Insulin-Like Growth Factor Binding Protein-5 Expression by Human Trabecular Meshwork

Mary K. Wirtz, Hong Xu, Kristal Rust, J. Preston Alexander, and Ted S. Acott

**PURPOSE.** The authors found transcript expression for insulin-like growth factor binding protein-5 (IGFBP-5) while screening for uniquely expressed trabecular meshwork (TM) mRNAs. Because the insulin-like growth factor (IGF) autocrine-paracrine system may provide an important signaling mechanism between TM cells and the outflow pathway, the expression of IGFBP-5 and IGF-I receptor in the TM was characterized.

**METHODS.** Poly(A+) RNA was isolated from cell cultures of human TM, ciliary body, retinal pigment epithelium, and skin fibroblasts and subjected to reverse transcription-polymerase chain reaction (RT-PCR) differential display analysis. A unique 980-bp band present in the TM was cloned and sequenced. Additional PCR and Northern analyses were used to define trabecular IGFBP-5 expression. Western immunoblots and confocal immunohistochemistry were used to evaluate the protein expression patterns of IGFBP-5 and the IGF-I receptor. IGFBP-5 and IGF-II were added to trabecular cells in culture, and matrix metalloproteinase production was evaluated.

**RESULTS.** A unique differential display band was identified in the TM. Sequencing of this band identified it as the 3'-untranslated region of IGFBP-5. RT-PCR, using a variety of specific primers for IGFBP-5, Northern analysis, Western immunoblots, and immunohistochemical analysis, confirmed that IGFBP-5 was expressed in the TM. However, IGFBP-5 was also present at low levels in the ciliary body and skin fibroblasts by Northern and Western analysis, in contrast with the differential display findings. In addition, the IGF-I receptor was expressed by the TM and showed cell-surface staining by immunohistochemistry. Trabecular IGFBP-5 was distributed throughout the meshwork in the extracellular matrix and the cells with more staining in the juxtacanalicular region than in the uveal meshwork. IGF-I, but not IGF-II, modestly increased trabecular stromelysin and gelatinase B but not collagenase, gelatinase A, or tissue inhibitor of metalloproteinases 1 or 2.

**CONCLUSIONS.** IGFBP-5 and IGF-I receptor were expressed at significant levels by TM cells and may serve an important role in trabecular function. (Invest Ophthalmol Vis Sci. 1998;39:45-53)

Trabecular meshwork (TM) has been implicated in the etiology of glaucoma. A molecular characterization of trabecular structure and function, particularly how it regulates aqueous humor outflow, is incomplete. Dissection of the meshwork from surrounding tissue for cell culture or for direct analysis is difficult, and there are few, if any, reliable markers to differentiate trabecular cells from those of the surrounding tissues. To further our molecular understanding of trabecular function and its putative role in the development of glaucoma and to find specific TM proteins, we undertook differential display analysis comparing human trabecular mRNA to that of the ciliary body, retinal pigment epithelium (RPE), corneal endothelium, and skin fibroblasts.

Differential display is a method of comparing tissue-specific expression of various genes. Tissue mRNAs are reverse transcribed into cDNAs, which are then amplified by polymerase chain reaction (PCR), using pairs of 10- to 13-oligonucleotide primers, from a set of primers with arbitrary but defined sequences. The resulting PCR products from the various tissues are compared by electrophoresis to identify tissue-specific or preferentially expressed bands. Then the bands of interest are analyzed in detail to verify the tissue expression pattern and identify the expressed genes. This technique provides a powerful means of identifying differentially expressed messages. Requiring little original tissue, it is particularly useful for analysis of trabecular gene expression patterns.

Insulin-like growth factors (IGF-I and IGF-II) have been studied extensively in a variety of ocular and nonocular tissues and are important in the regulation of normal, developmental, and pathological processes. They modulate both mitogenic and metabolic behavior in many different tissues. IGFs are often bound to specific, high-affinity insulin-like growth factor binding proteins (IGFBPs), and seven members of the IGFBP family have been identified. IGFBPs lengthen the extracellular half-life of the IGFs by protecting them from degradation. Moreover, the IGFBPs regulate IGF activity, by either enhancing or inhibiting ligand-receptor interactions, and provide storage for IGFs in the extracellular matrix. In addition, the IGFBPs themselves exhibit intrinsic bioactivity, which is independent of IGF effects. Recently, IGFBP-5-degrading proteinases were identified, including members of the matrix metalloproteinase family.

