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Accessibility
Androgen regulation of gene expression in human meibomian gland and conjunctival epithelial cells

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**Purpose:** Androgens exert a significant influence on the structure, function and/or pathophysiology of the meibomian gland and conjunctiva. We sought to determine whether this hormone action involves the regulation of epithelial cell gene expression in these tissues.

**Methods:** Immortalized human meibomian gland and conjunctival epithelial cells were treated with placebo or dihydrotestosterone (DHT) and processed for molecular biologic procedures. Gene expression was evaluated with BeadChips and data were analyzed with bioinformatic and statistical software.

**Results:** Androgen treatment significantly influenced the expression of approximately 3,000 genes in immortalized human meibomian gland and conjunctival epithelial cells. The nature of DHT action on gene activity was predominantly cell-specific. Similarly, DHT exerted a significant, but primarily cell-specific, influence on many gene ontologies and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways. These included groups of genes related, for example, to lipid dynamics, innate immunity, cell cycle, Janus kinase (JAK)-signal transducer and activator of transcription (stat) cascades, oxidative phosphorylation, the proteasome, and mammalian target of rapamycin (mTOR), Wnt, and peroxisome proliferator-activated receptor (PPAR) signaling.

**Conclusions:** Our findings support our hypothesis that androgens regulate gene expression in human meibomian gland and conjunctival epithelial cells. Our ongoing studies are designed to determine whether many of these genes are translated and play a role in the health and well being of the eye.

Androgens exert a significant influence on the structure, function and/or pathophysiology of many ocular tissues, including the meibomian gland, lacrimal gland, conjunctiva, and cornea [1-12]. These hormones regulate such ocular parameters as glandular architecture, protein synthesis and secretion, meibum production, mucus expression, aqueous tear output, tear film stability, immune activity, and epithelial cell dynamics [1-12]. Androgens have also been reported to correct defects, facilitate wound healing [6,7,13], suppress angiogenesis [14], and stimulate mitosis [9] in the corneal epithelium, to alter the development of allergic conjunctivitis [5], and to attenuate inflammation in autoimmune lacrimal tissue [8,11]. In addition, androgens have been proposed as a topical therapy for the treatment of aqueous-deficient and evaporative dry eye diseases [8,11]. However, despite these observations, the precise mechanisms underlying androgen-eye interactions in humans remain to be clarified.

We hypothesize that androgen action on the eye involves the local, intracrine synthesis of this sex steroid from adrenal precursors (e.g., dehydroepiandrosterone), binding to saturable, high-affinity and androgen-specific receptors, control of gene transcription, and ultimately modulation of translation. In support of this hypothesis, we have discovered that the human meibomian and lacrimal glands, and immortalized corneal and conjunctival epithelial cells, contain all the steroidogenic enzyme mRNAs necessary for the intracrine synthesis and metabolism of androgens [15]. Moreover, we have shown that androgen receptor mRNA and protein are present in epithelial cell nuclei of the human meibomian and lacrimal glands, cornea and conjunctiva [16, 17].

To continue to test our hypothesis, we examined the influence of androgens in gene expression in immortalized human meibomian gland and conjunctival epithelial cells.

**METHODS**

**Cell culture and hormone treatment:** Immortalized human meibomian gland epithelial cells, which were recently generated in our laboratory [2], were cultured in Keratinocyte Serum-Free Medium [KSM] supplemented with 50 μg/ml bovine pituitary extract (BPE), 5 ng/ml epidermal growth factor (EGF), and 100 U penicillin-streptomycin (Invitrogen, Carlsbad, CA). Cells were incubated in a humidified, 37 °C chamber under 5% CO₂/95% air. Immortalized human conjunctival epithelial cells, which were gifted by Dr. Ilene Gipson (Boston, MA), were cultured in serum-free conditions as previously described [18].

When approximately 80% confluent, cells were exposed to 10 nM dihydrotestosterone (DHT; Steraloids, Wilton, NH) or placebo for 3 (meibomian) or 4 (conjunctiva) days. These
time periods were previously shown to be optimal for the
generation of DHT-induced alterations in androgen receptor
mRNA levels in the different cell types [19]. For these studies
the DHT was dissolved in ethanol and aliquots were
evaporated in sterilized vials before the addition of medium.
The placebo was prepared by transferring media to vials
containing the residue of evaporated ethanol. After hormone
treatment, cells were harvested and processed for RNA
isolation.

Molecular biologic procedures: Total RNA was extracted
with RNAqueous Kits (Ambion, Austin, TX) and evaluated
on a RNA Nano 6000 Series II Chip with a 2100 Bioanalyzer
(Agilent Technologies, Palo Alto, CA) to confirm RNA
integrity. The RNA concentrations and associated 260/280 nm
ratios were determined using a NanoDrop 1000
Spectrophotometer (Thermo Scientific, Waltham MA).

The RNA (100 ng) samples were processed by Asuragen
(Austin, TX) for the determination of mRNA levels by using
Illumina HumanHT-12 v3 Expression BeadChips (San Diego,
CA). These BeadChips target more than 25,000 annotated
genes with over 48,000 probes derived from NCBI reference
sequences and the UniGene databases. In brief, biotin-labeled
cRNA samples were generated by using a MessageAmp™ II-
based protocol (Ambion Inc., Austin, TX), quantitated by UV
spectrophotometry and analyzed with an Agilent 2100
Bioanalyzer capillary electrophoresis system. The labeled
cRNAs were used to probe the BeadChips. Hybridization,
washing, and scanning of the Illumina arrays were conducted
according to the manufacturer’s instructions. Data were
processed with Illumina BeadStudio software v3 by using
both background subtraction and cubic spline normalization.
Standardized hybridization intensity values were adjusted by
adding a constant, so that the lowest intensity value for any
sample equaled 16 [20].

Normalized data were analyzed with GeneSifter.Net
software (Geospiza, Seattle, WA), a comprehensive program
that also produced gene ontology and z-score reports. Ontologies
included biologic processes, molecular functions and
cellular components and were organized according to the
guidelines of the Gene Ontology Consortium (GO) [21]. Gene
expression data were analyzed with and without log
transformation and statistical analyses of these data were
performed with Student’s t-test (two-tailed, unpaired). Genes
that were up- or down-regulated in the same direction in
different experiments were identified by using the
GeneSifter.Net intersector program (Geospiza). All data from
the Illumina BeadChips are accessible for download through the
National Center for Biotechnology Information’s Gene
Expression Omnibus (GEO) via series accession numbers
(GSE18091 and GSE18094).

Real time PCR procedures: The differential expression of
selected genes was verified by using quantitative real-time
PCR (qPCR) procedures. The cDNAs were transcribed by
employing SuperScript III Reverse Transcriptase (Invitrogen,
Grand Island, NY) and random hexamer primers (Invitrogen).
The qPCR reactions were performed in triplicate by using
TaqMan Gene Assays (Applied Biosystems, Inc., Foster City,
CA) and TaqMan-specific primers and probes for aldo-keto
reductase family 1, member c2 (Hs00413886_m1*), cdc28
protein kinase regulatory subunit 2 (Hs00244575_m1*), interferon α-inducible proteins 6
(Hs00242571_m1*), kallikrein related peptidase 11
(Hs01100849_m1*), keratin 16 (Hs00373910_g1*),
laminin, α3 (Hs00165042_m1*), leupaxin
(Hs00183105_m1*), micinchromosome maintenance
component 3 (Hs00172459_m1*), myosin light chain 6
(Hs00819642_m1; conjunctival epithelial cell endogenous
control), n (α) acetyltransferase 50 (Hs00363889_m1*;
meibomian gland epithelial cell endogenous control),
plasminogen activator, urokinase (Hs00170182_m1), serum
amyloid A1 (Hs00761940_s1), and uridine phosphorylase 1
(Hs00427695_m1*). Differential gene expression was
calculated according to the Comparative Ct method, as
outlined in Applied Biosystems User Bulletin 2 (updated
2001).

