Gene Structure and Properties of TIGR, an Olfactomedin-related Glycoprotein Cloned from Glucocorticoid-induced Trabecular Meshwork Cells

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Expression of the trabecular meshwork inducible glucocorticoid response (TIGR) gene progressively increases from barely detectable levels to greater than 2% of total cellular mRNA over 10 days exposure of trabecular meshwork (TM) cells to dexamethasone. Cycloheximide blocked most of the TIGR mRNA induction, suggesting a requirement for ongoing protein synthesis. The genomic structure of TIGR (~20 kilobases) consists of 3 exons, and a 5-kilobase promoter region that contains 13 predicted hormone response elements, including several glucocorticoid regulatory elements, and other potentially important regulatory motifs. TIGR cDNA encodes an olfactomedin-related glycoprotein of 504 amino acids with motifs for N- and O-linked glycosylation, glycosaminoglycan initiation, hyaluronic acid binding, and leucine zippers. Recombinant TIGR (rTIGR) showed oligomerization and specific binding to TM cells. Anti-rTIGR antibody detected multiple translatable/post-translational forms of TIGR produced by the cells (including secreted 66 kDa/55 kDa glycoproteins/proteins in the media and 55 kDa cellular proteins), whereas Northern blot showed a single mRNA species. The findings suggest potential mechanisms by which TIGR could obstruct the aqueous humor fluid flow and participate in the pathogenesis of glaucoma.

The trabecular meshwork inducible glucocorticoid response (TIGR) protein, which has significant homology in its C-terminal domain with olfactomedins, was initially cloned in our laboratories as a candidate gene for glaucoma using differential library screening in a trabecular meshwork cell culture model (1, 2). Mutations were recently found in this gene that co-segregated with both juvenile and adult forms of the disease (3).

Glucoma is a major cause of blindness, with its most prevalent form thought to involve the specialized endothelial cells lining the outflow pathway of the eye, termed the trabecular meshwork (TM) (4, 5). The synthesis and/or degradation of a variety of extracellular molecules in the meshwork are thought to be regulated by the TM cells, and alterations in the type or amount of connective tissue elements have been postulated to explain the increased outflow resistance seen in glaucoma cases (6). However, an understanding of the biochemical changes that actually contribute to this process has remained elusive.

Previously, we described a highly expressed protein and related glycoprotein (55 and 66 kDa, respectively) found in the media of TM cell culture, but not in other cell types examined, after a prolonged exposure to dexamethasone (DEX) (2). We used this observation to define a cell culture model for “steroid-induced glaucoma” and elevated intraocular pressure due to corticosteroids. The extracellular induced proteins appeared as reasonable candidates for being involved in steroid glaucoma since the time course and dose response of their induction mimicked the intraocular pressure elevation and increased outflow resistance seen in patients receiving glucocorticoid (GC) therapy (7, 8).

Coincident with our research, there was substantial interest in the glaucoma GLC1A locus (9–11) which was mapped on the basis of genetic linkage studies in patients with juvenile glaucoma, a relatively rare form of the disease. The identification of defects in the TIGR gene in glaucoma that map to the glaucoma GLC1A locus (3) has provided a strong impetus for research into the mechanisms by which the TIGR gene might be involved in outflow obstruction and glaucoma pathogenesis.

The following report describes our characterization of the TIGR gene structure, its induction properties, and potentially important aspects of its extracellular protein/glycoprotein. These findings reinforce the rationale used in our cloning strategy and provide specific leads to understand how TIGR could participate in the obstruction of fluid outflow in the trabecular meshwork.

