Expression Profile and Genome Location of cDNA Clones from an Infant Human Trabecular Meshwork Cell Library

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Purpose. To delineate the profile of genes expressed in infant human trabecular meshwork and identify candidate genes for glaucoma.

Methods. Human trabecular meshwork cell cultures were established from six young donors. A cDNA library was made from the combined trabecular meshwork mRNA. The end-sequence of random clones was determined by direct sequencing. These sequences were then analyzed by a National Center for Biotechnology Information (NCBI, Bethesda, MD) database search. Nucleotide searches were performed using the BLASTN (ver. 2.1.3; against the nonredundant nucleic acid sequence) and dbEST databases (both provided by NCBI in the public domain at www.ncbi.nlm.nih.gov).

Results. Sequences from 1118 clones from this nonamplified trabecular meshwork cDNA library were categorized. Of these, 877 expressed sequence tags (ESTs) (78.7%) were known genes. One hundred thirty-nine ESTs (12.5%) showed close identity to EST sequences reported in the public domain database (dbEST). Thirteen ESTs (1.2%) showed no significant similarity to known genes or ESTs in the public databases and were thus defined as novel ESTs. The most abundant genes expressed by the human trabecular meshwork included ferritin H, eukaryotic translation elongation factor 1-α, ferritin L, fibronectin, and TIMP-1. Ferritin H was the most abundant transcript, making up more than 4% of the genes expressed by the human trabecular meshwork. Extracellular matrix proteins were also highly expressed. The chromosome location of the trabecular meshwork ESTs is reported.

Conclusions. A profile of genes expressed by human trabecular meshwork is presented. Thirteen novel ESTs were identified. The combined information obtained from expression analysis and chromosomal localization of trabecular meshwork cDNAs should be valuable in identifying candidate genes for glaucoma. (*Invest Ophthalmol Vis Sci.* 2002;43:3698–3704)

Regulation of intraocular pressure is critical for maintaining the correct shape of the eye for vision.[1] Abnormal elevation of intraocular pressure often results in damage to the optic nerve and ultimately to glaucoma.[2] The human trabecular meshwork is a unique ocular tissue that controls intraocular pressure by regulating the outflow of aqueous humor from the anterior chamber into the venous system.[3] Perturbation of the trabecular meshwork and its ability to regulate intraocular pressure may result in glaucoma. The trabecular meshwork is a tiny tissue weighing only 100 to 150 μg and containing approximately 200,000 to 300,000 cells per eye.[4] Consequently, molecular analysis of the trabecular meshwork has been limited. Identification of genes active in the trabecular meshwork is central to further understanding of the unique functions of this tissue in normal and glaucomatous eyes.

Primary open-angle glaucoma (POAG) is one of the leading causes of blindness in the industrialized world.[5] A disease of the elderly, POAG will have a major impact on the quality of life of a population that is rapidly aging. The pathophysiology of POAG is not well understood. Six POAG genes have been mapped[6–12] and two of these have been identified, *MYOC* (*GLC1A*) and *OPTN* (*GLC1E*).[13,14] Developing better therapeutic strategies for this major blinding disease depends on breakthroughs in understanding the pathophysiology of POAG.

To identify the molecules expressed specifically in the human trabecular meshwork, we used a method called expression profiling of active genes. This method entails sequencing randomly selected clones from a 3′-directed cDNA library that faithfully represents the original composition of mRNA species.[15] The active genes are then identified by their sequences, and the relative abundance of each transcript can be estimated by the frequency of the corresponding EST in the cDNA library. The resultant gene expression profile provides a quantitative estimate of the profile of the relative transcript abundance in human trabecular meshwork. This approach is very useful in the identification of cell tissue-type–specific genes by comparing the expression profiles obtained with those from other cell and tissue types.[16,17]

This is the first analysis of a profile of gene expression in a human trabecular meshwork cell line to be reported. In this study, human trabecular meshwork cell lines from infant donors (age range, 0–2 years) were combined to prepare a cDNA library. An expression profile of the human trabecular meshwork was obtained. Identification of these human trabecular meshwork genes will help in developing a framework for characterizing gene expression in the normal trabecular meshwork. Candidate POAG genes (*GLC1*) were identified.

