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Tissue-selective regulation of androgen-responsive genes

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Abstract

Androgens regulate a wide array of physiological processes, including male sexual development, bone and muscle growth, and behavior and cognition. Because androgens play a vital role in so many tissues, changes in androgen signaling are associated with a plethora of diseases. How such varied responses are achieved by a single stimulus is not well understood. Androgens act primarily through the androgen receptor (AR), a hormone nuclear receptor that is expressed in a select variety of tissues. In order to gain a better understanding of how the tissue-selective effects of androgens are achieved, we performed a comparison of microarray data, using previously published datasets and several of our own microarray datasets. These datasets were derived from clinically relevant, AR-expressing tissues dissected from rodents treated with the full androgen dihydrotestosterone (DHT). We found that there is a diverse response to DHT, with very little overlap of androgen regulated genes in each tissue. Gene ontology analyses also indicated that, while several tissues regulate similar biological processes in response to DHT, most androgen regulated processes are specific to one or a few tissues. Thus, it appears that the disparate physiological effects mediated by androgens begin with widely varying effects on gene expression in different androgen-sensitive tissues. The analysis completed in this study will lead to an improved understanding of how androgens mediate diverse, tissue-specific processes and better ways to assess the tissue-selective effects of AR modulators during drug development.

Keywords

Androgens; selectivity; microarray; QPCR; gene ontology; prostate

Introduction

Androgens are best known for their ability to regulate male sexual development, but these hormones affect a number of other processes in a variety of peripheral target tissues. Androgens are necessary for the development of the prostate and testis, the latter being where most androgens are produced (1, 2). Androgens promote increases in skeletal muscle mass and bone mineral density, and lack of androgens leads to defects in both tissues (3–5). Androgens reduce fat mass and improve lean body mass by inhibiting adipogenesis (6). Androgens play important roles in the brain, affecting aggression and libido, and declining androgen levels are associated with neurodegenerative diseases (7, 8). Decreasing androgen levels are associated with increased risk of diabetes and cardiovascular disease (9). Androgens play a key role in androgenic alopecia and other disorders of the skin (10, 11). Androgens also play a role in many female physiological processes. In the ovary, excessive production of androgens by theca cells results in polycystic ovary syndrome (PCOS), which can cause virilization, and in severe cases, infertility (12). Androgens also play a role in the

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uterus, and especially in endometriosis, where anti-androgens can be used to treat endometrial growth (13). Androgens are increasingly being recognized as important players in the mammary gland and some forms of breast cancer (14). Thus, androgen action is vast and varied in both men and women.

Androgens act primarily through binding to and activating the androgen receptor (AR), a ligand-dependent transcription factor belonging to the nuclear receptor superfamily (15). AR is expressed in a select variety of tissues and mediates the varied physiological responses to androgens. Various mechanisms have been proposed to account for the tissue specific effects of androgens (and AR activation), including tissue specific expression of transcriptional cofactors and 5α-reductase, the enzyme which converts testosterone to the more potent dihydrotestosterone (DHT), but our molecular understanding of AR selectivity is poor (16). Because AR plays a vital role in so many tissues, changes in AR signaling are associated with several distinct diseases. Treatments for these diseases often have side-effects due to changes in AR activity in non-diseased tissues. For example, decreased levels of AR activity have been reported to result in osteoporosis, sarcopenia, and loss of libido (17, 18), and treatments that increase AR activity may lead to virilization in women and prostate hypertrophy in men (19). AR signaling is essential in the development of prostate cancer, the second most common cause of cancer death in US men (20). For metastatic prostate cancer, treatment typically involves the combined use of chemical castration agents and competitive AR antagonists to reduce AR activity. In addition to almost universal recurrence of the cancer (21), this treatment regimen causes debilitating side-effects due to decreased AR activity in non-diseased tissues, including osteoporosis, muscle wasting, fatigue, and decreases in cognitive function.