RESULTS

Androgen impact on gene expression in human ocular surface
and meibomian gland epithelial cells: To determine the effect
of DHT on gene expression in immortalized human
meibomian gland and conjunctival epithelial cells, cells were
exposed to placebo or DHT and processed for analysis by
using Illumina BeadChips and Geospiza software.

Our results demonstrate that DHT had a significant
impact on the expression of approximately 3,000 genes in
immortalized human meibomian gland and conjunctival
epithelial cells (Table 1). The relative direction of this
| Accession # | Gene                                                                 | Ratio | p value | Ontology                                      |
|------------|----------------------------------------------------------------------|-------|---------|-----------------------------------------------|
| DHT>Placebo| LEM domain containing 1                                              | 10.1  | 0.00000 | nuclear envelope                               |
| NM_001001552 | Late cornified envelope 3D                                             | 4.5   | 0.00024 | keratinization                                 |
| NM_152565 | ATPase, H+ transporting, lysosomal 38 kDa, V0 subunit d2              | 3.7   | 0.00048 | ion transport                                  |
| NM_021244 | Ras-related GTP binding D                                             | 3.5   | 0.00010 | positive regulation of TOR signaling cascade   |
| NM_001031615 | Aldehyde dehydrogenase 3 family, member B2                           | 3.0   | 0.00004 | alcohol metabolic process                      |
| NM_000435 | Notch homolog 3                                                       | 2.9   | 0.00015 | regulation of transcription, DNA-dependent     |
| NM_005218 | Defensin, β1                                                          | 2.6   | 0.00001 | chemotaxis                                     |
| NM_001003679 | Leptin receptor                                                       | 2.5   | 0.00553 | energy reserve metabolic process               |
| NM_000435 | Steroid-5α-reductase, α polypeptide 1                                 | 1.9   | 0.0097  | androgen biosynthetic process                  |
| NM_005063 | Stearoyl-CoA desaturase                                               | 1.7   | 0.00039 | fatty acid biosynthetic process                |
| NM_002015 | Forkhead box O1                                                       | 1.6   | 0.00006 | blood vessel development                       |
| Placebo>DHT | Ubiquitin-conjugating enzyme E2C                                      | 24.4  | 0.00000 | cell cycle checkpoint                          |
| NM_001800 | Topoisomerase (DNA) H α 170 kDa                                       | 16.7  | 0.00000 | resolution of meiotic recombination intermediates |
| NM_0015854 | Nucleolar and spindle associated protein 1                            | 15.6  | 0.00006 | mitotic sister chromatid segregation           |
| NM_000255 | Cell division cycle 20 homolog                                       | 13.4  | 0.00001 | cell cycle checkpoint                          |
| NM_001786 | Cyclin-dependent kinase 1                                             | 12.3  | 0.00000 | cell cycle checkpoint                          |
| NM_001168 | Baculoviral IAP repeat-containing 5                                   | 10.2  | 0.00000 | G2/M transition of mitotic cell cycle          |
| NM_004701 | Cyclin B2                                                             | 9.5   | 0.00002 | cell cycle checkpoint                          |
| NM_002263 | Kinesin family member C1                                              | 9.0   | 0.00000 | mitotic sister chromatid segregation           |
| NM_002994 | Chemokine (C-X-C motif) ligand 5                                      | 8.9   | 0.00019 | chemotaxis                                    |
| NM_003246 | Thrombospondin 1                                                      | 4.7   | 0.00001 | activation of MAPK activity                    |
| NM_002727 | Serglycin                                                             | 2.5   | 0.00012 | apoptosis                                      |
| NM_004994 | Matrix metalloproteinase domain 9                                     | 1.7   | 0.00001 | proteolysis                                   |

Relative ratios were calculated by comparing the degree of gene expression in meibomian gland epithelial cells treated with placebo or DHT. The mean gene intensity level in at least one group exceeded 100 BeadChip units.
### Table 3. Influence of DHT on Gene Expression Ratios in Immortalized Human Conjunctival Epithelial Cells.

| Accession # | Gene                                      | Ratio | p value     | Ontology                      |
|-------------|-------------------------------------------|-------|-------------|-------------------------------|
| **DHT>Placebo**                                      |       |             |                 |                               |
| NM_004994  | Matrix metallopeptidase 9                 | 10.9  | 0.00000     | proteolysis                   |
| NM_001012964 | Kallikrein-related peptidase 6             | 10.5  | 0.00001     | proteolysis                   |
| NM_001323  | Cystatin E/M                              | 10.4  | 0.00002     | epidermis development         |
| NM_003856  | Interleukin 1 receptor-like 1             | 9.0   | 0.00006     | immune response               |
| NM_018043  | Anoctamin 1, calcium activated chloride channel | 8.0   | 0.00001     | ion transport                 |
| NM_002153  | Hydroxysteroid (17β) dehydrogenase 2      | 7.8   | 0.00000     | steroid biosynthetic process  |
| NM_005416  | Small proline-rich protein 3              | 7.4   | 0.00023     | epidermis development         |
| NM_144947  | Kallikrein-related peptidase 11           | 7.2   | 0.00003     | proteolysis                   |
| NM_001077491 | Kallikrein-related peptidase 5            | 7.1   | 0.00018     | proteolysis                   |
| NM_198129  | Laminin, α3                              | 5.6   | 0.00001     | epidermis development         |
| **Placebo > DHT**                                    |       |             |                 |                               |
| NM_002993  | Chemokine (C-X-C motif) ligand 6          | 12.4  | 0.00005     | chemotaxis                    |
| NM_003186  | Transgelin                                | 10.8  | 0.00013     | muscle organ development      |
| NM_002974  | Serpin peptidase inhibitor, clade B, member 4 | 10.1  | 0.00000     | immune response               |
| NM_001733  | Complement component 1, r subcomponent   | 9.4   | 0.00012     | proteolysis                   |
| NM_005602  | Claudin 11                                | 8.8   | 0.00005     | cell adhesion                 |
| NM_006820  | Interferon-induced protein 44-like        | 8.5   | 0.00001     | immune response               |
| NM_016352  | Carboxypeptidase A4                       | 8.4   | 0.00033     | proteolysis                   |
| NM_003641  | Interferon induced transmembrane protein 1 | 8.0   | 0.00001     | cell surface receptor linked signaling pathway |
| NM_001710  | Complement factor B                       | 7.7   | 0.00000     | proteolysis                   |
| NM_001044391 | Mucin 1, cell surface associated         | 3.1   | 0.00127     | protein binding               |

Relative ratios were determined by comparing the degree of gene expression in conjunctival epithelial cells treated with placebo or DHT. The mean gene intensity level in at least one group was higher than 100 BeadChip units.
The hormone effect was about the same in both cell types, with DHT up- and down-regulating similar percentages of genes (i.e., meibomian: 49.8% ↑; conjunctiva: 44.8% ↑). Examples of genes that showed notable hormone-induced differences in terms of ratios are listed in Table 2 and Table 3. In addition, DHT significantly enhanced the expression of genes encoding mucin 16 (2.2 fold ↑, conjunctiva) and reduced the activity of genes for S100 calcium binding proteins A8 and A9 (2.1 and 1.4 fold ↓, respectively, conjunctiva). Analysis of BeadChip raw data also revealed that DHT caused an 8.0 and 39.7 fold decrease in the mRNA levels of the small proline-rich proteins 2F and 2A, respectively, in meibomian gland epithelial cells.

