Isoform-specific Changes in Scleral Transforming Growth Factor-β Expression and the Regulation of Collagen Synthesis during Myopia Progression*

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The development of high myopia is associated with altered scleral extracellular matrix biochemistry. Previous studies highlight the importance of collagen turnover in this process, yet it is unclear which factors control scleral remodeling. This study used a mammalian model of myopia to investigate the capacity of TGF (transforming growth factor)-β1, -β2, and -β3 to influence scleral remodeling in myopia. RT-PCR confirmed the presence of all mammalian TGF-β isoforms in scleral tissue and scleral fibroblasts. Myopia was experimentally induced via monocular deprivation of pattern vision, and animals were allocated to two groups depending on the duration of treatment (1 or 5 days). Down-regulation of each isoform was apparent after only 1 day of myopia development (TGF-β1, −32%; TGF-β2, −27%; TGF-β3, −42%). Whereas the decrease in TGF-β1 and -β3 expression was relatively constant between the two time points, differential down-regulation of TGF-β2 was found between days 1 (−27%) and 5 (−50%). In vitro experiments, using primary scleral fibroblasts, demonstrated the capacity of all isoforms to increase collagen production in a dose-dependent manner. Changes in TGF-β levels, which mimicked those during myopia induction, caused an −15% reduction in collagen synthesis, which is qualitatively similar to those previously reported in vivo. These data represent the first demonstration of TGF-β3 expression in the sclera and implicate all three TGF-β isoforms in the control of scleral remodeling during myopia development. In addition, the early alterations in TGF-β expression levels may reflect a role for these cytokines in mediating the retinoscleral signal that controls myopic eye growth.

Excessive enlargement of the eye, predominantly in the axial dimension, results in the development of high myopia both in humans and in animal models. High degrees of myopia are found in ∼3% of the general human population and result in a significantly increased risk of developing irreversible, sight-threatening pathology of the retina and choroid (1). Studies in animal models of high myopia have demonstrated that the increase in eye size is facilitated by the outer coat of the eye, the sclera, and it has been shown that active remodeling of the sclera, rather than passive stretch, plays a significant role in the enlargement of the ocular globe (2, 3).

The sclera is a fibrous connective tissue consisting largely of heterologous collagen fibrils that comprise mainly type I collagen with small amounts of other fibrillar and fibril-associated collagens (4). Previous studies using mammalian models of high myopia have shown that collagen production is down-regulated, largely because of reduced type I collagen gene expression (5, 6). Furthermore, collagen degradation is increased (5), with several studies reporting a concomitant increase in matrix metalloproteinase (MMP-2) activity (7, 8). In the longer term, these changes result in a reduced collagen fibril diameter (9). In addition to collagen alterations, changes have also been reported in the scleral proteoglycans, which undergo reduced synthesis and/or sulfation of their glycosaminoglycan side chains (10, 11). The overall result of such changes is that the sclera progressively thins and becomes less resistant to mechanical deformation by the intraocular pressure (12, 13).

Despite our knowledge of the changes in scleral extracellular matrix that occur during mammalian myopia development, we have relatively little understanding of the cellular and signaling factors that drive such changes. Recently, levels of the potent mitogen and inhibitor of collagen synthesis, fibroblast growth factor-2 (FGF-2), were found to be unaltered in the sclera of myopic eyes despite the presence of an anterior-posterior gradient in FGF-2 concentrations (14). Another potentially important candidate in the search for factors involved in the scleral remodeling in myopia is transforming growth factor-β (TGF-β), which, like FGF-2, is an important controller of cell cycle activity as well as collagen, proteoglycan, and MMP-2 production in the extracellular matrix (15).

The role of TGF-β in extracellular matrix (ECM) regulation has been well documented, particularly its coordination of changes during wound repair (16). Of the three highly conserved TGF-β isoforms found in mammals, each appears to have a specific role in coordinating ECM remodeling in vivo. Following wounding, the three TGF-β isoforms exhibit a distinct time-dependent alteration in expression levels that appears to be important for conferring organization and strength at wound sites. This is illustrated by the fact that an increase in the TGF-β3 levels relative to TGF-β1/β2, results in a scarless phenotype (17).

To date, many studies have demonstrated the presence of all three TGF-β isoforms in various structures within the mammalian eye (18). Specific knock-out mutations and corneal wound-healing studies have highlighted the importance of TGF-β and its isoforms to ocular development and repair (19).