In order to develop effective treatments for androgen-associated disorders, it is important that we understand exactly how androgens act in different tissues and how these signals are translated into different physiological outcomes. It would also be beneficial to have a means to measure effects of androgenic compounds in all clinically relevant AR-expressing tissues to prevent unwanted side-effects from drugs targeting the androgen-AR signaling axis. The first step in this process is to determine what effect androgens have on gene regulation in all clinically-relevant androgen-sensitive tissues. Therefore, we performed gene expression arrays to measure DHT-induced changes in four androgen-sensitive tissues in rats and compared these data to previously published gene expression array data from several other androgen-sensitive tissues from rats treated with DHT. We found that there is a diverse response to DHT, with very little overlap of androgen regulated genes in each tissue.

Methods

Animals

These studies were carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. All experiments were performed with UCSF or City of Hope regulatory approval. All Sprague Dawley rats were purchased from Jacksons Laboratory (Bar Harbor, Maine). Experiments were performed on sexually mature 10 week old rats. The first set of animal experiments (n=3/group) was performed in collaboration with the Preclinical Therapeutics core facility at UCSF. The second set of experiments (n=6/group) was performed in collaboration with the Animal Resource Center at the City of Hope. All male rats were castrated three days prior to drug treatment. All animals received a single IP injection of either vehicle (20% DMSO/ 80% PEG-400) or DHT (10mg/kg). Following a 16h treatment period animals were sacrificed and following organs harvested: in males, tibia, kidney, forebrain, skin, prostate, levator ani muscle and retroperitoneal fat pad; in females, mammary gland, ovary, uterus and tibia

Microarray Analysis

Tisssue was cooled on ice and RNA was extracted within an hour of sacrifice using an RNAEasy kit (Qiagen), according to manufacturer's protocol. Total RNA quality was assessed using a Pico Chip on an Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA). The following procedures were completed by the UCSF Microarray core facility. RNA was amplified and labeled with Cy3-CTP using the Agilent low RNA input fluorescent linear amplification kits following the manufacturers protocol (Agilent). Labeled RNA was assessed using the Nandrop ND-100 (Nanodrop Technologies, Inc., Wilmington DE), and equal amounts of Cy3 labeled target were hybridized to Agilent whole rat genome 4×44K Ink-jet arrays (Agilent). Hybridizations were performed for 14h, according to the manufacturers protocol (Agilent). Arrays were scanned using the Agilent microarray scanner (Agilent) and raw signal intensities were extracted with Feature Extraction v10.1 software (Agilent). This dataset was normalized using the *quantile* normalization method that is proposed by Bolstad et al (22). No background subtraction was performed, and the median feature pixel intensity was used as the raw signal before normalization. The microarray data discussed in this publication have been deposited in the National Center for Biotechnology Information's Gene Expression Omnibus and are accessible through GEO Series accession number GSE29170.

Gene Ontology Analysis

Functional annotation clustering was performed using DAVID (23), using GOTERM_BP-FAT to determine enrichment of related biological process subcategories. To reduce the redundancy associated with functional annotations, Functional Annotation Clustering groups/displays similar annotations together which makes the biology clearer and more focused to be read vs. traditional chart report. The grouping algorithm is based on the hypothesis that similar annotations should have similar gene members. The Functional Annotation Clustering integrates the same techniques of Kappa statistics to measure the degree of the common genes between two annotations, and fuzzy heuristic clustering (used in Gene Functional Classification Tool) to classify the groups of similar annotations according to kappa values. In this sense, the more common genes annotations share, the higher chance they will be grouped together. The p-values associated with each annotation terms inside each clusters are exactly the same meaning/values as p-values (Fisher Exact/EASE Score) shown in the regular chart report for the same terms. The Group Enrichentment Score, which is the geometric mean (in -log scale) of member's p-values in a corresponding annotation cluster, is used to rank their biological significance. Thus, the top ranked annotation groups most likely have consistent lower p-values for their annotation members.