Genes that demonstrated the greatest alterations in terms of statistical significance included those increased or decreased by DHT in immortalized human meibomian gland (aldo-keto reductase family 1, member C2 ↑, p<0.000001; DNA topoisomerase IIα ↓, p<0.000001), and conjunctival (uridine phosphorylase 1 ↑, p<0.000001; interferon, α-inducible protein 6 ↓, p<0.000001) epithelial cells.

The nature of androgen action on gene expression was predominantly cell-specific. Thus, 61.0 and 53.6% of upregulated genes, and 58.1 and 52.0% of downregulated genes, were unique to the meibomian gland and conjunctival epithelial cells, respectively. In addition, between 12.9 and 20.0% of genes showed opposite effects of DHT on gene expression in immortalized human meibomian gland and conjunctival epithelial cells.

**Table 4. Opposite effects of DHT on gene expression in immortalized human meibomian gland and conjunctival epithelial cells.**

| Cell 1   | Cell 2   | C1 ↑, C2 ↓ (Genes) | % | C1 ↓, C2 ↑ (Genes) | % |
|----------|----------|---------------------|---|---------------------|---|
| Meibomian| Conjunctiva| 199                 | 12.9–14.2 | 255                 | 18.3–20.0 |

Log transformed data were analyzed and the total number of genes with GEO sequence identities in each category was then determined. Gene expression was significantly (p<0.05) up (↑)- or down (↓)-regulated by DHT in the specific cell type. Abbreviations: “C” stands for “cell.”

**Table 5. Effect of DHT on chromosomal gene expression in immortalized human meibomian gland and conjunctival epithelial cells.**

| Chromosome | DHT Genes ↑ | Plac Genes ↑ | DHT z-score | Plac z-score |
|------------|-------------|-------------|-------------|-------------|
| Meibomian gland | | | | |
| 16         | 66          | 53          | 3.35        | 1.34        |
| 19         | 80          | 62          | 2.61        | 0.2         |
| 18         | 34          | 17          | 2.13        | -1.44       |
| 2          | 73          | 95          | -2.23       | 0.21        |
| 17         | 69          | 77          | 1.52        | 2.65        |
| 22         | 25          | 35          | 0.42        | 2.59        |
| 8          | 51          | 32          | -0.08       | -2.83       |
| 13         | 23          | 14          | -1.23       | -2.93       |

Conjunctiva | | | | |
| 16         | 66          | 45          | 4.08        | -0.49       |
| 20         | 51          | 37          | 3.84        | 1.77        |
| 1          | 143         | 147         | 3.51        | 1.77        |
| 19         | 73          | 73          | 2.42        | 0.94        |
| 2          | 32          | 40          | -2.33       | -2.22       |
| 13         | 15          | 19          | -2.39       | -2.37       |
| 3          | 47          | 94          | -2.57       | 1.77        |
| X          | 24          | 46          | -2.63       | -0.19       |
| 4          | 35          | 60          | -2.72       | -0.51       |
| 5          | 34          | 82          | -3.6        | 1.27        |
| 12         | 46          | 92          | -1.53       | 3.15        |
| 17         | 51          | 85          | -0.31       | 2.94        |
| 15         | 34          | 26          | 0.2         | -2.13       |
| 7          | 64          | 44          | 1.51        | -2.51       |
| 2          | 88          | 75          | 0.27        | -2.76       |

Chromosomes with the highest and lowest z-scores were selected after analysis of log-transformed Illumina BeadChip data. A z-score is a statistical rating of the relative expression of genes, and shows how much they are over- or under-represented in a given gene list [59]. Positive z scores represent a greater number of genes meeting the criterion than is expected by chance, whereas negative z scores reflect fewer genes meeting the criterion than expected by chance [59]. Z-scores with values >2.0 or <2.0 are quite significant and are highlighted in bold print. Terms: DHT Genes ↑ - number of genes upregulated in DHT-treated cells; Plac Genes ↑ - number of genes upregulated in placebo-treated cells; z-score - specific score for the upregulated genes in the DHT- and placebo-exposed cells.
20.0% of regulated genes were expressed in the opposite direction in these immortalized cells (Table 4).

The genes regulated by DHT were located on a variety of chromosomes. As shown in Table 5, the cellular pattern of this regulation showed some similarities and dissimilarities.

To confirm in part the Illumina BeadChip results, selected genes were analyzed by qPCR. This experimental approach verified the alterations of all tested genes (Table 6).

Androgen influence on the expression of gene ontologies and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways in human ocular surface and meibomian gland epithelial cells: Androgen administration had a significant impact on the biologic process, molecular function and cellular component gene ontologies, as well as on the KEGG pathways, in human ocular surface and meibomian gland epithelial cells.

As shown in Table 7, DHT significantly increased numerous ontologies in immortalized human meibomian gland epithelial cells, such as those associated with protein metabolism, signaling, tissue development, oxidoreductase and peptidase activities, intracellular organelles and peroxisomes. Treatment with DHT also stimulated 25 different ontologies (with ≥5 genes) concerned with lipid biosynthesis, homeostasis, transport and binding, as well as with cholesterol, fatty acid, phospholipid and steroid dynamics, as we recently reported [2]. In turn, DHT decreased ontologies linked to cell cycle, M phase, DNA metabolic processes, angiogenesis, innate immunity, RNA binding, and ribonucleoprotein complexes. These effects of DHT were paralleled by significant alterations in KEGG pathways. Androgen exposure upregulated pathways related to insulin, mTOR and peroxisome proliferator-activated receptor (PPAR) signaling, and downregulated those involved with the cell cycle, RNA transport and cancer (Table 8).

The influence of DHT on immortalized human conjunctival epithelial cells was quite different than that observed in human meibomian gland epithelial cells. As demonstrated in Table 9, DHT enhanced the expression of genes related to epithelium development, regeneration, wound healing, cell migration, Wnt receptor signaling, antioxidant activity and vacuoles, and reduced those associated with translation, RNA processing, mitotic cell cycle, immune response, JAK-STAT cascades, NADH dehydrogenase activity and ribosomes. In addition, androgen administration stimulated KEGG pathways linked to lysosomes, p53 signaling and endocytosis, and suppressed pathways involved with oxidative phosphorylation, the proteosome and RNA transport (Table 10).

Of interest, some ontologies were increased in both immortalized cell populations, regardless of treatment, such as cell death and apoptosis. In addition, there were ontologies that were decreased by androgens in both immortalized cells, including cellular component biogenesis, cellular location, innate immune response and nucleic acid metabolic processes. However, the majority of changes in gene ontologies and KEGG pathways appeared to be cell-specific.