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18121

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1 The abbreviations used are: MMP, matrix metalloproteinase; FGF, fibroblast growth factor; TGF, transforming growth factor; ECM, extracellular matrix.
Although, however, the ability of TGF-β to regulate fibroblast proliferation and type I collagen, MMP-2, and proteoglycan production implicates it in the control of scleral remodeling during myopic eye growth (15), few studies have investigated this possible role. Using the chick model of myopia, investigators demonstrated that although FGF-2 inhibited myopic eye growth, this effect was abolished when TGF-β1 was co-administered (20). Later studies provided further evidence that TGF-β may play a role in ocular growth, with TGF-β1 expression found to be suppressed in the retina/choroid of chicks developing myopia (19), whereas TGF-β2 content increased in both the retina/choroid and sclera (22, 23). However, there has been no study investigating individual changes in scleral TGF-β isoform expression during mammalian myopia development, nor is there any evidence as to how TGF-β might directly coordinate the scleral biochemical changes that occur.

The current study investigated four important factors to determine the likely extent of TGF-β involvement in the control of scleral matrix remodeling during mammalian myopia development. First, it was determined which of the three TGF-β isoforms were expressed in both tree shrew sclera and cultured scleral fibroblasts. Second, changes in scleral TGF-β isoform expression were characterized during the early and intermediate stages of myopia development. Third, the capacity of each of the three TGF-β subtypes to influence general collagen synthesis was determined using recombinant TGF-β and a scleral fibroblast cell line. Finally, the in vivo changes in TGF-β isoform expression during myopia progression were modeled in vitro to determine whether such changes were capable of bringing about the altered collagen synthesis previously reported in the sclera of eyes with induced myopia. The data collected provide important insights into the isoform-specific role of TGF-β in the control of scleral remodeling during the development of high (pathological) myopia.

EXPERIMENTAL PROCEDURES

**Experimental Subjects and in Vivo Procedures**—Maternally reared tree shrew (Tupaia belangeri) pups were removed from the maternal cage for experimental procedures 15 days after natural eye opening, which represents the start of the period during which tree shrews are most susceptible to the induction of myopia (24). Animals were assigned to one of two experimental groups to allow the investigation of TGF-β isoform expression during myopia development. Group 1 consisted of experimental animals that had myopia induced, monocularly, over a 1- (n = 6) or 5-day (n = 6) period using a translucent occluder attached to a head-mounted goggle as previously described (25). The untreated eye acted as a within-animal control, and right or left eye treatments were randomized within each group. Ocular refractive and biometric effects of such treatment paradigms are well documented (6, 7). Animals that were to wear the occluder for 1 day underwent the surgical procedure on the morning before the goggle was attached to discount possible confounding effects due to recovery from the anesthetic. During the experiment, animals were maintained on a 14/10-h light/dark cycle, and food and water were available ad libitum. All of the procedures were carried out in accordance with the National Health and Medical Research Council of Australia’s Guidelines for the Care and Use of Animals in Research.

At the end of the experimental period, animals were administered ketamine 90 mg/kg and xylazine 10 mg/kg before sodium pentobarbital (120 mg/kg) was used to induce a deep, intractable anesthesia. Ocular dissection was carried out as previously described (26) with a posterior scleral punch diameter of 7 mm finally isolated using a surgical trephine. All samples were frozen in liquid nitrogen within 6 min of enucleation. The left eye was always enucleated and dissected first to discount possible confounding effects due to recovery from the anesthetic. After 48 h, the medium was collected and [3H]proline incorporation to determine whether such changes were capable of bringing about the altered collagen synthesis previously reported in the sclera of eyes with induced myopia. The data collected provide important insights into the isoform-specific role of TGF-β in the control of scleral remodeling during the development of high (pathological) myopia.

**Materials**—MultiScreen filtration plates (Durapore, 0.65 μm pore size) were obtained from Millipore (Bedford, MA). Dulbecco’s modified Eagle’s medium and trypsin were obtained from Genset (La Jolla, CA). Guanidine thiocyanate, Dounce I, M-MLV reverse transcriptase, dNTPs, and RNASin were obtained from Promega (Madison, WI). FastStart DNA master mix was obtained from Roche Applied Science, RNeasy mini extraction and QIAquick PCR purification kits were obtained from Qiagen (Valencia, CA), ECG DTCS Quick Start sequencing kits were obtained from Beckman Coulter (Fullerton, CA). All other reagents were purchased from Sigma.