From the Casey Eye Institute, Oregon Health Sciences University, Portland, Oregon.

Supported by National Eye Institute grants EY10555, EY03279, EY08247, and EY10572 and by grants from the Glaucoma Research Foundation, American Health Assistance Foundation, Research to Prevent Blindness, and Alcon Laboratories.

Submitted for publication September 24, 1996; revised March 17, 1997 and July 30, 1997; accepted September 19, 1997.

Proprietary interest category: N.

Reprint requests: Mary K. Wirtz, Department of Ophthalmology, Casey Eye Institute, Oregon Health Sciences University, 3375 S. W. Terwilliger Blvd., Portland, OR 97201.
IGF-I has been identified in aqueous humor. Binding studies identified a single class of trabecular IGF receptors with $K_d = 0.37$ nM and 17,000 receptors per cell, which bind both IGF-I and IGF-II approximately equivalently. Additional detailed studies of trabecular IGFs have not appeared. Thus, when we identified trabecular IGFBP-5 by differential display, we proceeded to evaluate its expression by the meshwork and identified one trabecular IGF receptor. We also evaluated the effects of IGF-I and IGF-II on trabecular matrix metalloproteinase and tissue inhibitor of metalloproteinase (TIMP) production.

**Materials and Methods**

**Reagents**

Random hexamers were purchased from Promega (Madison, WI). Oligonucleotides were synthesized for us by Dr. Thomas Keller (Molecular Microbiology and Immunology (MMD) Core Facility, Oregon Health Sciences University, Portland, OR) or by Operon Technologies (Alameda, CA). Human poly(A$^+$) mRNA was isolated using a commercial kit (MicroFast Track; Invitrogen, San Diego, CA). The Original TA (thymidine adenosine) cloning kit from Invitrogen was used to clone the differential display product. Anti-IGFBP-5 antibody was purchased from Upstate Biotechnology (Lake Placid, NY), and anti-IGF-I receptor antibody was purchased from Santa Cruz Biotechnology (Santa Cruz, CA). The metalloproteinase and TIMP antibodies were from Tripletone Biologicals (Forest Grove, OR). Kits (Genius 2 and 3; Boehringer-Mannheim, Indianapolis, IN) were used for labeling the IGFBP-5 clone insert and for developing the Northern blot. The mRNAs were used to synthesize cDNAs using a commercial kit (SuperScript II; Life Technologies, Gaithersburg, MD). IGF-I and IGF-II were purchased from R&D Systems (Minneapolis, MN) or from Upstate Biotechnology.

**Cell Culture, Treatments, and Tissue Extractions**

Human cells from TM, RPE, ciliary body, and skin fibroblasts were grown in low, medium, or high glucose (1000, 2750, or 4500 mg/l, respectively) Dulbecco’s modified Eagle’s medium (Life Technologies) or from Upstate Biotechnology. The metalloproteinase and TIMP antibodies were from Tripletone Biologicals (Forest Grove, OR). Kits (Genius 2 and 3; Boehringer-Mannheim, Indianapolis, IN) were used for labeling the IGFBP-5 clone insert and for developing the Northern blot. The mRNAs were used to synthesize cDNAs using a commercial kit (SuperScript II; Life Technologies, Gaithersburg, MD). IGF-I and IGF-II were purchased from R&D Systems (Minneapolis, MN) or from Upstate Biotechnology.

