Cross-talk between miR-29 and Transforming Growth Factor-Betas in Trabecular Meshwork Cells

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Purpose. To investigate the interactions between microRNA-29 (miR-29), a negative regulator of extracellular matrix (ECM), and transforming growth factors (TGF)β-1 and TGFβ-2.

Methods. Changes in expression of the miR-29 family were analyzed by quantitative-PCR (Q-PCR) after treatment with *TGFβ1* and *TGFβ2* (1 ng/mL). *TGFβ1* and *TGFβ2* were evaluated at gene expression and protein levels by Q-PCR and ELISA, respectively, in human trabecular meshwork (HTM) cells transfected with miR-29b or scramble control. *TGFβ1* promoter activity was analyzed using an adenovirus with the reporter SEAP. The effects of miR-29b and *TGFβ2* on ECM gene expression were evaluated in cells transfected with miR-29b or scramble control and treated with *TGFβ2*, and the expression of ECM genes was analyzed by Q-PCR.

Results. *TGFβ2* but not *TGFβ1*, downregulated the three members of the miR-29 family. Overexpression of miR-29b antagonized the effects of *TGFβ2* on the expression of several ECM components. MiR-29b decreased the expression of *TGFβ1* at the promoter, transcript, and protein levels but had only a minor effect on the expression of active *TGFβ2*. The inhibition of *TGFβ1* by miR-29b was partially recovered after co-transfection with a plasmid-expressing bone morphogenetic protein 1.

Conclusions. Results showed some level of crosstalk between TGFβs and miR-29. Specifically, the downregulation of miR-29 by *TGFβ2* contributed to the induction of several ECM components by this cytokine in TM cells. This observation, together with the inhibitory effects of miR-29b on the expression of *TGFβ1*, suggests that the miR-29 family could play an important role in modulating TGFβs on the outflow pathway. (*Invest Ophthalmol Vis Sci.* 2011;52:3567–3572) DOI: 10.1167/iovs.10-6448

Glaucoma is second only to cataracts as the greatest blinding disorder worldwide, and it is estimated that it will affect 60.5 million people by 2010.[1] The main risk factor for primary open-angle glaucoma (POAG) is elevated intraocular pressure (IOP), presumably due to changes in the conventional outflow pathway (trabecular meshwork/Schlemm's canal). Alterations in the composition of the extracellular matrix (ECM) of the trabecular meshwork (TM) are known to be associated with POAG and are believed to play an important role in the abnormal increase in outflow resistance that leads to elevated IOP in this disease.[2,3]

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The transforming growth factor beta (TGFβ) subfamily of cytokines includes three isoforms, *TGFβ1*, β2, and β3, which regulate a wide range of essential cellular activities, including proliferation, differentiation, and ECM dynamics in many cell types.[4–6] Because of their pivotal role in cell regulation, alteration of TGFβs is a characteristic of many diseases and pathologies,[7–10] including glaucoma. *TGFβ2* has been found to be elevated in the aqueous humor of glaucoma patients,[11,12] and elevation of *TGFβ1* has been associated with pseudoexfoliative glaucoma.[13] Experiments in vitro and in vivo have shown that TGFβs might play an important role in the pathogenesis of the TM in glaucoma. For instance, TM cells treated with *TGFβ2* showed senescence-associated changes[14] and increased synthesis of several ECM components.[15,16] In addition, perfusion of human anterior segments with *TGFβ2* resulted in increased ECM and IOP,[17] and overexpression of the active form of *TGFβ2* increased IOP and reduced outflow facility in mice and rats.[18] Similarly, *TGFβ1* overexpression changed the morphology of the anterior segment of rat eyes and affected IOP.[19]

The miR-29 family of microRNAs (miRNAs) is composed of three highly similar orthologs (miR-29a, miR-29b, and miR-29c) that share identical seed sequences. The members of the miR-29 family are known to repress posttranscriptional expression of several mRNAs that encode proteins involved in fibrosis, including multiple ECM components such as collagens, fibrillins, and elastin.[20–23] We have previously demonstrated that miR-29b negatively regulates the expression of genes involved in ECM synthesis and deposition in TM cells and that downregulation of miR-29b, under chronic oxidative stress conditions, contributes to an increase in expression of multiple ECM components.[23] Some recent reports have suggested that downregulation of members of the miR-29 family by TGFβs might contribute to the fibrogenic effects of these cytokines. *TGFβ1* has been implicated in the reduction of the levels of miR-29a observed in fibroblasts from systemic sclerosis patients, which is believed to contribute to increased expression of multiple collagen genes targeted by miR-29a.[24] Similarly, *TGFβ1* has been shown to induce a significant downregulation of miR-29a in proximal tubule cells leading to an increase in collagen IV.[25] However, our knowledge about the interactions between miR-29 and TGFβs is still very limited. Therefore, we investigated the potential effects of *TGFβ1* and *TGFβ2* on the expression of the miR-29 family and evaluated whether alterations in miR-29 expression might contribute to the effects mediated by these cytokines on the expression of ECM genes in human TM cells. In addition, we analyzed whether miR-29 can, in turn, affect the expression of *TGFβ1* and *TGFβ2*.

