Gene Expression Profiles of Human Trabecular Meshwork Cells Induced by Triamcinolone and Dexamethasone

Bao Jian Fan,[1,2,3] *Dan Yi Wang,*[1,2,3] *Clement Chee Yung Tham,*[1] *Dennis Shun Chiu Lam,*[1] *and Chi Pui Pang*[1]

Purpose. Triamcinolone acetonide (TA) and dexamethasone (DEX) are corticosteroids commonly used for ocular inflammation, but both can cause ocular hypertension. In this study, the differential gene expression profile of human trabecular meshwork (TM) cells in response to treatment by TA in comparison with DEX was investigated.

Methods. Total RNA was extracted from cultured human TM cells treated with TA or DEX and used for microarray gene expression analysis. The microarray experiments were repeated three times. Differentially expressed genes were identified by an empiric Bayes approach and confirmed by real-time quantitative PCR.

Results. TA (0.1 mg/mL) treatment resulted in 15 genes upregulated and 12 genes downregulated, whereas 1 mg/mL TA resulted in 36 genes upregulated and 21 genes downregulated. These genes were mainly associated with acute-phase response, cell adhesion, cell cycle and growth, growth factor, ion binding, metabolism, proteolysis and transcription factor. Two genes, *MYOC* and *GAS1*, were upregulated, and three genes, *SENP1*, *ZNF343*, and *SOX30*, were downregulated by both TA and DEX treatment. Eight differentially expressed genes were located in known primary open-angle glaucoma (POAG) loci, including *MYOC*, *SOAT1*, *CYP27A1*, *SPOCK*, *SEMA6A*, *EGR1*, *GAS1*, and *ATP10A*.

Conclusions. Differential gene expression profiles of human TM cells treated by TA and DEX, and a dosage effect by TA, were revealed by microarray technology. TA and DEX treatment shared several differentially expressed genes, suggesting a common mechanism to cause ocular hypertension. Some differentially expressed genes located in the known POAG loci are potential candidates for glaucoma genes. (*Invest Ophthalmol Vis Sci.* 2008;49:1886–1897) DOI:10.1167/iovs.07-0414

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Corticosteroids such as triamcinolone acetonide (TA) and dexamethasone (DEX) are potent and effective modalities available for the treatment of ocular inflammation.[1,2] TA is a sustained-release crystalline form of cortisone, which may provide intraocularly available cortisone for a longer period than soluble cortisone.[3,4] TA crystals can be injected directly to the vitreous, and soluble TA can still be detected in the aqueous humor for 9 months or longer after intravitreous injection of 25 mg of TA.[5] However, TA treatment very often induces elevation in intraocular pressure (IOP), which is a risk factor for the development of glaucoma.[6,7] DEX is a widely used topical ocular anti-inflammatory drug,[2] but it can also cause an increase in IOP and subsequently, glaucoma.[8] Corticosteroids like TA and DEX are believed to decrease outflow of aqueous humor by inhibiting degradation of extracellular matrix (ECM) material in the trabecular meshwork (TM), leading to aggregation of an excessive amount of the material within the outflow channels and a subsequent increase in outflow resistance.[9,10] Ultimately, the elevated IOP increases the risk of optic nerve fiber damage indistinguishable from those associated with primary open-angle glaucoma (POAG).[2] Investigation into the molecular mechanism of this form of secondary ocular hypertension or glaucoma would provide new insights into the etiology of POAG.

Cultured human TM cells share many properties with human TM cells in situ[11] and are commonly used to study the biological effects of corticosteroids. Study of the prolonged biological effects of DEX on human TM cells in culture has helped to identify the first glaucoma gene, *MYOC*.[12,13] In recent years, microarray technology has been successfully used to identify changes in gene expression profiles of human TM cells in culture in response to DEX treatment.[14–17] There are both consistencies and differences in the gene expression profiles obtained in these studies. We have previously used a microarray with 2400 genes to study the effects of DEX on gene expression in human TM cells.[16] In the present study, we investigated for the first time the differential gene expression profile of human TM cells in response to TA in comparison with DEX. Microarrays containing 41,421 cDNA probes were used to study TA and DEX effects in parallel.

Methods

Cell Culture and Treatment

A human TM cell line was established from trabecular specimens obtained postmortem from a 52-year-old male Caucasian patient with no personal or family history of glaucoma.[11] The tissue was obtained and managed in conformity with the Declaration of Helsinki. The cells were grown in Dulbecco's modified Eagle's medium (DMEM), supplemented with 10% fetal bovine serum, 100 U/mL penicillin, and 100 μg/mL streptomycin sulfate and maintained in a humidified 5% CO_2 environment at 37°C. All culture reagents were obtained from Invitrogen Corp. (Carlsbad, CA). The eighth-passage cells at 80% confluence were used for corticosteroid treatment. The TA (Kenacort-A; Bristol-Myers-Squibb, New York, NY) dosage concentrations, 0.1 mg/mL and

TABLE 1. Intron-spanning Primers Used for RT-qPCR

Gene	GenBank	Primers	Amplicon (bp)
GAPDH	NM_002046	F: ACCACAGTCCATGCCATCAC R: TCCACCACCCTGTTGCTGTA	451
MYOC	NM_000261	F: CATCTGGCTATCTCAGGAGTGG R: TCTCGGCATCCACACACCATAC	124
GAS1	L13698	F: AAAGTCTTCAACGGGCTGCGCT R: TCCTTGACCGACTCGCAGATGG	137
SERPINA3	NM_001085	F: TCAACGACTACGTGAAGAATGG R: CCTTGACTGATGAGTATCTTGG	165
HIPK2	AF208291	F: ATGACCATGAAGCAGAGACAGG R: TCAGCATCTTCTTCAACAGGTC	171
SCD	Y13647	F: TTATGTCTCTGCTACACTTGGG R: GATGAGCTCCTGCTGTTATGC	140
MTIX	X76717	F: GCTCCTGCAAATGCAAAGAGTG R: AGCTGCACTTGTCTGACGTCC	136
IGFBP2	M35410	F: AGAAGGTCACTGAGCAGCACC R: ACCTGGTCCAGTTCCTGTTGGC	124
HNT	AF126426	F: TGCATAGCAACTGGTAGACCAG R: ACGTCATTGGAGGCACTGCACT	152
SENP1	AF149770	F: ATAGGATGAGGATGGATGCTGG R: TTGTGGAACATGTAAAAGATCGG	182
IGFBP3	M31159	F: CAGCGCTACAAAGTTGACTACG R: CAGGTGATTCAGTGTGTCTTCC	134
OPTN	AF061034	F: GTTGGAAGCGAAGTGGAAGCACTG R: TTTCGTTTCAAGGGCCTGAC	138
WDR36	AF385437	F: TTCTGTTCCACAGGATATCTGC R: TGTGCCAAATAATAAGAATGCC	206
CYP1B1	U03688	F: ACCGGCCACTATCACTGACATC R: ATCCAATTCTGCCTGCACTCG	115
APOE	M12529	F: CTGGGTCGCTTTTGGGATTACC R: TAGGCCTTCAACTCCTTCATGG	134
ANGPTL7	NM_021146	F: TGCCATCTACGACTGCTCTTCC R: GCTTGTACTGCTTCCAGTCCC	200
PEDF	U29953	F: AGCATTCTCCTTCTCGGTGTGG R: ACAAGAATTGCTTGAACCTGGG	187
APOD	NM_001647	F: CATTTCATCTTGGGAAGTGCCC R: TTCCATTAGTGAGTAGTTGGCC	141
TAGLN	NM_003186	F: TCTTCAAGCAGATGGAGCAGG R: TTCTTGGTCACTGCCAAGCTGC	163

F, forward; R, reverse.