**Differential Display of mRNA, Sequencing, Reverse Transcription–Polymerase Chain Reaction, and Northern Analysis**

A modification of the method of Sokolov and Prockop$^2$ was followed for the differential display. Cells from TM, RPE, ciliary body, and skin fibroblasts were grown to confluency in T75 flasks, trypsinized with 0.1% trypsin, neutralized with Dulbecco’s modified Eagle’s medium containing 10% fetal calf serum, and pelleted at 1000 g for 2 minutes. The cell pellets were resuspended in lysis buffer from the MicroFast Track kit, and poly(A$^+$) mRNA was isolated according to the manufacturer’s instructions. For the reverse transcription, the 20-μl reaction contained 100 ng of mRNA, 500 ng of random hexamer, and 125 μM concentration of each of the four nucleotide triphosphates. The reaction mixture was heated at 42°C for 1 minute, then 200 units of reverse transcriptase (Superscript II RNase H$^-$; Life Technologies) was added, and the reaction was incubated for 1 hour at 42°C. Two units of ribonuclease H was added to digest the RNA, and the reaction was incubated at room temperature for 5 minutes.

The PCR reaction for the differential display was conducted essentially as described previously.$^2$ The 25-μl reactions contained 1 μl of cDNA solution from the first step, 1 unit of Taq DNA polymerase (Perkin-Elmer Cetus, Norwalk, CT), 200 μM concentration of each of the four dinucleotide triphosphates, 3 μM MgCl$_2$, and 10 pmol each of primers BS71 (5’-GAGCTATGCGATC-3’) and BS58 (5’-CAGTGCCGC-3’) from Sokolow and Prockop. The reaction was amplified through 45 cycles, each consisting of 15 seconds at 94.5°C, 30 seconds at 34°C, and 30 seconds at 72°C. Then the reaction was incubated for 5 minutes at 72°C.

The PCR products were analyzed by electrophoresis in a 2% agarose gel containing 2 to 5 μg of ethidium bromide per 100 ml. The DNA bands were visualized by ultraviolet transillumination, and the band of interest was excised from the gel and cloned using the Original TA cloning kit. DNA sequencing was conducted by the MMI Core Facility using several different primers.

To verify the expression of IGFBP-5 in ocular tissues, the ciliary body, choroid, cornea, iris, lens, retina, sclera, and TM were carefully dissected from an eye aged less than 1 year and with no history of ocular disease. Two or more different cell lines from each tissue were analyzed to avoid individual variation effects. For comparisons of tissue IGFBP-5 mRNA expression, human donor eyes were obtained and dissected approximately 2 hours postmortem. Tissues were then subjected to RNA extraction using the Invitrogen MicroFast Track kit as directed by the manufacturers. For metalloproteinase induction studies, porcine trabecular cells, confluent and at passage 3, were maintained serum free for 48 hours before treatment. Because of the large number of cells required for these studies and the limited availability of human tissue, porcine trabecular cells were used. IGFBPs were added at 1 or 10 ng/ml, and media were collected for metalloproteinase analysis at 24, 48, and 72 hours. All human ocular tissues were obtained from the Lion’s Eye Bank; skin fibroblast cultures were obtained by skin biopsy from normal donors. These uses followed the guidelines of the Declaration of Helsinki and were approved by the Oregon Health Sciences University Human Subjects Institutional Review Board. Porcine eyes were obtained from a local abattoir and maintained on ice, and trabecular cells were cultured within 3 to 4 hours postmortem.

**Western Immunoblots and Zymography**

For IGFBP-5 analysis, human RPE, TM, ciliary body cells, and skin fibroblasts were grown to confluence, and the media were harvested, concentrated 10-fold using concentrators (Centriplus; Amicon, Beverly, MA), and electrophoresed on an 8%
**RESULTS**

**Differential Trabecular Gene Expression**

The differential display of poly(A<sup>+</sup>) RNA was conducted using the complete set of primers described by Sokolov and

sodium dodecyl sulfate-polyacrylamide gel electrophoresis gel. The same volume was used for each cell line. This blot was repeated four times with similar results each time. For analysis of the IGF-I receptor, similar cells were extracted with sample buffer and electrophoresed as described above. For metalloproteinase activity and protein assays, culture media after 72-hour treatment as indicated above were concentrated 10-fold and stored frozen until analysis. Western immunoblots and zymography were performed as previously described, and the gel and immunoblot densities were semiquantitated as described earlier.