Materials and Methods

Cell Culture and TGFβ Treatment

HTM cell cultures were generated from cadaver eyes, with no history of eye disease, within 48 hours post mortem, as previously reported.[26]

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All procedures involving human tissue were conducted in accordance with the tenets of the Declaration of Helsinski. Cell cultures were maintained at 37°C in 5% CO_2 in media (low-glucose Dulbecco's Modified Eagle Medium with L-glutamine, 110 mg/mL sodium pyruvate, 10% fetal bovine serum, 100 μM non-essential amino acids, 100 units/mL penicillin,100 μg/mL streptomicyn sulfate, and 0.25 μg/mL amphotericin B; all reagents were obtained from Invitrogen, Carlsbad, CA). For *TGFβ1* and *TGFβ2* (Sigma Aldrich, St. Louis, MO) treatment, the cells were serum starved for 24 hours and treated with 1 ng/mL *TGFβ1* or *TGFβ2* for 24 hours.

Transfections

HTM cells were plated 24 hours before transfection and transfected between 50% and 70% of confluence using reagent (lipofectamine 2000; Invitrogen), following the manufacturer's instructions. In brief, for transfection of cells in a 12-well plate, 40 picomoles mirna or mirna plus plasmids (Dharmacon, Chicago, IL) and 1 μL lipofectamine were diluted in 50 μL reduced serum medium (OPtiMem I; Invitrogen) each, incubated for 5 minutes at room temperature (RT), and then lipofectamine and mirna/plasmids were combined and incubated further for 20 minutes at RT and added to the cells in media without antibiotics. Cells were incubated overnight at 37°C in 5% CO_2 and changed to complete media after that. Cells were co-transfected with plasmids expressing a bone morphogenetic protein 1 (BMP1) open reading frame (Origene, Rockville, MD) or green fluorescent protein (GFP; 0.3 μg). The efficiency of the transfection with miRnas or plasmid was confirmed by quantitative-PCR (Q-PCR).

RNA Isolation and Q-PCR

Total RNA was isolated using one of two extraction methods (RNeasy kit; Qiagen, Valencia, CA; or Trizol; Invitrogen) according to the manufacturers' instructions. RNA yields were measured using fluorescent dye (RiboGreen; Invitrogen). First-strand cDNA was synthesized from total RNA (500 ng) by reverse transcription using reverse transcriptase (oligodT and SuperScript II; Invitrogen) according to the manufacturer's instructions. Q-PCR reactions were performed in 20 μL mixture containing 1 μL of the cDNA preparation (1X iQ SYBR Green Supermix; Bio-Rad, Hercules, CA), using the following PCR parameters: 95°C for 5 minutes followed by 50 cycles of 95°C for 15 seconds, 65°C for 15 seconds, and 72°C for 15 seconds. Here β-actin or *GADPH* were used as an internal standard of mRNA expression. Primers were designed using online available software (Primer 3′ Input Software, http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi),[27] and the annealing temperatures were determined from the above mentioned software and by Q-PCR using a thermal gradient in a single experiment with the same template for all primers (temperatures ranging from 55°C to 65°C; a CFX96 System and CFX Manager Sofware; Bio-Rad). The absence of nonspecific products was confirmed by both the analysis of the melt curves and by electrophoresis (3% Super AcrylAgarose gels; DNA Technologies, Gaithersburg, MD). The primers used for Q-PCR amplification are shown in Table 1. MicroRNAs were extracted using an miRNA isolation kit (RT^2 qPCR-Grade; SABiosciences, Frederick, MD). MiRNAs cDNA (25 ng) were amplified using a microRNA reverse transcription kit (TaqMan; Applied Biosystems, Foster City, CA) and specific primers for miR-29a, miR-29b, miR-29c, and U6B as a standard (all from Applied Biosystems). Q-PCR products were amplified following the manufacturer's instructions (TaqMan Universal PCR Master Mix; Applied Biosystems). The fluorescence threshold value (C_t) was calculated using commercially available system software (iCycle; Bio-Rad). The results were expressed as mean value ± SE in three independent experiments.

TGFβs Measurement

TGFβ1 and *TGFβ2* were measured (Quantikine Human *TGFβ1* and Human *TGFβ2*, respectively; R&D Systems, Minneapolis, MN) following the manufacturer's instructions. These are "sandwich" enzyme-linked immunoassays that measure activated *TGFβ1* and *TGFβ2*.

Promoter Activity Assay

The adenovirus Ad*TGFβ1* containing the *TGFβ1* promoter region and the reporter SEAP is described elsewhere.[28] Activation of *TGFβ1* promoter after transfection with miR-29b or scramble was quantified by the amount of SEAP released to the culture medium (Great EscAPeTM SEAP chemiluminescence kit 2; Clontech, Mountain View, CA) following the manufacturer's instructions.