**Primary Culture of Scleral Fibroblasts**—Scleral tissue was collected from animals 15 days after natural eye opening. Whole scleral tissue was taken from animals under terminal anesthesia as described above. Samples were cleaned of extraneous orbital tissue under a dissecting microscope and the explants incubated in culture dishes. Cells were grown in Dulbecco’s modified Eagle’s medium supplemented with 25 mM HEPES, penicillin-streptomycin (100 units/ml), and 10% fetal bovine serum and kept at 37 °C in a humidified incubator (5% CO₂ and 95% air). Cell growth was observed after 1 week in culture, and confluence was usually reached after 2.5 weeks. Cells were passaged using 0.25% trypsin, and subsequent experiments were restricted to passages 2–6 because cells demonstrated stable growth curves, morphology, and no sign of senescence during this period. When scleral fibroblast populations had reached 80% confluence, cells were passaged into 24-well plates for experiments or harvested for RNA extraction and analysis.

**Total RNA Extraction and Reverse Transcription**—Scleral tissue was homogenized using a tissue homogenizer and total RNA isolated using standard phenol-chloroform extraction procedure as previously described (5). Samples were treated with DNase I to remove any residual genomic DNA contaminants and the RNA re-extracted as above. Total RNA from cultured fibroblasts was prepared using the RNeasy spin columns according to the manufacturer’s protocol. Total RNA was quantified and checked for purity using spectrophotometry and gel electrophoresis.

First strand cDNA was prepared using 250 or 500 ng of total RNA. Reverse transcriptions were carried out using M-MLV reverse transcriptase (10 units) and oligo(dT₆₅₇) primer (1 μM). After cDNA synthesis, samples were heated to 94 °C for 2 min, diluted to appropriate levels, and stored at −20 °C until used.

**Quantitative Real-time PCR**—Quantitative PCR was carried out using the LightCycler (Roche Applied Science) real-time PCR machine and the fluorescent DNA-banding dye, SYBR green. Primers were optimized and melting peaks monitored to ensure unwanted dimer amplification did not affect PCR efficiency. Each cDNA sample was analyzed in triplicate.

Standards were generated using the tree shrew-specific primers for the three TGF-β isoforms and hypoxanthine-guanine phosphoribosyltransferase (Table I). Fragments were purified, quantified, and diluted to produce a four-point standard curve. All scleral samples were reverse-transcribed at the same time to control for between-experiment variation in reverse transcription efficiency. The standard reaction used the FastStart DNA Master SYBR Green I kit, 4 μl of cDNA and 0.5 μM primers. The specific amplification conditions are presented in Table I. TGF-β1 mRNA expression was expressed as copies/1000 copies of hypoxanthine-guanine phosphoribosyltransferase.

**LightCycler® Isoform Detection of Collagen Production**—Scleral fibroblasts were seeded at 5 × 10⁴ cells/well and maintained in growth media (described above) until confluent. Cells were then washed twice in a low-serum medium (Dulbecco’s modified Eagle’s medium supplemented with 1% fetal bovine serum, 25 mM HEPES, 100 units/ml penicillin-streptomycin) and incubated for a further 24 h. The medium was collected and supplemented with the required TGF-β isoform(s) (see below) and 0.5 μCi/ml [3H]proline. Cell incubations contained phosphate-buffered saline and 1% albumin (TGF-β diluted). After 48 h, the medium was collected and [3H]proline incorporation measured as described by Koyano et al. (28).

Dose-response curves were obtained by exposing the scleral fibroblasts to increasing concentrations (0.01–20 μg/ml) of the respective TGF-β isoforms. Calculations based on unpublished enzyme-linked immunosorbent assay data from this laboratory suggest concentrations of TGF-β1 in the posterior tree shrew sclera are ~0.1 ng/ml, which demonstrates that the isoforms used in this study are phys-
and the respective isoform concentrations altered to reflect the quantitative PCR data. For the first experiment, a combination dose approximating the EC50 response was chosen and the respective isoform concentrations altered to reflect the quantitative changes observed after 1 and 5 days of myopia induction.