1 mg/mL, were derived from reported experiments and clinical practice.[4,18] Intravitreous injection of 1 mg/mL TA has been widely applied in clinical practice in the treatment of various posterior segment diseases.[4] TA 0.1 mg/mL was used because the TA is less concentrated in aqueous humor after intravitreous injection. The vehicle, 0.0025% and 0.025% benzyl alcohol (BA; Sigma-Aldrich Chemie GmbH, Munich, Germany), was used as the control. The cells were maintained for 12 hours before harvesting. The time for TA treatment was determined by a parallel study on two selected index genes, *MYOC* and *GAS1*, which had been reported to be consistently upregulated in TM cells by DEX treatment.[14–17] Briefly, the human TM cells after exposure to TA (0.1 mg/mL, 1 mg/mL) or BA (0.0025%, 0.025%) were collected at 0, 10, 20, 30, 50, and 80 minutes and 2, 12, 24 and 48 hours for RNA extraction. The ratios of gene expression levels at each time point against those at 0 minutes were normalized with *GAPDH*. All experiments were performed in triplicate by RT-qPCR. An unpaired *t*-test was applied to compare the changes in gene expression between TA and BA treatment.

For DEX treatment, 100 nM DEX (Weimer Pharma GmbH, Rastatt, Germany) was used as in previous studies.[16,19] The vehicle, 1.1 μM BA (Sigma-Aldrich Chemie GmbH) was used as the control. The cells were maintained for 7 days before harvesting. The time for DEX treatment was based on clinical practice.[14] During DEX treatment, the medium was changed every other day. Three replicates consisting of individual sets of treated and control cells were prepared for each treatment ($n = 3$).

RNA Extraction

The human TM cells after TA or DEX treatment were harvested and homogenized using a shredder column (QIA column; Qiagen, Hilden, Germany). Total RNA was extracted (RNeasy mini kit; Qiagen) and RNA yield determined with a spectrophotometer (model ND-1000; NanoDrop Technologies, Wilmington, DE). The RNA quality was assessed by the ratio of ribosomal bands 28S and 18S (Gel Doc 2000 System; Bio-Rad Laboratories, Hercules, CA). The yield of total RNA extracted from TA- or DEX-treated human TM cells were comparable with that from control cells treated with benzyl alcohol, ranging from 67 to 233 μg RNA/plate (0.1 mg/mL TA: 104 $\pm$ 17 μg RNA/plate, $n = 3$; 0.0025% BA vehicle: 117 $\pm$ 47 μg RNA/plate, $n = 3$; 1 mg/mL TA: 134 $\pm$ 12 μg RNA/plate, $n = 3$; 0.025% BA vehicle: 167 $\pm$ 40 μg RNA/plate, $n = 3$; 100 nM DEX: 147 $\pm$ 52 μg RNA/plate, $n = 3$; 1.1 μM BA vehicle: 198 $\pm$ 55 μg RNA/plate, $n = 3$). The quality of RNA was also consistent among the samples, with rRNA ratio (28S/18S) ranging between 1.8 and 2.0. Thus, all RNA preparations in this study were deemed satisfactory for hybridization to the microarrays.

Microarray Experiments and Data Analysis

All the microarray experiments were performed in our laboratory. Coated human cDNA microarrays[20,21] (UltraGAPS; Stanford Functional Genomics Facility, Stanford, CA; http://www.microarray.org/sfgf/jsp/home.jsp) containing 41,421 cDNA probes representing 22,904 unique Unigene gene clusters (Unigene Build Number 173; http://www.ncbi.nlm.nih.gov/UniGene; provided in the public domain by the Na-

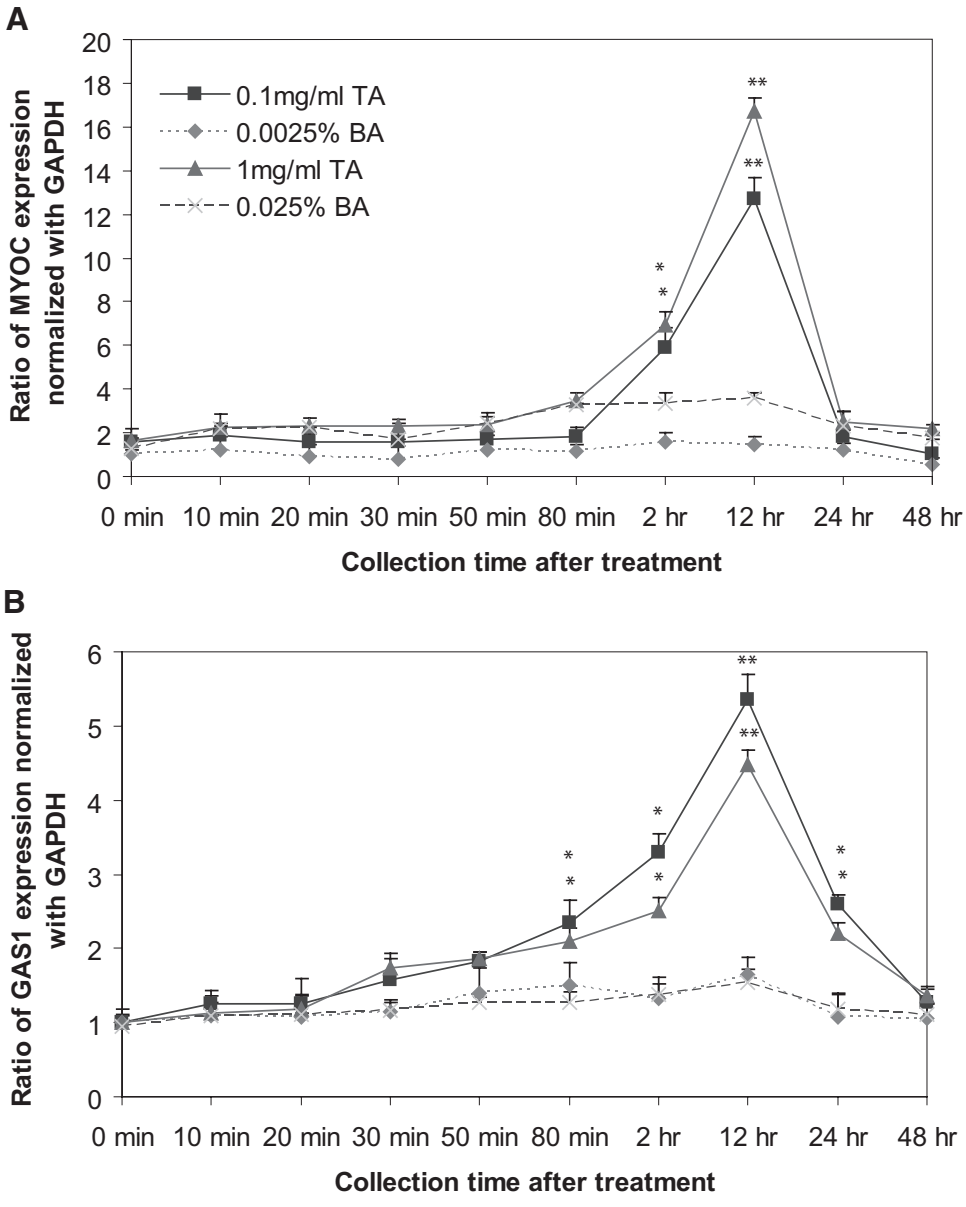

FIGURE 1. Changes in gene expression of *MYOC* (**A**) and *GAS1* (**B**) after TA treatment. The ratios of gene expression levels at each time point against those at 0 minutes were normalized with *GAPDH*. All experiments were performed in triplicate by RT-qPCR. Unpaired *t*-test was used to compare the gene expression changes between TA and BA treatment. $^{*}P < 0.05$; **(horizontal) $P < 0.01$; **(vertical) significant gene expression observed in both TA concentrations ($P < 0.05$).