Results

Effects of *TGFβ1* and *TGFβ2* on the Expression of miR-29

To evaluate the effects of *TGFβ1* and *TGFβ2* on the expression of the miR-29 family, three independent HTM cell lines were treated with either *TGFβ1* or *TGFβ2* (1 ng/mL), and the expression of miR-29a, miR-29b, and miR-29c was analyzed by Q-PCR. *TGFβ1* did not significantly affect the expression of miR-29b, increased the expression of miR-29a, and showed variable effects on the expression of miR-29c. On the other hand, *TGFβ2* significantly and consistently decreased the expression of all three miRNAs in the three cell lines analyzed (Fig. 1).

Effects of miR-29b on the Induction of ECM Genes by *TGFβ2*

To analyze the potential relevance of the downregulation of miR-29 mediated by *TGFβ2* on the induction of ECM-related genes by this cytokine, HTM cells were transfected with miR-29b or scramble control and split, and half of the cells were treated with *TGFβ2* (1 ng/mL) for 24 hours. To ensure that lipofectamine transfection was capable of delivering enough levels of miR-29 mimic, the efficiency of transfection was analyzed by Q-PCR in three HTM cell lines transfected with

Table 1. Primers Used for Q-PCR Amplification

Gene Symbol	Forward 5′–3′	Reverse 5′–3′
COL1A1	AGCCAGCAGATCGAGAACAT	TCTTGTCCTTGGGGTTCTTG
COL1A2	TGCAAGAACAGCATTGCATAC	GGCAGGCGAGATGGCTTATTTGTT
COL5A1	GGCTGTGCTACCAAGAAAGG	GAGGTCACGAGGTTGCTCT
LAMC1	AATGAAGCCAAGAAGCAGGA	ATGGACAGCAGCAGAGGAGT
SPARC	CCGGGACTTCGAGAAGAACT	CTCATCCAGGGCAATGTACT
TGFβ1	GTCCTCGAGCTCCATGGCGCTCTTCGTG	GTAAAGCTTCAAGCTAATGCTTCATCCT
TGFβ2	AGGGCGGCCGCCTGCAGCGCGAGAGGA	GGATATCTTTAGCTGCATTTGCAAGACT
CTGF	CCTGGTCCAGACCACAGAGT	TGGAGATTTTGGGAGTACGG
BMP1	GTGTGGCCCGATGGGGTCAT	CCCGCAAGGTCGATAGGTGAA
GAPDH	TCGACAGTCAGCCGCATCTTCTTT	ACCAAATCCGTTGACTCCGACCTT
ACTB	CCTCGCCTTTGCCGATCCG	GCCGGAGCCGTTGTCGACG

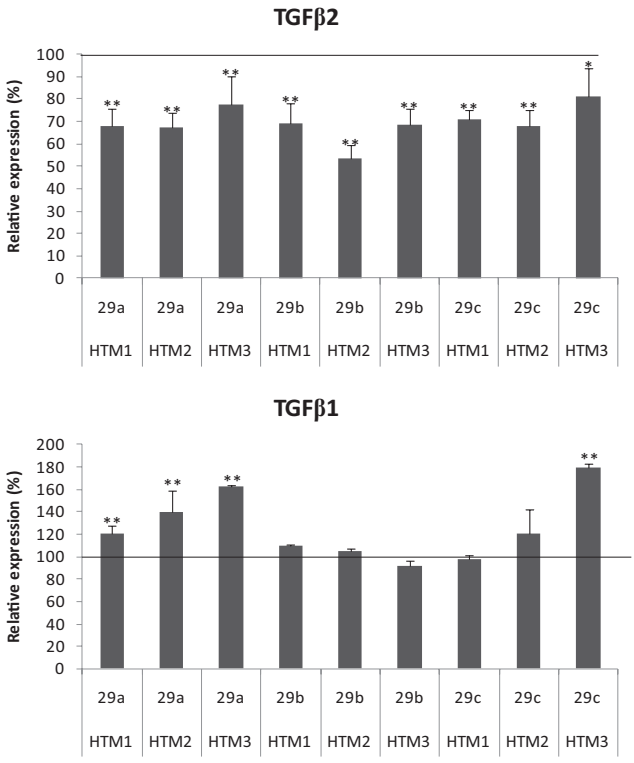

Figure 1. Effects of TGFβs on miR-29b expression. Three HTM cell lines were treated with *TGFβ1* or *TGFβ2* (1 ng/mL), and the expression of miR-29a, -b, and -c was analyzed by Q-PCR. The figures represent the relative expression of the fold change between cells treated with either *TGFβ1* or *TGFβ2* compared to controls (not treated cells). Bars represent SD from three different experiments. $^{*}P \leq 0.05$, $^{**}P \leq 0.01$.

scramble or miR29. Transfection with miR-29 mimic resulted in an average fold increase of 1917 ($P \leq 0.01$) in the presence of miR29 compared to the controls transfected with scramble microRNA. Transfection with miR-29b mimic resulted in significant downregulation of *COL1A1*, *COL1A2*, *LAMC-1*, *SPARC*, and *COL5A1*. *CTGF* was strongly upregulated by *TGFβ2*, and its upregulation was partially inhibited by miR-29b in only one cell line. Transfection of miR-29b significantly prevented the upregulation of collagens *LAMC-1* and *SPARC* induced by *TGFβ2* when compared with cells transfected with scramble and treated with *TGFβ2* (Fig. 2).

