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Specific Targeting of Gene Expression to a Subset of Human Trabecular Meshwork Cells Using the Chitinase 3-Like 1 Promoter

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Abstract

Purpose—To compare the gene expression profile of trabecular meshwork (TM) and Schlemm's canal (SC) primary cultures and to identify promoters for targeting gene expression to specific cells in the outflow pathway.

Methods—The differential gene expression profile of four human TM and three SC primary cultures was analyzed by gene microarrays (Affymetrix, Santa Clara, CA) and confirmed by quantitative real-time PCR. Based on the results, a recombinant adenovirus was constructed with the expression of the reporter gene *LacZ* driven by the 5' promoter region of the chitinase 3-like 1 (*Ch3L1*) gene (*AdCh3L1-LacZ*). The expression of the *Ch3L1* promoter was analyzed in human TM and SC cells and in human perfused anterior segments infected with *AdCh3L1-LacZ*.

Results— γ -Sarcoglycan, fibulin-2, and collagen XV were identified as the genes more highly expressed in SC than in TM cells. *Ch3L1* showed the highest levels of differential expression in TM versus SC cells. Expression analysis of the *Ch3L1* promoter demonstrated specific expression in a subset of the TM cells in cell culture and in perfused anterior segments.

Conclusions—Comparative analysis of gene expression between SC and TM primary cultures identified several genes with promoters potentially capable of targeting gene expression to specific cells within the outflow pathway. Results with the *Ch3L1* promoter indicated that two different cell subtypes may be present in the TM. This study provides a new potential tool to investigate the role of these different cell types in both normal and pathophysiological function of the outflow pathway, with implications for possible future glaucoma gene therapy.

Glaucoma is a group of blinding disorders characterized by damage to the optic nerve. The most common form of the disease, primary open-angle glaucoma (POAG), is frequently associated with elevated intraocular pressure (IOP) that results from an abnormal resistance to the outflow of aqueous humor through the conventional outflow pathway.^{1,2} The conventional outflow pathway is the route by which most aqueous humor exits the anterior chamber of the eye, and it includes the trabecular meshwork (TM) and Schlemm's canal (SC).³

The outflow pathway is a complex tissue composed of several different cell types that are morphologically and functionally different: (1) Schwalbe's line (SL) cells are located in the anterior, nonfiltering portion of the TM and have been proposed to be the progenitor cells of the TM; (2) TM cells cover the surface of the connective tissue beams in both the uveal and corneoscleral filtering meshwork and appear to be involved in phagocytosis and tissue remodeling; (3) juxtacanalicular tissue (JCT) cells are randomly distributed within the extracellular matrix of the juxtacanalicular meshwork; and (4) the cells of the inner wall endothelium of SC constitute the only continuous cell layer in the outflow pathway.^{4,5} The specific functional differences of these cells and their role in the physiology of the aqueous humor process are not clear. Although the locus of both normal outflow resistance and the abnormal resistance in POAG is believed to be at the level of the JCT and/or the inner wall of SC,⁶⁻⁸ there is uncertainty as to the exact location. Although one school of thought postulates that the extracellular material within the JCT is responsible for normal resistance, the other school of thought emphasizes the role of the cells of the inner wall of SC as the major locus of the outflow resistance.^{7,9-13}

A potential strategy for understanding these questions, which also has therapeutic implications, is directed gene delivery to specific cell types within the outflow pathway. We have recently demonstrated the feasibility of targeting gene expression to the outflow pathway with replication-deficient adenoviruses containing tissue-specific promoters that were identified from gene expression profile analyses of the TM.¹⁴ Analyses of the published TM libraries have provided important information about the genes preferentially expressed in the outflow pathway compared with other tissues¹⁸⁻²⁰; however, the specimens used in these analyses also include cells from the inner wall of SC. Therefore, the identification of genes differentially expressed in the TM and SC is the next logical step in planning further experiments targeting specific cell types.

One experimental approach for the identification of such differential markers is the comparison of gene expression profiles. However, because of the small size and complexity of the outflow pathway, it is impractical to dissect only the SC endothelia, making the construction of a direct SC cDNA library almost impossible. As an alternative approach, we present a comparative gene expression profile analyses between human primary cultured TM and SC cells. We identified genes with promoters potentially capable of targeting gene expression in specific cells in the outflow pathway. The expression of one of these promoters was tested in both TM cells and SC cells, and also in human perfused anterior segments.