tional Center for Biotechnology Information, Bethesda, MD) were used. The cDNA probes were derived from IMAGE (Integrated Molecular Analysis of Genomes and their Expression) Consortium clones from the Research Genetics Sequence Verified clone set (http://www.invitrogen.com/content/sfs/manuals/sequenceverifiedclones_man.pdf) and CGAP (Cancer Genome Anatomy Project, National Cancer Institute, Bethesda, MD) clone set (http://cgap.nci.nih.gov/Genes/PurchaseReagents; National Institutes of Health, Bethesda, MD). At the time of our experiments this array represented the largest number of genes compared with other type of arrays (e.g., U133A; Affymetrix, Santa Clara, CA). It was not capable of differentiating between splice variants. However, it was more sensitive than other oligonucleotide-based microarrays, because it possessed longer probes and more genes. The same lot of microarrays (print batch: SHEW) was used for all microarray experiments. An equal amount of total RNA obtained from TM cells treated with TA or DEX and their controls was reverse transcribed into cDNA and labeled with Cy3 and Cy5 respectively (CyScribe Post-Labeling kit; GE Healthcare, Amersham, UK). The first step involved the incorporation of amino allyl-dUTP during cDNA synthesis using an optimized nucleotide mix, and the second step involved chemically labeling the amino allyl-modified cDNA (CyDye NHS-ester; GE Healthcare). The labeled cDNA was hybridized to the microarray at 42°C for 16 hours, and scanned (ScanArray 4000 scanner; Packard Biochip Technologies, Billerica, MA).

Image acquisition and raw signal intensity extraction were performed (ScanArray ver. 2.1 and QuantArray ver. 3.0, respectively; Packard Biochip Technologies). The fluorescent images were visually inspected to flag and exclude the abnormal spots with irregular shape or dirt. To be consistent across arrays and under different experimental conditions, 62 (0.15%) abnormal spots found in any arrays were excluded from all subsequent analyses. Data analysis was performed using the Bioconductor (http://www.bioconductor.org)[22] statistics package Limma (ver. 2.7.9; http://bioinf.wehi.edu.qu/limma)[23] implemented on R ver. 2.3.1 for windows (http://www.r-project.org). The print-tip loess normalization method was used for within-array normalization. The quantile normalization method was used for between-array normalization.[24] Differentially expressed genes were identified by an empiric Bayes approach.[25] The B-statistic is the log-odds that a gene is differentially expressed that has been adjusted for multiple testing. We based our gene selection on a B-statistic >2, meaning that these were >100 times more likely to be differentially expressed than to remain unaffected. To be comparable with published data, the probability was computed based on the distribution of the moderated *t*-statistics. The probability was adjusted for multiple testing using the FDR method.[26]

TABLE 2. Differentially Expressed Genes in Human TM Cells Induced by 0.1 mg/mL TA

Gene Symbol	Gene Name	GenBank	Change (*x*-fold)	B-Statistic	*P*	Gene Function*	TA 0.1 mg/mL	TA 1 mg/mL	DEX
Upregulated Genes (n = 15)									
MYOC	Myocilin	NM_000261	9.86	9.58	0.000015	9	+	+	+
MT2A	Metallothionein 2A	J00271	3.89	9.38	0.000016	5	+	+	
GAS1	Growth arrest-specific 1	L13698	3.76	9.60	0.000014	3	+	+	+
MT1G	Metallothionein 1G	J03910	3.71	8.77	0.000028	5	+	+	
CSNK1G2	Casein kinase 1, gamma 2	U89896	3.19	6.21	0.00044	9	+	+	
MT1F	Metallothionein 1F	M10943	3.15	6.32	0.00040	5	+	+	
HIPK2	Homeodomain interacting protein kinase 2	AF208291	3.06	6.00	0.00051	3	+		+
SF1	Splicing factor 1	Y08765	2.98	6.15	0.00044	7	+	+	
MT1L	Metallothionein 1L	X76717	2.90	5.50	0.00091	5	+	+	
IRF7	Interferon regulatory factor 7	U73036	2.80	4.68	0.0022	7	+	+	
SERP1NA3	Serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 3	NM_001085	2.65	4.50	0.0027	1	+		+
AGXT	Alanine-glyoxylate aminotransferase	AJ292204	2.50	3.60	0.0068	8	+	+	
EDNRA	Endothelin receptor type A	NM_001957	2.48	3.16	0.011	9	+		
DNA2L	DNA replication helicase, yeast, homolog of	D42046	2.35	2.33	0.027	9	+	+	
MED6	Mediator of RNA polymerase II transcription, subunit 6 homolog (*S. cerevisiae*)	AF074723	2.33	2.57	0.021	7	+	+	
Downregulated Genes (n = 12)									
SENP1	SUMO1/sentrin specific peptidase 1	AF149770	−33.33	24.73	3.2×10^{-16}	6	+	+	+
ZNF343	Zinc finger protein 343	BC065009	−16.67	21.94	6.1×10^{-14}	7	+	+	+
SOX30	Transcription factor SOX-30	AB022083	−7.14	10.98	2.6×10^{-6}	7	+	+	+
HNT	Neurotrimin precursor	AF126426	−5.88	12.33	4.8×10^{-7}	2	+	+	
FOS	v-Fos FBJ murine osteosarcoma viral oncogene homolog	V01512	−3.92	8.76	0.000028	7	+	+	
MARVELD2	MARVEL domain containing 2	NM_001038603	−3.91	8.73	0.000028	9	+		
SPRY1	Sprouty homolog 1, antagonist of FGF signaling (*Drosophila*)	AY192146	−3.57	6.68	0.00029	9	+	+	
TCF7L2	Transcription factor 7-like 2 (T-cell specific, HMG-box)	NM_030756	−3.33	6.51	0.00033	7	+		+
TREH	Trehalase (brush-border membrane glycoprotein)	NM_007180	−2.63	4.44	0.0028	9	+	+	
COL13A1	Collagen, type XIII, alpha 1	NM_080815	−2.57	3.79	0.0056	2	+		
IRF2	Interferon regulatory factor 2	X15949	−2.33	2.08	0.034	7	+		
CD44	CD44 molecule (Indian blood group)	L05423	−2.27	2.28	0.028	2	+	+	
Housekeeping Gene and Other Reported Glaucoma-related Genes									
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase	NM_002046	−1.05	−3.86	0.98				
OPTN	Optineurin	AF061034	−1.05	−2.12	0.32				
WDR36	WD repeat protein 36	AF385437	−1.04	−2.38	0.36				
CYP1B1	Cytochrome P450 1B1	U03688	1.28	−2.03	0.25				
APOE	Apolipoprotein E precursor	M12529	−1.27	−2.06	0.26				

Genes are sorted in terms of multiples of change. +, Genes differentially expressed in various conditions.

* Gene functions are classified into nine categories according to biological process: 1, acute-phase response; 2, cell adhesion; 3, cell cycle and growth; 4, growth factor; 5, ion binding; 6, proteolysis; 7, transcription factor; 8, metabolism; and 9, others.

Genes with statistically significant difference were classified into gene families using the Ingenuity Pathways Knowledge Base database (Ingenuity Systems Inc., Mountain View, CA). Genes absent from the Ingenuity database were classified according to their reported functions or using the databases of Gene Ontology (http://www.geneontology.org/) or SwissProt (http://srs.ebi.ac.uk/srsbin/cgi-bin/wgetz).