Effects of miR-29b on the Expression of *TGFβ1* and *TGFβ2*

To analyze the effects of miR-29 on the expression of *TGFβ1* and *TGFβ2*, three independent HMT cell lines were transfected with either miR-29b mimic or scramble control. Expression of activated *TGFβ1* and *TGFβ2* proteins were quantified by ELISA, and changes in transcripts expression were analyzed by Q-PCR. Cells transfected with miR-29b showed a significant reduction in the levels of *TGFβ1* protein, 40% on average (Fig. 3A), and also downregulated mRNA levels (Fig. 3B) in three HTM primary cell lines. To study the effects of miR-29b on *TGFβ1* promoter, we infected three HTM cell lines with adenovirus expressing secreted luciferase under the control of the *TGFβ1* promoter. MiR-29b significantly reduced the luciferase expression driven by the *TGFβ1* promoter compared to controls (Fig. 3C). Mir-29b decreased *TGFβ2* mRNA in all three tested cell lines (Fig. 4A) and showed a significant, but small, decrease in *TGFβ2* at the protein level in two out of three cell lines transfected with miR-29b compared to the scramble control (Fig. 4B).

Effects of *BMP*1 Overexpression on the Inhibition of *TGFβ1* by miR-29b

Since we have previously identified *BMP*1, a known activator of *TGFβ1*,[29] as a direct target of miR-29b,[23] we investigated its potential involvement on the inhibition of *TGFβ1* mediated by miR-29. Three HTM cell lines co-transfected with (1) miR-29b and *BMP*1 ORF plasmid, (2) miR-29b and a plasmid-expressing GFP, or (3) scramble control and a plasmid-expressing GFP were analyzed for active *TGFβ1* protein expression by ELISA. Expression of *BMP*1 completely prevented the downregulation of *TGFβ1* induced by miR-29 in two cell lines and significantly decreased the level of *TGFβ1* downregulation in the third analyzed cell line (Fig. 5). The overexpression of *BMP*1 was confirmed by Q-PCR in cells transfected with miR29b and *BMP*1 compared to miR-29b and GFP (HTM-1, -2, and -3 showed 3.58, 2.53, and 1.83 folds, respectively; $P \leq 0.01$).

Discussion

Our results showed that although *TGFβ1* did not alter miR-29b and miR-29c and upregulated miR-29a, *TGFβ2* significantly

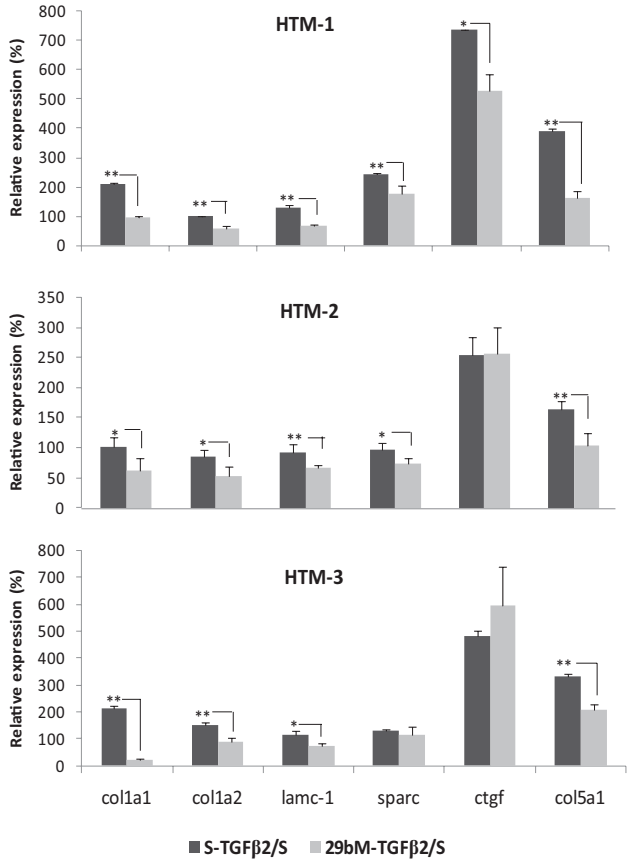

Figure 2. MiR-29b antagonized the effects of *TGFβ2* on ECM genes. HTM cell lines transfected with miR-29b mimic or scramble control were split, and half of the cells were treated with *TGFβ2* (1 ng/mL). The expression of *COL1A1*, *COL1A2*, *LAMC-1*, *SPARC*, *CTGF*, and *COL5A1* was analyzed by Q-PCR. The figure represents the relative expression of the fold change between cells transfected with scramble control and treated with *TGFβ2* and cells transfected with miR-29b and treated *TGFβ2*, both compared to scramble control without treatment. Bars represent SD from three different experiments. $^{*}P \leq 0.05$, $^{**}P \leq 0.01$.