MATERIALS AND METHODS

Cell Culture and Glucocorticoid Treatments—TM cells were propagated in tissue culture by techniques described previously (5). Confluent, stable monolayers were exposed to DEX to evaluate GC effects on mRNA expression over the calculated clinically relevant dose and time course (1). TM cells were treated with 500 nM DEX for 10 days for cDNA library construction and 100 nM DEX for 1, 4, 7, and 10 days for evaluating mRNA inductions.

cDNA Selection, Dot Blot Quantitation, and Sequencing Strategies—A cDNA library was constructed from total mRNA of 10 day-DEX-
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treated TM cells, using Lambda Zap II, and bacterial strain XL-1 from Stratagene (San Diego, CA) by standard protocols (12). Approximately 4 x 10⁶ cells were used for differential screening by hybridization of duplicate lifts with α-²⁵³P-labeled cDNA made from total mRNA of the 10-day DEX-treated and untreated TM cells as described (13). Clones differing in size fractionation on a 1% agarose gel. Primers used for the study included: 5'-CCTTCCTGGGCATGGAGTCCTG and 3'-CCTTCCTGGGCATGGAGTCCTG. The start site of the gene was identified by a combination of the Li-Cor automatic sequencer and/or by standard protocols (12). Approximately 4 x 10⁶ cells were used for differential screening by hybridization of duplicate lifts with α-²⁵³P-labeled cDNA made from total mRNA of the 10-day DEX-treated and untreated TM cells as described (13). Clones differing in size fractionation on a 1% agarose gel. Primers used for the study included: 5'-CCTTCCTGGGCATGGAGTCCTG and 3'-CCTTCCTGGGCATGGAGTCCTG. The start site of the gene was identified by a combination of the Li-Cor automatic sequencer and/or by standard protocols (12). Approximately 4 x 10⁶ cells were used for differential screening by hybridization of duplicate lifts with α-²⁵³P-labeled cDNA made from their TIGR clone PCI.4, and found that the TIGR induction seen with DEX alone, whereas control cultures had only minor effect in the mRNA levels of MT and 1-ACT. These findings demonstrate that the GC induction of TIGR was

(a) and (b). For amino acid sequencing, the proteins were transferred to membranes, cut out separately, eluted, and precipitated for sequencing by an Applied Biosciences 470A sequencer with on-line 1208 PTH. The results showed that the GC induction of TIGR matched the selection criteria we chose based on the steroid-induced glaucoma model (1, 2) and the clinical response (7, 8).

Results

Candidate Clone Selection and Induction Characteristics—Nine clones were confirmed to have progressive GC-induction characteristics when they were used as probes and hybridized to total RNA of 1, 4, 7, and 10 day-DEX-treated TM cells. These clones were found to have overlapping parts of the same sequence. The longest clone (2-kb insert) was used for sequencing of TIGR and synthesis of its recombinant protein after confirming that it had a full-length ORF. Other GC-regulated clones that were found included α1-ACT (3 clones, minor induction) and IGF-BP4 (8 clones, major reduction). Fig. 1a demonstrates the progressive induction of TIGR mRNA in a separate experiment. The results showed that the GC induction of TIGR matched the selection criteria we chose based on the steroid-induced glaucoma model (1, 2) and the clinical response (7, 8).

Fig. 2 shows a CH experiment in which the requirement of protein synthesis was examined. Two separate experiments were conducted with reproducible results obtained. Pre-treatment of the TM cells with CH for 6 h prior to addition of DEX blocked most of the TIGR induction seen with DEX alone, whereas control cultures had only minor effect in the mRNA levels of MT and α1-ACT. These findings demonstrate that the GC induction of TIGR was distinguishable from these other GC-regulated genes based on the dependence on protein synthesis shown for induction. TIGR Is a Leucine Zipper Glycoprotein with Homology to Olfactomedin—Structure-function analysis of TIGR cDNA sequence demonstrated it to be an extracellular molecule with homology to Olfactomedin (23) (see Fig. 7a). The predicted ORF for TIGR has two possible start (ATG) sites adjacent to one another. The TIGR gene codes for glycosylation and extracellular binding sites, including an N-glycosylation site, Asn-Glu-Ser (at aa 57–59) (24), seven potential O-glycosylation sites, including Ser-Pro, Pro-Ser, Thr-Xaa-Xaa-Pro, Ser-Xaa-Xaa-Pro, and Ser-Xaa-Xaa-Pro. The ORF for TIGR is to have at least one ATG site adjacent to another. The TIGR gene codes for glycosylation and extracellular binding sites, including an N-glycosylation site, Asn-Glu-Ser (at aa 57–59) (24), seven potential O-glycosylation sites, including Ser-Pro, Pro-Ser, Thr-Xaa-Xaa-Pro, Ser-Xaa-Xaa-Pro, and Ser-Xaa-Xaa-Pro. The ORF for TIGR is to have at least one ATG site adjacent to another. The TIGR gene codes for glycosylation and extracellular binding sites, including an N-glycosylation site, Asn-Glu-Ser (at aa 57–59) (24), seven potential O-glycosylation sites, including Ser-Pro, Pro-Ser, Thr-Xaa-Xaa-Pro, Ser-Xaa-Xaa-Pro, and Ser-Xaa-Xaa-Pro.
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DNAs—EcoRI, structural motifs are presented in Fig. 3.

