Genome-wide Expression Profiling and Pathway Analysis in Cyclic Stretched Human Trabecular Meshwork Cells

Michelle D. Drewry^{1†}, Jingwen Cai^{1†}, Inas Helwa¹, Eric Hu¹, Sabrina Liu¹, Hongmei Mu², Yanzhong Hu³, William M. Johnson⁴, Pedro Gonzalez⁴, W. Daniel Stamer⁴, Yutao Liu^{1,5,6}

¹ Department of Cellular Biology and Anatomy, Augusta University, Augusta, GA

² Kaifeng key lab of cataract and myopia, Institute of Eye Diseases, Kaifeng Centre Hospital, Kaifeng, Henan, China

³ Department of Cell Biology and Genetics, Henan University School of Medicine, Kaifeng, Henan, China

⁴ Department of Ophthalmology, Duke University, Durham, NC

⁵ James and Jean Culver Vision Discovery Institute, Medical College of Georgia, Augusta University, Augusta, GA

⁶ Center for Biotechnology and Genomic Medicine, Augusta University, Augusta, GA

[†] Equal contribution from these two authors

Corresponding Author

Yutao Liu, MD & PhD

Department of Cellular Biology and Anatomy

Medical College of Georgia, Augusta University

Augusta, GA, USA

Email: yutliu@augusta.edu

Conflict of Interest: None for all authors

Abstract

Purpose Regulation of intraocular pressure is dependent upon homeostatic responses of trabecular meshwork (TM) cells to mechanical stretch. Despite the important roles of miRNAs in regulating TM function and aqueous outflow, it remains unclear how miRNA and their target genes interact in response to physiological cyclic mechanical stretch. We aimed to identify differentially expressed miRNAs and their potential targets in human TM cells in response to cyclic mechanical stress.

Methods Monolayers of TM cells from non-glaucomatous donors (n=3-6) were cultured in the presence or absence of 15% mechanical stretch, 1 cycle/s, for 6 or 24-hours using computer-controlled Flexcell Unit. We profiled the expression of 800 miRNAs using NanoString Human miRNA assays and identified differentially expressed miRNAs using the Bioconductor Limma package. We identified differentially expressed genes using Operon Human Oligo Arrays with GeneSpring software. Pathway analysis with WebGestalt identified stretch-related pathways. We used Integrative miRNA Target Finder from Ingenuity Pathway Analysis to identify potential miRNA-mRNA regulations.

Results We identified 540 unique genes and 74 miRNAs with differential expression in TM cells upon cyclic mechanical stretch. Pathway analysis indicated the significant enrichment of genes involved in Wnt-signaling, receptor protein serine/threonine kinase signaling, TGF- β pathway, and response to unfolded protein. We also identified several miRNA master regulators, including miR-19b-3p and miR-93-5p, which may act as switches to control several mechano-responsive genes.

Conclusions This study suggests that cyclic mechanical stress of TM cells triggers alterations in the expression of both mRNAs and miRNAs implicated in glaucoma-associated pathways.

Introduction

Glaucoma is a group of optic neuropathies with progressive loss of retinal ganglion cells, optic atrophy, and visual field loss (1, 2). Glaucoma affects more than 70 million people worldwide (3, 4). Primary open-angle glaucoma (POAG) is the most common type of glaucoma without an identifiable secondary cause and affects approximately 3 million Americans in the US with more than half of these patients unaware of their glaucoma condition (3, 5). Often, POAG remains undiagnosed or unrecognized by patients until visual field loss is clinically severe (1). Known risk factors for POAG include advanced aging, elevated intraocular pressure (IOP), a positive family history of glaucoma, and/or African/Hispanic ancestry (5, 6). Elevated IOP is the only modifiable risk factor in the clinic currently (7). Lowering IOP in glaucoma patients with pharmacological interventions or surgeries, can delay the progression of vision loss (8).

IOP is regulated by the secretion of the aqueous humor (AH) into the posterior chamber and its drainage from the anterior chamber of the eye (9, 10). AH is secreted from the ciliary epithelium into the posterior chamber, travels through the pupil, and exits the anterior chamber primarily through the conventional (trabecular meshwork or TM) pathway, with a small portion leaving via the unconventional pathway (9, 11). The balance between the rate of secretion and resistance to drainage determines the IOP. Excessive resistance to AH outflow through the TM pathway causes elevated IOP, which may lead to compression, deformation, and damage of the retinal ganglion cell axons (8). This elevated pressure will then produce a loss in peripheral vision, and if not treated, may result in total irreversible vision loss (8).