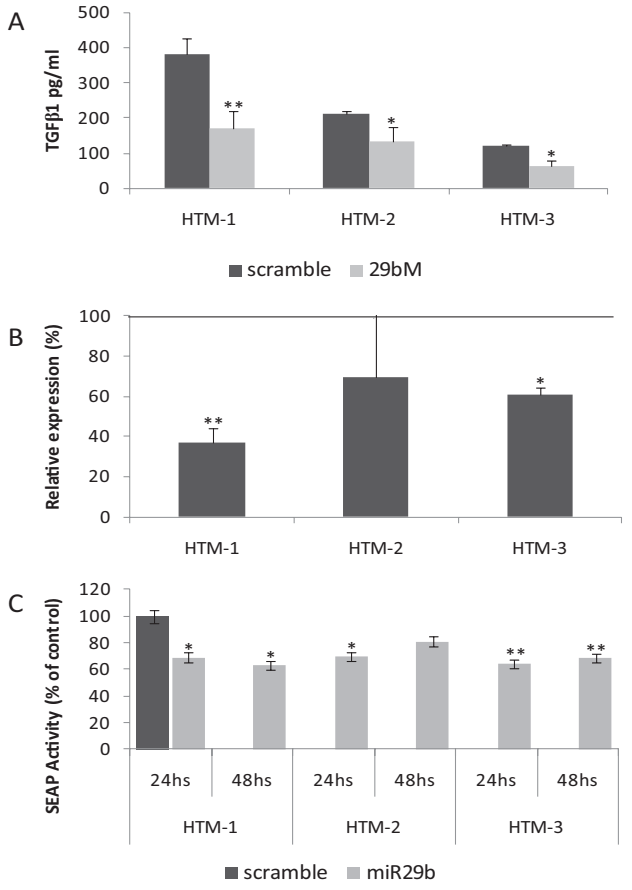

FIGURE 3. Effects of miR-29b on *TGFβ1*. The protein, mRNA, and promoter levels of *TGFβ1* were evaluated after transfection with miR-29b in three HTM cell lines. (**A**) Amount of activated *TGFβ1* on the supernatant of cells transfected with miR-29b compared to cells transfected with scramble control. (**B**) Relative expression of the fold change in *TGFβ1* between cells transfected with miR-29b compared to scramble control. (**C**) Percentage of SEAP activity of cells infected with adenovirus containing the *TGFβ1* promoter region and the reporter SEAP (25 pfu) and transfected with miR-29b compared to cells infected with the same virus and transfected with scramble control. Bars represent SD from three different experiments. $^{*}P \leq 0.05$, $^{**}P \leq 0.01$.

downregulated all members of the miR-29 family (29a, -b, and -c) at concentrations closer to those found in aqueous humor.[11,30] Although there are no reports about the effects of *TGFβ2* on miR-29 expression from other cell types, the lack of effects of *TGFβ1* on miR-29 expression contrasts with the results reported in human fibroblasts[24] and proximal tubule cells.[25] However, the inhibition of miR-29 by *TGFβ1* in these two cell types was observed at a concentration 10 times higher than those used in our experiments. In contrast, *TGFβ1* used at a concentration similar to that of our experimental model did not alter the expression of miR-29b in stellate cells.[31] These discrepancies could be the result of cell type specific responses or the different concentrations of *TGFβ1* used to treat the cells. Since our experiments were conducted with a concentration of *TGFβ1* higher than those believed to be present in the aqueous humor,[13,32,33] our results suggest that, at physiologic concentrations, *TGFβ1* might not significantly affect the expression of miR-29. However, the downregulation induced by *TGFβ2* in all members of the miR-29 family suggests that changes in expression of these miRNAs might contribute to the upregulation of ECM genes induced by *TGFβ2* in TM cells.

We have previously shown that miR-29b regulates the expression of various genes involved in ECM metabolism in HTM cells.[23] It is believed that all members of the miR-29 family target a similar set of genes because of their strong sequence similarities and identical seed sequences. The upregulation of ECM genes induced by *TGFβ2* in our experiments showed a relatively high degree of variability among the three HTM cell lines analyzed, suggesting that a high level of interindividual variability in the cellular responses to *TGFβ2* may exist in human populations. However, in spite of such high levels of variability, overexpression of miR-29b consistently inhibited the upregulation of *COL1A1*, *COL1A2*, *COL5A1*, *LAMC1*, and *SPARC* induced by *TGFβ2*. These results suggest that downregulation of miR-29 and the subsequent derepression of genes regulated by this family may indeed play an important role in the upregulation of ECM genes induced by *TGFβ2*.

The induction of ECM components by *TGFβ2* in optic nerve astrocytes[34] and trabecular meshwork[35] cell cultures has been reported to be mediated by its downstream mediator *CTGF*. However, miR-29b had only a minor effect on the upregulation of *CTGF* induced by *TGFβ2* in one cell line and had no significant effect in the other two cell lines analyzed. Therefore, the effects of miR-29b appear to be independent of *CTGF* and are more likely to be mediated by direct targeting of their 3′UTRs and downregulation of transcription factors such as SP1.[23]

In addition to the effects of TGFβs on miR-29 expression, we investigated whether miR-29 could, in turn, affect the expression of *TGFβ1* and *TGFβ2*. Although miR-29b had only a minor effect on *TGFβ2* protein, our results showed a significant and consistent downregulation of *TGFβ1* at pro-