Materials and Methods

Construction of the cDNA Library

All eyes used for cell culture were from patients without a history of ocular disease or surgery, diabetes, liver failure, sepsis, immune disease, or neurologic disease. Eyes were obtained from the Oregon Lions Sight and Hearing Foundation (Portland, OR), and protocols were approved by the Institutional Human Subjects Committee and conformed to the guidelines set forth in the Declaration of Helsinki and by the National Institutes of Health.

The human cDNA library was made from mRNA isolated from trabecular meshwork cells established from eyes of six individuals, aged 2 weeks to 2 years. These cell cultures were initiated and main-

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tained in medium with 10% fetal calf serum, as described earlier.[18] Confluent cells were harvested at passages 3 through 6. The cells were trypsinized, neutralized with media, flash frozen in liquid N_2 and stored at −70°C. The frozen cells (~96×10^6) were shipped on dry ice to Invitrogen (San Diego, CA) where a unidirectional cDNA library was constructed by using a pcDNA3 vector and TOP10F′ host cells. The isolated mRNA was reverse transcribed with an oligo-dT primer. To make the library unidirectional, the inserts were cut with the restriction enzymes *Bst*XI for the 5′ end and *Not*I for the 3′ end. The size of the inserts ranged from 0.5 to 1.6 kb, with the average size being 0.85 kb.

Template Preparation and Sequencing

Plasmid purifications were performed with one of three kits (Mini-prep 24 deom McConnell Research, San Diego, CA; the QIAwell 8 ultra plasmid kit from Qiagen, Valencia, CA; or the Perfectprep Plasmid Mini Kit from Eppendorf , Westbury, NY). The purified plasmid preps were then sequenced on an automated sequencer (model 377; Applied Biosystems, Foster City, CA) at the Molecular Microbiology and Immunology (MMI) Research Core Facility, Oregon Health and Sciences University, or the Veterans Administration Medical Center Molecular Biology Core Laboratory (Portland, OR) using the T7 primer. Because the trabecular meshwork cDNA library is unidirectional, all the ESTs were sequenced from the 5′ end of the cDNA clones.

Sequence Analysis

Nucleotide searches were first performed using BLASTN (ver. 2.1.3; unless otherwise noted databases used as resources in this study are provided in the public domain by the National Center for Biotechnology Information [NCBI], Bethesda, MD, and are available at the Internet addresses shown, in this case: http://www.ncbi.nlm.nih.gov/BLAST/) against the nonredundant nucleic acid sequence database. Matching sequences with an Expect (E)-value equaling zero were identified as known genes. Sequences with E-values close to zero, were manually analyzed for sequencing errors, alternative splicing, chimeric clones or contamination with vector sequences.

DNA sequences with no matches in the nonredundant GenBank database (http://www.ncbi.nlm.nih.gov/GenBank/) were subjected to a second search with BLASTN against the dbEST (http://www.ncbi.nlm.nih.gov/dbEST/) database. The dbESTs identified were searched for in the Unique Human Gene Sequence Collection database (UniGene; http://www.ncbi.nlm.nih.gov/UniGene/) to determine whether they had been placed in a gene cluster. BLAST searches were performed between September 2000 and September 2001. All the clones that had been identified as ESTs or unknown genes were BLASTed between August and September 2001. GeneRIF (http://www.ncbi.nlm.nih.gov/LocusLink/) was used to identify the functional role of each gene. Some genes had more than one function, and these were placed in one functional classification to avoid confusion.

The sequences of the 1118 cDNA clones were each mapped by BLASTing the sequences with BLASTN against the human genome database. Chromosome locations were also identified in UniGene, and more than 99% agreement was found between the two methods. Sequence data from this article has been deposited with the European Molecular Biology Laboratory (EMBL) and GenBank Data Libraries (the European Molecular Biology Laboratory library is available in the public domain at http://www.embl-heidelberg.de/).