**RESULTS**

**Expression of TGF-β Isoforms in Tree Shrew Sclera**—Although TGF-β1 and -β2 have been reported in the sclera (29), the existence of the third isoform has yet to be confirmed. To address this, tree shrew-specific primers for each of the three mammalian isoforms were designed and RT-PCR performed. As observed in Fig. 1, the predicted size fragments were amplified for TGF-β1, -β2, and -β3 (161, 200, and 210 bp, respectively) in both tree shrew sclera and cultured tree shrew scleral fibroblasts. The cultured tree shrew skin fibroblast cell line served as a positive control because skin fibroblasts have previously been shown to express the three isoforms (30). The addition of total RNA (lanes 2, 4, 6) controlled for genomic DNA contamination. This is, to our knowledge, the first demonstration of TGF-β3 in the mammalian sclera.

The use of quantitative real-time PCR data from unoccluded (control) eyes allowed the expression levels of the individual isoforms to be investigated. Fig. 2, A and B demonstrate that TGF-β2 is the predominant isoform of the sclera (~1,930 copies/1000 copies hypoxanthine-guanine phosphoribosyltransferase), whereas TGF-β1 (~17% (1 day), ~6% (5 day) of TGF-β2 levels) and TGF-β3 (~3% of TGF-β2 levels) are expressed to a lesser extent. Higher the TGF-β2 expression in the sclera correlates well with previous studies in other ocular tissues where this isoform has been shown to predominate (31).

**TGF-β Isoform mRNA Expression in Myopia**—Analysis of scleral mRNA expression in treated and control eyes of tree shrews developing myopia revealed isoform-specific alterations of TGF-β. After 1 day of treatment, there was a significant decrease in TGF-β2 and -β3 expression of 27%, (p < 0.05) and 42%, (p < 0.05), respectively (Fig. 2C). No significant change was found in the expression of TGF-β1 despite it showing a decrease (32%, p = 0.11). After 5 days of myopia induction, TGF-β3 expression was still significantly reduced by 36% (p < 0.05) when compared with the contralateral control eye. In addition, TGF-β1 showed a similar mean decrease in expression (37%, p < 0.05), whereas TGF-β2 showed an additional down-regulation (50%, p < 0.001) when compared with the 1-day data. This further reduction of 23% between the 1- and 5-day data was found to be significant (p = 0.05).

**TGF-β Isoform Control of Collagen Synthesis**—Each of the TGF-β isoforms has been reported to alter collagen expression in various cell types. To assess the effects of TGF-β on collagen expression in scleral fibroblasts, the cells were exposed to increasing concentrations of each of the isoforms. Proline incorporation was assayed because it is considered a relatively specific marker of collagen production. A dose-dependent increase in [3H]proline incorporation was observed for each of the three TGF-β isoforms (Figs. 3, A, B, and C). A maximum increase in [3H]proline incorporation of 264, 196, and 221% was seen for TGF-β1, -β2, and -β3, respectively. Mean dose-response curves, plotted from two separate experiments, enabled the EC50 value to be determined for each isoform. TGF-β2 was found to be the most potent of the three isoforms in scleral fibroblast cells with an EC50 of 0.75 ng/ml, followed by TGF-β1 (EC50, 0.81 ng/ml), with TGF-β3 being the least potent isoform (EC50, 1.84 ng/ml). Although TGF-β3 had previously been reported to be the most potent isoform in lung fibroblasts (32), little difference is found between isoform potency in renal fibroblasts (33), indicating that TGF-β regulation of collagen synthesis may be tissue-specific. Thus, given that TGF-β2 is the major isoform of the sclera, it is perhaps unsurprising that it exhibits an increased potency.

**In vivo**, scleral fibroblasts are not exposed to individual TGF-β isoforms but rather a balanced mixture of the three. As mentioned earlier in this report, the combination of TGF-β isoforms has a critical role in tissue development and wound healing. An estimate was made of the in vivo scleral ratios based on the quantitative PCR results (Fig. 2B) for the 5-day treatment group. The scleral fibroblast dose-response experiment was repeated; however, each dose included a TGF-β1: TGF-β2:TGF-β3 ratio of 2:3:1. The dose-response relationship

### Table 1

| Gene     | Primer sequence | PCR conditions | ANN value | EXT time | SIGNAL temperature |
|----------|-----------------|----------------|-----------|----------|-------------------|
| TGF-β1   | gcctgtaacccacatgatcc | 3               | 72 → 60   | 87       |
| TGF-β2   | tgacccacacatacatacag | 3               | 72 → 58   | 82       |
| TGF-β3   | cttgcacccacagtatgcgacac | 3               | 72 → 58   | 85       |
| HPRT     | ggacacccacagctttcc     | 4               | 72 → 55   | 80       |