The molecule includes clusters of two and seven leucine zippers described for proteoglycans (26, 27). Other important features of the consensus motifs Asp-Xss-Ser-Gly and Ser-Gly-Xaa-Gly de-

45) and Ser-Gly-Glu-Gly (at aa 238–241) sequences that match the GAG initiation sites Asp-Gln-Ser-Gly (at aa 42–45). Heart and skeletal muscle of skeleton (lane 6) but is not found in tissues of brain (lane 2), placenta (lane 3), lung (lane 4), liver (lane 5), and kidney (lane 7). Control hybridization with actin was used to confirm the presence of RNA in all lanes. Lane 8 shows Northern blot of the 2.5 kb mRNA of TIGR from DEX-treated TM cells obtained from a separate experiment.

HA binding sequence Arg-Arg-Gly-Gln-Cys-Pro-Ser-Thre-Arg (at aa 181–189) that resembles the consensus motif B(X 7)B, in the untreated control to 0.5, 0.75, 1.75, and 2.70% of total cellular mRNA after 1, 4, 7, and 10 days. Northern blot analysis. Hybridization of TIGR cDNA probe to multiple tissue mRNA filter from CLONTECH shows TIGR mRNA of approximately 2.5 kb is expressed in tissues of heart (lane 1) and smooth muscle of skeleton (lane 6) but is not found in tissues of brain (lane 2), placenta (lane 3), lung (lane 4), liver (lane 5), and kidney (lane 7). Control hybridization with actin was used to confirm the presence of RNA in all lanes. Lane 8 shows Northern blot of the 2.5 kb mRNA of TIGR from DEX-treated TM cells obtained from a separate experiment.

the doublet 55-kDa (Fig. 2). The DEX induction of TIGR in the absence of CH is also shown (lane DEX). Concurrent DEX and CH treatments (lane DEX + CH) for 6 h shows some TIGR expression. No TIGR expression is seen in the untreated controls (lane CONTROL) or with CH only (lane CH). MT and a1-ACT induction by DEX are not affected by CH treatments. PCR of β-actin levels was used for the normalization of the different samples.

Fig. 2. Protein synthesis requirement for TIGR expression. Cycloheximide treatments were as described under “Materials and Methods.” As shown, a 6-h CH incubation followed by a 24-h 100 nM DEX incubation (lane CH . . . DEX) greatly diminished the DEX induction of TIGR. The DEX induction of TIGR in the absence of CH is also shown (lane DEX). Concurrent DEX and CH treatments (lane DEX + CH) for 6 h shows some TIGR expression. No TIGR expression is seen in the untreated controls (lane CONTROL) or with CH only (lane CH). MT and a1-ACT induction by DEX are not affected by CH treatments. PCR of β-actin levels was used for the normalization of the different samples.