Semiquantitative RT-PCR Analysis of IGFBP-5, -6, and -7

Human trabecular meshwork cDNA was harvested from infant human trabecular meshwork cell cultures containing 10% fetal calf serum. Isolation of mRNA and reverse transcription of the mRNA was performed, as described previously.[19] PCR amplification was performed in a final volume of 25 μL containing 10 mM Tris-HCl (pH 8.3); 50 mM KCl; 1.5 mM $MgCl_2$; 0.001% gelatin; 10 pmol of each primer; 200 μM of dATP, dCTP, dGTP, dTTP; 5 ng of trabecular meshwork cDNA; and 1.0 U *Taq* polymerase (RED*Taq*; Sigma, St. Louis, MO) using a commercial PCR system (model 9700; Applied Biosystems) with initial denaturation of 94°C for 10 seconds, 70°C to 60°C for 20 seconds, and 72°C for 40 seconds; 23 cycles of 94°C for 10 seconds, 55°C for 20 seconds, and 72°C for 40 seconds; and a final incubation at 72°C for 5 minutes. The PCR primers used to amplify each of the specific IGFBP mRNAs are shown in Table 1.

TABLE 1. IGFBP Primers Used to RT-PCR the IGFBP cDNA

Primer Name	Primer Sequence	Expected PCR Product Size (bp)
IGFBP5U	5′TGA AGA AGG ACC GCA GAA 3′	285
IGFBP5L	5′GTC CAC GCA CCA GCA GAT 3′	
IGFBP6U2	5′ACA GGA TGT GAA CCG CAG AG 3′	314
IGFBP6L2	5′AGG AGG AGC TTC CAT TGC CA 3′	
IGFBP7U2	5′AGC AAG GTC CTT CCA TAG TG 3′	231
IGFBP7L2	5′GCA CCC AGC CAG TTA CTT CA 3′	

Touchdown amplification was used to eliminate nonspecific annealing. Different template concentrations and cycle numbers were used to make certain that reactions were in the linear phase of amplification. Different template concentrations, including 0.05, 0.5, 5, and 25 ng were used, whereas other parameters were kept constant. Five nanograms of template was the optimal concentration, with reactions in the linear phase. To further refine the reaction, different numbers of cycles from 20 to 25 were tested after 11 cycles of touchdown amplification. Optimal results within the linear range were established, with 23 cycles used for all three IGFBP PCR products.

Identification of Glaucoma Candidate Genes

The genomic locations of the trabecular meshwork cDNA clones were identified as described above. If a clone mapped to chromosomes 2, 3, 8, or 7 (*GLC1B, GLC1C*, *GLC1D*, or *GLC1F*, respectively), the contig containing that sequence was noted in our database. The initial Human Genome Database sequencing the entire human genome was completed after the *GLC1* genes were mapped.[20,21] With this new sequence data, the flanking markers of each of the *GLC1* loci were identified in their respective contigs and the contigs encompassing each *GLC1* locus were aligned. The contigs containing sequences from the trabecular meshwork clones were then compared with the *GLC1* contigs. If a contig containing a gene identified by a trabecular meshwork clone was also in a *GLC1* contig, then the gene was considered to be a glaucoma candidate gene.

A second method used to determine whether a trabecular meshwork EST was a glaucoma candidate gene was to identify the *GLC1* cytogenetic location using the Online Mendelian Inheritance in Man (OMIM) Morbid Map (http://www.ncbi.nlm.nih.gov/Omim/searchmorbid/). LocusLink was then queried with the gene's accession number to determine whether the gene was within the *GLC1* region as specified by the OMIM Morbid Map.