Even though dysfunction of the trabecular outflow pathway is responsible for elevated IOP, clinically, most of IOP-lowering medications target the unconventional outflow pathway or secretion. New medications targeting the predominant drainage pathway (the TM pathway) are in high demand, including two recently approved medications (8, 12, 13). Thus, rho kinase inhibitors and nitric oxide

drugs are the results of research over the last 25 years that identified important regulatory processes in the trabecular outflow pathway (8).

Due to high levels of fluctuations in IOP from blinking, eye movement and ocular pulse, TM cells are constantly under mechanical forces and strain (9, 14, 15). TM cells react to this stress, by maintaining cellular and tissue homeostasis, and preventing injury (16, 17). Strain causes profound changes to cell morphology, affecting motility, stiffness, contraction, orientation, and cell alignment (14-16). Recent studies have indicated autophagy as one of the relevant stress response adaptive mechanisms (16, 18, 19). Other genes that are impacted by the mechanical strain include those involved in extracellular matrix synthesis/remodeling, cytoskeletal organization, and cell adhesion (16, 20-25). It remains unknown how these genes are regulated (16). Since miRNAs regulate gene expression, we aimed here to identify the miRNA profile changes in response to mechanical strain in TM cells and to determine how these specific miRNAs target the gene expression in the stretched TM cells.

Our study focuses on discovering specific pathways that may induce elevated IOP. We stretched cultures of human TM cells by 15% with 1 cycle/second and measured genome-wide gene and miRNA expression profiles and their respective signal pathways in response to the mechanical stretch. Our hypothesis for this study is that mechanical stress-responsive pathways in human TM are critical for maintaining AH outflow resistance homeostasis and thus IOP. The identification of such pathways will enable further study into finding therapeutic targets that regulate IOP in glaucoma patients.

Methods

Cell Culture

Within 48 hours post mortem, primary human TM cells were obtained from cadaver eyes without a history of eye disease (26, 27). Tissues were processed in accordance with the Declaration of Helsinki. Secondary cultures of cells expanded from primary isolates of human TM cells were grown at 37°C in 5%

CO₂ in low glucose Dulbecco's Modified Eagle Medium (DMEM) with L-glutamine, 110 mg/ml sodium pyruvate, 10% fetal bovine serum – premium select (Atlanta Biologicals, Flowery Branch, GA, USA), 100 μ M non-essential amino acids, 100 units/ml penicillin, and 100 μ g/ml streptomycin from Invitrogen (ThermoFisher Scientific, Waltham, MA, USA), as previously described (26).

Gene Expression and Analysis

Primary human TM cells from three non-glaucoma donors (n=3) were plated on collagen-coated flexible silicone bottom plates from Flexcell (Flexcell International Corporation, Burlington, NC, USA). After reaching confluence, TM cells were switched to serum-free DMEM for 3 hours, followed by cyclic mechanical stress for 6 hours (15% stretching, 1 cycle/second) using the computer-controlled FX-3000 Flexercell Strain Unit from Flexcell (25). Control cells were cultured under the same condition but without mechanical stress. Total RNA was extracted using the RNeasy kit (Qiagen, Germantown, MD, USA) according to the recommended procedures by the manufacturer. Quality and quantity of the RNA were evaluated using Agilent Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA, USA). RNA from three stretched and three control TM cells were amplified and labeled with Cy5 dye, and a universal Reference human RNA from Stratagene, Agilent Technologies, was also labeled with Cy3 dye. Each TM sample and the reference RNA sample were hybridized to Operon Human Oligo Arrays version 3.0 (35k probes) at the Duke University Microarray Core Facility.

