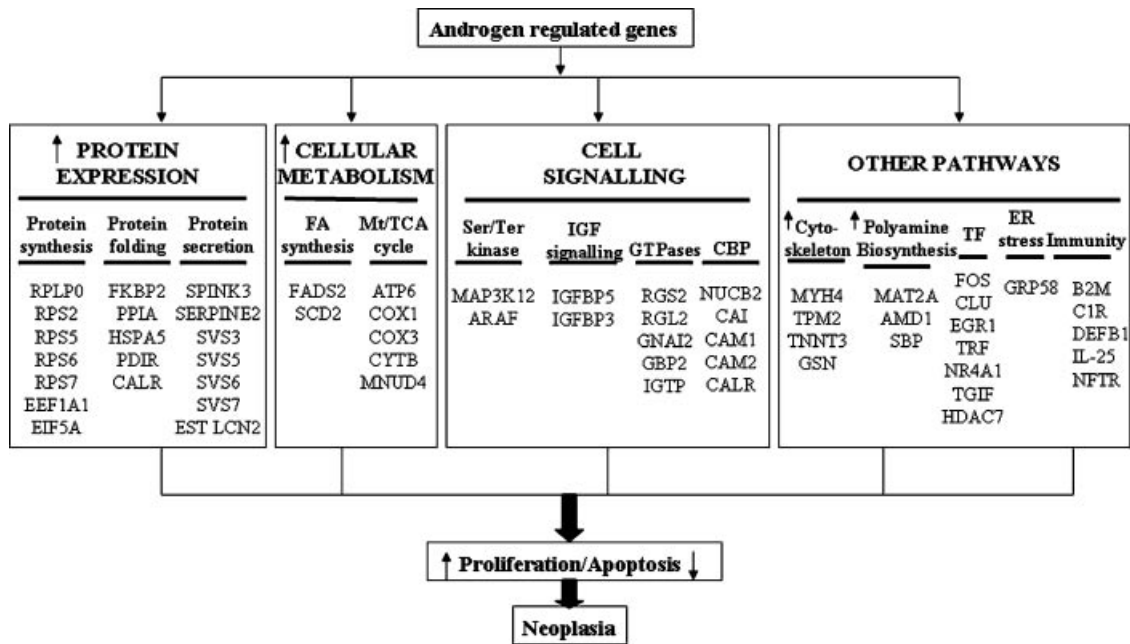
### Validation of ARGs

ARGs in the prostate have been identified previously by studies using different methods, such as

cDNA subtraction, differential display, RT-PCR, microarray and SAGE [4–7,19,20,51,80,82]. However, relatively few genes were overlapping between these studies. The difference between these studies could be caused by multiple factors, such as the use of different androgens, androgen dose, in vitro and in vivo models. Our data have shown that many androgen-induced genes observed by previous in vitro studies are also induced in vivo. The current study with sufficiently high tag numbers complements previously published SAGE studies on ARGs by both validating recently identified ARGs and building upon existing gene expression data with respect to androgen action in prostate tissue. Although the specific mechanisms by which androgens alter cellular growth in prostate disease states such as benign hyperplasia and cancer remain to be delineated, investigating ARGs in normal/benign prostate should extend our knowledge on prostate physiology and elucidate the influence of

androgens on prostate differentiation and transformation to adenocarcinoma. This identification of genes modulated by DHT in prostate supplied many ARGs that could be responsible for susceptibility to androgen-induced prostate neoplasia. Therefore, this functional genomic profile will be potentially useful for the diagnosis, prevention and treatment in androgen-induced prostate neoplasia, especially in the androgen activation pathways.