RESULTS

cDNA Library Analysis

A cDNA library was constructed from human trabecular meshwork cells, as has been described. A listing of the genes in the library is available online (see "cDNA Library of Trabecular Meshwork Genes" at http://www.iovs.org/cgi/content/full/43/12/3698/DC1). No amplification of the mRNA was required, and the library was not screened or subtracted. Therefore, the recurrence of specific genes should represent the actual gene expression level in the human trabecular meshwork. A total of 1118 ESTs were categorized into five classes to determine the

TABLE 2. Summary of ESTs Found in the HTM Library

	cDNA Category	Clones (*n*)	Clones (%)
I	Known genes	883	78.9
II	Known ESTs-unknown genes	137	12.3
III	No match in databases	13	1.2
IV	Mitochondrial transcripts	43	3.8
V	Uninformative sequences	42	3.8
Total		1118	100

fidelity of the library (Table 2). Class I (883 ESTs, 79.6%) showed identity with the sequences of the nonredundant GenBank database (using BLASTN and E values of zero). These ESTs were labeled as known genes. Class II (137 ESTs, 12.3%) showed close identity to EST sequences reported in the public-domain database (dbEST). These ESTs were named known ESTs-unknown genes. Class III (13 ESTs, 1.2%) showed no significant similarity to known genes or known ESTs in the public databases and were thus defined as novel ESTs. Class IV (43 ESTs, 3.8%) contained transcripts of mitochondrial origin. Class V (42 ESTs, 3.8%) included vector, ribosomal RNA (three sequences), or repetitive elements. The low number of class IV and V elements indicates that genomic and mitochondrial contamination of the cDNA library was low.

The 883 known genes expressed by the human trabecular meshwork cell lines were categorized into eight subgroups based on their functions, according to the format of Adams et al.[22] The 883 clones representing known genes are listed online at the Internet address provided in the prior paragraph with their RefSeq accession numbers, how many times they were observed (frequency), and their chromosome location. With the near completion of the Human Genome Project, more than 95% of the genes have been mapped.

Unknown Human Trabecular Meshwork Genes

Of the 1118 clones sequenced, 137 (12.3%) produced sequences matching ESTs from unknown genes previously reported in the dbEST databank. A listing of these ESTs is also available online (see "ESTs from Unknown Genes in the Trabecular Meshwork Library" at http://www.iovs.org/cgi/content/full/43/12/3698/DC1). UniGene clusters have been established for 122 of these ESTs. The number of ESTs in each of these clusters is shown in the online listing of ESTs from unknown genes. The ESTs are sorted by their abundance in UniGene. The ESTs not matching UniGene clusters are shown at the bottom of the table. Sixteen of these clones matched an EST in dbEST but have no UniGene cluster. The remaining 13 clones were unique sequences not found in BLASTN, dbEST, or UniGene, which implies that these genes are expressed only in trabecular meshwork or are very rare transcripts that have not been found in previous EST projects. All the unknown genes were relatively rare trabecular meshwork transcripts, with only one or two clones being found. The majority (91.5%) of the unknown human trabecular meshwork sequences have been mapped. Thus, ESTs that were *GLC* candidate genes were established.

Highly Expressed ESTs

The ESTs more highly represented (by six or more clones) in the human trabecular meshwork library are summarized in a listing of highly expressed genes (Table 3). The three most abundant transcripts are ferritin H, eukaryotic translation elongation factor 1-α, and ferritin L chain. Ferritin H comprises 4.28% of the clones in the human trabecular meshwork library. ESTs shared with the organ culture human trabecular meshwork cDNA library[23] are indicated by asterisks. Of the 17 highly expressed genes, 7 were also present in the organ culture trabecular meshwork cDNA library.[23]

Interleukin-1β, which may mediate the response of trabecular meshwork cells to laser trabeculoplasty as shown by our group,[24] was expressed in the trabecular meshwork library (See Trabecular Meshwork Gene listing online).