TIGR in the media of DEX-treated TM cells labeled with [35S]methionine. As expected, the 66-kDa glycoprotein was found as the major extracellular form, and doublet proteins near 55-kDa range were found in smaller amounts (Fig. 4A). These precipitated proteins support the idea that glycosylated and non-glycosylated forms of TIGR are expressed extracellularly after prolonged DEX treatment of TM cells. Amino terminal sequencing of the 55-kDa doublet forms of the rTIGR protein (Fig. 4C) confirmed our proposed ORF for its cDNA sequence. The MRFFCA sequence obtained for the higher molecular mass doublet protein (a) confirmed the utilization of the first ATG. The RTAGL sequence obtained for the lower molecular mass doublet protein (b) agreed with the predicted cleavage site Ala-Arg (at aa 32–33) (28) for the proposed ORF. Based on the protein sequencing and antibody results, TIGR cDNA appears to code for the doublet 55 kDa (a) and (b) proteins of 504 and 472 aa, respectively. The finding that both the 66-kDa glycoprotein and 55-kDa doublet proteins are precipitated by the anti-rTIGR antibody indicates that the 66-kDa could be the glycosylated form of one (or both) forms of the 55-kDa protein. The putative motifs for secretion and glycosylation identified for the TIGR cDNA sequence also go along with this view. Our prior biochemical studies of these proteins using tunicamycin had suggested that N-glycosylation might be an important component of the 66-kDa glycosylated form of TIGR (29). The 55-kDa proteins could also be found in the DEX-treated TM cells, in a much lesser amount. The multiple forms of TIGR appear to be the result of translational and post-translational events of TIGR gene expression since only a single gene copy and transcript were found for TIGR. Proposed structures for the doublet 55-kDa (a) and (b) and the 66-kDa glycosylated (c) forms of TIGR found in the media are described graphically in Fig. 5 (iii).

TIGR Gene Structure—Two genomic clones, PAC-59-F4 and PAC-63-B19, were obtained in a screening of approximately 10,000 PAC clones using TIGR cDNA. The clones appeared to be similar to each other based on identical mapping by the HindIII digestion of their DNAs and on a Southern assay using TIGR cDNA probe to show identical banding patterns. The clones were estimated to be about 100 kb and termed P1-TIGR. The subclones (that showed positive signals when hybridized to TIGR cDNA) included six EcoRI clones (namely 4A, 6A, 9B, 10B, 12B, and 2C), five HindIII clones (12C, 1D, 1E, 9E and 12E),
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and one Pst-I clone (1F). Our mapping results showed clones 12B (7 kb), 1E (6 kb), and 4A, 6A, 6B, 9B, 2C (5 kb) were overlapping and clones 9E, 12E, 1F, (2 kb), 10B (2.5 kb), and 12C (4 kb) were overlapping. Positions of these subclones are shown in Fig. 5(i). Based on the mapping and sequencing of these subclones, the TIGR gene structure was established. This structure includes three exons interrupted by two introns, plus 5’ and 3’ untranslated regions regions (Fig. 5(ii)). The transcriptional initiation sites include two cytosine residues adjacent to the TATA and CAT boxes.

Due to the possibility that GCs could initiate gene expression from far upstream GRE elements (30, 31), we sequenced a 5-kb region of the P1-TIGR genomic clone to characterize its promoter structure (Fig. 6). Putative sequences found for the TIGR gene included a potential TATA box, a CCAAT box, a GC box, and a TATA box. The 5’ flanking region of the gene includes a stop codon, two polyadenylation signals, a GT repeat unit, and a polythymidine sequence. Sequences established from the TIGR cDNA clone are shown in the brackets. The initiation sites, 5’ sequence, intronic sequences, and 3’ sequence were established from genomic subclones of the P1-TIGR gene.