RT-QPCR

Dissected tissue was stored in RNA Later (Qiagen) for less than an hour before being mechanically homogenized prior to RNA isolation with an RNAEasy kit (Qiagen). RNA was reverse transcribed (promega) and amplified (Qiagen Taq and reagents) on a 7300 Real Time PCR System (Applied Biosystems), using SYBR green (Invitrogen) as the detecting dye and Rox (Invitrogen) as the reference dye. Differences between experimental (x) and vehicle control (y) samples were normalized to RPL19 transcript levels (androgen unresponsive {Jones, 2009 #420}) and determined with the following calculation: $(2\text{^}(Ct_{xgene1} - Ct_{ygene1}))/(2\text{^}(Ct_{xRPL19} - Ct_{yRPL19}))$.

Statistics

For the analysis of the microarray data, an one-way ANOVA linear model was fit to the comparison to estimate the mean M values and calculated moderated t-statistic, B statistic,

false discovery rate (24), and adjusted p-value (25) for each gene for the comparison of interest. All procedures were carried out using functions in the R package *limma in Bioconductor* (26, 27). For most analyses, the B statistic (B>0) was least stringent and was used to compile lists of candidate androgen-regulated genes for each tissue. To test for differences in means of our qPCR data, we used non-parametric Wilcoxin Rank Sum analysis with a cutoff for significance of $p<0.1$.

Results

Comparison of androgen regulated gene profiles in different tissues

In order to identify potential DHT-regulated genes, we searched published microarray data and performed our own microarrays on several important androgen regulated tissues (Table 1). We castrated 10 week old Sprague Dawley (SD) male rats and three days later treated them with vehicle or 10mg/kg DHT (IP) for 16hrs (n=3). Likewise, we treated intact female rats with vehicle or 10mg/kg DHT (IP) for 16hrs (n=3). We then sacrificed the animals and, in males, dissected the prostate, levator ani (LA) muscle, tibia, kidney, forebrain, retroperitoneal fat pad, and skin; in females we harvested mammary gland, ovary, uterus, and tibia (Supplementary Figure 1). Datasets included in our primary comparisons were derived from experiments with designs very similar to ours. Experiments were performed in SD rats treated with a similar concentration of DHT (3 or 10mg/kg) for a similar period of time (16 to 24hrs). In some cases, the timing of castration or ovariectomy was slightly different than what was used in our study, but these constraints should provide comparable datasets. DHT and not testosterone was used as the stimulus to avoid complications due to aromatization of testosterone to estrogens. Two datasets included publically available data on the NCBI Gene Expression Omnibus, which allowed us to reanalyze raw data with filtering and statistical criteria that more closely matched our array experiments. However, raw data from three datasets were not publically available, so we could only evaluate published lists of genes using author-defined statistical criteria. For our arrays, we evaluated the significance of differences in gene regulation using several statistical methods but chose to use the B statistic (B>0) for cross dataset comparisons because it was the least stringent, thus allowing the most possible overlap. Our raw data is publicly available for re-analysis (GSE29170). Filtered lists of significantly regulated genes are available in supplemental information.

From the lists of published data and data generated by our microarray experiments, we created a matrix in which to compare androgen-regulated genes in the prostate, uterus, skin, fat, ovary, muscle, and forebrain. We also used datasets from mouse prostate (GSE5901) (28), uterus (GSE6237) (29), retroperitoneal fat pad (GSE9631)(30), bone (GSE5776)(31) and testis (GSE438) (32) as secondary comparisons because the choice of rodent and design of experiments was different from ours. We sought to determine only whether genes were present or absent in each tissue; no attempt was made to compare the strength of regulation or rank, as several datasets used in this analysis did not provide that information. The two datasets from prostate tissue (ours and (33) were combined via Venn analysis to create a non-overlapping list of DHT-regulated genes in the prostate, as were the two datasets from uterine tissue (GSE6237; (29) and (34). A comparison of the two prostate datasets demonstrated only a partial overlap of genes identified as DHT-regulated in each, which may be attributable to the different statistical methods used to determine significance and the use of only the ventral prostate versus the whole prostate. Likewise, a comparison of the two uterus datasets revealed only a partial overlap, which may be attributable to differences in species (rat vs. mouse) and the different statistical methods used to determine significance. Regardless, the overlap in DHT-regulated genes between like tissues was still far greater than that with unlike tissues as we found very little overlap of androgen-regulated genes among androgen-sensitive tissues (Table 2). FKBP5 was the only gene to score positive in