### Table 6. Confirmation of selected Illumina BeadChip chip results by qPCR.

| Gene                                      | Illumina ratio | qPCR ratio |
|-------------------------------------------|----------------|------------|
| **Meibomian Gland Epithelial Cells**      |                |            |
| DHT>Placebo                               |                |            |
| Keratin 16                                | 2.4            | 5.7        |
| Aldo-keto reductase family 1, member C2   | 2.6            | 1.7        |
| Kallikrein-related peptidase 11           | 1.8            | 2.4        |
| Placebo>DHT                               |                |            |
| CDC28 protein kinase regulatory subunit 2 | 3.1            | 1.5        |
| Minichromosome maintenance component 3    | 3.5            | 19.3       |
| Leupaxin                                  | 3.1            | 57.8       |
| **Conjunctival Epithelial Cells**         |                |            |
| DHT>Placebo                               |                |            |
| Laminin, α3                               | 5.6            | 6.9        |
| Plasminogen activator, urokinase          | 5.3            | 1.1        |
| Uridine phosphorylase 1                   | 4.2            | 2.7        |
| Placebo>DHT                               |                |            |
| Interferon, α-inducible protein 6          | 4.8            | 1.6        |
| Serum amyloid A1                          | 5.7            | 3.1        |
| EGF-containing fibulin-like extracellular matrix protein 1 | 4.9 | 2.5 |

The expression of designated genes, that were shown to be significantly altered in DHT-treated cells by using Illumina BeadChips, were re-examined with qPCR procedures. The qPCR data from meibomian gland cells were standardized to N (α) acetyltransferase B complex 50 and data from conjunctival cells were normalized to myosin, light chain 6, alkali, smooth muscle and non-muscle. Neither of the genes used for standardization responded to DHT exposure. The relative ratios of gene expression in 3 separate experiments are listed in the Illumina and qPCR “Ratio” columns.
Table 7. Influence of DHT on the expression of gene ontologies in human meibomian gland epithelial cells.

| Ontology                                   | DHT Genes ↑ | Plac Genes ↑ | DHT z-score | Plac z-score |
|--------------------------------------------|--------------|--------------|-------------|--------------|
| **Biologic Process**                       |              |              |             |              |
| programmed cell death                      | 136          | 143          | 5.18        | 5.09         |
| oxidation-reduction process                | 95           | 61           | 4.85        | −0.42        |
| protein metabolic process                  | 280          | 278          | 4.35        | 2.85         |
| purine ribonucleotide metabolic process    | 50           | 37           | 3.8         | 0.87         |
| nerve growth factor receptor signaling pathway | 28         | 22           | 3.38        | 1.44         |
| response to hormone stimulus              | 61           | 48           | 3.36        | 0.7          |
| regulation of signaling                    | 132          | 106          | 3.14        | −0.45        |
| tissue development                         | 80           | 69           | 2.58        | 0.46         |
| cell cycle                                 | 75           | 202          | −0.65       | 13.58        |
| M phase                                    | 14           | 107          | −3.29       | 13.34        |
| DNA metabolic process                      | 38           | 118          | −0.87       | 11.17        |
| organelle fission                          | 12           | 81           | −2.41       | 11.94        |
| RNA processing                             | 39           | 111          | −0.49       | 10.47        |
| angiogenesis                               | 21           | 30           | 1.05        | 2.95         |
| immune system process                      | 99           | 122          | 0.86        | 2.66         |
| innate immune response                     | 27           | 41           | −0.08       | 2.28         |
| blood vessel development                   | 30           | 35           | 1.49        | 2.15         |
| regulation of cellular biosynthetic process | 139         | 148          | −2.24       | −2.39        |
| regulation of transcription                | 111          | 119          | −2.26       | −2.33        |
| neurologic system process                  | 59           | 33           | −3.68       | −7.15        |
| **Molecular Function**                     |              |              |             |              |
| protein binding                            | 572          | 707          | 6.41        | 13.12        |
| hydrogen ion transmembrane transporter activity | 21       | 3            | 5.82        | −1.58        |
| oxidoreductase activity, acting on CH-OH group of donors | 23 | 9 | 5.47 | 0.17 |
| catalytic activity                         | 423          | 403          | 5.11        | 2.27         |
| translation factor activity, nucleic acid binding | 16 | 8 | 4.47 | 0.82 |
| peptidase activity                         | 55           | 36           | 3.01        | −0.63        |
| RNA binding                                | 61           | 127          | 1.12        | 10.03        |
| nucleotide binding                         | 164          | 243          | 1.32        | 7.58         |
| ATPase activity                            | 31           | 42           | 1.61        | 3.59         |
| helicase activity                          | 7            | 20           | −0.89       | 3.2          |
| ion channel activity                       | 14           | 12           | −2.51       | −3.15        |
| DNA binding                                | 115          | 149          | −2.82       | −0.43        |
| zinc ion binding                           | 121          | 99           | −0.68       | −3.47        |
| transporter activity                       | 82           | 50           | 0.41        | −3.94        |
| transmembrane receptor activity            | 33           | 26           | −5.76       | −6.98        |
| **Cellular Component**                     |              |              |             |              |
| vacuole                                    | 56           | 20           | 7.82        | −0.56        |
| lysosome                                   | 45           | 20           | 6.54        | 0.19         |
| intracellular organelle                    | 721          | 852          | 5.76        | 11.82        |
| organelle membrane                         | 191          | 152          | 5.26        | 0.86         |
| endoplasmic reticulum                      | 109          | 76           | 4.97        | 0.28         |
| peroxisome                                 | 19           | 4            | 4.85        | −1.23        |
| nuclear part                               | 165          | 335          | −0.05       | 13.77        |
| organelle lumen                            | 195          | 344          | 1.62        | 13.26        |
| ribonucleoprotein complex                  | 37           | 104          | 0.52        | 12.01        |
| chromosome                                 | 37           | 97           | −1.62       | 10.08        |
| spindle                                    | 9            | 47           | −0.99       | 9.87         |
| extracellular space                        | 37           | 57           | −2.23       | 0.31         |
| cell junction                              | 38           | 28           | −0.19       | −2.17        |
| extracellular region                       | 98           | 105          | −3.36       | −3.26        |
| intrinsic to membrane                      | 307          | 246          | −2.55       | −7.7         |

Designated ontologies were selected after the analyses of log-transformed data. Criteria for inclusion were an ontology containing ≥8 genes and having a z-score >2.0 or <−2.0. High and low values for the placebo (Plac) and DHT groups in specific ontologies are highlighted in bold print. Androgen administration also stimulated over 25 different ontologies related to lipid biosynthesis, homeostasis, transport and binding, as previously shown [2].
The present study demonstrates that androgen treatment significantly influences the expression of thousands of genes in immortalized human meibomian gland and conjunctival epithelial cells. The nature of this DHT action is predominantly cell-specific: some androgen responses are shared by both cell types, the majority are unique, and others are completely opposite. Depending upon the cell type, DHT exerts a significant effect on many gene ontologies and KEGG pathways, including those related to lipid dynamics, innate immunity, cell cycle, JAK-stat cascades, oxidative phosphorylation, the proteasome, and mTOR, Wnt and PPAR signaling. Our findings support our hypothesis that androgens regulate gene expression in human meibomian gland and conjunctival epithelial cells.

Our finding that the nature of DHT action on ocular surface and adnexal cells is predominantly cell-specific is not surprising. It is well established that androgen effects are not necessarily the same in different tissues. For example, androgens increase immunoglobulin A (IgA) and secretory component (SC) expression in the lacrimal gland, appear to have no influence on IgA or SC levels in salivary, respiratory, intestinal, uterine or bladder tissues, and actually decrease IgA amounts in the mammary gland [22,23]. In addition, we have found that testosterone induces a 7.8- to 13-fold increase in epidermal growth factor and nerve growth factor mRNA levels in the submandibular gland [24] but has no effect on these factors in the lacrimal gland (unpublished). Conversely, testosterone stimulates the expression of submandibular androgen-repressed protein (SMARP) in the lacrimal gland, but suppresses SMARP levels in the submandibular gland [25]. As another example, androgens promote the angiogenic activity of prostate epithelial cells, but reduce such activity by prostate stromal cells [26]. In effect, the nature of androgen influence is generally cell- and tissue-specific.