Real-Time Quantitative PCR

Gene-specific RT-qPCR was used to confirm the differentially expressed genes identified from the microarray experiments. First-strand cDNA for RT-qPCR was synthesized from 500 ng of RNA using random primer p[dN]6 (Roche Diagnostics, Mannheim, Germany) and a reverse transcriptase kit with RNase inhibitor (Superscript III Reverse Transcriptase kit and RNase OUT inhibitor; Invitrogen). The amount of cDNA corresponding to 25 ng of RNA was amplified with intron-spanning primers (Table 1). RT-qPCR was performed using SYBR green PCR master mix (Bio-Rad Laboratories), according to the manufacturer's instructions, on a sequence detection system (prism 7000; Applied Biosystems, Inc. [ABI], Foster City, CA). The thermocycler parameters were 95°C for 2 minutes, 40

TABLE 3. Differentially Expressed Genes in Human TM Cells Induced by 1 mg/mL TA

Gene Symbol	Gene Name	GenBank	Change (*x*-fold)	B Statistic	*P*	Gene Function*	TA 0.1 mg/mL	TA 1 mg/mL	DEX
Upregulated Genes (n = 36)									
MYOC	Myocilin	NM_000261	12.60	22.36	5.8×10^{-12}	9	+	+	+
MT1G	Metallothionein 1G	J03910	5.44	21.45	1.1×10^{-10}	5	+	+	
CASP4	Caspase 4	Z48810	5.12	19.57	7.0×10^{-10}	6		+	
SEMA6A	Semaphorin 6A	NM_020796	4.43	15.25	6.3×10^{-8}	9		+	
MED6	Mediator of RNA polymerase II transcription, subunit 6 homolog (*S. cerevisiae*)	AF074723	4.33	14.58	1.2×10^{-7}	7	+	+	
MT1L	Metallothionein 1L	X76717	4.09	13.08	4.8×10^{-7}	5	+	+	
MT2A	Metallothionein 2A	J00271	3.97	12.25	1.1×10^{-6}	5	+	+	
IRF7	Interferon regulatory factor 7	U73036	3.87	11.60	2.0×10^{-6}	7	+	+	
AGXT	Alanine-glyoxylate aminotransferase	AJ292204	3.80	11.15	3.0×10^{-6}	8	+	+	
DNA2L	DNA replication helicase, yeast, homolog of	D42046	3.80	11.13	3.0×10^{-6}	9	+	+	
SF1	Splicing factor 1	Y08765	3.69	10.39	6.2×10^{-6}	7	+	+	
MT1F	Metallothionein 1F	M10943	3.59	9.72	0.000012	5	+	+	
PLXNC1	Plexin C1	AF030339	3.57	9.62	0.000012	2		+	
SCD	Stearoyl-CoA desaturase	Y13647	3.53	9.32	0.000016	5		+	
GAS1	Growth arrest-specific 1	L13698	3.39	8.37	0.000038	3	+	+	+
MT1K	Metallothionein 1K	/	3.33	7.96	0.000058	5		+	
MT1E	Metallothionein 1E	M10942	3.27	7.56	0.000089	5		+	
MT1H	Metallothionein 1H	X64834	3.22	7.16	0.00012	5		+	
RNF41	Ring finger protein 41	AF077599	3.21	7.13	0.00012	5		+	
MT1X	Metallothionein 1X	X76717	3.05	6.01	0.00041	5		+	+
RPP14	ribonuclease P 14kDa subunit	AF077599	2.95	5.34	0.00075	9		+	
ATP10A	ATPase, class V, type 10A	AB051358	2.92	5.11	0.00091	9		+	
PRF1	Perforin 1	X13224	2.91	5.06	0.00094	5		+	
KCND1	Potassium voltage-gated channel, Shal-related subfamily, member 1	AF166003	2.78	4.16	0.0024	5		+	
B4GALNT1	Beta-1,4-*N*-acetylgalactosaminyl-transferase 1	NM_001478	2.76	4.02	0.0028	9		+	
DSIPI	Delta sleep-inducing peptide, immunoreactor	CR533450	2.75	3.95	0.0030	9		+	
OSBP	Oxysterol binding protein	M86917	2.73	3.81	0.0033	8		+	
ZNF263	Zinc finger protein 263	D88827	2.66	3.33	0.0055	7		+	
B3GNT6	Beta-1,3-*N*-acetylglucosaminyl-transferase 6	AF029893	2.66	3.29	0.0056	9		+	
SOAT1	Sterol O-acyltransferase 1	BC028940	2.61	2.98	0.0076	8		+	
SCNN1D	Sodium channel, nonvoltage-gated 1, delta	U38254	2.61	2.92	0.0079	5		+	
CSNK1G2	Casein kinase 1, gamma 2	U89896	2.58	2.73	0.0092	9	+	+	
VWF	von Willebrand factor	X04385	2.55	2.51	0.011	2		+	
PEX6	Peroxisomal biogenesis factor 6	U56602	2.54	2.43	0.012	9		+	
CGI-07	Glutamate [NMDA] receptor subunit 3A precursor	AJ416950	2.53	2.41	0.012	9		+	
ALDH5A1	Aldehyde dehydrogenase 5 family, member A1	Y11192	2.49	2.13	0.016	9		+	
Downregulated Genes (n = 21)									
SENP1	SUMO1/sentrin specific peptidase 1	AF149770	−11.11	52.08	6.8×10^{-25}	6	+	+	+
ZNF343	Zinc finger protein 343	BC065009	−8.33	38.04	1.3×10^{-18}	7	+	+	+
FOS	v-fos FBJ murine osteosarcoma viral oncogene homolog	V01512	−4.55	16.53	1.7×10^{-8}	7	+	+	
IGFBP3	Insulin-like growth factor binding protein 3	M31159	−4.00	13.04	4.8×10^{-7}	4		+	+
HNT	Neurotrimin precursor	AF126426	−3.70	10.89	3.7×10^{-6}	2	+	+	
EPHA4	EPH receptor A4	NM_004438	−3.57	10.05	8.6×10^{-6}	9		+	
PALLD	Palladin	NM_016081	−3.44	8.69	0.000030	2		+	
CD44	CD44 molecule (Indian blood group)	L05423	−3.45	8.49	0.000034	2	+	+	
SOX30	Transcription factor SOX-30	AB022083	−3.23	7.32	0.00011	7	+	+	+
SFRP2	Secreted frizzled-related protein 2	AF311912	−3.13	6.62	0.00021	3		+	

(*continues*)

TABLE 3 (*continued*). Differentially Expressed Genes in Human TM Cells Induced by 1 mg/mL TA

Gene Symbol	Gene Name	GenBank	Change (*x*-fold)	B Statistic	*P*	Gene Function*	TA 0.1 mg/mL	TA 1 mg/mL	DEX
Downregulated Genes (n = 21)									
TREH	Trehalase (brush-border membrane glycoprotein)	NM_007180	−3.03	5.99	0.00041	9	+	+	
DCBLD2	Discoidin, CUB and LCCL domain-containing protein 2	AF387547	−3.03	5.87	0.00045	3		+	
SPRY1	Sprouty homolog 1, antagonist of FGF signaling (*Drosophila*)	AY192146	−3.03	5.65	0.00055	9	+	+	
EGR1	Early growth response 1	X52541	−2.94	5.16	0.00088	7		+	
PLK2	Polo-like kinase 2 (*Drosophila*)	AF059617	−2.86	4.47	0.0018	9		+	
NEK7	Never in mitosis gene a-related kinase 7	AB062450	−2.70	3.72	0.0037	9		+	
TRIM21	Tripartite motif-containing 21	U01882	−2.63	3.14	0.0066	5		+	
SCG2	Secretogranin II (chromogranin C)	M25756	−2.63	2.97	0.0076	5		+	
JUNB	Jun B proto-oncogene	M29039	−2.56	2.54	0.0085	7		+	
ELK3	ELK3, ETS-domain protein	Z36715	−2.50	2.06	0.017	7		+	
STC2	Stanniocalcin-2 precursor	AF055460	−2.50	2.02	0.017	8		+	
Housekeeping Gene and Other Reported Glaucoma-related Genes									
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase	NM_002046	1.09	−3.62	0.91				
OPTN	Optineurin	AF061034	−1.19	−2.78	0.52				
WDR36	WD repeat protein 36	AF385437	1.09	−2.07	0.27				
CYP1B1	Cytochrome P450 1B1	U03688	1.19	−2.35	0.38				
APOE	Apolipoprotein E precursor	M12529	−1.30	−2.01	0.24				

Genes are sorted in terms of multiples of change. +, genes differentially expressed in various conditions.