**Immunohistochemistry and Confocal Microscopy**

Human anterior segments from two subjects (aged 48 and 83 years) with no history of ocular disease were embedded in 4% agarose and fixed overnight at 4°C in 4% paraformaldehyde. Sections (200 μm) were cut and subjected to digestion with 1:1000 bovine testicular hyaluronidase in 100 mM Tris-HCl, pH 8.0 (1.5 units/well; Sigma Chemical, St. Louis, MO) for 45 minutes at 37°C. Sections were washed for 20 minutes with 100 mM Tris-HCl, pH 7.6, and 0.1% Triton X-100, blocked with 2% goat serum in 100 mM Tris-HCl, pH 7.6, for 30 minutes at room temperature, incubated with primary antibody for 24 hours at 4°C, washed three times with 100 mM Tris-HCl, pH 7.6, at room temperature for 10 minutes, and incubated for 24 hours at 4°C with a fluorescein isothiocyanate-conjugated, goat anti-rabbit immunoglobulin G (Sigma). After washing three times with 100 mM Tris-HCl, pH 7.6, at room temperature for 10 minutes, the nuclei were stained with propidium iodide (PI, 5 g/ml; Molecular Probes, Eugene, OR) for 20 minutes at room temperature in the dark and then washed three times with 100 mM Tris-HCl, pH 7.6, at room temperature. To avoid the fluorescence fading with exposure to light, sections were incubated with SLOWFADE (Molecular Probes), and subjected to confocal microscopic analysis at the MMI Core Facility (Oregon Health Sciences University).

Tissue specimens were viewed using a confocal laser scanning microscope and analysis system (Leica 500; Nussloch, Germany) consisting of an inverted microscope (fluovert-FU; Leitz, Heidelberg, Germany), a krypton-argon laser, a simultaneous dual-channel photomultiplier tube detector, and a 24-bit imaging system. Filter sets were selected to differentiate between fluorescein isothiocyanate and PI excitation, and emissions and data were collected separately. The two data sets were then merged using a software function (Scanware; Leica). A 40x-1.3 NA oil objective lens was used, and pinhole settings were maintained at optimal levels for minimum slice thickness resolution. For the optical z-series, slices were taken every 6 μm over a 20-μm thickness, and merged images were photographed. Intensity span settings were optimized using the positive tissue and maintained unchanged for scanning the respective control slides.

**FIGURE 1.** Differential display comparison of mRNAs from the trabecular meshwork (TM) and several other cell lines. The mRNA was extracted from retinal pigment epithelium (RPE), ciliary body (CB), TM, and skin fibroblast (FIB) cells, reverse transcribed with random hexamers and subjected to polymerase chain reaction (PCR) amplification using BS58 and BS71 as primers. PCR products were electrophoresed with a 123-bp DNA ladder as the size markers (MARKER). The size of the PCR products is indicated on the right side of the gel.

**FIGURE 2.** Northern analysis of ciliary body (CB), skin fibroblast (FIB), and trabecular meshwork (TM) mRNA probed with cloned insulin-like growth factor binding protein-5 (IGFBP-5). RNA from these cell lines and from retinal pigment epithelium (not shown) was electrophoresed, blotted, and probed with the IGFBP-5 probe (band at ~6 kb). The blot was stripped and reprobed with a cyclophilin probe (CyP band). Asterisks show ribosomal band positions. RNA markers were also run to verify band positions (not shown).
Primer pairs were used to analyze the coding region and the 3'-untranslated region of IGFBP-5. A map of these primer pairs is shown in Figure 4. The expected band sizes were compared across different tissues.