Insulin-like Growth Factor–Binding Proteins

Most of the known insulin-like growth factor binding proteins (IGFBPs)[25] were expressed in the human trabecular meshwork cDNA library. Two of the IGFBPs were highly expressed: IGFBP7 and IGFBP2 (Table 3). Lower amounts of IGFBP4, IGFBP5, IGFBP6, and IGFBP8 were present. To determine whether the number of clones reflects the abundance of mRNA in the human trabecular meshwork, RT-PCR of IGFBP7, IGFBP6, and IGFBP5 was performed. All three IGFBP messages were present (Fig. 1). The quantity of IGFBP7 mRNA appeared to be at least two to four times higher than IGFBP6 and IGBP5 mRNAs, reflecting the fourfold increase in IGFBP7 found in the trabecular meshwork cDNA library.

Candidate Glaucoma Genes Expressed in the Human Trabecular Meshwork Library

Six loci for POAG have been mapped.[7–12] The genes for *GLC1A* and *GLC1E* have been identified, *MYOC* and *OPTN*, respectively.[13,14] The genomic location of all 1118 ESTs in the human trabecular meshwork library was established to determine whether any of these were glaucoma candidate genes for the four remaining *GLC1* loci. As shown in Table 4, 10 of the ESTs mapped to the *GLC* loci. Two different methods were used to determine whether the ESTs from the human trabecular meshwork library were *GLC1* candidate genes. Agreement between the two methods was found for the *GLC1C*, *GLC1D*, and *GLC1F* candidate genes (see Table 4, OMIM Morbid Map versus MapView; http://www.ncbi.nlm.nih.gov/cgi-bin/Entrez/map_search). However, discrepancy between the two methods was observed for three of the *GLC1B* candidate genes. This most

TABLE 3. Highly Expressed Genes in TM cDNA Library

Gene	Accession No.	Frequency	Total (%)
Ferritin H*	NM_002032	44	4.28
Eukaryotic translation elongation factor 1, α†	NM_001402	15	1.46
Ferritin L†	NM_000146	13	1.26
Fibronectin	M10905	11	1.07
Ribosomal protein L13a	NM_012423	11	1.07
TIMP-1†	NM_003254	9	0.87
Prothymosin β4*	NM_021109	9	0.87
Ribosomal protein L18a	NM_000980	9	0.87
Ribosomal protein L17	NM_000985	8	0.78
Ribosomal protein S2*	NM_002952	8	0.78
Ribosomal protein S18	NM_022551	8	0.78
Insulin-like growth factor–binding protein 7	NM_001553	8	0.78
Tumor protein, translationally controlled 1	NM_003295	7	0.68
Glyceraldehyde-3-phosphate dehydrogenase†	NM_002046	7	0.68
Insulin-like growth factor–binding protein 2	NM_000597	6	0.58
Amyloid β (A4) precursor-like protein 2	NM_001642	6	0.58
Ribosomal protein S23	NM_001025	6	0.58

* Expressed or † highly expressed[23] in HTM organ culture explants.

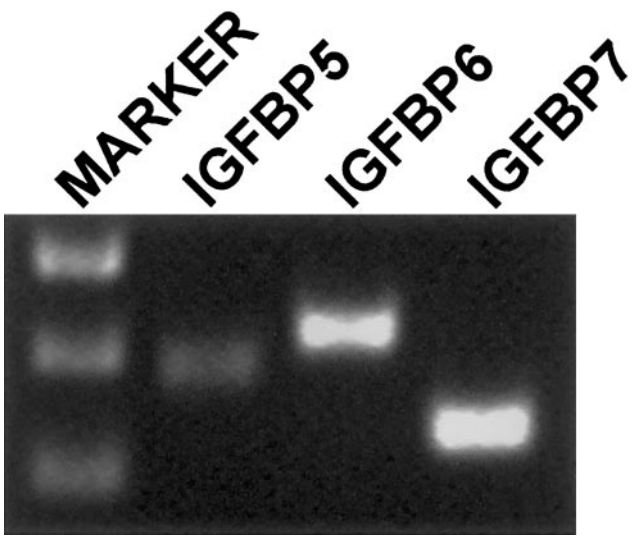

FIGURE 1. RT-PCR of IGFBP5, IGFBP6, and IGFBP7 from cDNA isolated from confluent human infant trabecular meshwork cells grown in 10% fetal calf serum.