Materials and Methods

Cell Cultures

Primary cultures of human TM and SC cells were prepared from donor eyes as previously described^{15,16} and maintained at 37°C in 5% CO₂ in low-glucose Dulbecco's modified Eagle's medium (DMEM) with L-glutamine and 110 mg/L sodium pyruvate, supplemented with 10% fetal bovine serum (FBS), 100 µM nonessential amino acids, 100 U/mL penicillin, 100 µg/mL streptomycin sulfate, and 0.25 µg/mL amphotericin B. All the reagents were obtained from Invitrogen Corp. (Carlsbad, CA). The protocols involving the use of human tissue were consistent with the tenets of the Declaration of Helsinki.

RNA Extraction

Human TM and SC primary cultures at passage three were grown to confluence. After the culture medium was removed, cells were immediately immersed in preservative (RNA later; Ambion Inc., Austin, TX) to maintain the integrity of the RNA. Total RNA was then

isolated (RNeasy kit; Qiagen Inc., Valencia, CA), according to the manufacturer's protocol and treated with DNase. RNA yields were determined with a fluorescent dye (RiboGreen; Molecular Probes, Inc., Eugene, OR).

Gene Microarray Analysis

Total RNA (10 μ g) from four different human TM cultures and three different SC cultures was hybridized independently to U95Av2 human arrays (Affymetrix, Santa Clara, CA) at the Duke University Microarray Facility, according to the manufacturer's protocol.

The signal values for each specific gene were normalized using the mean signal value from all probe sets in the array. The difference in expression (x -fold) between human TM (HTM) and SC cells for each gene were calculated by comparing the mean normalized signal values of the gene from the four arrays hybridized with HTM cell probes and those from the three arrays hybridized with SC cell probes.

Quantitative Real-Time PCR

First-strand cDNA was synthesized from total RNA (1 μ g) by reverse transcription using oligodT primer and reverse transcriptase (Superscript II; Invitrogen) according to the manufacturer's instructions. Real-time PCR reactions were performed in a 20- μ L mixture containing 1 μ L of the cDNA preparation, 1 $\times$ PCR mix (iQ SYBR Green Supermix; Bio-Rad, Hercules, CA), and 500 nm of each primer, in a thermocycler (iCycler iQ system; Bio-Rad) using the following PCR parameters: 95°C for 5 minutes followed by 50 cycles at 95°C for 15 seconds, 65°C for 15 seconds, and 72°C for 15 seconds. The fluorescence threshold (C_t) was calculated with the system software. The absence of nonspecific products was confirmed by both the analysis of the melting-point agarose curves and by electrophoresis in 3% gels. β -Actin served as an internal standard of mRNA expression. The sequences of the primers used for the amplifications are shown in Table 1.

Generation of the Recombinant Adenovirus

For the generation of the replication-deficient recombinant adenovirus AdCh3L1-LacZ, a 1059-bp DNA fragment containing the 5' region of the *Ch3L1* gene was amplified by PCR from 100 ng of human genomic DNA (BD-Clontech, Palo Alto, CA) using the specific primers: Chi3L1-forward 5'-CTCGAGTTAAGCCTGCAAAGAATGGAGT-3', and Chi3L1-reverse 5'-CAAGCTTGCCACGGCTCCTGGTGCCAGCT-3', which contain the restriction sites for *Xho*I and *Hind*III, respectively. The PCR reaction was performed at 94°C for 15 seconds followed by 35 cycles of 68°C for 15 seconds and 72°C for 60 seconds (Advantage-HF PCR kit; BD-Clontech). The PCR product was purified and cloned into TOPO TA (Invitrogen) for sequencing. The product with the correct sequence was released by digestion with *Xho*I and *Hind*III (New England BioLabs, Beverly, MA) and introduced into a modified pShuttle (Stratagene, La Jolla, CA) containing the *LacZ* gene and the SV40 polyadenylation signal (pShuttle-LacZ). This pShuttle-LacZ with the *Ch3L1* promoter was used to generate the replication-deficient recombinant adenovirus (AdCh3L1-LacZ) using a commercial system (Ad-Easy; Stratagene). The generation of the AdMGP-LacZ containing a matrix Gla protein (MGP) promoter fragment (550 to +27) has been described.¹⁴

Analysis of *Ch3L1* Promoter Expression in Primary Cultures of HTM and SC Cells

Confluent cultures of human TM or SC cells at passage three were infected with 100 pfu/cell of either the AdCh3L1-LacZ or the AdMGP-LacZ recombinant adenovirus. Two days after infection, β -galactosidase expression was analyzed.