**FIG. 1. TGF-β isoform expression in the tree shrew sclera.**

Using areas of tree shrew sequence, primers were designed for the respective TGF-β isoforms enabling specific products to be amplified (TGF-β1, 161 bp, TGF-β2, 200 bp, TGF-β3, 210 bp). RT-PCR was performed on tree shrew scleral tissue (lane 1) and primary cultures of tree shrew scleral (lane 3) and skin fibroblasts (lane 5). Total DNA from scleral tissue (lane 2) and the scleral and skin fibroblast cell lines (lanes 4 and 6, respectively) were used as controls for genomic DNA contamination.
A combination (Fig. 4A) displayed an increased potency compared with the individual isoforms with a calculated EC$_{50}$ of 0.55 ng/ml.

Previous studies of collagen synthesis during myopia induction have reported decreases in the order of 28% in whole scleral samples (5). To determine whether the observed TGF-$
\beta$ decreases during myopia induction (Fig. 2, C and D) could result in such a collagen decrease, [H]$\text{proline}$ incorporation was assayed after reducing each isoform in line with the 1- and 5-day data. Using the combination dose-response curve, the EC$_{50}$ concentration (0.55 ng/ml) was selected as a control level.

The experiments reduced TGF-$
\beta$1 levels by 32%, TGF-$
\beta$2 by 27%, and TGF-$
\beta$3 by 42% to mimic the in vivo situation after day 1 of myopia development, whereas a decrease in TGF-$
\beta$1, -$\beta$2, and -$\beta$3 levels of 36, 37, and 50%, respectively, approximated the situation at 5 days of myopia progression. As can be seen in Fig. 4B, by altering isoform levels collagen synthesis was reduced by $-13$% (1 day) and $-15$% (5 days) relative to the 0.55 ng/ml control.

**DISCUSSION**

The current study makes four important contributions to the literature on scleral changes in mammalian high (pathological) myopia. First, this is, to our knowledge, the first demonstration of the expression of all three TGF-$\beta$ isoforms in the mammalian sclera. This is important because the isoform profile affects the rate and final composition of a remodeled ECM, as occurs during myopia induction. Second, scleral expression levels of the TGF-$\beta$ isoforms were differentially reduced in an isoform-
and time-dependent manner, possibly reflecting isoform-specific roles in the remodeling of the scleral ECM at different stages of myopia development. Third, the current study demonstrated that all three isoforms were capable of up-regulating collagen synthesis in scleral fibroblasts and that, of the three, TGF-$\beta$2 was the most potent. Finally, changes in TGF-$\beta$ concentrations, which approximated the gene expression changes found in vivo, were capable of down-regulating collagen synthesis in scleral fibroblasts as has previously been reported from the sclerae of myopic eyes (5, 6). Collectively, these data demonstrate a mechanism whereby changes in the TGF-$\beta$ isoform profile result in an altered scleral ECM composition similar to that of the myopic phenotype.

All three mammalian isoforms of TGF-$\beta$ are expressed in the mammalian sclera and, although TGF-$\beta$1 and -$\beta$2 have previously been reported (29), this is, to our knowledge, the first explicit demonstration of TGF-$\beta$3 expression in this tissue. Because this isoform plays a role in producing a more ordered ECM following remodeling after wounding (17), it is possible that it fills a similar role in the sclera. The scleral TGF-$\beta$ isoform expression profile is in keeping with previous studies in other ocular structures, confirming that TGF-$\beta$2 predominates, whereas the quantitative data indicate that TGF-$\beta$3 is expressed at significantly lower levels than the other isoforms. This ocular profile is relatively unusual compared with the rest of the body, excepting tissues such as amniotic fluid (31), and it has been suggested that it is important to the immune privilege that the eye enjoys (34). However, the potential significance of

**Fig. 2.** Alterations in TGF-$\beta$ isoform gene expression during myopia development. Monocular deprivation of form vision was used to induce myopia in tree shrews, and TGF-$\beta$ isoform expression was quantified after 1 (A, C) and 5 (B, D) days of deprivation. Copies of individual isoforms were quantified in scleral samples ($n$ = 6) with reference to an external standard (see "Experimental Procedures") and were expressed per 1000 copies of the housekeeping gene, hypoxanthine-guanine phosphoribosyltransferase (A, B). Data are also presented as the percentage difference in gene expression (treated eye-control eye) ± S.E. (C, D). * indicates a statistically significant result.
this isoform profile in guiding postnatal ocular growth is less clear with only limited studies performed (20). The isoform-specific changes observed in the current study provide further information on the possible role of TGF-β in ocular growth.