### Table 1. Reverse Transcription-Polymerase Chain Reaction Amplification of IGFBP-5 mRNA in Several Tissues Using Different PCR

<table>
<thead>
<tr>
<th>Primer Pairs</th>
<th>Sequence Position</th>
<th>Size of PCR Product</th>
<th>RPE</th>
<th>CB</th>
<th>Fibroblasts</th>
<th>TM</th>
</tr>
</thead>
<tbody>
<tr>
<td>IGFBP-5(R/F)</td>
<td>4993-778</td>
<td>280</td>
<td></td>
<td></td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>980(R/F)</td>
<td>3803-4525</td>
<td>722</td>
<td></td>
<td>+</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>BS71/980F</td>
<td>3798-4525</td>
<td>727</td>
<td></td>
<td></td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>980R/BS58</td>
<td>3803-4761</td>
<td>958</td>
<td></td>
<td></td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>BP5tailR1/BP5tailF1</td>
<td>4659-5151</td>
<td>492</td>
<td></td>
<td></td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>BP5tailR1/BP5tailF2</td>
<td>4659-5954</td>
<td>1295</td>
<td></td>
<td></td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>BP5tailR2/BP5tailF1</td>
<td>4854-5151</td>
<td>297</td>
<td></td>
<td></td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>BP5tailR2/BP5tailF2</td>
<td>4854-5954</td>
<td>1100</td>
<td></td>
<td></td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>980R/BPtailF</td>
<td>3803-5151</td>
<td>1348</td>
<td></td>
<td></td>
<td></td>
<td>+</td>
</tr>
</tbody>
</table>

*Abbreviations: IGFBP-5, insulin-like growth factor binding protein-5; PCR, polymerase chain reaction; RPE, retinal pigment epithelium; CB, ciliary body; TM, trabecular meshwork.*
combination with the specific primers 980F and 980R, respectively, only the TM showed a band. When additional primers spanning the region 3' from the clone were used, the primer pairs resulting in larger PCR products, namely, BP5tailR1/BP5tailF1, BP5tailR1/BP5tailF2, and BP5tailR2/BP5tailF2, gave the expected size band only in the TM. No product was seen in the RPE, ciliary body, or fibroblasts. However, when a set of primers was used in the same region, but resulting in a much smaller PCR product (298 bp), the predicted product was observed in ciliary body, fibroblasts, and TM but not in the RPE.

**Western Immunoblot Analysis of Trabecular IGFBP-5 and IGF-I Receptor**

Although IGFBP-5 was apparently not differentially expressed in the TM, the potential importance of IGFBP-5 in regulation of the TM suggested that further characterization of IGFBP-5 in the TM would be valuable. The expression of IGFBP-5 protein was determined by Western immunoblot analysis of concentrated culture medium (Fig. 5A). A strong 31-kDa band was present in the TM medium; a light band at this position was detectable in the ciliary body and in skin fibroblasts but not in the RPE. There was apparently much more IGFBP-5 protein in the TM than in the ciliary body, a difference we consistently observed in four separate experiments. The doublet at 29 and 31 kDa represents the glycosylated and deglycosylated forms of IGFBP-5. In addition, a smaller band at 23 kDa was observed in all three cell lines; this was the proteolytic processed form of IGFBP-5 (data not shown).

If IGFBP-5 interacts in a complete IGF autocrine-paracrine loop, an IGF receptor and IGFs must be present. Because IGF-I is 20-fold greater in aqueous humor than in IGF-II, we tested for the presence of the IGF-I receptor. A single IGF receptor has been identified in the TM, but the identity of the receptor was not determined. Thus, we subjected cellular extracts of the TM, RPE, ciliary body, and skin fibroblasts to Western immunoblot analysis of insulin-like growth factor binding protein-5 (IGFBP-5) and of insulin-like growth factor-I (IGF-I) receptor. (A) Culture media from two human cell lines of retinal pigment epithelium (RPE), trabecular meshwork (TM), skin fibroblasts (FIB), and ciliary body (CB) cells were concentrated 10-fold and subjected to Western immunoblot with antibody to IGFBP-5. The expected position (~31 kDa) of the band is shown by the arrow at the left. (B) Cellular extracts from similar cell lines were subjected to similar analysis using an antibody to the IGF-I receptor. The arrow shows the position of the observed band (~150 kDa).
FIGURE 6. Confocal immunohistochemical analysis of trabecular insulin-like growth factor binding protein-5 (IGFBP-5) and insulin-like growth factor-I (IGF-I) receptor. Trabecular meshwork (TM) sections were incubated with primary antibody to IGFBP-5 (A), to IGF-I receptor (B), or to no primary antibody (C). Sections were then incubated with fluorescein isothiocyanate-conjugated second antibody and subjected to confocal analysis. Fluorescein isothiocyanate immunostaining is shown as yellow-green, nuclei are stained with propidium iodide, Schlemm's canal is labeled SC, and the anterior chamber is located in the upper portion of each panel. The total area of each image is 250 μm² (15.6 μm x 16 μm). Sections are from an eye aged 48 years; similar results were seen in sections from an eye aged 83 years.