In order to validate the role of ARGs in prostate cancer, we further performed knockdown of ARG in polyamine metabolism which is closely related to cell proliferation. Our results showed that knockdown of AMD1 gene expression by siRNA decreased LNCaP cell growth, which was not caused by interferon response. Remarkably, the level of inhibition of prostate cancer cell growth by AMD1 siRNA was comparable to AR siRNA. Moreover, this gene is abnormally highly expressed in endometrial adenocarcinomas [83] and is



**Fig. 2.** Overview of the effects of dihydrotestosterone on different pathways in prostate tissue. AMD1, S-adenosylmethionine decarboxylase 1; ARAF, raf-related oncogene; ATP6, ATP synthase F0 subunit 6; CAI, calcium binding protein, intestinal; CALR, calreticulin; CAM1/2, calmodulin 1/2; CBP, calcium binding protein; CLU, clusterin; COX1/3, cytochrome c oxidase subunit 1/3; CYTB, cytochrome b; EEF1A1, eukaryotic translation elongation factor 1 alpha 1; EGR1, early growth response 1; EIF5A, eukaryotic translation initiation factor 5A; EST LCN2, EST lipocalin 2; FA, fatty acid; FADS2, fatty acid desaturase 2; FKBP2, FK506 binding protein 2; FOS, FBJ osteosarcoma oncogene; GBP2, guanylate nucleotide binding protein 2; GNAI2, guanine nucleotide binding protein, alpha inhibiting 2; GSN, gelsolin; HDAC7, histone deacetylase 7A; HSPA5, heat shock 70 kDa protein 5 (glucose-regulated protein); IGFBP3/5, insulin-like growth factor binding protein 3/5; IGTP, interferon gamma induced GTPase; MAP3K12, mitogen activated protein kinase kinase kinase 12; MAT2A, methionine adenosyltransferase II, alpha; Mt/TCA cycle, mitochondrial energy/TCA cycle; MNUD4, NADH dehydrogenase subunit 4; MYH4, myosin, heavy polypeptide 4, skeletal muscle; NR4A1, nuclear receptor subfamily 4, group A, member 1; NUCB2, nucleobindin 2; PDIR, protein disulfide isomerase-related protein; PPIA, peptidylprolyl isomerase A; RGL2, ral guanine nucleotide dissociation stimulator,-like 2; RGS2, regulator of G-protein signaling 2; RPLP0, acidic ribosomal phosphoprotein PO; RPS2/5/6/7, ribosomal protein S2/5/6/7; Ser/Ter kinase, serine/threonine protein kinase family; SBP, spermine binding protein; SCD2, stearoyl-Coenzyme A desaturase 2; SERPINE2, serine (or cysteine) proteinase inhibitor, clade E, member 2; SPINK3, serine protease inhibitor, Kazal type 3; SVS3/5/6/7, seminal vesicle secretion 3/5/6/7; TF, transcriptional factors; TGIF, transforming growth factor-beta-induced factor; TNNT3, troponin T3, skeletal, fast; TPM2, tropomyosin 2, beta; TRF, transferrin.

induced by the androgen (R1881) in LNCaP cells (unpublished data). Furthermore, we have recently demonstrated the similar growth-suppressive effect of two other ARGs, namely HSPA5 and MAT2A on LNCaP cells [84]. These evidences support the idea that ARGs are essential for the normal development of the prostate and can also be responsible for the pathogenesis of the prostate cancer.

### CONCLUSIONS

ARGs are essential for the normal development of the prostate. Moreover, ARGs are also responsible for the pathogenesis of the prostate cancer. Due to their importance in normal prostate development and prostate cancer initiation and progression, we have focused the present study on the investigation of ARGs in the normal mice prostate *in vivo*. Figure 2 provides a complete compendium of the analysis of the transcripts discussed in this manuscript. Here, we have identified 187 transcripts which rapidly responded to DHT within 24 hr. Forty-seven of them are known ARGs, whereas most of them were not previously known as androgen target genes. Our results clarify DHT effects on prostate transcriptome in the areas of protein metabolism, cell proliferation and apoptosis. In addition, we detected gene expression changes in metabolic pathways, cytoskeleton, immunity and endoplasmic reticulum stress. Furthermore, knockdown of *S*-adenosylmethionine decarboxylase 1 in LNCaP cells confirmed the importance of ARGs in prostate cancer cell growth. These data support the idea that ARGs are essential for the normal development of the prostate. Further studies investigating their protein expression and protein localization within specific lobes/regions and cell types of the prostate as well as in different prostate diseases will emphasize the importance of ARGs in the pathogenesis of the prostate cancer.

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