Analysis of β -Galactosidase Activity

Cells were fixed in 1% paraformaldehyde, 0.2% glutaraldehyde, 0.02% NP40, and 0.01% sodium deoxycholate in PBS. After two washes with PBS, β -galactosidase activity was detected by overnight incubation at 37°C in 1 mg/mL 5-bromo-4-chloro-3 β -D-galactoside, 5 mM K₃Fe(CN), 5 mM K₄Fe(CN)6-3H₂O, and 2 mM Mg₂Cl in PBS. For the detection of β -galactosidase in organ cultures, anterior segments were fixed by perfusion at 15 mm Hg, removed from the perfusion system, and stained overnight in the staining solution. After color development, the segments were postfixed in 10% neutral buffered formalin, dehydrated in an ethanol and xylene series, and embedded in paraffin. Sections (5–6 μ m) were then counterstained (Hematoxylin QS; Vector Laboratories, Burlingame, CA).

Perfusion of Human Eye Anterior Segments

Organ cultures of human anterior segments were performed using the method described by Johnson and Tschumper.¹⁷ Briefly, human cadaveric eyes (ages, 33–74 years) <48 hours after death were bisected at the equator, and the lens, iris, and vitreous were removed. The anterior segments were then clamped to a modified Petri dish and perfused by a microinfusion pump at a constant flow of 3 μ L per minute with serum-free, high-glucose DMEM supplemented with 110 mg/L sodium pyruvate, 100 U/mL penicillin, 0.1 mg/mL streptomycin, 170 μ g/mL gentamicin, and 250 μ g/mL amphotericin B. Perfused anterior segments were incubated at 37°C in 5% CO₂. Intraocular pressures were continuously monitored with a pressure transducer connected to the dish's second cannula and recorded with an automated computerized system. Only anterior segments with a stable outflow facility between 0.09 and 0.40 μ L/min per mm Hg that remained unchanged after viral infection were used.

Analysis of *Ch3L1* Promoter Expression in Perfused Organ Cultures

Anterior segments of human eyes were cultured as described earlier. After 48 hours of perfusion, the pumps were stopped, and the pressure dropped to <5 mm Hg. The segments were inoculated with 10⁷ pfu of Ad*Ch3L1-LacZ* in 100 μ L of perfusion medium at 3 μ L/min. Once the total volume was inoculated, the normal perfusion regimen was resumed. β -Galactosidase activity was examined at 2 days after infection. Six eyes from different donors were analyzed for the expression of the *Ch3L1* promoter.

Results

Genes Differentially Expressed in TM and SC Cells

The gene expression profiles of four primary TM cultures and three primary SC cultures were analyzed in parallel by oligo-nucleotide gene microarray (Affymetrix). Data resulting from these arrays are available on request. The genes more significantly expressed in SC than in the TM are shown in Table 2, and the genes more significantly expressed in the TM than in the SC are shown in Table 3. To confirm the experimental results of the arrays, we performed quantitative real-time PCR on *Ch3L1*, *MGP*, and *CDT6*, as representative of TM genes, and on γ -sarcoglycan (*SGCG*), collagen XV (*COL15*), and fibulin-2 (*FBLN2*), as representative of SC cells. The differential expression (*x*-fold) was calculated from the C_t values (Fig. 1).

Analysis of the Gene Expression Profile of Primary HTM and SC Cultures

The gene expression profiles of primary TM and SC cell cultures were compared with the previously published cDNA libraries from TM tissue and infant TM cell culture.^{18–20} In Table 4, the genes with a signal expression higher than 20,000 in either the TM or SC are compared with the genes more highly represented in the libraries. To simplify the data, we

excluded the generally highly expressed ribosomal proteins from the analysis. *EF-1 α* was the only common gene found to be among the most highly expressed genes in the four studies. Table 5 shows genes reported to be highly expressed in human TM tissue, either in revived or nonrevived organs, whose expression was found to be significantly lost in the TM and SC cultured cells.