Raw data was normalized using LOWESS (locally weighted scatterplot smoothing), and the logRatio for each sample was downloaded from the GSE14768 dataset deposited in the NCBI GEO database (<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE14768>) (25). We analyzed the expression data using the GeneSpring version 14.9 software from Agilent, performing the Welch's t-test to derive the significance of differential expression and the fold change between stretched and non-stretch samples. Significantly differentially expressed genes were identified with a Benjamini-Hochberg (BH)

false discovery rate (FDR) < 0.05 and a fold change ≥ 2 or ≤ -2 . The list of genes was categorized using the online PANTHER function classification system (28). The list of the significantly differentially expressed genes was uploaded into the WEB-based Gene Set Analysis Toolkit (WebGestalt) for gene ontology and pathway analysis (29). The gene ontology analysis examined potentially enriched pathways or categories in biological processes, cellular components, and molecular function. These differentially expressed genes were also uploaded to Ingenuity Pathway Analysis (IPA) to search for potential upstream regulators.

miRNA Expression and Analysis

Monolayers of human TM cells from six donors (n=6) were maintained in low glucose DMEM media with 10% FBS and underwent cyclic mechanical stretch (15%, 1 cycle/second) for 24 hours, as described previously (30). Controls were under the same condition for 24 hours but without the cyclic mechanical stretch. Total RNA was extracted using the mirVana miRNA isolation kit from Thermo Fisher Scientific. RNA quantity was measured using the Tecan microplate reader M200 (Tecan Group Ltd., Mannedorf, Zurich, Switzerland), and RNA quality was evaluated using the Agilent Bioanalyzer 2100 with RNA Pico Chips. 100 ng of total RNA was used to measure the expression of 800 pre-selected human miRNAs using the nCounter Human v3 miRNA expression assay kit from NanoString Technologies (Seattle, WA, USA), as previously described (31). After specific sample preparation and overnight hybridization, digital readouts of the relative miRNA abundance were obtained, translating to miRNA expression.

The raw data RCC files produced by nCounter were processed with the NanoString nSolver 3.0 software using a previously described analysis pipeline (31). Each sample was run through several quality control checks before analysis. These checks examined the quality of imaging, binding density, positive control linearity, and positive control limit of detection. Based on RNA content, the raw miRNA counts

were normalized using the trimmed geometric mean with the nSolver software. The geometric mean calculated the average number of counts for each sample using the miRNAs with the median 40% of the counts. This particular technique is similar to the Trimmed Mean of M method, which was shown to be successful in normalizing miRNA counts (32). To account for technology-associated sources of variation, the miRNA counts were normalized further using the geometric mean of the positive control probes.

After normalization, the data was exported from nSolver into CSV files and imported into the R Language and Environment for Statistical Computing. Using NanoString's recommended methods, the background level probes for the control and stretched samples were identified within R. The negative control probes for each sample were extracted and multiplied by the corresponding normalization factors produced by nSolver. A Welch's t-test was performed for each probe to compare the normalized negative control counts to the sample counts. If the counts for a probe were not significantly different than the negative controls ($p > 0.05$), then it was considered background. Finally, the limma Bioconductor package was used to perform the differential analysis with paired samples after the background level probes were identified for each sample type. The differentially expressed probes were those with an absolute log2 fold change ≥ 1.5 , a p-value ≤ 0.05 , and an absolute difference in counts ≥ 5 . Since the laboratory culture condition may change the miRNA expression in the cultures of human TM cells isolated from TM tissues, we examined whether these differentially expressed miRNAs were present in human TM tissues using our published miRNA expression profile from seven non-glaucoma human TM tissues with miRNA-Seq (33).

Integrative Analysis of miRNA and mRNAs

miRNAs are known to regulate mRNA expression of many target genes. One miRNA may target many genes, and one gene could be regulated by many miRNAs. To identify miRNAs that potentially regulate the genes differentially expressed during the cyclic mechanical stretch of human TM cells, we used the miRNA Target Filter function in the Ingenuity Pathway Analysis (IPA, Qiagen). Briefly, we

uploaded the list of differentially expressed miRNAs with expression fold changes and the list of differentially expressed mRNAs with expression fold changes to the IPA. The miRNA Target Filter in IPA detects target genes from multiple sources (TargetScan, TarBase, miRecords, and Ingenuity Knowledge Base) (34-36) and identifies which genes in the uploaded mRNA gene list are potential targets of the selected miRNAs. We required that the expression of interested miRNAs be negatively paired with their potential mRNA targets. If the miRNA expression is increased, then the expression of its target genes is decreased and vice versa. The target genes could include those experimentally validated in the literature or those predicted with high or moderate confidence. For accuracy, we limited our miRNA targets to those experimentally validated.

Although the cultures of human TM cells were derived from human trabecular meshwork tissues, gene expression profile may change due to laboratory culture conditions. Using the miRNA expression data from eight non-glaucomatous human TM tissue, we examined whether these specific miRNAs in TM cells were expressed in human TM tissue (33). Their expression in normal human TM tissues might suggest their potential physiological role in aqueous outflow dynamics.