Androgen exposure caused a striking impact on gene expression in immortalized human meibomian gland epithelial cells. Most notable were the effects of DHT on lipid- and keratin-related genes. Androgen treatment induced a significant increase in the activity of numerous genes associated with lipogenesis and cholesterol biosynthesis [2]. This hormone response is analogous to the androgen influence on meibomian glands in vivo [27-30], wherein testosterone stimulates many genes linked to lipid metabolic pathways. Androgen administration also led to a 40 fold decrease in the mRNA level of small proline-rich protein 2A (SPPR2A). This gene, which is significantly upregulated in human meibomian gland dysfunction (MGD) [31], encodes a protein that promotes keratinization [32]; keratinization, in turn, is believed to be a primary cause of MGD and the consequent tear film hyperosmolarity and evaporative dry eye [3]. The SPPR2A gene is also significantly downregulated by androgens in meibomian glands of male and female mice [27,28]. These combined DHT effects, increasing lipogenesis and suppressing keratinization, may begin to explain how topical androgens enhance the synthesis and secretion of meibomian gland lipids, prolong the tear film breakup time and alleviate evaporative dry eye disease [32,33]. In addition, these DHT effects may account for why androgen insufficiency (e.g., during anti-androgen treatment, complete androgen insensitivity syndrome and/or aging) is associated with keratinization of the meibomian gland ductal epithelium (i.e., orifice metaplasia), altered meibum lipid profiles, and a reduced quality of meibomian gland secretions [34-38].

Androgen treatment also led to a significant change in the expression of many other genes in immortalized human meibomian gland epithelial cells, such as those associated with steroidogenesis, microbial protection, tissue development, oxidative stress, mTOR and PPAR signaling, cell cycle, innate immunity and angiogenesis. Androgen administration upregulated the mRNA levels of defensin b1, an antimicrobial peptide implicated in epithelial surface resistance to microbial colonization [39], as well as steroid-5α-reductase, α polypeptide 1, which catalyzes the

### Table 8. DHT Impact on KEGG Pathways in Human Meibomian Gland Epithelial Cells.

| KEGG Pathway | DHT Genes ↑ | Plac Genes ↑ | DHT z-score | Plac z-score |
|--------------|-------------|--------------|--------------|--------------|
| Lysosome     | 26          | 7            | 5.76         | -1.06        |
| Oxidative phosphorylation | 21          | 8            | 4.3          | -0.57        |
| Peroxisome   | 16          | 3            | 4.24         | -1.28        |
| Aldosterone-regulated sodium reabsorption | 9          | 2            | 3.41         | -0.83        |
| Insulin signaling pathway | 21          | 9            | 3.35         | -0.32        |
| mTOR signaling pathway | 10          | 3            | 3.18         | -0.67        |
| PPAR signaling pathway | 12          | 3            | 3.01         | 9.94         |
| DNA replication | 1           | 19           | -1.05        | 9.22         |
| Splicosome    | 4           | 39           | -1.93        | 8.41         |
| Cell cycle    | 5           | 36           | -1.53        | 7.22         |
| Mismatch repair | 1           | 11           | -0.51        | 11.22        |
| RNA transport | 12          | 27           | 0.14         | 4.27         |
| p53 signaling pathway | 7           | 15           | 0.79         | 4.03         |
| Small cell lung cancer | 8           | 17           | 0.61         | 3.89         |

Pathways were selected after the analysis of log-transformed data. The criterion for inclusion was a pathway having a z-score >2.0 or <-2.0. High and low values for the placebo (Plac) and DHT groups in specific pathways are highlighted in bold print.

### DISCUSSION

The present study demonstrates that androgen treatment significantly influences the expression of thousands of genes in immortalized human meibomian gland and conjunctival epithelial cells. The nature of this DHT action is predominantly cell-specific: some androgen responses are shared by both cell types, the majority are unique, and others are completely opposite. Depending upon the cell type, DHT exerts a significant effect on many gene ontologies and KEGG pathways, including those related to lipid dynamics, innate immunity, cell cycle, JAK-stat cascades, oxidative phosphorylation, the proteasome, and mTOR, Wnt and PPAR signaling. Our findings support our hypothesis that androgens regulate gene expression in human meibomian gland and conjunctival epithelial cells.

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Androgen treatment also led to a significant change in the expression of many other genes in immortalized human meibomian gland epithelial cells, such as those associated with steroidogenesis, microbial protection, tissue development, oxidative stress, mTOR and PPAR signaling, cell cycle, innate immunity and angiogenesis. Androgen administration upregulated the mRNA levels of defensin b1, an antimicrobial peptide implicated in epithelial surface resistance to microbial colonization [39], as well as steroid-5α-reductase, α polypeptide 1, which catalyzes the
| Ontology                                      | DHT Genes ↑ | Plac Genes ↑ | DHT z-score | Plac z-score |
|----------------------------------------------|-------------|--------------|-------------|--------------|
| Biologic Process                             |             |              |             |              |
| apoptosis                                    | 141         | 152          | 6.33        | 4.91         |
| response to stress                           | 215         | 252          | 5.14        | 4.87         |
| regulation of signal transduction            | 129         | 100          | 5.02        | -0.55        |
| epithelium development                       | 50          | 33           | 4.95        | 0.25         |
| nerve growth factor receptor signaling pathway | 33         | 21           | 4.95        | 0.74         |
| regeneration                                 | 18          | 9            | 4.87        | 0.51         |
| wound healing                                | 61          | 47           | 3.95        | 0.1          |
| cell proliferation                           | 106         | 144          | 3.17        | 5.15         |
| cytokine production                          | 32          | 30           | 2.99        | 1.36         |
| cell migration                               | 53          | 41           | 2.61        | -0.81        |
| cell adhesion                                | 69          | 58           | 2.46        | -0.79        |
| MAPK cascade                                 | 35          | 19           | 2.59        | -1.84        |
| canonical Wnt receptor signaling pathway     | 15          | 14           | 2.32        | 1.18         |
| toll-like receptor 4 signaling pathway       | 10          | 10           | 2.06        | 1.39         |
| translation                                  | 27          | 109          | -0.12       | 13.76        |
| viral reproduction                           | 19          | 103          | -1.64       | 12.88        |
| innate immune response                       | 33          | 68           | 1.36        | 6.69         |
| RNA processing                               | 26          | 93           | -2.41       | 6.63         |
| cell cycle checkpoint                        | 16          | 43           | 0.43        | 6.36         |
| macromolecule metabolic process              | 411         | 583          | 0.7         | 5.9          |
| gene expression                              | 189         | 331          | -2.00       | 5.18         |
| immune response                              | 69          | 103          | 1.66        | 4.27         |
| mitotic cell cycle                           | 54          | 82           | 1.65        | 4.2          |
| JAK-STAT cascade                             | 5           | 14           | 0.27        | 3.85         |
| antigen processing and presentation          | 2           | 12           | -0.98       | 3.53         |
| system process                               | 69          | 64           | -4.59       | -7.03        |
| neurologic system process                    | 57          | 40           | -3.59       | -7.13        |
| regulation of transcription                  | 105         | 117          | -2.41       | -3.68        |
| Molecular Function                           |             |              |             |              |
| protein binding                              | 562         | 709          | 6.93        | 9.48         |
| enzyme inhibitor activity                    | 44          | 22           | 6.2         | -0.08        |
| SH3 domain binding                           | 21          | 9            | 5.25        | 0.05         |
| cell adhesion molecule binding               | 9           | 4            | 3.85        | 0.35         |
| antioxidant activity                         | 10          | 8            | 3.57        | 1.89         |
| catalytic activity                           | 374         | 450          | 2.59        | 2.73         |
| translation initiation factor activity       | 7           | 11           | 2.09        | 3.63         |
| voltage-gated ion channel activity           | 3           | 9            | -2.8        | -1.65        |
| structural constituent of ribosome           | 5           | 69           | -1.71       | 16.86        |
| RNA binding                                 | 32          | 153          | -2.89       | 12.32        |
| threonine-type endopeptidase activity        | 0           | 14           | -1.22       | 9.88         |
| NADH dehydrogenase activity                  | 0           | 16           | -1.56       | 8.37         |
| small GTPase regulator activity              | 22          | 8            | 0.82        | -3.22        |
| zinc ion binding                             | 95          | 98           | -2.84       | -4.69        |
| DNA binding                                 | 103         | 137          | -3.53       | -2.83        |
| G-protein coupled receptor activity          | 8           | 13           | -6.46       | -6.7         |
| Cellular Component                           |             |              |             |              |
| vacuole                                      | 43          | 32           | 5.27        | 1.48         |
| cytoplasm                                    | 642         | 854          | 8.33        | 13.06        |
| intracellular                                | 792         | 1059         | 5.01        | 11.05        |
| cell surface                                 | 43          | 30           | 3.99        | 0.03         |
| lysosome                                     | 34          | 32           | 4.2         | 2.45         |
| intracellular organelle part                 | 389         | 592          | 2.42        | 9.7          |
| organelle                                    | 631         | 924          | 2.15        | 11.22        |
| intrinsic to membrane                        | 293         | 281          | -2.3        | -7.61        |
| ribonucleoprotein complex                    | 18          | 128          | -2.64       | 14.84        |
| ribosome                                     | 6           | 68           | -1.74       | 14.78        |
| mitochondrion                                | 80          | 224          | -0.25       | 13.3         |
| membrane part                                | 361         | 366          | -1.26       | -6.2         |