Expression of the *Ch3L1* Promoter in Primary TM and SC Cultures

To test the specificity of *Ch3L1*, we constructed a replication-deficient recombinant adenovirus in which the expression of the *LacZ* reporter gene was driven by the 5' promoter region of the *Ch3L1* gene (*AdCh3L1-LacZ*). Human TM and SC cells were infected with the recombinant adenovirus *AdCh3L1-LacZ*, and β -galactosidase activity was investigated. Consistent with the arrays, no β -galactosidase staining was detected in SC cells (Fig. 2A). Despite the high multiplicity of infection (MOI = 100 pfu/cell), only approximately 50% of cells in the TM culture stained positively for β -galactosidase (Fig. 2B). All the TM cells, however, showed strong β -galactosidase activity when infected with *AdMGP-LacZ*, a reported positive marker for TM cells, using the same MOI of 100 pfu/cell (Fig. 2C).

Expression of the *Ch3L1* Promoter in the Outflow Pathway

Injection of anterior segments with *AdCh3L1-LacZ* shows β -galactosidase expression restricted to the TM region (Fig. 2D). In the six pairs of eyes analyzed, *Ch3L1* promoter activity was detectable in the most anterior and posterior regions of the TM next to the sclera spur and SL, but not in the middle area (Fig. 2E). Most of the staining was found to be in the trabecular cells, although some β -galactosidase activity was observed in the juxtacanalicular cells of some of the eyes (Fig. 2F).

Discussion

Given the important role of the aqueous humor outflow pathway in modulating IOP, there has been increasing interest in the development of methods for gene transfer to the cells of this tissue for both therapeutic and experimental purposes.^{21–28} We have recently demonstrated the feasibility of specific gene delivery to the cells of the outflow pathway using an adenoviral vector containing a tissue specific promoter.¹⁴ In the present study, we have focused on the identification of differential markers by comparing the gene expression profile of human TM and SC cells. Our objective was to identify promoters capable of targeting gene expression in specific cell types within the outflow pathway.

The data from the comparative analysis show a surprisingly low number of potential specific markers for SC cells, perhaps partially because of the presence of cells from the inner wall of the SC in the TM primary cultures, which thus may have resulted in the expression of SC-specific genes in the TM cultures. The genes most differentially expressed in SC cells compared to TM cells were *SGCG*, *COL15*, and *FBLN2*. Given the lack of information about the function of *SGCG*, a glycoprotein mainly expressed in muscle cells, it is difficult to speculate about its possible role in SC function.^{29–31} In addition, this gene shows a relatively low signal value and high variability among the samples.

Potentially more relevant to the specific functions of SC may be the expression of *COL15* and *FBLN2*. *COL15* is present in the basement membrane of many mesenchymally derived cells, including endothelial cells.^{32–34} The proteolytically processed C-terminal fragment of *COL15* functions as an endostatin, an inhibitor of endothelial cell migration and angiogenesis.^{35–38} This fragment also appears to interact with *FBLN2*,³⁹ an extracellular matrix protein abundant in vessel walls and basement membranes,^{40,41} whose expression has been reported to be modulated by dexamethasone,⁴² an agent known to cause temporary

glaucoma. Although the specific role of FBLN2 and the biological consequences of its interaction with the endostatin domain of COL15 are still unknown, the expression of these two genes in SC cells may support the vascular origin theory of SC.^{43,44}

The comparative analysis of the gene expression profile of TM and SC cells revealed *Ch3L1* as a potential differential marker for TM cells, because it was one of the more abundant transcripts in TM cells and was practically absent in SC cultures. *Ch3L1*, also called human cartilage glycoprotein-39 (HC-gp39 or YKL-40), is a mammalian glycoprotein member of family 18 glycosyl hydrolases.^{45,46} High production of Ch3L1 is detected in the cartilage of rheumatoid arthritis patients,^{46–50} in various inflammatory disorders,^{51–53} in the liver of patients with alcoholic cirrhosis,^{54,55} in several types of cancer,^{56–60} and in macrophages in atherosclerotic plaques.^{61–63} Although the biological function of this protein is still unknown, it is thought to be involved in tissue remodeling and inflammation, acting as a growth factor for connective tissue cells and as a potent migration factor for endothelial cells.^{64–66} Therefore, Ch3L1 could play a role in both the normal physiology of the TM and the abnormalities that occur in glaucoma.