Availability of Data and Materials

The miRNA dataset supporting the conclusions of this article is available in the NCBI Gene Expression Omnibus and are accessible through the GEO Series accession number GSE113755 (<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE113755>).

Results

mRNA Differential Expression Analysis

From gene differential analysis, using an absolute fold change ≥ 2 and a FDR value ≤ 0.05 , we identified a total of 540 unique genes with significant differential expression (**Supplemental Table S1**).

Of these, 276 genes were down-regulated and 264 genes up-regulated in the mechanically stretched human TM cells compared to non-stretched human TM cells. We have selected the top 20 down-regulated and top 20 up-regulated genes with the largest fold changes, listed in **Table 1**. Using the online PANTHER classification system, we found that the top functional categories of these genes were related to binding, catalytic activity, structural molecule activity, and transporter activity. The main classes of these proteins were related to nucleic acid binding, transcription factor, enzyme modulator, hydrolase, transferase, and cytoskeletal proteins. The top related pathways included gonadotropin-releasing hormone receptor pathway, CCKR signaling, apoptosis pathway, angiogenesis, Wnt-signaling pathway, integrin-signaling, and inflammation.

To identify enriched functional categories in the differentially expressed mRNAs via gene ontology, the list of these 540 genes was uploaded to WebGestalt. A number of these genes were identified as being significantly involved in many biological processes, such as regulation of transmembrane receptor protein serine/threonine kinase signaling pathway, canonical Wnt receptor signaling pathway, response to unfolded protein, and ribosome biogenesis (**Supplemental Figure S1**). Pathway analysis based on KEGG Pathway database (Kyoto Encyclopedia of Genes and Genomes) indicated the following enriched KEGG pathways in response to cyclic mechanic stretch: ribosome biogenesis, MAPK signaling, protein processing in ER (endoplasmic reticulum), metabolic pathways, P53 signaling pathway, and TGF- β signaling pathway (**Supplemental Table S2**). Ingenuity Pathway Analysis indicated that the top upstream regulators to the differentially expressed genes included *TGF- β 1*, *HDAC*, *PDGF-BB*, and *MYC*.

miRNA Differential Analysis

Using NanoString nCounter Human miRNA Assay for 800 pre-selected human miRNAs, we detected the expression of 373 different miRNAs in the cultures of human TM cells (**Supplemental Table S3**). Using a p-value cutoff < 0.05 and absolute fold change > 1.5, 74 miRNAs (10 down- and 64

up-regulated) were differentially expressed in cyclic mechanically stretched TM cells (**Table 2**). The top most significantly expressed miRNAs were miR-32-5p, miR-4286, miR-135-5p, miR-136-5p, miR-93-5p, miR-19b-3p, and miR-27a-3p (p value = 1.2×10^{-5} , 1.3×10^{-5} , 4.5×10^{-5} , 7.0×10^{-5} , 2.8×10^{-4} , 3.1×10^{-4} , and 9.1×10^{-4} , respectively). The miRNAs with the greatest fold change in response to stretch included miR-4286, miR-32-5p, miR-136-5p, miR-93-5p, miR-135-5p, miR-21-5p, and miR-19b-3p, with fold changes ranging from 1.98 to 2.42. Consistent with previous reports, miR-100-5p, miR-27a-3p, miR-27b-3p, miR-24-3p, miR-16-5p, let-7f-5p, and let-7i-5p were significantly upregulated in stretched versus control TM cells (37). IPA showed that these miRNAs are mostly associated with cellular functions related to proliferation, development, cell cycle regulation, and cellular movement. We found that 29 of these 74 miRNAs were expressed in non-glaucomatous human trabecular meshwork tissues with normalized sequencing counts ≥ 10 (**Figure 1**).