immunoblot analysis for the IGF-I receptor. This receptor is expressed at detectable levels by all five of these cell types (Fig. 5B), exhibiting a band at approximately the predicted size (~135 kDa). Immunohistochemical Identification of Trabecular IGFBP-5 and IGF-I Receptor

To confirm the expression of IGFBP-5 and IGF-I receptor in the TM, sections were probed with these antibodies or no first antibody, and nuclei were stained with PI (Fig. 6). IGFBP-5 (Fig. 6A) was expressed throughout the TM with the highest level exhibited in the juxtacanalicular region next to Schlemm's canal. Significant immunostaining was seen surrounding Schlemm's canal and in the sclera below it. IGFBP-5 appeared to be associated with cells and extracellular matrix and produced a punctate appearance in the trabecular extracellular matrix.

The IGF-I receptor showed a dramatically different pattern of staining in the TM. No scleral immunostaining with the IGF-I antibody was seen, and only light staining on the lower wall of Schlemm's canal was observed (Fig. 6B). The IGF-I receptor appeared to be found only when associated with the trabecular cell surface and showed a very different pattern when compared with the IGFBP-5 antibody. The negative control (no first antibody) showed very light background immunostaining (Fig. 6C).

Effects of IGF Treatment on Trabecular Matrix Metalloproteinase Production

It has been hypothesized that trabecular extracellular matrix turnover regulates aqueous humor outflow and that the disruption of the process causes glaucoma. IGFBP-5 may participate in this turnover by modulating the levels of IGF-I and IGF-II, which in turn may regulate the levels of the matrix metalloproteinases. In the current study, treatment of porcine trabecular cells with IGF-I induced a moderate increase in production of stromelysin (~2.5-fold) and gelatinase B (~4-fold) (Fig. 7). This increase was dependent on dose and time.
FIGURE 7. Effects of IGF-I and IGF-II treatment on trabecular matrix metalloproteinase and inhibitor production. Porcine trabecular cells, serum free for 24 hours, were treated with 1 or 10 ng/ml IGF-I or -II, and media were collected after 72 hours and analyzed. Zymography, using gelatin substrate in the gels, was used to evaluate gelatinase A and B activity. A Western immunoblot, probed with a mixture of stromelysin and interstitial collagenase antibodies, was used to analyze for changes in their levels. A separate Western immunoblot, probed with a mixture of tissue inhibitor of metalloproteinase-1 and -2 antibodies, was used to evaluate inhibitor levels. This figure is a composite of the zymogram and the two Western immunoblots. Stromelysin and collagenase protein standards were included in the Western blots to show the relative migration positions of each protein. On several separate blots (not shown) each protein was analyzed individually and no cross-reactivity was observed between bands.

Treatment with IGF-II produced a smaller increase in gelatinase B (~2.5-fold) and did not produce an appreciable stromelysin change. Trabecular production of gelatinase A, interstitial collagenase, TIMP-1, and TIMP-2 were not significantly affected by either growth factor treatment; only low levels of interstitial collagenase were produced (undetectable in Fig. 7) even with the highest doses.

DISCUSSION

Using differential display of mRNA, we identified IGFBP-5 expression in the TM. This is the first IGFBP to be identified in the TM, although others may be present as well. Although the results from the differential display suggested that IGFBP-5 was only present in the TM, Western immunoblots, Northern analysis, and PCR with other primer pairs demonstrated that IGFBP-5 was also synthesized by ciliary body and skin fibroblasts, although not by RPE. The Northern blot showed similar amounts of IGFBP-5 mRNA in the three cell lines, whereas the Western immunoblot showed that higher levels of IGFBP-5 protein were observed in the TM. The simplest explanation for this paradox is that this transcript is more stable in trabecular cells than in the ciliary body or skin fibroblasts.