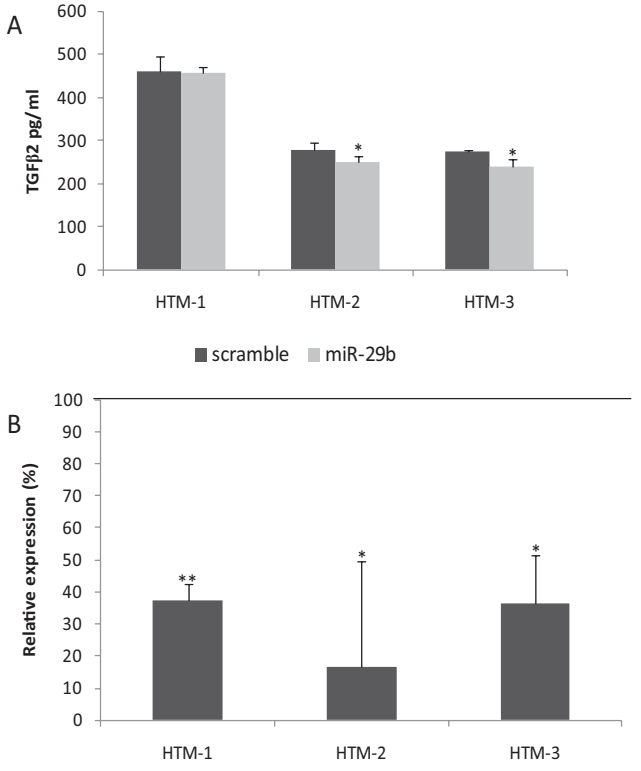

FIGURE 4. Effects of miR-29b on *TGFβ2*. The protein and mRNA levels of *TGFβ2* were evaluated after transfection with miR-29b in three HTM cell lines. (**A**) Amount of activated *TGFβ2* on the supernatant of cells transfected with miR-29b compared to cells transfected with scramble control. (**B**) Relative expression of the fold change in *TGFβ2* between cells transfected with miR-29b compared to scramble control. Bars represent SD from three different experiments. $^{*}P \leq 0.05$, $^{**}P \leq 0.01$.

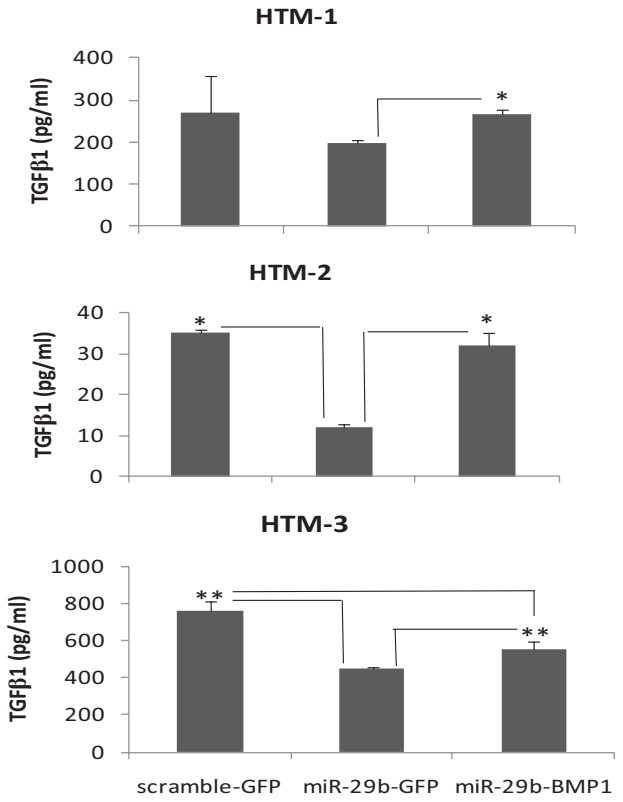

FIGURE 5. BMP1 expression partially recovers *TGFβ1* protein levels. HTM cells were transfected with miR-29b or scramble and plasmids expressing BMP1 ORF or GFP and the amount of active *TGFβ1* protein was analyzed by ELISA. Bars represent SD from three different experiments. $^{*}P \leq 0.05$, $^{**}P \leq 0.01$.

tein, transcript, and promoter levels. We have previously reported the targeting of *BMP1* by miR-29b in HTM cells,[23] which is one of the known regulators of *TGFβ1* protein activation. *BMP1* has been shown to activate latent *TGFβ1* by MMP2-dependent cleavage of latent TGFβ binding protein 1 and to form an amplification loop with *TGFβ1*.[29] Consistent with this role in *TGFβ1* activation, overexpression of *BMP1*, lacking the 3′UTR that contains the binding site for miR-29, partially prevented the decrease in active *TGFβ1* induced by miR-29 in HTM cells. These results support a contributing role of *BMP1* targeting the regulation of *TGFβ1* expression by miR-29, but they do not explain the observed decrease in promoter activity and downregulation of *TGFβ1* transcript induced by miR-29b. MiR-29b decreased the activity of the *TGFβ1* promoter by mechanisms yet to be defined. There are few examples of miRNAs that regulate gene expression at the promoter level. MiR-520b downregulated MHC class I–related chain A at the 3′UTR and promoter levels,[36] and miR-373 has been shown to target the promoters of E-cadherin and *CSDC2* genes.[37] Therefore, we used two available online tools (RegRNA software [http://regrna.mbc.nctu.edu.tw]; and miRBase [http://www.mirbase.org/search.shtml])[38,39] to search for putative target sequences in the promoter region of *TGFβ1*. However, no putative binding sites for the miR-29 family were found (data not shown). Therefore, the downregulation of *TGFβ1* promoter may be mediated by alterations of other genes involved in the regulation of the *TGFβ1* promoter activity. The combined effects of miR-29b on the inhibition of *TGFβ1* activation through BMP1 targeting and the repression of the transcriptional activity of the *TGFβ1* promoter suggest that miR-29 regulates *TGFβ1* through redundant and potentially additive mechanisms.