The gene expression profiles deduced from our gene array analysis of both TM and SC primary cultures show relatively low consistency with previous studies, which notably also exhibited important discrepancies among themselves.^{18–20} These inconsistencies may result from differences in both the methodology and the specific models used for the analysis. While all the other studies were performed using the cDNA library technology, the study presented herein was performed by gene microarrays (Affymetrix). To date, there is still not enough information regarding how these two platforms compare with each other; however, discrepancies have been observed, for instance, when comparing the most highly expressed genes in pancreatic adenocarcinoma, by different profiling technologies.⁶⁷

Another factor is that none of these prior studies were based on the same model. Since our study used cultured TM and SC cells, the study of Gonzalez et al.¹⁸ was based on one perfused human eye; Tomarev et al.¹⁹ analyzed the expression profile of a pool of native donor eyes; and Wirzt et al.²⁰ used primary cultures of TM from infant donors. Differences in gene expression among cultured cells, organ culture, and donor tissue should be anticipated and have, in fact, been reported.⁶⁸ These differences may be due to several factors, including changes in the tissue microenvironment, differences between culture medium and aqueous humor, and the ages of the donors. Given this level of discrepancy, further analyses with both intact and individual tissues are necessary to obtain a more accurate gene expression profile for outflow pathway cells.

Based on our results from these arrays, we selected *Ch3L1*, the gene with the highest level of differential expression between TM and SC cells, as a potential promoter for specific gene expression delivery to the TM cells. At the time the experiments were performed, the promoter of *Ch3L1* had not been characterized; however, a recently published work has confirmed that the 5' fragment that we amplified to make the construct contains the fully functional *Ch3L1* promoter.⁶⁹

The lack of expression of the *Ch3L1* promoter in SC cells from both primary cultured cells and organ culture was clearly consistent with the data from the arrays. However, a surprising result that contrasted with previously reported data on the expression of the *MGP* promoter in the outflow pathway¹⁴ was the heterogeneity in the expression of the *Ch3L1* promoter in TM cells. This may suggest the existence of at least two sub-populations of cells in human TM primary cultures. The pattern of β -galactosidase activity in histologic sections from eyes perfused with the *AdCh3L1-LacZ* showed that these two subtypes of cells do not correspond simply to trabecular or juxtacanalicular cells, because, in the six pairs of eyes analyzed, the

β -galactosidase staining was detectable in the most anterior and posterior regions of the TM next to the sclera spur and SL, but not in the middle area, regardless of cell type.

As mentioned earlier, *Ch3LI* protein is commonly produced in pathologic conditions involving inflammation and tissue remodeling. The distribution of positive-stained cells in the outflow pathway could reflect the areas that are subject to strong mechanical stress and, therefore, are involved in induced changes in extracellular matrix composition.⁷⁰

We hypothesize that changes in the expression of *Ch3LI* protein could be involved in the mechanism of abnormal resistance found in many forms of open-angle glaucoma. *Ch3LI* has been reported to be upregulated after treatment of human TM cells with dexamethasone,⁷¹ and also in the retina in a monkey glaucoma model.⁷² The observation that *Ch3LI* was not detected among the clones analyzed in the cDNA library of cultured TM cells from infant donors²⁰ is consistent with the suggested correlation between *Ch3LI* expression and aging,⁷³ and we hypothesize that many glaucomas involve accelerated cellular processes of aging.

In conclusion, the comparative analyses of gene expression profiles of SC and the TM cells provide new insight on the differences between these two cell types that could lead to a new understanding of their respective physiologic roles. Our study identified several genes with promoters potentially useful for targeting gene expression in specific cell types within the outflow pathway. In addition, the expression analysis of a promoter fragment from one such gene, *Ch3LI*, indicates that the TM may have different cell subtypes distinguishable at the molecular level. This would suggest that the cellular composition of this tissue may be even more complex than initially thought.

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Figure 1. Confirmation of the differential expression between human TM and SC cultured cells by real-time PCR. Expression differences (x -fold) based on the fluorescence threshold values (C_t) calculated using the thermocycler software are presented for three genes with promoters potentially useful for targeting gene expression to TM but not SC cells: *Ch3L1*, *MGP*, and *CDT6*; and three genes with promoters potentially useful for targeting gene expression to SC but not TM cells: *COLXV*, *FBLN2*, and *SGCG*.

Figure 2.