In some other tissues, two IGFBP-5 transcripts, 1.7 kb and 6 kb, have been reported. In our Northern blots, only the 6-kb transcript was evident in the three tissues. However, because the cloned probe was from the 3'-untranslated region of the IGFBP-5 mRNA, bp 3782 through 4753, it did not
recognize the 1.7-kb mRNA form. Therefore, from the PCR analysis with several primers (Table 1), some primers were only successful on some of the tissues, but all were successful on the TM. Even though the 6-kb transcript was present in the ciliary body and fibroblasts, this mRNA may be less stable than in the TM. The large amplicons in this region (Table 1) could be synthesized in the TM, but not in the ciliary body and fibroblasts. Previous work shows that stability of messages is often determined by how fast the RNA degrades from the 3' end. The 3'-untranslated region of IGFBP-5 contains four "AUUUAA" motifs, which are thought to promote mRNA degradation. Thus, the TM may possess a stabilizing factor for IGFBP-5 that lengthens the half-life of the IGFBP-5. On the other hand, there may just be more IGFBP-5 message present in the TM and, thus, amplification of long stretches >800 bp is more efficient. Work is now in progress to further clarify this possibility.

The IGF-I receptor was also present in the TM, suggesting that IGFBP-5 may act in an autocrine manner. IGF-I and IGF-II are important local regulators of cell metabolism. They are present in the aqueous humor in the subnanomolar range at twice the levels found in the vitreous. Arnold et al suggested that, because the aqueous and vitreous are functionally distinct with respect to the abundance of IGFs and specific IGFBPs, the physiologic action(s) of the IGFs may be different in these two compartments. The major IGFBP in the ciliary body is IGFBP-2. In this study we demonstrated that the TM cells expressed the IGF-I receptor and produced IGFBP-5. This raises the question of what the role of IGFBP-5 in the TM may be. IGFBPs are important regulators of IGF's biological actions. When IGFBPs are present in a soluble, high-affinity state they reduce the amount of IGF-I or -II available for receptor interaction and inhibit IGF bioactivity. IGFBP-5 is unique in that it binds to both cell surfaces and extracellular matrix, properties that IGFBP-1, -2, and -4 do not share. When IGFBP-5 is associated with the extracellular matrix, its affinity for IGF-I is substantially lowered. IGF-I may be more readily accessible to cell-surface receptors. Our observation that trabecular stromelysin was induced by IGF-I, but not by IGF-II, is compatible with this concept. Binding of IGFBP-5 to the extracellular matrix also protects it from degradation. IGFBP-5 localization in the extracellular matrix may provide a mechanism for concentrating IGF-I in discrete areas, making it available to stimulate specific cellular responses.

Another interesting observation suggests that IGFBP-5 and IGF-I play an important role in trabecular function. Stromelysin, which we found to be induced modestly by IGF-I in the TM, cleaves IGFBP-5 thereby releasing the amino-terminal end to which IGF is bound. IGFBP-5 has an affinity for both IGF-I and IGF-II that is about 10- to 40-fold greater than the IGFBP-5 affinity for type I IGF receptor. By degrading IGFBP-5, stromelysin may release IGF-I, allowing it to bind to other IGFBPs and/or cell-surface receptors during crucial periods of wounding or remodeling of the TM. Although further work is needed, the role of IGFBP-5 on IGF-I suggests that it could have an important role in regulation of outflow by the TM.

An insulin-dependent diabetes mellitus susceptibility gene has recently been mapped to the 4-megabase region containing IGFBP-5 on chromosome 2q34. Given the possible association between diabetes and glaucoma, as well as our finding that IGFBP-5 was expressed at high levels in the TM, particularly the juxtacanalicular region, IGFBP-5 may be a predisposing gene for certain subclasses of primary open-angle glaucoma.

In conclusion, the TM has been shown to contain an IGF-I receptor and IGFBP-5. In addition, IGF-I is present in the anterior chamber. Therefore, this is the first demonstration of a complete IGF autocrine-paracrine system in the anterior segment.

References