FIG. 3. Exon and intronic sequences of the TIGR gene. Amino acid sequence and structural motifs for TIGR protein are based on the ORF proposed for TIGR cDNA. The 55-kDa doublet proteins, (a) and (b) forms, as described in the text, are proposed based on amino-terminal sequencing of the rTIGR proteins that showed two protein products in the 55-kDa range. Two major transcriptional start sites are identified by primer at C*C* sequence; the intronic sequences have consensus splicing sites gt, ag, and thymidine track at the 3’ end of each intron. The 3’ flanking region of the gene includes a stop codon, two polyadenylation signals, a GT repeat unit, and a polythymidine sequence. Sequences established from the TIGR cDNA clone are shown in the brackets. The initiation sites, 5’ sequence, intronic sequences, and 3’ sequence were established from genomic subclones of the P1-TIGR gene.
promoter include (a) consensus sequences TATA, CAT boxes, start sites; (b) multiple hormone and cell signaling response elements including seven GREs, three nGREs (four GREs and two nGRE are proximal, within 2.5-kb promoter region), three ERE (estrogen response element), a PRE (progesterone response element), and a TRE (proximal thyroid response element); (c) early and immediate gene response elements including an SRE (serum response element), three AP-1 sites and an AP-2 site, and an ICS (interferon consensus sequence); (d) elements that could relate to oxidative damage, DNA damage, shear stress, and heat shock responses, including one NF-kB, two PEAs, four SSRE (shear stress response elements (32), and two HSPRE (heat shock protein response element); (e) repressor sequences, including PRE (plasma repressor factor element); (f) sequences thought to be regulated in a tissue-specific manner, including GC2, Prl-FPII and Prl-FPIII (for pituitary), HNF-1 (hepatoma nuclear factor-1) and VBP (vitellogenin gene-binding protein) (for liver), and KTF (for epidermal cells) sites; (g) uncommon features, including a proximal (GT)\textsuperscript{13} repeat unit (based on genotyping random genomic DNAs of 58 unrelated individuals, the GT repeat could range from 8 to 22 GT units); a proximal (CA)\textsuperscript{6} repeat unit; a proximal MIR repeat region (at nt 2\textsuperscript{514} to 2\textsuperscript{319}) (33) and a distal Alu repeat region (at nt 2\textsuperscript{3,942} to 2\textsuperscript{3,823}). The roles of Alu and MIR repeats in promoter functions are not known, but the region of the MIR repeat could be of interest in evaluating TIGR gene expression, due to its proximity to the promoter and potential regulatory motifs identified. The absence of an Sp-1 site could help explain the observation that TIGR is not as widely expressed as other common extracellular matrix components.

**Homology—Using the Blast search, the best fit of TIGR**
cDNA sequence was found for mucus olfactomedin family members at the carboxyl terminus. Its leucine zipper domain has homology to various known leucine zipper proteins. The alignment presented in Fig. 7a shows the TIGR homology to olfactomedin of bullfrogs (23, 34), the Z domain of a neuron-specific olfactomedin-related glycoprotein from rat brain (35), and an EST sequence from human brain (36). These domains share very similar aa positions to one another, indicating a closely related family gene (with the exception being the truncated human clone in which the position with respect to its full-length sequence has not been established). A consensus motif for TIGR and olfactomedins is also proposed. Of particular interest is the cysteine residue 433 within the most conserved region of 11 aa found for species. This cysteine residue was thought to be involved in the protein oligomerization by disulfide-linked polymer formation in other olfactomedins.

The leucine zipper domain of TIGR was found to have ho-
mology to various proteins. Some of these appear to have a greater degree of homology to TIGR than myosin in Kobuta’s report (37), a topic discussed in greater detail under “Discussion.” Fig. 7b shows that domain of seven leucine zippers (aa 117–166) of TIGR was quite similar to the α subunit of RNA polymerase (38) in which it shared 17 identical aa (with 6 leucine zippers) and 18 similar aa; M protein (a cell surface and antigenic protein of Streptococcus pyogenes) (39) had 15 identical aa (with all 7 leucine zippers) and 21 similar aa when compared with TIGR; TIGR had 15 identical aa (with 1 leucine zipper and 6 leucine zipper equivalent) and 21 similar aa with a neural differentiation factor C2-HC type zinc finger protein r-MyT2 (40); and for nonmuscle myosin, TIGR had 15 identical aa (with 1 leucine zipper and no leucine zipper equivalent) and 23 similar aa (37, 41). No significant homology was found for TIGR’s leucine zipper domains for oligomerization. Western analysis of the cross-linked products for diluted rTIGR or conditioned media of DEX-treated TM cells demonstrated that TIGR could exist as a dimer or oligomer (seen as 110 and 200 kDa in equal amounts in Fig. 8b). It is possible that both leucine zippers and the conserved cysteine 433 residue contribute to this high molecular mass formation as discussed later.