likely is due to the refined mapping of the flanking marker, *D2S2161*, which, according to the Human Genome Database, is within NT_015805, which maps to 2p12-p11.1. Originally, *D2S2161* was mapped to 2qcen.[11] *GLC1B* had the most candidate genes including endobrevin, DKFzp586M0122, a homologue of mouse DNA-directed RNA polymerase I, ribosomal protein L31, and thymosin-β.[10] The gene for *GLC1A*, myocilin, was not present in the trabecular meshwork library, however, previous reports have shown that myocilin is expressed at low levels in trabecular meshwork cell lines unless the cells are stressed or exposed to glucocorticoids.[26–30] *OPTN*, the *GLC1E* gene, was also not present in the screened library.[14] Ten candidate genes for glaucoma have been identified in this human trabecular meshwork library. In addition, *PITX2*, which causes Reiger syndrome type I (*RIEG1*) was identified in the library.[31–33] However, *CYP1B1*, which is associated with congenital glaucoma was not found.[34]

DISCUSSION

Composition of Human Trabecular Meshwork cDNA Library

The majority (78.9%) of ESTs identified in the human trabecular meshwork library are known genes. With each succeeding cDNA library added to the public database, a greater number of genes are being identified. Thus, the high number of known genes is to be expected. Conversely, fewer (1.2%) unique genes were identified in our human trabecular meshwork library, again reflecting the growing database. However, these unique genes are probably of specific importance to trabecular meshwork function. The low number of unique genes also illustrates that this library reflects the total transcript level within the trabecular meshwork, because it is not subtracted. The low number (3.8%) of mitochondrial ESTs as well as of repetitive sequences, vector or ribosomal RNA (3.8%) shows that this library primarily consists of cDNA transcripts.

Characterization of ESTs Present in Normal Human Trabecular Meshwork Cell Lines

The trabecular meshwork morphology is still developing in the period after birth up to 4 years of age.[35] Thus, the cell lines, which were established from individuals aged 2 weeks to 2 years may represent a transitional stage with some fundamental differences in gene expression compared with adult cells. Of note, the two major transcripts in our cDNA library were also the two major transcripts in a mouse blastocyst cDNA library.[36]

Glaucoma Genes

The transcript for myocilin, the *GLC1A* gene, was not observed in the sequenced clones. This is not unexpected, because myocilin is expressed at low levels in trabecular meshwork cell lines, unless they have been stressed or induced with glucocorticoids.[26–30] Candidate genes for *GLC1B*, *GLC1C*, *GLC1D*, and *GLC1F* were identified. Hepatocellular carcinoma-associated antigen 112 has been analyzed in affected members of *GLC1F*; however, no disease-causing mutations have been found. We are in the process of analyzing the remaining *GLC1C* and *GLC1F* candidate genes in affected family members. *PITX2*, the *RIEG1* gene, was expressed in our library.[31–33]

Abundant Human Trabecular Meshwork Transcripts

The most abundantly expressed transcript in the human trabecular meshwork library was ferritin H, representing 4.28% of the library clones sequenced. The ferritin L gene was the third highest expressed in the trabecular meshwork library. Ferritin is the major iron storage protein of eukaryotes and appears to be ubiquitously expressed.[37] The ferritin protein is a spherical shell composed of 24 subunits of varying proportions of H and L chains, depending on the tissue type.[38] H-rich ferritin predominates in erythropoietic tissues, where iron availability is

TABLE 4. Candidate *GLC1* Genes Expressed in the Trabecular Meshwork cDNA Library