Integrative Analysis of miRNA and mRNAs

Using the miRNA Target Finder in IPA and restricting the analysis to only experimentally validated miRNA targets, we identified 8 unique miRNAs that target 17 mRNA genes in response to cyclic mechanical stretch in the cultures of human TM cells (**Table 3**). Most of these miRNAs targeted more than one gene, and several genes were targeted with more than one miRNAs (**Figure 2**). For example, increased expression of miR-15-5p inhibited the expression of *NT5DC1*, *OGT*, *OSGEPL1*, *RHOT1*, *SLC35A1*, and *WEE1*, while increased expression of miR-93-5p inhibited the expression of *BAMBI*, *HBPI1*, *MAP3K12*, and *MYLIP*. The reduced expression of *MYLIP* was associated with the increased expression of miR-32-5p, miR-93-5p, and miR-19b-3p. The reduced expression of *WEE1* was related to the increased expression of miR-15a-5p and miR-27b-3p. Using our previous data on miRNA expression profile with 7 non-glaucomatous TM tissues (33), we found that six miRNAs (miR-125a-5p, miR-27b-3p, miR-93-5p, miR-29a-3p, miR-19b-3p, and miR-30a-5p), but not miR-15a-5p or miR-32-5p,

were expressed in human TM tissue. This integrative miRNA target analysis identified potential regulatory expression networks in response to the cyclic mechanical stress in human primary TM cells.

Discussion

Overview

We have identified 540 genes and 74 miRNAs differentially expressed in primary human trabecular meshwork cells in response to cyclic mechanical stress. Pathway analysis using these genes and miRNAs indicated potential involvement of Wnt receptor signaling pathway, unfolded protein response, serine/threonine kinase signaling pathway, TGF- β signaling pathway, mTOR signaling pathway, and apoptosis pathway. Our miRNA analysis suggests alterations in miRNAs potentially related to cell proliferation, development, cell cycle regulation, and cellular movement. Although the main findings of the expression responses to cyclic mechanical stress are similar to other mechanotransduction studies (22, 38), this is the first time that the Wnt-signaling pathway, unfolded protein response, and the serine/threonine kinase signaling pathway have been associated with cyclic mechanical stretch in monolayers of human TM cells.

Wnt-signaling

Our analysis indicated significant enrichment of the canonical Wnt receptor signaling pathway (15 differentially expressed genes) in response to cyclic mechanical stretch in primary TM cells. Many studies have identified the activation of Wnt signaling as a normal physiological response to mechanical loading in bone and periodontal tissue (39). For example, Wnt signaling has been demonstrated to act downstream of mechanical activity and is required for joint patterning and chondrocyte maturation (40). Interestingly, the response of Wnt signaling to cyclic mechanical stress could be impaired by the aging process, i.e. the response of Wnt signaling to the mechanical stress is age-dependent, with a previous study showing more response in young adult mice (5-12 month old) and less or no response in old adult

mice (22-month old) (41). Wnt inhibitor can induce the increased cellular stiffness in human trabecular meshwork cells (9), which might be related to the glaucoma progression (42). Wnt is present in the outflow facility as a local mediator (9). Importantly, an antagonist to Wnt signaling, sFRP-1 increases outflow facility in perfused human anterior segments, and the overexpression of sFRP-1 leads to elevated IOP in mice (11, 43). Together this evidence suggests a homeostatic role for Wnt signaling in the trabecular outflow pathway and in regulation of IOP. Future studies will be needed to examine how this mechanical stress-induced Wnt signaling functions in glaucomatous TM cells or aged human TM cells. The Wnt signaling pathway could be regulated by several differentially expressed miRNAs, including miR-374a (44), miR-34a targeting p53 (45), let-7, and miR-31-5p (46). In summary, the interaction of Wnt signaling and miRNA regulation clearly contributes to the molecular response of human TM cells to cyclic mechanical stress.

Unfolded protein response/ER stress

Unfolded protein response and related ER (endoplasmic reticulum) stress can be induced by cyclic mechanical stretching in smooth muscle cells and cardiomyocytes (47, 48), in which ER stress can lead to apoptosis and inflammation (49). ER stress and unfolded protein response have been implicated in the pathogenesis of glaucoma through the failure to eliminate misfolded/damaged proteins, leading to functional impairment of TM cells and increased outflow resistance (49, 50). Reducing ER stress and unfolded protein response in TM cells chemically can prevent dexamethasone-induced ocular hypertension in mice (49). Rare variants in key regulators of unfolded protein response (*ACADVL*, *EXOSC3*, *KDELRL3*, *SHC1*, *SRPRB*, *SYVN1*, *TATDN2*, and *TPP1*) were found to be significantly enriched in POAG patients with elevated IOP (51). Differentially expressed miRNAs miR-455-5p, miR-34a-5p, miR-199a-5p, miR-221-5p, miR-494-3p, miR-29a-3p, miR-93-5p, miR-25-3p, and miR-30a have been known to target the regulators of unfolded protein response (52, 53). This evidence in conjunction with the differential expression of mRNAs and miRNAs associated with unfolded protein response provide additional support for its potential contribution to IOP regulation through TM cells. Further investigation

into how unfolded protein response is damaged in glaucomatous TM cells from human donors will be necessary.