Characterization of TIGR Binding Affinities to TM Cells—Fig. 9 shows evidence for two sites of rTIGR protein binding to TM cells. A saturable site with high affinity \( (k_d = 4.3 \times 10^{-9} M) \) and a non-saturable site with lower affinity \( (k_d = 2.3 \times 10^{-8} M) \) were observed. The high affinity site in TM cells was not observed in similar analyses conducted using fibroblasts or Matrigel, which did show the low binding affinity site (data not shown). The findings were verified by two additional studies. The high affinity site suggests a possible ligand-receptor type for TIGR-TM cell interactions. It is likely that this interaction does not involve integrin receptor since no RGD motif (42) was identified for TIGR.

**DEX-induced Organ Culture Expression of TIGR—DEX produced a progressive increase of TIGR expression in the trabecular meshwork organ culture system as shown in Fig. 4B.** This finding demonstrated that the GC-induction of TIGR is not a cell culture artifact. The 55-kDa doublet proteins were detected by Western assay in the perfusate from the organ culture eye, using the rTIGR polyclonal antibody. The 66-kDa form of TIGR might be retained in the meshwork due to predicted interactions with other extracellular matrix molecules. An in vivo role for TIGR (and its potential effect in obstructing outflow) is reinforced by recent findings of increased TIGR gene products in DEX-treated organ culture and glaucoma eyes (43).

**DISCUSSION**

In this paper, we have shown that the induction of the TIGR gene expression in TM cells by prolonged DEX treatment results in high levels of new extracellular proteins. We have presented evidence that the TIGR gene products interact with TM cells and are capable of forming oligomeric complexes. The promoter regulatory elements and protein synthesis requirements found for TIGR may be related to the unusual, prolonged time course of the protein’s induction. Evaluations of these
FIG. 6. Major characteristics of the 5' region of the TIGR gene. The basic promoter sequences and major putative regulatory motifs are underlined. The sequence for each motif is included, and its unmatched sequence is shown in boldface. The numbers are the nucleotide positions relative to the first cytosine start sites (C*). Unusual features include proximal (GT)_{10-20} repeats, no SP-1 site, and a distal Alu repeat (nt 23,942 to 23,723), and proximal MIR repeats (nt 2514 to 2319) are underlined with broken lines.

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data support the original model that led us to the cloning of TIGR as a glaucoma gene and also provide new insights into the biochemical and cellular mechanisms that could explain the molecule’s effects.

The cycloheximide study, which demonstrated a need for protein synthesis for the induction of TIGR, suggested a requirement for new or continuous production of transcription factors. The finding may also help to explain the progressive nature of the TIGR induction which is distinct from more common GC-inductions (such as MT and alpha-1 ACT shown here) that reach maximal levels by 24 h (44).

The high copy number of GREs, sGREs, and other hormone response elements found throughout the promoter region suggests the importance of Cis-acting elements for the observed progressive GC-induction of TIGR. Our recent studies2 have identified a sequence alteration adjacent to one putative GRE composite site (45) that co-segregated with glaucoma in a chronic open-angle glaucoma pedigree. The role of this or other potential promoter mutations in glaucoma pathogenesis is currently being explored. The variety of cellular regulators that might interact with the TIGR gene’s promoter based on the motifs present suggest potential physiological as well as stress-response roles for the TIGR protein/glycoprotein.

A large amount of the 66-kDa glycoprotein was found in the media of DEX-treated TM cultures, with some of the 55-kDa form detected in both media and cells. The ability of anti-rTIGR antibody to precipitate both forms agrees with our previous suggestion that the 66-kDa form could be due to glycosylation of the 55-kDa protein (1, 2). The predicted N- and O-glycosylation sites identified in the TIGR sequence also support this possibility. The potential GAG initiation and GAG binding sites could also be of relevance to the molecule’s extracellular interactions. GAGs, including HA, are distributed throughout the TM, and HA has been of substantial interest concerning its influence on fluid dynamics in the meshwork (46).