Loci	OMIM Morbid Map*	Accession No.	Gene Name	LocusLink†	Contig	MapView‡
GLC1B	2qcen-q13	NM_003761	Endobrevin DKFzp586M0122, homologous to mouse RNA	2p12-p11.2	NT_015805	2p12-p11.1
		NM_015425	Polymerase I, DNA-directed	2p24.3-p24.1	NT_015805	2p12-p11.1
		NM_000993	Ribosomal protein L31	2q11.2	NT_022171	2p11.1-q12.1
		NM_021103	Thymosin-β 10	2p11.2	NT_005120	2pcen
GLC1C	3q21-q24	M90657	Transmembrane 4 superfamily, member 1	3q21-q25	NT_033349	3q21-q25
		NM_006286	Transcription factor Dp-2	3q23	NT_0022502	3q23
GLC1D	8q23	NM_020189	DC6	8q23.2	NT_008048	8q23.2
GLC1F	7q35-q36	AI344746	Est	—	NT_007914	7q33-q36.1
		NM_018487	Hepatocellular carcinoma–associated antigen 112	7q35	NT_007914	7q33-q36.1
		NM_005614	Ras-related GTP-binding protein	7q36	NT_007914	7q33-q36.1

* OMIM Morbid Map gives the cytogenetic location of the disease loci.
† LocusLink displays the cytogenetic location of the genes. AI344746 has no reported map location in LocusLink.
‡ MapView was used to correlate the contig's physical position with the cytogenetic map.

required both for heme synthesis and host defense. Ferritin H accumulates and releases iron faster than L-rich ferritin.[38] For long-term iron storage in the liver and spleen, L-rich ferritin is the preferred form. The human trabecular meshwork cells were cultured in 10% fetal calf serum, which is rich in iron. The levels of ferritin H transcripts may result from exposure of the cells to a high concentration of iron and/or serum, because both are known to induce synthesis of ferritin H.[39] Potential functions for ferritin in the anterior chamber may include providing iron to apoenzymes, boosting host defense and neutralization of oxidative stress by the trabecular meshwork. Myocilin (*GLC1A*) may be a heat shock or oxidative stress protein. The similar role of ferritin H in macrophages during oxidative stress suggests that, under certain environmental stresses, ferritin H and myocilin may both be important in protecting the trabecular meshwork from reactive radicals. Numerous heat shock proteins, including αB crystallin, HSPB1, HSPA5, HSPCA, and chaperonin were found in the trabecular meshwork library in relatively abundant numbers.

Ferritin was not as highly expressed in the human trabecular meshwork library synthesized from organ culture message,[23] although ferritin L was an abundant transcript. This difference between the two human trabecular meshwork libraries could either reflect cell culture versus organ culture, the age difference between the samples, or serum versus serum-free conditions. The ratio of H to L chains varies during development.[38,40] Our cDNA library is from cells from infants in whom the trabecular meshwork is still maturing, which may be why this ratio is observed.

Another gene, known for its role in protecting motoneurons from iron toxicity, macrophage inhibitory cytokine-1, was expressed in the trabecular meshwork library.[41]

IGFBP7 and IGFBP2 were highly expressed in the trabecular meshwork cDNA library, with eight and six clones, respectively. Lower expression of IGFBP5, IGFBP6, IGFBP8, and IGFBP4 was observed. In an earlier article, we described the expression of IGFBP5 in trabecular meshwork.[42] In the organ culture human trabecular meshwork, IGFBP3 and IGFBP5 were expressed.[23] Thus, all the known IGFBPs with the exception of IGFBP1 have been shown to be expressed in human trabecular meshwork.

Gene Expression and Mechanisms of Trabecular Meshwork Outflow

The trabecular meshwork has been implicated as the site of obstruction of aqueous outflow, which in turn leads to increased intraocular pressure and potentially to glaucoma.[4,43,44] Several of the genes expressed in our trabecular meshwork library have been explored by us and others as possible modifiers of aqueous outflow through the trabecular meshwork. Interleukin-1β, which appears to mediate the response of trabecular meshwork cells to laser trabeculoplasty as shown by our group,[24] was expressed in the trabecular meshwork library (see Trabecular Meshwork Gene listing online). Endogenous production of interleukin-1β by glaucomatous trabecular meshwork cells also appears to protect the cells from responding to oxidative stress by induction of apoptosis.[45]