Expression analysis of the *Ch3L1* promoter. Cells from primary cultures of SC and TM cells and from perfused human anterior segments were infected with the Ad*Ch3L1-LacZ* recombinant adenovirus expressing the reporter gene *LacZ* under the control of a 1059-bp fragment of the *Ch3L1* promoter, and analyzed for β -galactosidase expression 48 hours after infection. (A) Primary cultures of human SC cells did not show any detectable expression of the *Ch3L1* promoter. (B) Primary cultures of HTM cells showed a mixture of *Ch3L1* positive and negative cells indicative of the presence of at least two subpopulations with distinct patterns of gene expression. (C) Control cultures infected with the AdMGP-*LacZ* virus demonstrated that virtually all the cells in the culture expressed this TM marker. (D) Perfused anterior segments from human eyes showed preferential expression in the TM and in blood vessels of the sclera. (E) Low-magnification view of paraffin sections showed *Ch3L1* expression preferentially located in the anterior and posterior regions of the TM. (F) Higher magnification views of paraffin sections also demonstrated the presence of a mixture of positive and negative *Ch3L1* cells in the tissue. Similar results including lack of detectable expression in SC cells and heterogeneous staining of TM cells were obtained in both, primary cultures from three different cell lines, and in organ culture from six perfused anterior segments.

Table 1

Primer Sequences Used for the Real-Time PCR Analysis

Gene	Primers
Ch3L1	Forward 5'-TCCCCTATGCCACCAAGGGCAAC-3' Reverse 5'-CCATACCATGGCGCCTGCCAGC-3'
Matrix Gla protein	Forward 5'-CCAAGAGAGGATCCGAGAACG-3' Reverse 5'-ATCCATAAACCATGGCGTAGC-3'
Angiopoietin-like factor	Forward 5'-AACGAACACATCCACCGGCTCTC-3' Reverse 5'-GTGGCTATACTCAGCGTAGCGCAG-3'
γ -Sarcoglycan	Forward 5'-CGCTGGGAAAATTGAGGCGCTTTC-3' Reverse 5'-CCACGTCCCCTGCACCAGCTTGG-3'
Collagen XV	Forward 5'-CCTGCTCTGCATTTGGCTGCTCTG-3' Reverse 5'-AACAGTCCTGCAGCTCTGGCCTG-3'
Fibulin-2	Forward 5'-TCCACCCTAGCTTCCGCTGCCTG-3' Reverse 5'-AAGTCATGGCACGTGGTGCCTCG-3'
β -Actin	Forward 5'-CCTCGCCTTTGCCGATCCG-3' Reverse 5'-GCCGGAGCCGTTGTCGACG-3'

Table 2

Genes More Differentially Expressed in SC Cells than in TM Cells

Gene Name	Unigene*	SC	TM	x-Fold
γ -Sarcoglycan	Hs.37167	974 $\pm$ 1202	12 $\pm$ 3	82
Collagen, type XV, alpha 1	Hs.409034	1024 $\pm$ 723	17 $\pm$ 12	59
Fibulin 2	Hs.198862	4645 $\pm$ 601	153 $\pm$ 82	30
Cysteine-rich protein 1 (intestinal)	Hs.423190	1858 $\pm$ 890	90 $\pm$ 48	21
Wingless-type MMTV integration site family member 2	Hs.89791	1078 $\pm$ 823	61 $\pm$ 21	18
Phosphoserine phosphatase-like	Hs.369508	338 $\pm$ 264	26 $\pm$ 19	13
Meningioma 1	Hs.268515	1565 $\pm$ 461	120 $\pm$ 128	13
Similar to rat myomegalin	Hs.390449	235 $\pm$ 106	19 $\pm$ 20	12
Dermatopontin	Hs.80552	295 $\pm$ 168	24 $\pm$ 16	12
Cysteine knot superfamily 1	Hs.40098	1496 $\pm$ 1352	150 $\pm$ 93	10
Calcium channel, voltage-dependent, beta 4 subunit	Hs.284800	316 $\pm$ 25	35 $\pm$ 21	9
Microfibril-associated glycoprotein-2	Hs.300946	1357 $\pm$ 762	156 $\pm$ 82	9
Neuronal PAS domain protein 1	Hs.79564	451 $\pm$ 389	55 $\pm$ 27	8
Chemokine (C-X-C motif) ligand 12	Hs.436042	5481 $\pm$ 3195	677 $\pm$ 485	8
Insulin-like growth factor 2	Hs.349109	347 $\pm$ 246	43 $\pm$ 47	8
Phospholipase A2, group IIA	Hs.76422	447 $\pm$ 484	56 $\pm$ 37	8
Neurotrophin 3	Hs.99171	217 $\pm$ 57	27 $\pm$ 16	8
WNT1 inducible signaling pathway protein 2	Hs.194679	2436 $\pm$ 737	372 $\pm$ 35	7
D component of complement	Hs.155597	1221 $\pm$ 825	202 $\pm$ 30	6
Keratin 7	Hs.23881	1542 $\pm$ 1080	268 $\pm$ 177	6

* <http://www.ncbi.nlm.nih.gov/UniGene>; provided in the public domain by the National Center for Biotechnology Information, Bethesda, MD.