* Gene functions are classified into nine categories according to biological process: 1, acute-phase response; 2, cell adhesion; 3, cell cycle and growth; 4, growth factor; 5, ion binding; 6, proteolysis; 7, transcription factor; 8, metabolism; and 9, others.

cycles of 95°C for 15 seconds, and 60°C for 1 minute. All PCR reactions were performed in triplicate.

Relative quantification of gene expression was performed using the standard curve method (User Bulletin 2; Prism 7000 Sequence Detection System; ABI) and normalized to the housekeeping gene *GAPDH* expression level. Mean C_t (threshold cycle: the cycle at which the increase in signal associated with exponential growth of PCR product was first detected) of the TA or DEX treated sample was compared to that of the untreated control sample by using the C_t of *GAPDH* as an internal control. ΔC_t was calculated as the difference in C_t values derived from the target gene (in each sample assayed) and the *GAPDH* gene, while $\Delta\Delta C_t$ represented the difference between the paired samples. The changes (*x*-fold) of expression level for upregulated genes were expressed as $2^{-\Delta\Delta Ct}$, whereas those for downregulated genes were expressed as $-2^{\Delta\Delta Ct}$. All data were expressed as the mean ± SD.

Replication from a Different TM Cell Line Treated with DEX

Gene expressions induced by DEX were further studied in a second TM cell line from a different source.[27] It was obtained from a 56-year-old male Caucasian patient with no personal or family history of glaucoma. After treatment by 100 nM DEX for 7 days, 19 genes were investigated by RT-qPCR (Table 1), including an additional four genes previously reported to be highly upregulated by DEX, *ANGPTL7*,[17] *PEDF*,[14,15] *APOD*,[15,17] and *TAGLN*.[16,17] The conditions for cell culture, DEX treatment, RNA extraction and RT-qPCR were the same as those for the first TM cell line.

RESULTS

Determination of the Time for TA Treatment

Compared with the vehicles, both 0.1- and 1-mg/mL TA treatments induced expression of *MYOC* at 2 hours ($P < 0.05$) and reached the highest expression at 12 hours ($P < 0.01$; Fig. 1A). Similarly, both TA concentrations induced expression of *GAS1* at 80 minutes and 2 hours ($P < 0.05$) and reached the peak expression at 12 hours ($P < 0.01$). Elevated expression of *GAS1* remained significant at 24 hours ($P < 0.05$; Fig. 1B). Our results showed that both *MYOC* and *GAS1* were most upregulated at 12 hours in both TA concentrations. Although the time for their highest gene expression may not be the same for other genes, this time point should be appropriate and relevant, since they were reported to be consistently upregulated in TM cells by DEX treatment.[14–17] We therefore used 12 hours for TA treatment in the present study.

Differentially Expressed Genes in 0.1 and 1 mg/mL TA

The human TM cells treated with 0.1 mg/mL TA resulted in a significant expression level change in 27 genes (B-statis-

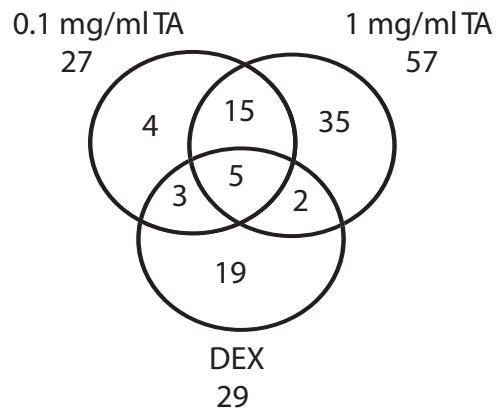

FIGURE 2. Venn diagrams showing the differentially expressed genes between 0.1 mg/mL TA, 1 mg/mL TA, and DEX treatments.

TABLE 4. Differentially Expressed Genes in Human TM Cells Induced by DEX

Gene Symbol	Gene Name	GenBank	Change (*x*-fold)	B-Statistic	*P*	Gene Function*	TA 0.1 mg/mL	TA 1 mg/mL	DEX
Upregulated Genes (n = 14)									
MYOC	Myocilin	NM_000261	10.62	3.86	0.0023	9	+	+	+
GAS1	Growth arrest-specific 1	L13698	8.60	3.47	0.0025	3	+	+	+
SAA2	Serum amyloid A2	NM_030754	8.55	2.52	0.0082	1			+
SERPINA3	Serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 3	NM_001085	4.24	3.45	0.0021	1	+		+
HIPK2	Homeodomain interacting protein kinase 2	AF208291	4.10	2.74	0.0077	3	+		+
IGFBP2	Insulin-like growth factor binding protein 2	M35410	4.08	2.00	0.019	4			+
SAA1	Serum amyloid A1	M23699	3.69	2.34	0.0093	1			+
S100A12	S100 calcium binding protein A12	X97859	2.85	2.26	0.0096	5			+
DDIT4	DNA-damage-inducible transcript 4-like	NM_019058	2.70	2.37	0.0091	9			+
DACH2	Dachshund homolog 2 (*Drosophila*)	AF428101	2.66	2.06	0.015	7			+
MT1X	Metallothionein 1X	X76717	2.62	2.42	0.0086	5		+	+
DNMT3L	DNA (cytosine-5-)-methyltransferase 3-like	AF194032	2.51	2.28	0.0095	5			+
CYP27A1	Cytochrome P450, family 27, subfamily A, polypeptide 1	M62401	2.50	2.22	0.0099	5			+
SUPT3H	Suppressor of Ty 3 homolog (*S. cerevisiae*)	AF064804	2.36	2.12	0.011	3			+
Downregulated Genes (n = 15)									
SENP1	SUMO1/sentrin specific peptidase 1	AF149770	−14.93	2.81	0.0052	6	+	+	+
ZNF343	Zinc finger protein 343	BC065009	−11.36	3.56	0.0027	7	+	+	+
SOX30	Transcription factor SOX-30	AB022083	−8.85	3.45	0.0022	7	+	+	+
TCF7L2	Transcription factor 7-like 2	Y11306	−5.43	2.43	0.0085	7	+		+
IL1B	Interleukin 1, beta	K02770	−5.18	3.49	0.0024	3			+
CCL2	Chemokine (C-C motif) ligand 2	M24545	−3.70	2.91	0.0072	9			+
SPOCK	Sparc/osteonectin, cwcv and kazal-like domains proteoglycan (testican) 1	X73608	−3.34	2.31	0.0094	5			+
IGFBP3	Insulin-like growth factor binding protein 3	M31159	−2.97	2.51	0.0083	4		+	+
ESM1	Endothelial cell-specific molecule 1	X89426	−2.93	2.75	0.0075	4			+
CSPG2	Chondroitin sulfate protcoglycan 2 (versican)	X15998	−2.92	2.70	0.0072	2			+
LAMA4	Laminin, alpha 4	S78569	−2.80	2.35	0.0092	2			+
PLAU	Plasminogen activator, urokinase	X02419	−2.63	2.01	0.018	6			+
THBS2	Thrombospondin 2	L12350	−2.62	2.32	0.0093	2			+
IER3	Immediate early response 3	S81914	−2.57	2.16	0.010	9			+
ANGPT1	Angiopoietin 1	U83508	−2.54	2.30	0.0095	9			+
Housekeeping Gene and Other Reported Glaucoma-related Genes									
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase	NM_002046	−1.05	−3.69	0.94				
OPTN	Optineurin	AF061034	−1.31	−2.46	0.51				
WDR36	WD repeat protein 36	AF385437	−1.12	−2.12	0.33				
CYP1B1	Cytochrome P450 1B1	U03688	1.34	−2.09	0.26				
APOE	Apolipoprotein E precursor	M12529	1.63	−2.04	0.27				

Genes are sorted in terms of multiples of change. +, Genes differentially expressed in various conditions.