more than three tissues (prostate, uterus, muscle, skin, fat). Most, but not all, genes were similarly up or down-regulated when found in multiple tissues. This suggests that androgens regulate different sets of genes in different tissues, likely accounting for the vastly different physiological roles androgens play in different tissues.

Insights into androgen regulated pathways in the prostate, skin, ovary, and forebrain

We sought to understand the biological role of DHT-induced genes in the four tissues we examined by microarray. To do so, we performed gene ontology analyses on each of our array datasets. In the prostate, we found many of the same genes and biological pathways to be DHT-regulated that had been previously reported in mice (28) and rats (33). DHT treatment significantly increased the expression of genes associated with steroid and amino acid synthesis, as well as genes associated with protein turnover and intracellular/vesicle transport (Table 3). DHT treatment decreased the expression of cell death genes associated with castration, as well as some specific biosynthetic and kinase pathways (Table 4). DHT treatment also decreased genes associated with response to infection/wounding as well as angiogenesis. While these observations have been made previously, the effects of androgen on angiogenesis and response to infection/wounding could be very interesting and warrant further investigation.

Of the 167 androgen regulated genes identified by microarray in the forebrain, only 40 up-regulated and 80 down-regulated had functional annotation. As the forebrain contains a mixture of different AR-expressing cell populations (7, 8), the lack of a more defined anatomical specificity might have contributed to the paucity of androgen-regulated genes identified in our analysis. Nonetheless, it was evident that DHT treatment significantly affected genes associated with synapse formation and gated-channel activity, as well as cation and lipid transport (Tables 5 and 6). DHT also appears to increase the expression of genes responsible for cell-cell adhesion and macromolecule complex assembly. Each of these warrants further investigation, as understanding AR activity in the brain could help explain developmental behavior and may help with development of drugs for neurodegenerative conditions associated with decreasing androgen levels (35).

In the ovary microarray data, only 15 of the 51 genes identified as up-regulated in response to DHT were functionally annotated, so a clustering analysis by biological function could not be performed. 136 down-regulated genes were functionally annotated, and many were involved in biological processes normally associated with brain function, including synapse formation, neuronal development, and behavior and learning (Table 7). There were also four olfactory receptors that were down-regulated. To the best of our knowledge, ovary and brain samples were not switched and no extraneous nervous tissue was extracted with the ovaries. In support of this, microarray data from forebrain samples is also enriched for neuronal genes, and the ovary data is enriched for genes associated with cell signaling and secretion, which would be expected. There have been recent discoveries of neuronal associated genes and olfactory receptors being expressed in other tissues (36), which makes confirming these finding by other means essential. If confirmed, it will be very interesting to determine what role these genes play in the ovary.

In the skin, DHT appears to drastically decrease cell cycle progression, cell division, and DNA replication, with a very strong enrichment for down-regulated genes associated with these biological categories (Tables 8 and 9). Excess androgens are known to play a role in several skin disorders, including seborrhea, hirsutism, and androgenic alopecia (10, 11). AR is expressed in the dermal papillar cells of hair follicles and in sebaceous glands (37) and while our understanding of how androgens cause these disorders is far from complete, it could be imagined that inhibiting the cell cycle and cell division could be responsible for the loss of the normal hair cycle that leads to baldness (38). The genes identified from this array

analysis could provide insight into the mechanism of androgen action in the skin and in androgen related skin disorders.