Specific ontologies were selected after the analyses of log-transformed data. Criteria for inclusion in the Table were an ontology containing ≥8 genes and having a z-score >2.0 or <-2.0. High and low values for the placebo (Plac) and DHT groups in designated ontologies are highlighted in bold print.
conversion of testosterone into the more potent androgen, DHT [39]. This steroid regulation appears to be a form of feed-forward control exerted by DHT on its own biosynthesis [40]. Androgen increased the gene expression of leptin receptor, involved in the regulation of fat metabolism, glucose homeostasis, wound healing and the immune system [39]; FOXO1, a transcription factor that mediates cell responses to oxidative stress [39] and is known to interact with androgen receptors [41]; and stearoyl-CoA desaturase, an iron-containing enzyme that catalyzes the synthesis of unsaturated fatty acids. Testosterone enhances stearoyl-CoA desaturase mRNA levels in mouse male and female meibomian glands [27,28], and the targeted disruption of this rate-limiting enzyme causes meibomian gland atrophy [42]. Androgen exposure also increased ontologies and pathways related to peroxisomes, which are organelles involved in metabolism of fatty acids and other metabolites [39]; PPAR, which may promote tissue differentiation [43,44]; and mTOR, a serine/threonine protein kinase that may modulate cell growth, cell proliferation, cell motility, cell survival, protein synthesis and transcription [39,45,46], and is also activated by androgens in the prostate [47]. Androgen administration downregulated genes related to cell cycle regulation (e.g., ubiquitin-conjugating enzyme E2C, cyclin-dependent kinase 1 and cyclin B2), innate immunity (e.g., chemokine (C-X-C motif) ligand 5 and thrombospondin 1) [39,48] and angiogenesis (e.g., thrombospondin 1). Thrombospondin 1 mRNA content is also decreased by androgens in the prostate, bladder and breast cancer cells [49-52]. Also notable was the DHT suppression of gene expression for matrix metalloproteinase 9, an enzyme that is increased in the tear film in dry eye and is known to promote corneal inflammation [53].

The effect of DHT on immortalized human conjunctival epithelial cells was quite different than that observed in human meibomian gland epithelial cells. For example, androgen administration enhanced the expression of genes involved in epithelium development, regeneration, wound healing and cell migration (e.g., matrix metalloproteinase, kallikrein-related peptidases 5, 6 & 11, cystatin E/M, laminin, α3), and suppressed those related to the immune response (e.g., chemokine (C-X-C motif) ligand 6, serpin peptidase inhibitor, clade B, member 4, complement component 1, r subcomponent, interferon-induced protein 44-like, interferon induced transmembrane protein, complement factor B) and mitotic cell cycle (e.g., sepin 4, endothelin 1, F-box protein 6 and proteasome subunit, β type, 9). The decrease in immune-related gene activity may play a role in the reported androgen ability to alter the development of allergic conjunctivitis [5] and to attenuate the immune effect of lipopolysaccharide in both conjunctival and meibomian gland epithelial cells [54]. The downregulation of conjunctival genes associated with the cell cycle, which was also found in immortalized human meibomian gland epithelial cells, may reflect a hormone-induced bias toward cell differentiation as compared to proliferation. Androgens are also known to inhibit the cell cycle in other tissues [55-57].

Of particular interest was the DHT upregulation of mucin 16 (MUC16), and downregulation of mucin 1 (MUC1), gene expression in the conjunctival epithelial cells. These transmembrane mucins help to prevent pathogen penetration into the eye and to maintain a wet ocular surface phenotype [18]. The mucin gene intensities in our study were relatively low, especially for MUC16. This finding may reflect the fact that we cultured cells in serum-free media: exposure of conjunctival epithelial cells to serum, which leads to their stratification, has been reported to promote mucin expression [18]. It is possible that the lack of serum may also have influenced the nature of the MUC1 response to DHT. Thus, others have shown that androgen increases MUC1 expression when breast and prostate cell lines are cultured in serum [58]. This observation would be consistent with the decreased MUC1 levels found in conjunctiva an individual with complete androgen insensitivity syndrome [12]. We are currently investigating whether the presence or absence of serum causes significant variations in the molecular biologic
response of ocular surface and adnexal cells to androgen administration.

Ultimately, it is very important to demonstrate that cellular responses in vitro duplicate those in vivo. Such demonstrations, as we have recently done with androgens and the meibomian gland [2,27-31,34-38], may provide new and meaningful insight into the regulation of ocular surface cells in health and disease.

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REFERENCES

1. Sullivan DA. Tearful relationships? Sex, hormones and aqueous-deficient dry eye. Ocul Surf 2004; 2:92-123. [PMID: 17216082]

2. Liu S, Khandelwal P, Hatton M, Sullivan DA. Culture, immortalization and characterization of human meibomian gland epithelial cells. Invest Ophthal Vis Sci 2010; 51:3993-4005. [PMID: 20335607]

3. Knop E, Knop N, Millar T, Obata H, Sullivan DA. The International Workshop on Meibomian Gland Dysfunction: Report of the Subcommittee on Anatomy, Physiology, and Pathophysiology of the Meibomian Gland. Invest Ophthal Vis Sci 2011; 52:1938-78. [PMID: 21450915]

4. Sullivan DA. Ocular mucosal immunity. In: Ogra PL, Mestecky J, Muecksch FF, Stites DP, Stollar BD, editors. Handbook of Mucosal Immunology. 2nd Edition. Orlando, FL: Academic Press, 1999. p.1241–1281.