* Gene functions are classified into 9 categories according to biological process: 1, acute-phase response; 2, cell adhesion; 3, cell cycle and growth; 4, growth factor; 5, ion binding; 6, proteolysis; 7, transcription factor; 8, metabolism; and 9, others.

tic > 2; Table 2). Among them, 15 genes were upregulated with changes multiples ranging from 2.33- to 9.86-fold, whereas 12 genes were downregulated, with changes ranging from −2.27- to −33.33-fold. The *MYOC* gene was the most upregulated gene, increased by 9.86-fold. The housekeeping gene and the other four glaucoma-related genes were not differentially expressed (B-statistic < −2). TA (1 mg/mL) caused a significant expression level change in 57 genes (B-statistic > 2; Table 3). Among them, 36 genes were upregulated ranging from 2.49- to 12.60-fold, whereas 21 genes were downregulated from −2.50- to −11.11-fold. *MYOC* was upregulated by the largest change 12.60-fold. The housekeeping gene and other four glaucoma-related genes were not differentially expressed (B-statistic < −2).

Intriguingly, when compared to 0.1 mg/mL TA, 1 mg/mL TA resulted in an additional 24 genes upregulated and 13 genes

TABLE 5. Validation of Microarray Gene Expression by RT-qPCR

	0.1 mg/mL TA		1 mg/mL TA		DEX		
Gene Symbol	**Microarray**	**RT-qPCR**	**Microarray**	**RT-qPCR**	**Microarray**	**RT-qPCR**	**RT-qPCR***
Upregulated Genes							
MYOC	9.86	11.69	12.60	14.92	10.62	12.81	15.49
GAS1	3.76	5.28	3.39	3.94	8.60	9.97	13.86
SERPINA3	2.65	5.05			4.24	10.33	8.34
HIPK2	3.06	4.47			4.10	5.50	7.81
IGFBP2					4.08	3.05	6.25
SCD			3.53	4.08	1.56		1.68
MT1X			3.05	3.12	2.62	1.94	3.16
Downregulated Genes							
SENP1	−33.33	−13.78	−11.11	−7.15	−14.93	−7.21	−20.24
HNT	−5.88	−2.11	−3.70	−1.72	−1.24		−1.12
IGFBP3			−4.00	−5.62	−2.97	−3.20	−4.48
Housekeeping Gene and Other Nonregulated Genes							
GAPDH	−1.05	1.00	1.09	1.00	−1.05	1.00	1.00
OPTN	−1.05	−1.04	−1.19	−1.02	−1.31	−1.14	−1.27
WDR36	−1.04	−1.33	1.09	1.25	−1.12	−1.18	−1.25
CYPIB1	1.28	1.36	1.19	1.35	1.34	1.79	1.56
APOE	−1.27	−1.03	−1.30	−1.25	1.63	1.84	1.52
ANGPTL7					1.11		1.38
PEDF					1.42		1.47
APOD					1.35		1.51
TAGLN					1.26		1.34

Numbers are average multiples of change of gene expression ($n = 3$).
* For the replication study of a different cell line treated with DEX.

downregulated. On the contrary, only three upregulated genes (*HIPK2*, *SERPINA3*, and *EDNRA*) and four downregulated genes (*MARVELD2*, *TCF7L2*, *COL13A1*, and *IRF2*) were induced by 0.1 mg/mL TA, but not by 1 mg/mL TA. This suggests a strong dosage effect of TA on gene expression of human TM cells (Fig. 2, Tables 2, 3).

Differentially Expressed Genes in DEX

DEX resulted in a significant change in expression levels of 29 genes (B-statistic > 2; Table 4): 14 genes upregulated at 2.36- to 10.62-fold and 15 genes downregulated at −2.54- to −14.93-fold. *MYOC* was the most upregulated gene at 10.62-fold. The housekeeping gene and the other four glaucoma-related genes were not differentially expressed (B-statistic < -2).

Differentially Expressed Genes Induced by Both TA and DEX

Five genes were differentially expressed at both concentrations (0.1 and 1 mg/mL) of TA and DEX (Fig. 2): two upregulated genes (*MYOC* and *GAS1*) and three downregulated genes (*SENP1*, *ZNF343*, and *SOX30*). Another three genes (*SERPINA3*, *HIPK2*, and *TCF7L2*) were differentially expressed at 0.1 mg/mL TA and DEX. Two other genes (*MT1X* and *IGFBP3*) were differentially expressed in 1 mg/mL TA and DEX (Fig. 2). Notably, the up- or downregulations for these genes were consistent in TA or DEX (Tables 2, 3, 4). Twenty genes were differentially expressed in both 0.1 and 1 mg/mL TA: 12 upregulated genes (*MYOC*, *MT2A*, *GAS1*, *MT1G*, *CSNK1G2*, *MT1F*, *SF1*, *MT1L*, *IRF7*, *AGXT*, *DNA2L*, and *MED6*) and 8 downregulated genes (*SENP1*, *ZNF343*, *SOX30*, *HNT*, *FOS*, *SPRY1*, *TREH*, and *CD44*; Tables 2, 3).

RT-qPCR Confirmation of Differentially Expressed Genes

To confirm the microarray results, we chose a subset of 10 genes for validation by RT-qPCR. These genes were either highly upregulated (*MYOC*, *GAS1*, *SERPINA3*, *HIPK2*, *SCD*, *MT1X*, and *IGFBP2*) or highly downregulated (*HNT*, *SENP1*, and *IGFBP3*) in the microarray analysis. In addition, four glaucoma-related genes (*OPTN*, *WDR36*, *CYP1B1*, and *APOE*) were also included for RT-qPCR.[28] Consistent results were obtained (Table 5, Fig. 3). Microarray experiments demonstrated that the housekeeping gene *GAPDH* had no significant change in expression between treated TM cells and control cells under various conditions (changes were −1.05-, 1.09-, and −1.05-fold in 0.1 mg/mL TA–, 1 mg/mL TA–, and DEX-treated TM cells, respectively; B-statistic < -3.6).

Consistent results were obtained from replicating DEX induction effects on gene expressions in a different TM cell line. All eight differentially expressed genes due to DEX treatment in the first TM cell line were also upregulated (*MYOC*, *GAS1*, *SERPINA3*, *HIPK2*, *MT1X*, and *IGFBP2*) or downregulated (*SENP1* and *IGFBP3*). The other 10 genes with expression that was not affected by DEX treatment in the first cell line (*SCD*, *HNT*, *OPTN*, *WDR36*, *CYP1B1*, *APOE*, *ANGPTL7*, *PEDF*, *APOD*, and *TAGLN*) were also not differentially expressed in the second cell line (Table 5).

DISCUSSION

In the present study, the differential gene expression profile of human TM cells in response to TA was investigated for the first time. We found expressions of several genes affected both by TA and DEX treatment. The microarray results were confirmed by RT-qPCR. The microarray raw data have been submitted to NCBI Gene Expression Omnibus (GEO, series accession number: GSE6298).