Several of the publications used in our comparison conducted their own gene ontological analyses. It appears that there is little overlap in the biological functions regulated by androgens in different tissues (28–31, 33, 34, 39, 40). Most tissues have androgen-induced pathways that are very specific for that tissue; adipogenesis in fat, myogenesis in muscle, osteogenesis in bone. However, there are a few pathways that appear to be commonly regulated in several tissues. Lipid/cholesterol metabolism is common among many, but not all tissues. Likewise, androgens increase many biosynthetic pathways in most, but not all tissues. Cell proliferation in response to DHT occurs in many tissues (muscle, prostate, fat, bone), but in other tissues androgens appear to inhibit cell cycle progression (skin, uterus). Androgens also decrease apoptotic/cell death genes in several tissues (prostate, bone), but not others. There appears to be some similarity in androgen gene regulation in male and female reproductive tissues, as both we and others have seen significant overlap in DHT-induced gene expression in prostate and uterus (34). Unexpectedly, DHT appears to induce genes involved in neuronal development and synapse function in several tissues other than the forebrain, including the ovary and muscle; it remains to be seen if these findings are biologically relevant.

Confirmation of tissue-selective androgen regulation

We selected a subset of genes from each tissue to validate by RT-QPCR. We chose several genes that were found by array to be regulated in multiple tissues as well as many genes that were regulated in only one tissue. We performed initial experiments using RNA samples from the three vehicle and three DHT-treated rats used in our microarray studies but we repeated the experiment using six animals per group to increase the statistical power of the analysis. We found that the results of the two studies were highly similar for most genes examined, suggesting that DHT-induced changes in each tissue were reproducible. In the second experiment, we included an intact control group, so that we could determine which genes regulated by castration in addition to those that were regulated by subsequent addition of DHT. In general, we found that the microarray and QPCR results were qualitatively similar but fewer genes were found to be significantly regulated by DHT upon QPCR analysis, due in large part to the use of a more stringent statistical analysis to determine the significance of expression differences (Table 10). We next cross-examined the expression of genes found to be significantly regulated in one tissue in each of the other tissues. We found that more genes were commonly regulated in multiple tissues than the microarrays suggested (Table 10), but there was still a high degree of tissue-selective regulation.

Discussion

In this study, we created novel datasets of DHT-regulated genes in the rat prostate, skin, ovary, and forebrain. We chose to use whole organs for our analyses instead of more detailed tissue dissections to enable easy reproduction of our experiments and to ascertain the response to androgens in the entire organ, because physiological changes take place on that scale. Hypotheses generated from gene regulation data on the organ scale can be investigated later in subgross detail. The published data used for the microarray comparisons were also derived from whole organs, other than the published rat prostate study (33), which used only the ventral prostate for analysis. The lack of overlap between microarray datasets was striking. The lack of similarity is likely due to several factors; the different statistical analyses used to determine significance of gene regulation, the difference in DHT dosage (3 vs. 10mg/kg) in some studies, the length of treatment (16 vs. 24hrs), and general inter-lab experimental variability. As mentioned, the microarray data is likely an underestimation of the real similarity of DHT-regulated genes across tissues, as QPCR experiments

demonstrated that a higher percentage of genes were commonly regulated than observed by microarray. Furthermore, more rigorous comparison across tissues might result in increased observation of commonly regulated genes, but this would require additional publically available datasets. Nonetheless, it is clear that DHT regulates distinct sets of genes in different androgen-sensitive tissues.

The physiological effects of androgens like DHT are mediated by the AR. Our microarray comparisons suggest that the disparate physiological effects mediated by androgens through AR begin with widely disparate effects on gene expression in each of the AR-expressing tissues. While this study does not suggest a mechanism by which tissue-specific effects of androgens are achieved, it does provide an excellent dataset/model that can be used to address this question. Future studies will use bioinformatic analyses and promoter mutation experiments to determine which genes are directly regulated by AR. Bioinformatic analyses of promoters of tissue-specific AR-regulated genes and genes that are commonly regulated by AR in several tissues may shed light on how different genes are regulated in response to a single stimulus. For instance, a binding site for a particular transcription factor may be enriched in the promoters of muscle-specific androgen regulated genes, thus suggesting that the combined action of that transcription factor and AR provide muscle-selective androgen effects.

