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

Mary K. Wirtz, John R. Samples, Hong Xu, Tesa Severson, and Ted S. Acott

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 databases (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-a, 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. Abnormal elevation of intraocular pressure often results in damage to the optic nerve and ultimately to glaucoma. 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. 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. 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. 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 and two of these have been identified, MYOC (GLC1A) and OPTN (GLC1E). 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. 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 type-specific genes by comparing the expression profiles obtained with those from other cell and tissue types.

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-
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 N2 and stored at −70°C. The frozen cells (−96 × 106) 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 BstXI for the 5’ end and NcoI 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 QIAwWell 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/) or in the human genome (http://www.ncbi.nlm.nih.gov/Omim/search-morbid/) 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/GenBank/) were subjected to analysis for sequencing errors, alternative splicing, chimeric clones or contamination with vector sequences.

The genomic locations of the trabecular meshwork cDNA clones were determined 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/search-morbid/). 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/43/12/3608/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 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 5’ | 285 |
| IGFBP5L | 5’GTC CAC GCA GCA GAT 3’ | 314 |
| IGFBP6U | 5’AGA GGA TGT GAA CCG CAG AG 3’ | 231 |
| IGFBP6L | 5’AGG AGG AGG TTC TTG CAT TGG CA 3’ | |
| IGFBP7U | 5’AGC AGG GTC CTT CCA TAG TG 3’ | |
| IGFBP7L | 5’GCC CCA AGC CAG TTA CTT CA 3’ | |

1.0 U Taq polymerase (RedTaq; 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. 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 25 cycles used for all three IGFBP PCR products.
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 database. 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 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-a, 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 library23 are indicated by asterisks. Of the 17 highly expressed genes, 7 were also present in the organ culture trabecular meshwork cDNA library.24

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 IGFBP5 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 GLC1F, GLC1G, and GLC1F candidate genes (see Table 4, OMIM Morbid Map versus MapView; http://www.ncbi.nlm.nih.gov/entrez/mapsearch). However, discrepancy between the two methods was observed for three of the GLC1B candidate genes. This most

<table>
<thead>
<tr>
<th>Table 2. Summary of ESTs Found in the HTM Library</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>cDNA Category</strong></td>
</tr>
<tr>
<td>-------------------</td>
</tr>
<tr>
<td>I</td>
</tr>
<tr>
<td>II</td>
</tr>
<tr>
<td>III</td>
</tr>
<tr>
<td>IV</td>
</tr>
<tr>
<td>V</td>
</tr>
<tr>
<td><strong>Total</strong></td>
</tr>
</tbody>
</table>

**Table 3.** Highly Expressed Genes in TM cDNA Library

<table>
<thead>
<tr>
<th>Gene</th>
<th>Accession No.</th>
<th>Frequency</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ferritin H*A</td>
<td>NM_002032</td>
<td>44</td>
<td>4.28</td>
</tr>
<tr>
<td>Eukaryotic translation elongation factor 1, α†</td>
<td>NM_001402</td>
<td>15</td>
<td>1.46</td>
</tr>
<tr>
<td>Ferritin L†</td>
<td>NM_000146</td>
<td>13</td>
<td>1.26</td>
</tr>
<tr>
<td>Ribonuclease M10905</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ribosomal protein L15a</td>
<td>NM_012423</td>
<td>11</td>
<td>1.07</td>
</tr>
<tr>
<td>TIMP-1†</td>
<td>NM_002545</td>
<td>9</td>
<td>0.87</td>
</tr>
<tr>
<td>Prothymosin β4*</td>
<td>NM_021109</td>
<td>9</td>
<td>0.87</td>
</tr>
<tr>
<td>Ribosomal protein L18a</td>
<td>NM_000980</td>
<td>9</td>
<td>0.87</td>
</tr>
<tr>
<td>Ribosomal protein L1†</td>
<td>NM_000985</td>
<td>8</td>
<td>0.78</td>
</tr>
<tr>
<td>Ribosomal protein S2</td>
<td>NM_002952</td>
<td>8</td>
<td>0.78</td>
</tr>
<tr>
<td>Ribosomal protein S18</td>
<td>NM_022551</td>
<td>8</td>
<td>0.78</td>
</tr>
<tr>
<td>Insulin-like growth factor-binding protein 7</td>
<td>NM_001553</td>
<td>8</td>
<td>0.78</td>
</tr>
<tr>
<td>Tumor protein, translationally controlled 1</td>
<td>NM_003295</td>
<td>7</td>
<td>0.68</td>
</tr>
<tr>
<td>Glyceraldehyde-3-phosphate dehydrogenase†</td>
<td>NM_002046</td>
<td>7</td>
<td>0.68</td>
</tr>
<tr>
<td>Insulin-like growth factor-binding protein 2</td>
<td>NM_000597</td>
<td>6</td>
<td>0.58</td>
</tr>
<tr>
<td>Amyloid β (A4) precursor-like protein 2</td>
<td>NM_001642</td>
<td>6</td>
<td>0.58</td>
</tr>
<tr>
<td>Ribosomal protein S25</td>
<td>NM_001025</td>
<td>6</td>
<td>0.58</td>
</tr>
</tbody>
</table>