5. Saruya S. Studies on allergic conjunctivitis. Effects of castration and sex hormone administration on experimental allergic conjunctivitis. Nippon Ganka Gakkai zasshi 1968; 72:833-45. [PMID: 5749618]

6. Hiwatari S. Protein anabolic steroids in ophthalmology. Ber Zusammenkunft Dtsc Ophthalmol Ges 1964; 65:424-6. [PMID: 14260569]

7. Schumacher H, Machemer R. Experimental investigations on the treatment of corisone lesions of the cornea. Klin Mbl Augenheilk 1966; 148:121-6. [PMID: 427217]

8. Sullivan DA, Wickham LA, Krenzer KL, Rocha EM, Toda I. Aqueous tear deficiency in SJögren’s syndrome: Possible causes and potential treatment. In: Pleyer U, Hartmann C, Sterry W, editors. Oculardermal Diseases - Immunology of Bullous Oculo-Muco-Cutaneous Disorders. Buren, The Netherlands: Aelours Press, 1997. p.95–152.

9. Tsai TH, Schieving LE, Schieving LA, Paul J. Sex differences in circadian rhythms of several variables in lymphorecticular organs, liver, kidney, and corneal epithelium in adult CD2F1 mice. Anat Rec 1985; 211:263-70. [PMID: 2581477]

10. Zeligs MA, Gordon K. Dehydroepiandosterone therapy for the treatment of dry eye disorders. Int Patent Application WO 94/04155, March, 1994.

11. Sullivan DA, Wickham LA, Rocha EM, Krenzer KL, Sullivan BD, Steagall R, Cermak JM, Davis MR, Ullman MD, Sato EH, Gao J, Rocha FJ, Ono M, Silveira LA, Lambert RW, Kelleher RS, Tolls BD, Toda I. Androgens and dry eye in SJögren’s syndrome. Ann N Y Acad Sci 1999; 876:312-24. [PMID: 10415627]

12. Mantelli F, Moretti C, Micera A, Bonini S. Conjunctival mucus deficiency in complete androgen insensitivity syndrome (CAIS). Graefes Arch Clin Exp Ophthalmol 2007; 245:899-902. [PMID: 1712009]

13. Hildebrandt PG. Experience in local anabolic therapy of corneal diseases. Med Monatsschr 1974; 28:359-60. [PMID: 4437474]

14. Yamamoto T, Terada N, Nishizawa Y, Petrov T. Angiostatic activities of medroxyprogesterone acetate and its analogues. Int J Cancer 1994; 56:393-9. [PMID: 7508892]

15. Schirra F, Suzuki T, Dickinson DP, Townsend DJ, Gipson IK, Sullivan DA. Identification of steroidogenic enzyme mRNAs in the human lacrimal gland, meibomian gland, cornea and conjunctiva. Cornea 2006; 25:438-42. [PMID: 16670482]

16. Wickham LA, Gao J, Toda I, Rocha EM, Ono M, Sullivan DA. Identification of androgen, estrogen and progesterone receptor mRNAs in the eye. Acta Ophthalmol Scand 2000; 78:146–53. [PMID: 10794246]

17. Rocha EM, Wickham LA, Silveira LA, Krenzer KL, Yu FS, Toda I, Sullivan BD, Sullivan DA. Identification of androgen receptor protein and 5α-reductase mRNA in human ocular tissues. Br J Ophthalmol 2000; 84:76-84. [PMID: 10611104]

18. Gipson IK, Spurr-Michaud S, Argüeso P, Tisdale A, Ng TF, Russo CL. Mucin gene expression in immortalized human corneal-limbal and conjunctival epithelial cell lines. Invest Ophthal Vis Sci 2003; 44:2496-506. [PMID: 12766048]

19. Khandelwal P, Liu S, Sullivan DA. Dihydrotestosterone regulation of androgen receptor mRNA in human ocular surface epithelial cells. ARVO Annual Meeting; 2009 May 3-7; Fort Lauderdale (FL).

20. MAQC Consortium. Shi L, Reid LH, Jones WD, Shippy R, Warrington JA, Baker SC, Collins PJ, de Longueville F, Kawasaki ES, Lee KY, Luo Y, Sun YA, Willey JC, Setterquist RA, Fischer GM, Tong W, Dragan YP, Dij D, Fruhe FW, Goodsoad FM, Herman D, Jensen RV, Johnson CD, Lobenhoffer EK, Puri RK, Schirra F, Suzuki T, Kipps T, Thompson KL, Townsend DJ, Gipson IK, Trujillo ME, Jackman RW, Yue H, Brown BD, Brunner A, Canales R, Cao XM, Cebula TA, Chen JJ, Cheng J, Chu TM, Chudin E, Corson J, Corton JC, Croner LJ, Davies C, Davisson TS, Delenstrarr G, Deng X, Dorris D, Eklund AC, Fan XH, Fang H, Fulmer-Smentek S, Fuscoe JC, Gallagher K, Ge W, Guo L, Guo X, Hager J, Haje PK, Han J, Han T, Harbottle HC, Harris SC, Hatchwell E, Hauser CA, Hester S, Hong H, Hurban P, Jackson SA, Jiao H, Knight CR, Hester J, Harbottle HC, Harris SC, Hatchwell E, Hester S, Hong H, Hurban P, Jackson SA, Ji H, Knight CR, Kuo WP, LeClerc JE, Levy S, Li QZ, Liu C, Liu Y, Lombardi MJ, Ma Y, Magnuson SR, Marqson B, McDaniel T, Mei N, Myklebost O, Ning B, Novoradovskaya N, Orr MS, Osborn TW, Papallo A, Patterson TA, Perkins RG, Peters EH, Peterson R, Philips KL, Pine PS, Pusztai L, Qian F, Ren H, Rosen M, Rosenzweig BA, Samaha RR, Schena M, Schroth GP, Shchegrova S, Smith DD, Stauderler F, Su Z, Sun H, Szallasi Z, Tezak Z, Thierry-Mieg D, Thompson KL, Tikhonova I, Turpaz Y, Vallanat B, Varn C, Wang CR, Wang Y, Wang W, Walfinger R, Wang A, Wu J, Xiao C, Xie Q, Xu J, Yang W, Zhang L, Zhang S, Zong Y, Slikker W Jr. The MicroArray Quality Control (MAQC) project shows inter-
24. Treister NS, Richards SM, Jensen RV, Sullivan DA. Influence of androgens on the ocular secretory system. Immunol Invest 1988; 17:183-94. [PMID: 3410512]

23. Weisz-Carrington P, Roux ME, McWilliams M, Phillips Quagliata JM, Lamm ME. Hormonal induction of the secretory immune system in the mammary gland. Proc Natl Acad Sci USA 1978; 75:2928-32. [PMID: 275864]

22. Sullivan DA, Hann LE, Vaerman JP. Selectivity, specificity and kinetics of the androgen regulation of the ocular secretory immune system. Immunol Invest 1988; 17:183-94. [PMID: 3410512]

21. Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, Davis AP, Dolinski K, Dwight SS, Eppig JT, Harris MA, Hill DP, Issel-Tarver L, Kasarskis A, Lewis S, Matesec JC, Richardson JE, Ringwald M, Rubin GM, Sherlock G. Gene ontology: tool for the unification of biology. The Gene Ontology Consortium. Nat Genet 2000; 25:25-9. [PMID: 1080265]