This list of genes could also provide the basis for evaluating the tissue selective activity of androgenic/anabolic or anti-androgenic compounds. There is increasing clinical demand for drugs that can regulate AR activity in a tissue selective manner. These selective AR modulators (SARMs) are finding use in the treatment of osteoporosis, muscle wasting disease, andropause, and other androgen dependent disorders (41). However, there is not an ideal animal model for testing the selective activity of these compounds in all important androgen sensitive tissues (16). We suggest that a model utilizing changes in androgen-dependent gene transcription as an endpoint may provide an excellent way to assess the tissue-specific activity of putative SARMs. Such a model would be a boon to the burgeoning field of SARM research and would accelerate drug discovery for several important androgen dependent diseases.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Microarray datasets used for analysis. All animals were between 10 and 12 weeks of age at the start of the study. Animals were oriechtomized (ORX) or ovariectomized (OVX) and then treated with DHT after the indicated amount of time. The tissues were harvested following DHT treatment for the indicated amount of time.

Species/Age/Tissue	Treatment	Reference
SD rat/10wks/Prostate	ORX 3d; 10mg/kg DHT IP 16h	GSE29170
SD rat/10wks/Forebrain	ORX 3d; 10mg/kg DHT IP 16h	GSE29170
SD rat/10wks/Ovary	ORX 3d; 10mg/kg DHT IP 16h	GSE29170
SD rat/10wks/Skin	ORX 3d; 10mg/kg DHT IP 16h	GSE29170
SD rat/10wks/Prostate	ORX 3d; 3mg/kg DHT IP 24h	(33)
SD rat/12wks/Uterus	OVX 3w; 3mg/kg DHT IP 24h	(34)
SD rat/10wks/Muscle	ORX 11w; 3mg/kg DHT IP 24h	(39)

SD=Sprague Dawley

Table 2

Overlap of microarray-identified androgen-regulated genes:

Tissue	Total # of DHT-regulated genes	# of genes identified in 1 other tissue**	# of genes identified in 2 or more other tissues***
Prostate (in house) *	822	61	8
Prostate (33)	216	31	3
Uterus (34)	97	32	3
Uterus (29)	232	56	2
Skin (in house) *	309	36	5
Forebrain (in house) *	165	18	0
Ovary (in house) *	294	18	1
Muscle (39)	70	54	5
Fat (30)	94	25	4

* in house total # of regulated genes based on B statistics.

** not including prostate-prostate or uterus-uterus overlap: 40 and 22 genes were similarly regulated between prostate and uterus datasets respectively.

*** FKBP5 was the only gene to score positive in more than three tissues (prostate, uterus, muscle, skin, fat). Most, but not all, genes were similarly up or down-regulated when found in multiple tissues.

Table 3

Gene ontology analysis of genes up-regulated in the prostate in response to DHT. (420 genes available for analysis).

Biological Process Cluster	Contributing ontological subcategories	Enrichment score
Steroid synthesis	9	6.71
Proteasomal degradation	43	4.35
Amino acid biosynthesis	8	4.22
Intracellular/vesicle transport	12	3.53
Unfolded protein/response to stress	7	2.41

Table 4

Gene ontology analysis of genes down-regulated in the prostate in response to DHT. (258 genes available for analysis).

Biological Process Cluster	Contributing ontological subcategories	Enrichment score
Biosynthesis/transcription	17	2.87
Angiogenesis	5	2.81
Response to infection/wounding	8	2.77
Phosphorylation/protein kinase	24	2.74
Apoptosis/cell death	21	2.12

Table 5

Gene ontology analysis of genes up-regulated in the forebrain in response to DHT. (40 genes available for analysis).

Biological Process Cluster	Contributing ontological subcategories	Enrichment score
Cell-cell adhesion	3	1.04
Macromolecule complex assembly	4	0.90
Biosynthesis/transcription	15	0.59
Cation transport	3	0.56

Table 6

Gene ontology analysis of genes down-regulated in the forebrain in response to DHT. (80 genes available for analysis).