* Expressed or † highly expressed23 in HTM organ culture explants.
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. GLC1B had the most candidate genes including endobrevin, DKFzp586M0122, a homologue of mouse DNA-directed RNA polymerase I, ribosomal protein L31, and thymosin-β. 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. OPTN, the GLC1E gene, was also not present in the screened library. 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. However, CYP1B1, which is associated with congenital glaucoma was not found.

**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. 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.

**Glucoma 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. 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 RIEGT gene, was expressed in our library.

**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. The ferritin protein is a spherical shell composed of 24 subunits of varying proportions of H and L chains, depending on the tissue type. H-rich ferritin predominates in erythropoietic tissues, where iron availability is

---

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

<table>
<thead>
<tr>
<th>Loci</th>
<th>Accession No.</th>
<th>Gene Name</th>
<th>LocusLink‡</th>
<th>Contig</th>
<th>MapView‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>GLC1B</td>
<td>NM_003761</td>
<td>Endobrevin</td>
<td>2p12-p11.1</td>
<td>NT_015805</td>
<td>2p12-p11.1</td>
</tr>
<tr>
<td></td>
<td>NM_015425</td>
<td>Polymerase I, DNA-directed</td>
<td>2p24.3-p24.1</td>
<td>NT_015805</td>
<td>2p12-p11.1</td>
</tr>
<tr>
<td></td>
<td>NM_000993</td>
<td>Ribosomal protein L31</td>
<td>2q11.2</td>
<td>NT_022171</td>
<td>2p11.1-q12.1</td>
</tr>
<tr>
<td></td>
<td>NM_021103</td>
<td>Thymosin-β 10</td>
<td>2p11.2</td>
<td>NT_005120</td>
<td>2qcen</td>
</tr>
<tr>
<td></td>
<td>NM_006286</td>
<td>Transmembrane 4 superfamily, member 1</td>
<td>5q21-q25</td>
<td>NT_035549</td>
<td>5q21-q25</td>
</tr>
<tr>
<td></td>
<td>NM_006286</td>
<td>Transcription factor Dp-2</td>
<td>5q25</td>
<td>NT_0022502</td>
<td>3q25</td>
</tr>
<tr>
<td>GLC1C</td>
<td>M90657</td>
<td>DC6</td>
<td>8q23.2</td>
<td>NT_008048</td>
<td>8q23.2</td>
</tr>
<tr>
<td>GLC1D</td>
<td>NM_020189</td>
<td>Ras-related GTP-binding protein</td>
<td>7q35</td>
<td>NT_007914</td>
<td>7q35-q36.1</td>
</tr>
<tr>
<td>GLC1F</td>
<td>NM_018487</td>
<td>Hepatocellular carcinoma-associated antigen 112</td>
<td>7q35</td>
<td>NT_007914</td>
<td>7q35-q36.1</td>
</tr>
<tr>
<td></td>
<td>NM_005614</td>
<td>Est</td>
<td>7q36</td>
<td>NT_007914</td>
<td>7q35-q36.1</td>
</tr>
</tbody>
</table>

* 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. 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 iron serum, because both are known to induce synthesis of ferritin H. 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 aβ 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 as in the organ culture 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. In the organ culture human trabecular meshwork, IGFBP3 and IGFBP5 were expressed. Thus, all the known IGFBPs with the exception of IGFBP1 have been shown to be expressed in human trabecular meshwork cells also appears to protect the cells from responding to oxidative stress suggests that, under certain environmental stresses, ferritin H and myocilin may both be important in protecting the trabecular meshwork from reactive radicals.

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. 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. 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 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.

Acknowledgments

The authors thank the Oregon Lions Sight and Hearing Foundation for providing eyes and the MMI Research Core Facility at Oregon Health Sciences University and the Portland VAMC Molecular Biology Core Laboratory for sequencing.

References