Although our experiments were conducted in vitro and only short-term effects were evaluated, the results indicate that interactions between the TGFβ family of cytokines and the miR-29 family of miRNAs might contribute to the modulation of ECM synthesis in TM cells. Specifically, the downregulation of the miR-29 family by *TGFβ2* and the subsequent derepression of genes targeted by this family of miRNAs appear to be an important regulatory event that contributes to the upregulation of several ECM components induced by *TGFβ2*. This observation, together with the inhibitory effects of miR-29b on the expression of *TGFβ1*, suggests that the miR-29 family could play an important role in modulating the pathogenic effects of TGFβs on the outflow pathway in glaucoma.

References

1. Quigley HA, Broman AT. The number of people with glaucoma worldwide in 2010 and 2020. *Br J Ophthalmol.* 2006;90:262–267.
2. Lutjen-Drecoll E. Morphological changes in glaucomatous eyes and the role of TGFbeta2 for the pathogenesis of the disease. *Exp Eye Res.* 2005;81:1–4.
3. Tektas OY, Lutjen-Drecoll E. Structural changes of the trabecular meshwork in different kinds of glaucoma. *Exp Eye Res.* 2009;88:769–775.
4. Roberts AB, Heine UI, Flanders KC, Sporn MB. Transforming growth factor-beta. Major role in regulation of extracellular matrix. *Ann N Y Acad Sci.* 1990;580:225–232.
5. Hynes RO. The extracellular matrix: not just pretty fibrils. *Science.* 2009;326:1216–1219.
6. Schuster N, Krieglstein K. Mechanisms of TGF-beta-mediated apoptosis. *Cell Tissue Res.* 2002;307:1–14.
7. Lee HS, Song CY. Effects of TGF-beta on podocyte growth and disease progression in proliferative podocytopathies. *Kidney Blood Press Res*. 33:24–29.
8. Jinnin M. Mechanisms of skin fibrosis in systemic sclerosis. *J Dermatol*. 37:11–25.
9. Rosenbloom J, Castro SV, Jimenez SA. Narrative review: fibrotic diseases: cellular and molecular mechanisms and novel therapies. *Ann Intern Med*. 152:159–166.
10. Jeon HS, Jen J. TGF-beta signaling and the role of inhibitory Smads in non-small cell lung cancer. *J Thorac Oncol*. 5:417–419.
11. Tripathi RC, Li J, Chan WF, Tripathi BJ. Aqueous humor in glaucomatous eyes contains an increased level of TGF-beta 2. *Exp Eye Res.* 1994;59:723–727.
12. Ozcan AA, Ozdemir N, Canataroglu A. The aqueous levels of TGF-beta2 in patients with glaucoma. *Int Ophthalmol.* 2004;25:19–22.
13. Schlotzer-Schrehardt U, Zenkel M, Kuchle M, Sakai LY, Naumann GO. Role of transforming growth factor-beta1 and its latent form binding protein in pseudoexfoliation syndrome. *Exp Eye Res.* 2001;73:765–780.
14. Yu AL, Birke K, Moriniere J, Welge-Lussen U. TGF-beta2 induces senescence-associated changes in human trabecular meshwork cells. *Invest Ophthalmol Vis Sci*. 2010;51:5718–5723.
15. Fatma N, Kubo E, Toris CB, Stamer WD, Camras CB, Singh DP. PRDX6 attenuates oxidative stress- and TGFbeta-induced abnormalities of human trabecular meshwork cells. *Free Radic Res.* 2009;43:783–795.
16. Fuchshofer R, Stephan DA, Russell P, Tamm ER. Gene expression profiling of TGFbeta2- and/or BMP7-treated trabecular meshwork cells: Identification of Smad7 as a critical inhibitor of TGF-beta2 signaling. *Exp Eye Res.* 2009;88:1020–1032.
17. Gottanka J, Chan D, Eichhorn M, Lutjen-Drecoll E, Ethier CR. Effects of TGF-beta2 in perfused human eyes. *Invest Ophthalmol Vis Sci.* 2004;45:153–158.
18. Shepard AR, Millar JC, Pang IH, Jacobson N, Wang WH, Clark AF. Adenoviral gene transfer of active human transforming growth factor-{beta}2 elevates intraocular pressure and reduces outflow facility in rodent eyes. *Invest Ophthalmol Vis Sci*. 51:2067–2076.