The rapidity of the decrease in TGF-β expression reported in this study, combined with maintenance of the reduced expression levels, indicates that this family of cytokines may have roles as both a primary intrascleral mediator as well as a continuous regulator of the remodeling process. In vivo wound healing studies have shown that TGF-β isoform expression does alter within 24 h of the initial insult, seemingly coordinating the ECM repair process (35). In fact, changes in TGF-β1

expression have been reported in corneal fibroblasts 30 min after the initial injury (36). However, although the changes in scleral TGF-β expression occur before the structural changes can be observed that ultimately produce the myopia, earlier time points need to be investigated before the precise role of these isoforms as an intrascleral mediator of remodeling could be confirmed.

In addition to the above, the early decrease in scleral TGF-β isoforms may also be indicative of a role for the TGF-β in the retinoscleral signal guiding ocular growth. It is well established that the retina, under the influence of a change in the visual environment, is the site of initiation of a signaling process that traverses the retina and choroid and drives scleral remodeling to facilitate myopic eye growth (37). The potential role of TGF-β in this process was highlighted by a report that TGF-β abolished the inhibitory effect of FGF-2 on ocular growth (20). Furthermore, urokinase plasminogen activator, an activator of latent TGF-β, was found to inhibit ocular growth, whereas an inhibitor of TGF-β activation, plasminogen activator inhibitor-1, increased ocular growth rates (21). However, despite the fact that both studies used the chick model of myopia, the respective results are difficult to reconcile with TGF-β both increasing and decreasing ocular growth.

When the current data are compared with those reported for avian models of myopia, scleral TGF-β expression is regulated in the opposite direction (22). This observation is perhaps unsurprising given the differing structure of the chick sclera, which is largely cartilaginous with a thin fibrous outer layer, and the fact that the chick sclera thickens overall during my-
opla development (38). As a result, scleral findings in myopic birds and mammals are often opposite in direction. For example, scleral glycosaminoglycan synthesis is found to be up-regulated in birds and down-regulated in mammals developing myopia (39).

Although the precise role of TGF-β in initiating any remodeling response is speculative, the isoform quantitative data, in conjunction with the proline incorporation data, confirm the role of TGF-β in the ongoing scleral remodeling process. In this study, all three isoforms were found to stimulate collagen synthesis as measured by proline incorporation. This is in agreement with a previous study that reported that TGF-β could up-regulate collagen I and III mRNA in bovine scleral fibroblasts (40). The same study also showed that scleral biglycan mRNA and protein levels were increased after TGF-β exposure. As mentioned previously, decreases in proteoglycan side chains (10, 11) and collagen (5) are reported in sclera of myopic eyes; this would correlate with the TGF-β decreases reported in this study.

TGF-β1 and -β2 are typically profibrotic in that they encourage the laying down of new matrix in a generally disorganized fashion and inhibit MMP activity. In contrast, TGF-β3 has been reported to play an anti-fibrotic role in promoting organo-scleral remodeling (30). The fact that only the TGF-β1 and -β3 isoforms are known to have varying effects on the ECM, whether it be regulating different genes or exhibiting different complexity with respect to scleral remodeling. Each of the TGF-β isoforms is known to have varying effects on the ECM, which collagen subtypes are specifically regulated and whether these alterations occur prior to significant changes in eye size imply these molecules as potential mediators of the retinoscleral signal that initiates the scleral changes in high myopia.

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In summary, the data from this study demonstrate that scleral TGF-β expression is reduced during myopia development in an isoform- and time-specific manner. These changes are consistent with the concurrent reductions in scleral collagen synthesis that have been shown to drive remodeling of the scleral ECM in myopia. An important conclusion from these findings is that coordinated changes in expression levels of all three isoforms may control scleral ECM remodeling in myopia. In addition, the fact that TGF-β alterations do occur prior to significant changes in eye size implicate these molecules as potential mediators of the retinoscleral signal that initiates the scleral changes in high myopia.
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