Biological Process Cluster	Contributing ontological subcategories	Enrichment score
Lipid transport	7	1.64
Cation transport	6	1.62
Response to endogenous/hormone stimulus	7	1.14
Homeostasis	9	1.01
Synapse formation/gated ion channel activity	13	0.94

Table 7

Gene ontology analysis of genes down-regulated in the ovary in response to DHT. (134 genes available for analysis).

Biological Process Cluster	Contributing ontological subcategories	Enrichment score
Synapse/neuronal development	18	2.08
Cell signaling/secretion	4	1.25
Homeostasis	9	1.25
G-protein signaling	6	1.06

Table 8

Gene ontology analysis of genes up-regulated in the skin in response to DHT. (75 genes available for analysis).

Biological Process Cluster	Contributing ontological subcategories	Enrichment score
Muscle development	5	2.0
Phosphorylation	5	1.57
Fatty acid metabolism	3	1.51
Response to hormone/other stimuli	10	1.36

Table 9

Gene ontology analysis of genes down-regulated in the skin in response to DHT. (152 genes available for analysis).

Biological Process Cluster	Contributing ontological subcategories	Enrichment score
Cell cycle/M phase/cell division	18	7.64
DNA replication	12	2.78
Phosphorylation	4	2.68
Reproduction	9	1.43

Table 10

QPCR-validated androgen-regulated genes.

Tissue	Gene	QPCR fold change	Other similarly regulated tissues
Prostate	Odc1	↓4.5 fold with castration, ↑3.3 fold with DHT	Muscle, skin
	Sqle	↓6.3 fold with castration, ↑4.1 fold with DHT	Mammary
	Gadd45g	↓3.0 fold with castration, ↑4.2 fold with DHT	
	Fkbp5	↓12.2 fold with castration, ↑13.4 fold with DHT	Muscle
	Nkx3.1	↓3.6 fold with castration, ↑4.5 fold with DHT	
	Zfp36l	↑5.8 fold with castration, ↓2.8 fold with DHT	
	Sox4	↓5.7 fold with castration, ↑4.8 fold with DHT	Muscle, kidney, tibia
	Myom1	↓6.5 fold with castration	Kidney
	Pvalb	↓3.2 fold with castration	Muscle, kidney
	Ptgis	↓2.2 fold with castration	Muscle, skin
	Eno3	↓3.5 fold with castration	Fat, uterus
	Cox6a2	↓4.2 fold with castration	Fat
	Acta1	↓10.7 fold with castration	Muscle, uterus, tibia, skin, mammary
	Pou2af1	↓2.4 fold with castration	Tibia, ovary, mammary
	Col1a1	↓2.7 fold with castration	Muscle, tibia
	Fut4	↓21.8 fold with castration	Forebrain
	Cldn3	↑2.4 fold with castration, ↓3.5 fold with DHT	Forebrain, uterus
	Aytl2	↑1.9 fold with castration	Kidney
	P21	↑2.1 fold with DHT	Muscle, fat, forebrain
Forebrain	Lrrc4c	↓2.5 fold with castration	
	Cldn3	↑3.5 fold with castration, ↓3.4 fold with DHT	Prostate, uterus
	P21	↑2.2 fold with castration	Prostate, muscle, fat
	Fut4	↑1.7 fold with castration, ↓2.2 fold with DHT	Prostate
	Igf1	↓3.0 fold with castration	Muscle, fat
Ovary	Pou2af1	↓2.3 fold with DHT	Prostate, tibia, mammary
Skin	Acta1	↓2.9 fold with castration	Prostate, muscle, uterus, tibia, mammary
	Ccnb1	↓1.8 fold with castration, ↑2.5 fold with DHT	Fat
	Pdk4	↓2.5 fold with castration, ↑1.8 fold with DHT	
	Myf6	↓2.4 fold with castration, ↑2.0 fold with DHT	Fat
	Odc1	↑2.1 fold with castration, ↓3.0 fold with DHT	Prostate, muscle
	Wnt5a	↑1.9 fold with DHT	
	Ptgis	↓1.5 fold with castration, ↑2.9 fold with DHT	Prostate, muscle
Muscle *	Acta1	↓3.7 fold with castration	Prostate, uterus, tibia, skin, mammary
	Fkbp5	↑3.2 fold with DHT	Prostate
	Pvalb	↓4.9 fold with castration	Prostate, kidney
	Myot	↑2.8 fold with DHT	
	Axin1	↑5.6 fold with DHT	