The cytoskeletal proteins may also affect aqueous outflow. Several pharmacologic agents that cause changes in cytoskeletal structure in trabecular meshwork cells are used clinically to increase outflow facility.[46,47] Several cytoskeleton proteins, including α-smooth muscle actin, γ1 actin, three isoforms of myosin, and vinculin were expressed in our trabecular meshwork library. Expression of α-smooth muscle actin has previously been demonstrated in trabecular meshwork organ culture and cells.[48–50]

Fibronectin, also highly expressed in our library, has been known to be a major component of trabecular meshwork extracellular matrix.[51,52] Increased fibronectin deposition in the aqueous outflow pathway has been shown to occur in elderly and glaucomatous eyes,[51] although a recent study found no differences between normal and glaucomatous eyes.[53] The potential for fibronectin to influence the flow of aqueous humor through the juxtacanalicular trabecular meshwork and the inner walls of Schlemm's canal has intrigued many glaucoma researchers over the years.[51–57]

Another abundant gene expressed in the trabecular meshwork was GAPDH, which is known for its role in glycolysis and oxidative phosphorylation. Considering that glycolysis is the major energy pathway for the trabecular meshwork,[58] expression of the glycolytic enzymes would be expected. However, although high levels of GAPDH transcript were found, other genes for glycolytic enzymes were expressed at lower levels: glucose-6-phosphate dehydrogenase and enolase 1, each of which had two clones compared with seven clones for GAPDH. Once considered a housekeeping protein, many new functions for GAPDH have been ascribed, including membrane, cytoplasmic and nuclear functions in endocytosis, mRNA regulation, tRNA export, DNA replication, and DNA repair.[59] Thus, the presence of GAPDH in our cDNA library may reflect additional roles for GAPDH in the trabecular meshwork in addition to glycolysis.

Comparison of Genes Highly Represented in Cell Line and Organ Culture Human Trabecular Meshwork cDNA Libraries

Four genes were highly expressed in both the organ culture library reported by Gonzalez et al.[23] and our cell culture human trabecular meshwork cDNA library. These include ferritin L, translation elongation factor 1-α, GAPDH, and TIMP1. Approximately 25% of the transcripts were shared between the two libraries. Glycolysis provides the major source of energy to the trabecular meshwork; thus, high levels of GAPDH would be expected in both human trabecular meshwork cDNA libraries.[58] The high levels of translation elongation factor 1-α in both cDNA libraries suggests that translational control may be an important method for regulating gene expression in the trabecular meshwork.

Primary cultured cells and organ cultures each have their unique advantages and disadvantages. One advantage of using primary cultured cells is that more cells can be obtained, thus allowing RNA work without need for amplification. In addition, the cells can be frozen for storage and thus are readily available for experimentation, whereas experiments with organ cultures are dependent on the supply of eyes. Because the trabecular meshwork cells have been isolated, changes occurring within these cells are solely a result of the trabecular meshwork's physiology and are not influenced by adjacent tissues. However, some limitations of the cell culture system do exist. One of these is that mechanical forces acting on the outflow pathway tissue are not present in traditional cell culture. In addition, changes in gene expression may occur in cultured cells that do not reflect in vivo conditions. Organ cultures are probably a closer approximation of what is actually happening in the eye, because this model preserves the architecture of the outflow tissue. However, a limited amount of tissue necessitates amplification of the isolated message. Thus, the resultant library may not accurately reflect actual levels of specific messages, because some may be amplified better than others. Both primary cultured cells and organ cultures have unique advantages and disadvantages that must be taken into consideration in analysis of resultant data. Analysis of the two systems will give a much fuller picture of what is actually occurring in vivo, then either one alone could do.

Additional research is needed to generate a comprehensive profile of genes expressed in the trabecular meshwork during development and aging. The study reported herein and another recent publication[23] provide a comprehensive reference point and an important resource of cloned "trabecular meshwork-expressed genes" that can be used for biological and functional investigations. Expression and chromosomal localization of trabecular meshwork ESTs should facilitate identification of mutations that result in or contribute to glaucoma.

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