The genes affected by TA could be grouped into nine categories according to their functions: acute-phase response, cell adhesion, cell cycle and growth, growth factor, ion binding, metabolism, proteolysis, transcription factors, and others. Such diversity is consistent with the existence of numerous

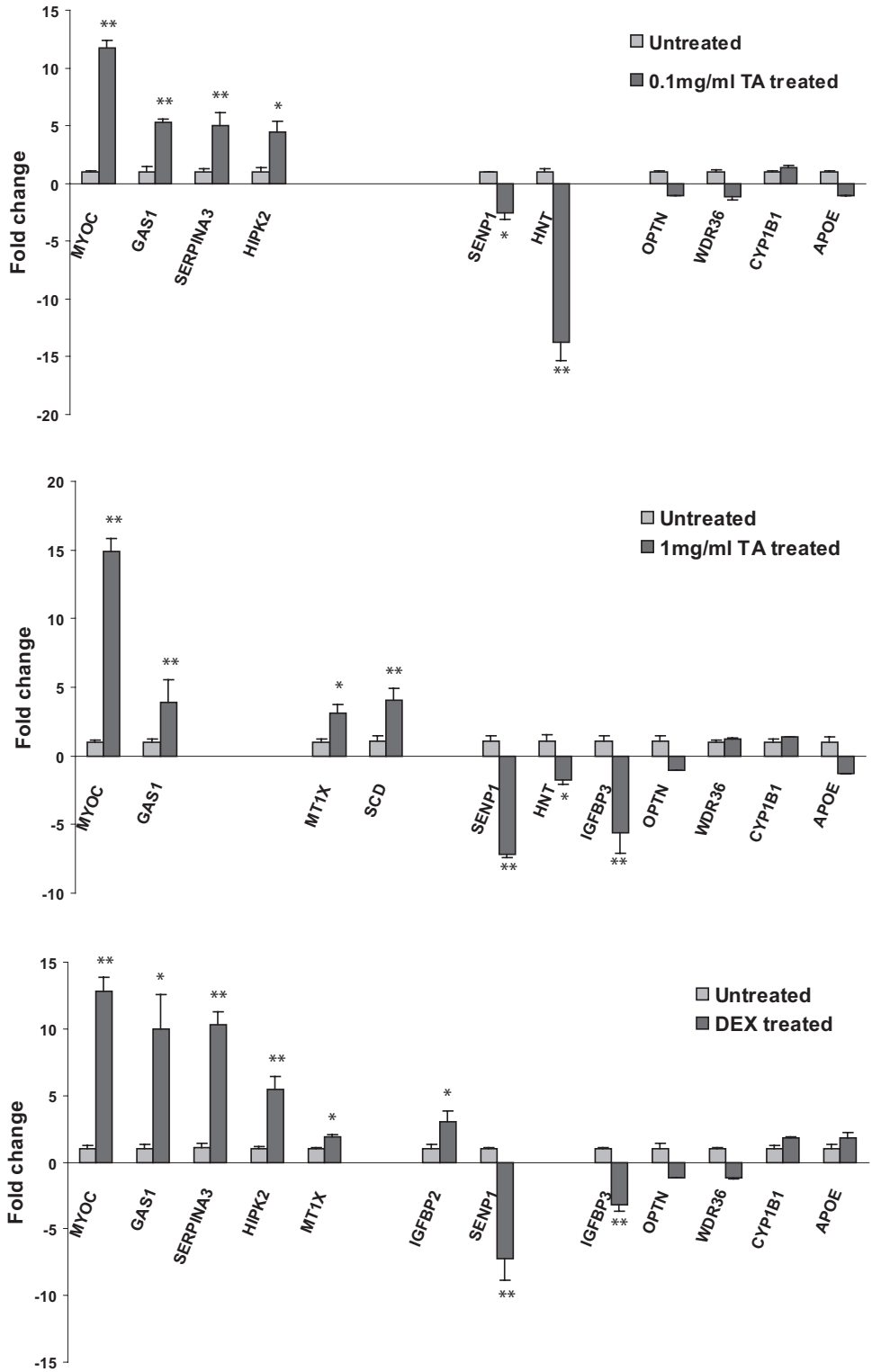

FIGURE 3. RT-qPCR confirmation of expression changes for selected genes between treated and untreated human TM cells. The data were normalized with *GAPDH*, and the changes were calculated. $^{*}P < 0.05$; $^{**}P < 0.01$ (*t*-test, $n = 3$).

regulatory mechanisms in the human TM in response to biochemical disturbances.[29] Some of these genes are potentially associated with ocular hypertension and subsequently glaucoma. They added to the pool of putative genes for glaucoma. Many genes attributed to glaucoma are still to be identified, since mutations in all known glaucoma genes can only account for no more than 10% of patients with glaucoma.[28]

GAS1, *HIPK2*, *DCBLD2*, and *SFRP2* are involved in cell cycle and growth. *GAS1* encodes an integral membrane protein and suppresses cell proliferation by blocking entry to the S-phase.[30] GAS1 may interact with integrins and modify the attachment of cells to the ECM.[31] Therefore its upregulation may inhibit cell proliferation, raise cell adhesion, and hence increase the outflow resistance of aqueous humor. Intriguingly, *GAS1* was downregulated in human TM cells by treatment with transforming growth factor (TGF)-β1[32] and with overexpression of MYOC.[33] TGF-β1 is a cytokine that alters ECM metabolism, and excess ECM has been shown to increase aqueous outflow resistance in the TM of glaucomatous eyes.[34] However, the gene expression of *TGFB1* was not altered by treat-

TABLE 6. Differentially Expressed Genes Located in Known POAG Loci

Gene Symbol	Gene Name	POAG Loci	TA 0.1 mg/mL	TA 1 mg/mL	DEX
MYOC	Myocilin	1q21-q31 (*GLC1A*)	+	+	+
SOAT1	Sterol O-acyltransferase 1	1q21-q31 (*GLC1A*)		+	
CYP27A1	Cytochrome P450, family 27, subfamily A, polypeptide 1	2q33-q34			+
SPOCK	Sparc/osteonectin, cwcv and kazal-like domains proteoglycan (testican) 1	5q22.1-q32 (*GLC1M*)			+
SEMA6A	Semaphorin 6A	5q22.1-q32 (*GLC1M*)		+	
EGR1	Early growth response 1	5q22.1-q32 (*GLC1M*)		+	
GAS1	Growth arrest-specific 1	9q22 (*GLC1J*)	+	+	+
ATP10A	ATPase, class V, type 10A	15q11-q13 (*GLC1I*)		+	

+, Genes differentially expressed in various conditions.

ment with TA or DEX in the present study. On the other hand, *MYOC* was the most upregulated gene by TA or DEX treatment, whereas *GAS1* was also upregulated in our study. This is consistent with previous reports.[15,16] Although *MYOC* and *GAS1* are most likely simultaneously involved in regulation of IOP, the underlying mechanism is unclear. *HIPK2* encodes a conserved serine/threonine nuclear kinase that interacts with homeodomain transcription factors and inhibits cell growth.[35] HIPK2 is an upstream protein kinase for PAX6 modulating PAX6-mediated transcriptional regulation which involves in organogenesis of the eye and central nervous system.[36] However, its role in increasing IOP remains to be elucidated.

A group of genes encoding metallothioneins (MTs) were upregulated by TA: *MT1E*, *MT1F*, *MT1G*, *MT1H*, *MT1K*, *MT1L*, *MT1X*, and *MT2A*. But only *MT1X* was upregulated by DEX. MTs are a large family of proteins playing multiple roles including binding of toxic metals, free radical scavenging, and oxidative stress.[37] In the TM, MTs are upregulated by DEX treatment[13] and elevated IOP.[38]

We also found that TA affects upregulation of *OSBP*, *SOAT1*, *AGXT*, and downregulation of *STC2*, which regulate steroid hormone metabolism. Abnormal steroid metabolism in the human TM is associated with elevated IOP and glaucomatous optic neuropathy.[15] Other genes encoding enzymes that regulate steroid metabolism, such as *AKR1C1* and *AKR1C3*, have been reported to be induced by DEX in human TM.[15] However, in the present study, *OSBP*, *SOAT1*, *AGXT*, and *STC2* were affected by TA, but not by DEX.