Tissue	Gene	QPCR fold change	Other similarly regulated tissues
	Igf1	↓4.2 fold with castration, ↑7.0 fold with DHT	Fat, forebrain
	Odc1	↓2.7 fold with castration, ↑6.2 fold with DHT	Prostate, skin
	Sox4	↑2.2 fold with DHT	Prostate, kidney, tibia
	Ptgis	↓2.1 fold with castration	Prostate, skin
	Ldhc	↓2.2 fold with castration	Fat
	Mt1a	↑4.1 fold with DHT	Fat, mammary
	Col1a1	↓3.2 fold with castration	Prostate, tibia
	P21	↑3.5 fold with DHT	Prostate, fat, forebrain
Uterus	Zfp36l	↑2.7 fold with DHT	Fat
	Eno3	↓3.0 fold with DHT	Prostate, fat
	Fkbp5	↓2.5 fold with DHT	
	Acta1	↑4.7 fold with DHT	Prostate, muscle, tibia, skin, mammary
	Cldn3	↑2.2 fold with DHT	Prostate, brain
	Mmp11	↑3.5 fold with DHT	
	Col5a2	↓2.4 fold with DHT	
Tibia bone **	Pou2af1	↓2.3 fold with castration, ↑2.0 fold with DHT	Prostate, ovary, mammary
	Col1a1	↓2.3 fold with castration	Prostate, muscle
	Acta1	↓1.9 fold with castration, ↑1.8 fold with DHT	Prostate, muscle, uterus, skin, mammary
	Wap	↑2.7 fold with castration, ↓2.5 fold with DHT	Kidney
	Sox4	↑3.5 fold with DHT	Prostate, muscle, kidney
Fat **	Eno3	↓6.8 fold with castration, ↑2.6 fold with DHT	Prostate, uterus
	Cox6a2	↓4.0 fold with castration	Prostate
	Ldhc	↓1.7 fold with castration, ↑2.1 fold with DHT	Muscle
	P21	↓2.2 fold with castration, ↑2.2 fold with DHT	Prostate, muscle, forebrain
	Mt1a	↑4.6 fold with DHT	Muscle, mammary
	Igf1	↓2.6 fold with castration	Muscle, forebrain
	Myf6	↑2.9 fold with DHT	Skin
	Zfp36l	↓2.0 fold with castration, ↑1.7 fold with DHT	Uterus
	Ccnb1	↓2.0 fold with castration, ↑2.2 fold with DHT	Skin
Kidney ***	Mmp14	↓2.4 fold with castration	
	Pvalb	↑9.4 fold with DHT	Prostate, muscle
	Aytl2	↑2.4 fold with DHT	Prostate
	Sox4	↑3.3 fold with DHT	Prostate, muscle, tibia
	Myom1	↑17.0 fold with DHT	Prostate
	Wap	↓7.6 fold with castration, ↑33.9 fold with DHT	Tibia
Mammary ***	Sqle	↑7.5 fold with DHT	Prostate
	Acta1	↑9.7 fold with DHT	Prostate, muscle, uterus, tibia, skin
	Mt1a	↓4.3 fold with DHT	Muscle, fat
	Pou2af1	↓8.5 fold with DHT	Prostate, tibia, ovary

*Array data from soleus muscle while QPCR data from LA muscle.

**From secondary analysis datasets in mice (30, 31).

***No array data associated with tissue; results from cross-check.