19. Robertson JV, Golesic E, Gauldie J, West-Mays JA. Ocular gene transfer of active TGF-beta induces changes in anterior segment morphology and elevated IOP in rats. *Invest Ophthalmol Vis Sci*. 51:308–318.
20. Sengupta S, den Boon JA, Chen IH, et al. MicroRNA 29c is down-regulated in nasopharyngeal carcinomas, up-regulating mRNAs encoding extracellular matrix proteins. *Proc Natl Acad Sci U S A.* 2008;105:5874–5878.
21. van Rooij E, Sutherland LB, Thatcher JE, et al. Dysregulation of microRNAs after myocardial infarction reveals a role of miR-29 in cardiac fibrosis. *Proc Natl Acad Sci U S A.* 2008;105:13027–13032.
22. Kapinas K, Kessler CB, Delany AM. miR-29 suppression of osteonectin in osteoblasts: Regulation during differentiation and by canonical Wnt signaling. *J Cell Biochem.* 2009;108:216–224.
23. Luna C, Li G, Qiu J, Epstein DL, Gonzalez P. Role of miR-29b on the regulation of the extracellular matrix in human trabecular meshwork cells under chronic oxidative stress. *Mol Vis.* 2009;15:2488–2497.
24. Maurer B, Stanczyk J, Jungel A, et al. MicroRNA-29, a key regulator of collagen expression in systemic sclerosis. *Arthritis Rheum*. 62:1733–1743.
25. Du B, Ma LM, Huang MB, et al. High glucose down-regulates miR-29a to increase collagen IV production in HK-2 cells. *FEBS Lett*. 2010;584:811–816.
26. Stamer WD, Seftor RE, Williams SK, Samaha HA, Snyder RW. Isolation and culture of human trabecular meshwork cells by extracellular matrix digestion. *Curr Eye Res.* 1995;14:611–617.
27. Rozen S, Skaletsky H. Primer3 on the WWW for general users and for biologist programmers. *Methods Mol Biol.* 2000;132:365–386.
28. Liton PB, Liu X, Challa P, Epstein DL, Gonzalez P. Induction of TGF-beta1 in the trabecular meshwork under cyclic mechanical stress. *J Cell Physiol.* 2005;205:364–371.
29. Ge G, Greenspan DS. BMP1 controls TGFbeta1 activation via cleavage of latent TGFbeta-binding protein. *J Cell Biol.* 2006;175:111–120.
30. Welge-Lussen U, May CA, Lutjen-Drecoll E. Induction of tissue transglutaminase in the trabecular meshwork by TGF-beta1 and TGF-beta2. *Invest Ophthalmol Vis Sci.* 2000;41:2229–2238.
31. Ogawa T, Iizuka M, Sekiya Y, Yoshizato K, Ikeda K, Kawada N. Suppression of type I collagen production by microRNA-29b in cultured human stellate cells. *Biochem Biophys Res Commun*. 391:316–321.
32. Koliakos GG, Schlotzer-Schrehardt U, Konstas AG, Bufidis T, Georgiadis N, Dimitriadou A. Transforming and insulin-like growth factors in the aqueous humour of patients with exfoliation syndrome. *Graefes Arch Clin Exp Ophthalmol.* 2001;239:482–487.
33. Schlotzer-Schrehardt U, Lommatzsch J, Kuchle M, Konstas AG, Naumann GO. Matrix metalloproteinases and their inhibitors in aqueous humor of patients with pseudoexfoliation syndrome/glaucoma and primary open-angle glaucoma. *Invest Ophthalmol Vis Sci.* 2003;44:1117–1125.
34. Fuchshofer R, Birke M, Welge-Lussen U, Kook D, Lutjen-Drecoll E. Transforming growth factor-beta 2 modulated extracellular matrix component expression in cultured human optic nerve head astrocytes. *Invest Ophthalmol Vis Sci.* 2005;46:568–578.
35. Junglas B, Yu AH, Welge-Lussen U, Tamm ER, Fuchshofer R. Connective tissue growth factor induces extracellular matrix deposition in human trabecular meshwork cells. *Exp Eye Res.* 2009;88:1065–1075.
36. Yadav D, Ngolab J, Lim RS, Krishnamurthy S, Bui JD. Cutting edge: down-regulation of MHC class I-related chain A on tumor cells by IFN-gamma-induced microRNA. *J Immunol.* 2009;182:39–43.
37. Place RF, Li LC, Pookot D, Noonan EJ, Dahiya R. MicroRNA-373 induces expression of genes with complementary promoter sequences. *Proc Natl Acad Sci U S A.* 2008;105:1608–1613.
38. Huang HY, Chien CH, Jen KH, Huang HD. RegRNA: an integrated web server for identifying regulatory RNA motifs and elements. *Nucleic Acids Res.* 2006;34:W429–434.
39. Griffiths-Jones S, Saini HK, van Dongen S, Enright AJ. miRBase: tools for microRNA genomics. *Nucleic Acids Res.* 2008;36:D154–158.