Table 3

Genes More Differentially Expressed in TM Cells than in SC Cells

Gene Name	UniGene*	TM	SC	x-Fold
Chitinase 3-like 1	Hs.382202	23881 ± 12927	147 ± 134	162
Myocilin	Hs.78454	2033 ± 1545	18 ± 17	115
Vascular cell adhesion molecule 1 [†]	Hs.109225	739 ± 481	10 ± 1	77
Interleukin 11	Hs.1721	427 ± 733	6 ± 5	74
Apolipoprotein E	Hs.169401	2759 ± 2424	50 ± 23	55
Cyclooxygenase-2 (hCox-2)	Hs.196384	1291 ± 1201	26 ± 14	51
Stanniocalcin 1	Hs.25590	3516 ± 5286	86 ± 71	41
Retinoic acid receptor responder 1	Hs.82547	3564 ± 2764	99 ± 92	36
Matrix Gla protein	Hs.365706	2425 ± 3187	76 ± 61	32
Regulator of G-protein signalling 5	Hs.24950	924 ± 832	39 ± 9	24
Angiopoietin-like factor	Hs.146559	223 ± 271	10 ± 11	23
Prostaglandin E receptor 2	Hs.2090	978 ± 569	46 ± 21	21
Vascular cell adhesion molecule 1 [†]	Hs.109225	603 ± 417	30 ± 19	20
Tumor necrosis factor (ligand) superfamily, member 10	Hs.387871	874 ± 793	46 ± 18	19
EphB2	Hs.125124	652 ± 210	36 ± 19	18
Guanylate cyclase 1, soluble, alpha 3	Hs.433488	515 ± 642	35 ± 32	15
Aldo-keto reductase family 1, member B10	Hs.116724	2772 ± 839	199 ± 78	14
Fibroblast growth factor receptor 2	Hs.404081	527 ± 303	38 ± 26	14
Neuronal Shc adaptor homolog	Hs.30965	392 ± 57	31 ± 18	13
Growth differentiation factor 5	Hs.1573	527 ± 423	44 ± 23	12

* See Table 2.

[†] Results correspond to two different probe sets for vascular cell adhesion molecule 1 present in the array.

Table 4

Summary of the Genes Highly Expressed in Human TM and SC Cells

Gene Name	UniGene ID*	Signal Values $\pm$ SD			Tomarev et al. ¹⁸	Wirtz et al. ²⁰
		TM	SC			
Ferritin, light polypeptide	Hs.433670	34375 $\pm$ 11034	36156 $\pm$ 13589	†		†
Elongation factor EF-1 α	Hs.439552	29910 $\pm$ 9533	34764 $\pm$ 14285	†	†	†
Tissue inhibitor of metalloproteinases 1 (TIMP1)	Hs.446641	33826 $\pm$ 11139	30003 $\pm$ 11521	†		†
Actin, β	Hs.426930	28991 $\pm$ 10727	32043 $\pm$ 20571			
Glyceraldehyde-3-phosphate dehydrogenase	Hs.169476	31614 $\pm$ 10521	31739 $\pm$ 16310	†		†
Vimentin	Hs.439800	26015 $\pm$ 6654	31593 $\pm$ 14506		†	
Ferritin, heavy polypeptide 1	Hs.418650	25700 $\pm$ 7010	31519 $\pm$ 10687			†
Thymosin, β 4	Hs.79968	24290 $\pm$ 7485	28860 $\pm$ 10761		†	†
Interferon induced transmembrane protein 1 (9-27)	Hs.374690	28277 $\pm$ 7902	25567 $\pm$ 7671			
Insulin-like growth factor binding protein 4	Hs.1916	27961 $\pm$ 8632	18958 $\pm$ 6426			
Clusterin	Hs.436657	27838 $\pm$ 11408	13288 $\pm$ 5795			
Interferon-induced transmembrane protein 3 (1-8U)	Hs.374690	26719 $\pm$ 8925	24778 $\pm$ 7492			
Guanine nucleotide binding protein (G protein)	Hs.5662	22201 $\pm$ 7451	26601 $\pm$ 10629			
Laminin receptor 1	Hs.356261	21332 $\pm$ 6765	26547 $\pm$ 10797			
Lectin, galactoside-binding, soluble, 1	Hs.407909	21658 $\pm$ 4720	25810 $\pm$ 13272			
Annexin A2	Hs.437110	21806 $\pm$ 3573	25309 $\pm$ 8783			
β -2-microglobulin	Hs.48916	25199 $\pm$ 9487	24499 $\pm$ 4905	†		
Actin, γ 1	Hs.14376	21377 $\pm$ 7608	24787 $\pm$ 12811			
Chitinase 3-like 1	Hs.382202	23881 $\pm$ 12927	147 $\pm$ 134	†		
Ubiquitin C	Hs.183704	20307 $\pm$ 3715	22451 $\pm$ 8342			
Fibronectin 1	Hs.418138	18032 $\pm$ 10516	20822 $\pm$ 9363			†
Nonmuscle/smooth muscle alkali myosin light chain	Hs.77385	18285 $\pm$ 3683	20624 $\pm$ 8476			