20. Sullivan DA, Hann LE, Vaerman JP. Selectivity, specificity and kinetics of the androgen regulation of the ocular secretory immune system. Immunol Invest 1988; 17:183-94. [PMID: 3410512]

19. Sullivan DA, Hann LE, Vaerman JP. Selectivity, specificity and kinetics of the androgen regulation of the ocular secretory immune system. Immunol Invest 1988; 17:183-94. [PMID: 3410512]

18. Sullivan DA, Hann LE, Vaerman JP. Selectivity, specificity and kinetics of the androgen regulation of the ocular secretory immune system. Immunol Invest 1988; 17:183-94. [PMID: 3410512]

17. Sullivan DA, Hann LE, Vaerman JP. Selectivity, specificity and kinetics of the androgen regulation of the ocular secretory immune system. Immunol Invest 1988; 17:183-94. [PMID: 3410512]

16. Sullivan DA, Hann LE, Vaerman JP. Selectivity, specificity and kinetics of the androgen regulation of the ocular secretory immune system. Immunol Invest 1988; 17:183-94. [PMID: 3410512]

15. Sullivan DA, Hann LE, Vaerman JP. Selectivity, specificity and kinetics of the androgen regulation of the ocular secretory immune system. Immunol Invest 1988; 17:183-94. [PMID: 3410512]

14. Sullivan DA, Hann LE, Vaerman JP. Selectivity, specificity and kinetics of the androgen regulation of the ocular secretory immune system. Immunol Invest 1988; 17:183-94. [PMID: 3410512]

13. Sullivan DA, Hann LE, Vaerman JP. Selectivity, specificity and kinetics of the androgen regulation of the ocular secretory immune system. Immunol Invest 1988; 17:183-94. [PMID: 3410512]

12. Sullivan DA, Hann LE, Vaerman JP. Selectivity, specificity and kinetics of the androgen regulation of the ocular secretory immune system. Immunol Invest 1988; 17:183-94. [PMID: 3410512]

11. Sullivan DA, Hann LE, Vaerman JP. Selectivity, specificity and kinetics of the androgen regulation of the ocular secretory immune system. Immunol Invest 1988; 17:183-94. [PMID: 3410512]

10. Sullivan DA, Hann LE, Vaerman JP. Selectivity, specificity and kinetics of the androgen regulation of the ocular secretory immune system. Immunol Invest 1988; 17:183-94. [PMID: 3410512]

9. Sullivan DA, Hann LE, Vaerman JP. Selectivity, specificity and kinetics of the androgen regulation of the ocular secretory immune system. Immunol Invest 1988; 17:183-94. [PMID: 3410512]

8. Sullivan DA, Hann LE, Vaerman JP. Selectivity, specificity and kinetics of the androgen regulation of the ocular secretory immune system. Immunol Invest 1988; 17:183-94. [PMID: 3410512]

7. Sullivan DA, Hann LE, Vaerman JP. Selectivity, specificity and kinetics of the androgen regulation of the ocular secretory immune system. Immunol Invest 1988; 17:183-94. [PMID: 3410512]

6. Sullivan DA, Hann LE, Vaerman JP. Selectivity, specificity and kinetics of the androgen regulation of the ocular secretory immune system. Immunol Invest 1988; 17:183-94. [PMID: 3410512]

5. Sullivan DA, Hann LE, Vaerman JP. Selectivity, specificity and kinetics of the androgen regulation of the ocular secretory immune system. Immunol Invest 1988; 17:183-94. [PMID: 3410512]

4. Sullivan DA, Hann LE, Vaerman JP. Selectivity, specificity and kinetics of the androgen regulation of the ocular secretory immune system. Immunol Invest 1988; 17:183-94. [PMID: 3410512]

3. Sullivan DA, Hann LE, Vaerman JP. Selectivity, specificity and kinetics of the androgen regulation of the ocular secretory immune system. Immunol Invest 1988; 17:183-94. [PMID: 3410512]

2. Sullivan DA, Hann LE, Vaerman JP. Selectivity, specificity and kinetics of the androgen regulation of the ocular secretory immune system. Immunol Invest 1988; 17:183-94. [PMID: 3410512]

1. Sullivan DA, Hann LE, Vaerman JP. Selectivity, specificity and kinetics of the androgen regulation of the ocular secretory immune system. Immunol Invest 1988; 17:183-94. [PMID: 3410512]
50. Johnson AM, O'Connell MJ, Miyamoto H, Huang J, Yao JL, Messing EM, Reeder JE. Androgenic dependence of exophytic tumor growth in a transgenic mouse model of bladder cancer: a role for thrombospondin-1. BMC Urol 2008; 8:7. [PMID: 18433501]

51. Mattila MM, Tarkkonen KM, Seppänen JA, Ruohola JK, Valve EM, Härkönen PL. Androgen and fibroblast growth factor 8 (FGF8) downregulation of thrombospondin 1 (TSP1) in mouse breast cancer cells. Mol Cell Endocrinol 2006; 253:36-43. [PMID: 16723184]

52. Colombel M, Filleur S, Fournier P, Merle C, Guglielmi J, Courtin A, Degeorges A, Serre CM, Bouvier R, Clézardin P, Cabon F. Androgens repress the expression of the angiogenesis inhibitor thrombospondin-1 in normal and neoplastic prostate. Cancer Res 2005; 65:300-8. [PMID: 15665307]

53. Li DQ, Pflugfelder SC. Matrix metalloproteinases in corneal inflammation. Ocul Surf 2005; 3:S198-202. [PMID: 17216119]

54. Sahin A, Kam WR, Rahimi Darabad R, Topilow K, Sullivan DA. Regulation of leukotriene B4 secretion by human corneal, conjunctival and meibomian gland epithelial cells. Arch Ophthalmol. 2012 In press

55. Pradeep PK, Li X, Peegel H, Menon KM. Dihydrotestosterone inhibits granulosa cell proliferation by decreasing the cyclin D2 mRNA expression and cell cycle arrest at G1 phase. Endocrinology 2002; 143:2930-5. [PMID: 12130558]

56. Heisler LE, Evangelou A, Lew AM, Trachtenberg J, Elsholtz HP, Brown TJ. Androgen-dependent cell cycle arrest and apoptotic death in PC-3 prostatic cell cultures expressing a full-length human androgen receptor. Mol Cell Endocrinol 1997; 126:59-73. [PMID: 9027364]

57. de Launoit Y, Dauvois S, Dufour M, Simard J, Labrie F. Inhibition of cell cycle kinetics and proliferation by the androgen 5 alpha-dihydrotestosterone and antiestrogen N,n-butyln-methyl-11-[16' alpha-chloro-3',17 beta-dihydroxy-esta-1', 3',5'-tetraene-7 alpha-y] undecanamide in human breast cancer ZR-75–1 cells. Cancer Res 1991; 51:2797-802. [PMID: 2032219]

58. Mitchell S, Abel P, Madaan S, Jeffs J, Chaudhary K, Stamp G, Lalani el-N. Androgen-dependent regulation of human MUC1 mucin expression. Neoplasia 2002; 4:9-18. [PMID: 11922395]

59. Doniger SW, Salomonis N, Dahlquist KD, Vranizan K, Lawlor SC, Conklin BR. MAPPFinder: using Gene Ontology and GenMAPP to create a global gene-expression profile from microarray data. Genome Biol 2003; 4:R7. [PMID: 12540299]