Receptor serine/threonine kinase pathway/TGF- β Pathway

Receptor serine/threonine kinase pathway are mediated by the TGF- β type I and type II receptors (54). Cyclic mechanical stress has been shown to potentially activate TGF- β /BMP pathway through the SMAD-mediated Runx2 pathway or the non-SMAD-mediated MAPK pathway (55, 56). TGF- β and its related pathways have been strongly implicated in the pathogenesis of glaucoma and IOP regulation (54, 57-60). TGF- β may increase outflow resistance by altering extracellular matrix homeostasis and cell contractility in the TM through interactions with other proteins and signaling molecules (54, 58, 60). TGF- β treatment results in the regulation of many miRNAs, including the up-regulation of miR-21, miR-181, miR-494, miR-10b, miR-27a, miR-183, miR-182, miR-155, and miR-451 and the down-regulation of miR-200, miR-34a, miR-203, miR-584, and miR-450b-5p (61). Most members of TGF- β pathway may be targeted by a number of miRNAs, including miR-18a, miR-24, let-7, miR-744, miR-30, miR-200, miR-128a, miR-21, miR-17, miR-148a, miR-99a/b, and miR-92b (61, 62). Our study identified the differential expression of miR-24-3p, miR-27a, miR-21, miR-4286, and miR-29a, which all potentially target known genes involved in TGF- β pathway-mediated extracellular matrix homeostasis in TM cells.

Conclusions and Future Work

Our study not only identified new miRNAs related to cyclic mechanical stretch in TM cells, but also replicated many previously reported miRNAs involved in this process. Several of our identified miRNAs, including miR-16, miR-27a, miR-27b, let-7f, miR-26a, miR-24 and miR-7, have been reported to be differentially expressed in TM cells after 3-hour cyclic mechanical stretching (63). miR-151 can regulate the expression of MMP13 (64), and miR-21 may regulate the expression of MMP9 and TIMP-1 (65). Both MMPs and TIMPs have been implicated in outflow resistance (66). An IOP- and POAG-associated gene *ABCA1* may be regulated by two differentially expressed miRNAs: miR-135 and miR-

33a (67-69). Current IOP lowering therapies focus on reducing IOP without addressing extracellular matrix homeostasis processes in the TM (11). More studies would be beneficial to understand the role of TGF- β in POAG at the levels of production, activation, downstream signaling, and homeostatic regulation (58).

Our integrative miRNA-mRNA expression analysis identified several miRNA-mRNA regulatory expression interactions in the TM cells, highlighting several miRNA master regulators, such as miR-125a-5p, miR-15a-5p, miR-93-5p, miR-19b, miR-30a, and miR-32-5p. miR-15a has been reported to be down-regulated in stress-induced senescent human TM cells (70). miR-93 was found to have increased expression in glaucomatous TM cells and induced apoptosis in these TM cells (71). Since our integrative analysis was restricted to only those experimentally validated miRNA targets, additional master miRNA regulators may be identified if we expanded our analysis to include predicted targets with high or moderate confidence, though this would potentially increase the number of false positive findings.

Despite the strength of our experimental and bioinformatics analyses, our study has several limitations. First, the sample size of primary human TM cells was limited, derived from cells isolated from only 3 or 6 donor eyes. It would be better to have more donor-derived TM cells from both males and females, with similar ethnical background and similar age distributions. Second, the cyclic mechanical stretch experiment was done at single time point with 6 or 24 hours. Including a time-series design with many different time points would be beneficial in identifying time-dependent molecular responses, and it would be better to have the expression of miRNAs and mRNAs done at the same time point. Third, all the TM cells used in our study were derived from unaffected non-glaucomatous postmortem donors. Including TM cells derived from glaucoma-affected postmortem donors will be necessary to further explore mechanical stress-induced changes in miRNA and mRNA expression. These factors will be considered and included in our future experiments.