TM treated with corticosteroids leads to a reduction in extracellular proteolytic activity of stromelysin and tissue plasminogen activator.[39] Our results showed a large decrease in the expression of some genes that encode proteases, especially *SENP1*, suggesting induced regulation of proteolysis in human TM cells by TA. SENP1 is a sentrin-specific protease.[40] In this study, a decrease in SENP1 expression is accompanied by an increase in expression in protease inhibitors such as SERPINA3, which is a plasma protease inhibitor and a member of the serine protease inhibitor class.[41] Variations in *SERPINA3* have been implicated in Alzheimer's disease and Parkinson's disease for their antichymotrypsin effects.[42,43]

In addition, more than 10 genes encoding transcription factors were differentially expressed under TA treatment (Tables 2, 3). *SF1*, *ZNF263*, *EGR1*, and *ZNF343* are zinc finger proteins that bind nucleic acids and regulate gene transcriptions.[44] *EGR1* was downregulated. Known as early growth response 1 and as nerve growth factor-induced clone A (NGF1A), it directly controls *TGFB1* gene expression.[45] Therefore, reduced expression of EGR1 by TA may lead to disruption in TGF-B1 activity in ECM metabolism and subsequent impairment of aqueous outflow which causes elevation in IOP.

Five genes were commonly differentially expressed by both TA and DEX: *MYOC*, *GAS1*, *SENP1*, *ZNF343* and *SOX30*. Among them, *MYOC* was upregulated as expected. *GAS1*, encoding the growth arrest specific 1 protein, which suppresses cell proliferation in lung carcinoma cell lines, was also upregulated. GAS1 disrupts the attachment of cells to the ECM.[31] The expression of *SENP1*, *ZNF343*, and *SOX30* was reduced. *SENP1* is involved in the degradation of ECM.[40] *ZNF343* and *SOX30* are members of transcription factor genes. Like *EGR1*, *ZNF343* is a zinc finger protein involved in ECM metabolism. Since excess ECM has been shown to increase aqueous outflow resistance in the TM of glaucomatous eyes,[34] alteration in expressions of these genes and subsequent changes in the activities of their encoded proteins in the ECM may cause disruption of aqueous outflow through the TM.

Eight differentially expressed genes in human TM cells treated with TA or DEX were located in known POAG loci (Table 6). Among them, *SPOCK* and *EGR1* were downregulated and the rest upregulated. *GAS1* was located in one locus (*GLC1J*) for juvenile-onset POAG.[46] *SPOCK*, *SEMA6A*, and *EGR1* were in another locus (*GLC1M*) for juvenile-onset POAG.[47] Since high IOP is a characteristic feature of juvenile-onset POAG, these genes are potential candidates for this severe type of hypertensive glaucoma. The fact that four other known glaucoma related genes (*OPTN, WDR36, CYP1B1*, and *APOE*) were not differentially expressed in human TM cells treated with TA or DEX indicates that they may not be the cause of elevated IOP in high-tension glaucoma (Tables 2, 3, 4).

Among the differentially expressed genes in this study, nine genes (*MYOC*, *GAS1*, *SAA2*, *SERPINA3*, *HIPK2*, *IGFBP2*, *SAA1*, *MT1X*, and *CSPG2*) have been reported in other microarray studies (Table 7).[14–17] *SAA1* and *SAA2* are serum amyloid genes that are arranged in a head-to-head transcriptional orientation.[48] *SAA1* and *SAA2* are members of an acute-phase response family of proteins whose systemic concentrations dramatically change during the initial inflammatory process.[49] The increased expression of *IGFBP2* can modulate the biological actions of insulin-like growth factors (IGFs) by either enhancing[50] or inhibiting[51] ligand-receptor interactions, and provide storage for IGFs in the ECM. These genes are involved in the degradation of ECM, inflammation, and acute-phase response and ultimately may affect ECM formation in TM. Increased expressions of these genes may thus enhance the degradation of ECM that regulates the outflow resistance of aqueous humor.[2] However, expressions of several genes which had been reported to be highly upregulated by DEX, such as, *ANGPTL7*,[17] *PEDF*,[14,15] *APOD*,[15,17] and *TAGLN*,[16,17] were found not to be affected by DEX in this study (B-statistic < -3). Notably, consistent results of unaffected expression of these genes were obtained from a different cell line in the present study (Table 5). Experimental variations, such as the differences in the sources of the human TM, number of cell passages, exposure time to DEX, array type, and methodologies for data analysis may be reasons for such discrepancies.[17] In this study, we used an empiric Bayes approach to identify differentially expressed genes. All significant genes had a B-

TABLE 7. Commonly Differentially Expressed Genes in Human TM Cells Induced by DEX between This Study and Published Studies

Gene Symbol	Gene Name	This Study (DEX/ Control)	Lo et al.[15] (hTM/ONH Astrocytes)	Isibashi et al.[14] (DEX/ Control)	Leung et al.[16] (DEX/ Control)	Rozsa et al.[17] (DEX/ Control)
MYOC	Myocilin	12.8	50.0	24	4.3	16.7
GAS1	Growth arrest-specific 1	8.6	24.0		3.3	
SAA2	Serum amyloid A2	8.6				114.0
SERPINA3	Serpin peptidase inhibitor, clade A (alpha-1 anti-proteinase, antitrypsin), member 3	4.2	165.0			48.2
HIPK2	Homeodomain interacting protein kinase 2	4.1				4.3
IGFBP2	Insulin-like growth factor binding protein 2	4.1	57.0	4.9	1.7	2.1
SAA1	Serum amyloid A1	3.7				219.0
MT1X	Metallothionein 1X	2.6				3.7
CSPG2	Chondroitin sulfate proteoglycan 2 (versican)	−2.9	−27.0			

Data are average multiples of change of gene expression, and genes are sorted according to the average change.

statistic of at least 2 which approximately corresponded to $P < 0.02$, derived by *t*-test (Tables 2, 3, 4).

In one study, *MYOC* change was 191.3-fold with qPCR and 16.7-fold with microarray.[17] However, Lo et al.[15] reported a similar change in *MYOC* between RT-PCR and microarray. The *MYOC* change was 315-fold with RT-PCR and 148-fold with micorarray. An even lower *MYOC* change in RT-PCR has been reported: the respective *MYOC* mRNA expression ratios for the four sample pairs were 4.1, 2.4, 1.5, and 1.7 with RT-PCR, and 3.2, 42.7, 47.3, and 2.4 with microarray.[14] In this study, the *MYOC* change induced by DEX was 12.81-fold with RT-qPCR and 10.62-fold with microarray. We compared detailed changes between RT-qPCR and microarray and found that the RT-qPCR identified greater changes than microarray for most genes (Table 5). The difference in array type and methodologies for image analysis may be the major reasons for such discrepancies between studies. Intriguingly, we found similar changes in *MYOC* between 12 hours of TA treatment and 7 days of DEX treatment (Table 5). This suggests that TA could induce a *MYOC* increase in a shorter time than DEX. However, the underlying mechanism remains to be elucidated.

We found some differences in the effects of DEX on human TM gene expression from our previous study,[16] although the same TM cell line was used. The culture conditions that we used previously[16] followed those for establishment of this cell line.[11] The time for DEX treatment was 10 days, and the cells were grown until 100% confluence before DEX treatment.[16] In the present study, the time for DEX treatment was 7 days, and the cells were grown to 80% confluence before DEX treatment. We wanted to improve the culture conditions analogous to clinical practice and to apply the commonly used conditions for cell culture. Also, microarrays of 2400 genes were used,[16] but, in the present study, we used microarrays of 41,421 probes. Consequently, more differentially expressed genes were identified. Despite such differences, three genes (*MYOC*, *GAS1*, and *IGFBP2*) were commonly identified by two studies.

In summary, the simultaneous investigation of gene expression profiles of human TM cells treated with TA and DEX provides a technical approach to the identification of candidate genes for glaucoma. Most of the genes identified from the present study are novel candidates that have not been directly implicated in IOP regulation. Some genes particularly merit attention, including the genes commonly differentially expressed under TA and DEX treatment (e.g., *GAS1*, *SENP1*, *ZNF343*, and *SOX30*) and the genes located in known POAG loci (e.g., *SPOCK*, *SEMA6A*, and *EGR1*). Future sequence analysis, association study, and functional analysis of these genes should be helpful in identifying glaucoma genes.

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