* See Table 2.

† Transcripts highly represented in libraries from perfused TM,¹⁸ postmortem TM,¹⁹ and infant primary TM culture.²⁰

Table 5
Summary of the Genes That Appear to be Downregulated in TM and SC Primary Cultures Compared with the TM Tissue

Gene Name	UniGene ID*	HTM		SC		Gonzalez et al. ¹⁸		Tonarev et al. ¹⁹	
		Rank	SV ± SD	Rank	SV ± SD	Rank	n	Rank	n
SPARC-like 1	Hs.75445	8375	64 ± 59	10528	25 ± 12			23	10
Matrix metalloproteinase 3	Hs.375129	7729	86 ± 100	8884	54 ± 22	17	4		
Purkinje cell protein 4	Hs.80296	6411	151 ± 88	9567	40 ± 14			9	14
Small inducible cytokine	Hs.105656	5739	198 ± 31	4353	334 ± 70	13	4		
Angiopoietin like factor	Hs.146559	5395	223 ± 271	11929	10 ± 10			15	13
v-Fos	Hs.25647	4475	314 ± 155	5989	182 ± 20			36	8
Apolipoprotein D	Hs.75736	3809	408 ± 55	1438	1308 ± 1567	8	6	8	15
Decorin	Hs.156316	2066	696 ± 273	2542	704 ± 233			21	10
Regulator of G-protein signal	Hs.24950	2896	924 ± 832	7539	39 ± 9	14	4	14	13
Myocilin	Hs.78454	982	2033 ± 1546	11095	18 ± 17			3	28
β -Tubulin	Hs.300701	979	2041 ± 908	581	3427 ± 1147	16	4		
Matrix Gla Protein	Hs.365706	841	2425 ± 3187	8107	76 ± 60	4	9	5	22
CREBBP/EP300 inhibitory protein	Hs.381137	751	2724 ± 283	893	2176 ± 781			31	9
Prothymosin α	Hs.264317	633	3145 ± 1002	694	2828 ± 562			29	9
Phosphatase type 2B	Hs.432840	483	4116 ± 1086	349	5651 ± 4523			45	7
Triosephosphate isomerase	Hs.83848	440	4462 ± 1922	1072	1788 ± 425	12	5		
Cytochrome c oxidase VIIc	Hs.430075	240	5287 ± 1003	456	4412 ± 751			40	7
Tropomyosin α	Hs.133892	349	5501 ± 2324	242	7739 ± 6489			6	18
α -Tubulin	Hs.446608	303	6212 ± 2688	309	6465 ± 2032	15	4		
Tumor protein TPST1	Hs.374596	257	6880 ± 1156	180	9806 ± 3325			4	27
Nascent-polypeptide-associated	Hs.32916	219	7870 ± 1744	189	9632 ± 2444			11	14
NADH dehydrogenase 1 α	Hs.83916	204	8322 ± 1552	269	7216 ± 754			32	9
Lysosomal-associated protein	Hs.111894	161	8608 ± 1569	171	7964 ± 432			38	8
Heat Shock Protein 90	Hs.446579	183	9152 ± 2154	276	7003 ± 1977	9	5		
CTGF	Hs.410037	150	10960 ± 5109	250	7546 ± 1885			43	7
Lactate dehydrogenase	Hs.2795	138	11284 ± 1411	178	9979 ± 3746	7	7		
Actin $\alpha 2$	Hs.208641	114	12526 ± 7959	346	5671 ± 2907			19	11

SV, signal value; n, number of individual clones.

* See Table 2.

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