The sequence of TIGR cDNA, including two ATG sites near the amino terminus, provides interesting clues to the multiple forms of TIGR expression in TM cells. Although the ATG sites do not have the consensus Kozak sequence (47), criteria for a consensus signal sequence (i.e., an 18–20 aa leucine-rich sequence from its putative cleavage site Arg-Ala) (28) suggests that the 55 kDa (b) form (472 aa) could have been made from a protein in which the second ATG site served as the point of initiation of translation. The presence of this mature form (b) in the cells, and some of the larger form (a) in the media without its signal sequence being cleaved, indicates that the protein trafficking studies of TIGR gene products in the TM cells would be useful.

The significant homology of TIGR to the mucus glycoprotein olfactomedin could be relevant to an understanding of TIGR’s postulated extracellular interactions. Importantly, this domain is the region in which several structural mutations have been linked with glaucoma phenotypes (3, 48–50). The observation that this domain is highly conserved when the olfactomedin genes of fish, frog, rat, mouse, and human are compared (51) supports its functional significance.

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2 Nguyen et al., unpublished data.
FIG. 8. TIGR dimerization and oligomerization. a, G-150 gel filtration profile shows TIGR is excluded and appears in the void volume of the column, most likely due to existing as a high molecular weight oligomer. b, cross-linking study, showing two high molecular mass complexes of TIGR in equal amounts, one at approximately 110 kDa as a dimer and one at 200 kDa, likely to be a tetramer (A). Recombinant TIGR is a monomer at 55 kDa if examined without cross-linking (B).

The olfactomedin domain may play a distinct role compared with other domains of the molecule. This is supported by studies of the olfactomedin-related brain glycoprotein gene which produced four different molecules (AMY, BMY, AMZ, and BMZ), two of which contain an olfactomedin-like domain within their Z regions (35). Oligomerization is known to be a characteristic of olfactomedins, but not much is known about this function. In the case of TIGR, oligomerization could be a particular important feature in the obstruction of the trabecular meshwork.

On a mechanistic level, both the apparent ligand-receptor interaction of TIGR with the TM cells and the oligomerization of TIGR could have an important impact on the outflow. Polymerization of the molecule is also suggested by the elution of rTIGR above 150 kDa during isolation by Sephadex G-150. It is possible that TIGR dimers or polymers are linked to form a higher molecular mass complex via a cysteine-cysteine formation similar to that predicted in olfactomedins (23). Mutations affecting this domain might alter TIGR oligomerization in a way that could increase its cell surface and/or matrix interactions and contribute to intraocular pressure elevation. Independently, Raymond et al. (50) postulated a role for TIGR oligomerization to help explain their genetic findings, in which homozygotes with TIGR mutations were normal while heterozygotes showed the disease for some glaucoma family members.

While most of our findings and prior concepts of outflow resistance in the eye direct attention at the extracellular function of TIGR, we cannot exclude intracellular interactions with cytoskeleton or other cellular structures as playing a role. The report of Kobuta et al. (37) that described a myosin-like protein (called myocilin or MYOC, GenBank accession no. D88214, which has cDNA sequence that differs from that of TIGR by only a 2-base pair GA insertion at nt 104–105) supports a structural role for the 55-kDa gene products in the cilium of photoreceptor cells. The authors emphasized that myocilin could have myosin-related properties, due to the similarity of the leucine zipper domain for myocilin and myosin. This idea was also brought forward by Coca-Prados et al. (52) in a report showing a high expression of TIGR in iris muscle. However, as we pointed out in Fig. 7b, the leucine zipper domain also has substantial homology with other proteins. This raises the question of whether TIGR’s biological properties should be linked to myosin on the basis of the leucine zippers. Although we have not evaluated whether TIGR is glycosylated or exists extracellularly in muscle or photoreceptors, our cell culture findings support the idea that TIGR’s extracellular forms could be TM cell-specific. If confirmed by further evaluations, the cell type-specific expression of the TIGR gene could be of importance to understanding its roles in glaucoma.

In summary, our findings have shown that TIGR is primarily expressed as a major extracellular glycoprotein/protein after sustained GC exposure. Characterization of the gene and its products has provided clues as to the mechanisms by which the TIGR could produce biochemical changes that result in physiologic and pathogenic effects.

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