In summary, our genome-wide mRNA and miRNA expression profiling has identified a large number of differentially expressed genes and miRNAs in response to cyclic mechanical stretch in primary human TM cells. Our analysis has identified several important signaling pathways involved in this response, such as TGF- β , Wnt signaling, and ER stress. The miRNA-mRNA integrative analysis found several miRNA master regulators, suggesting their potential role in TM cellular function in response to cyclic mechanical stress. More functional studies are needed to further validate the role of these miRNAs and mRNAs in relation to TM cellular function.

Acknowledgements

We thank the financial support from The Glaucoma Foundation, The Glaucoma Research Foundation, The BrightFocus Foundation, NIH R01EY023242, R21EY028671, R01EY022359, and R01EY023287. Financial support from Fight for Sight is gratefully acknowledged. The funding sources have no influences on the experimental design, data generation and analysis. We sincerely thank all the donors for their ocular samples. This study would not be feasible without these precious samples. This manuscript has been presented at the ISER/BrightFocus 2017 Glaucoma Symposium in Atlanta, Georgia, USA, by Y Liu.

References

1. Allingham RR, Shields MB. Shields' textbook of glaucoma. 6th ed. Philadelphia: Wolters Kluwer/Lippincott Williams & Wilkins Health; 2011. p. p.
2. Allingham RR, Liu Y, Rhee DJ. The genetics of primary open-angle glaucoma: a review. *Experimental eye research*. 2009;88(4):837-44.
3. Quigley HA. Glaucoma. *Lancet*. 2011;377(9774):1367-77.
4. Quigley HA, Broman AT. The number of people with glaucoma worldwide in 2010 and 2020. *The British journal of ophthalmology*. 2006;90(3):262-7.
5. Fan BJ, Wiggs JL. Glaucoma: genes, phenotypes, and new directions for therapy. *The Journal of clinical investigation*. 2010;120(9):3064-72.
6. Liu Y, Allingham RR. Major review: Molecular genetics of primary open-angle glaucoma. *Experimental eye research*. 2017;160:62-84.
7. Fingert JH. Primary open-angle glaucoma genes. *Eye (Lond)*. 2011;25(5):587-95.
8. Weinreb RN, Aung T, Medeiros FA. The pathophysiology and treatment of glaucoma: a review. *JAMA : the journal of the American Medical Association*. 2014;311(18):1901-11.
9. Stamer WD. The cell and molecular biology of glaucoma: mechanisms in the conventional outflow pathway. *Investigative ophthalmology & visual science*. 2012;53(5):2470-2.
10. Ito YA, Walter MA. Genetics and Environmental Stress Factor Contributions to Anterior Segment Malformations and Glaucoma. In: Rumelt S, editor. *Glaucoma - Basic and Clinical Aspects*: InTech; 2013.
11. Stamer WD, Acott TS. Current understanding of conventional outflow dysfunction in glaucoma. *Curr Opin Ophthalmol*. 2012;23(2):135-43.
12. Kazemi A, McLaren JW, Kopczynski CC, Heah TG, Novack GD, Sit AJ. The Effects of Netarsudil Ophthalmic Solution on Aqueous Humor Dynamics in a Randomized Study in Humans. *Journal of ocular pharmacology and therapeutics : the official journal of the Association for Ocular Pharmacology and Therapeutics*. 2018;34(5):380-6.
13. Serle JB, Katz LJ, McLaurin E, Heah T, Ramirez-Davis N, Usner DW, et al. Two Phase 3 Clinical Trials Comparing the Safety and Efficacy of Netarsudil to Timolol in Patients With Elevated Intraocular Pressure: Rho Kinase Elevated IOP Treatment Trial 1 and 2 (ROCKET-1 and ROCKET-2). *American journal of ophthalmology*. 2018;186:116-27.
14. Johnstone MA. Intraocular pressure regulation: findings of pulse-dependent trabecular meshwork motion lead to unifying concepts of intraocular pressure homeostasis. *Journal of ocular pharmacology and therapeutics : the official journal of the Association for Ocular Pharmacology and Therapeutics*. 2014;30(2-3):88-93.
15. Xin C, Wang RK, Song S, Shen T, Wen J, Martin E, et al. Aqueous outflow regulation: Optical coherence tomography implicates pressure-dependent tissue motion. *Experimental eye research*. 2017;158:171-86.
16. Liton PB. The autophagic lysosomal system in outflow pathway physiology and pathophysiology. *Experimental eye research*. 2016;144:29-37.
17. Liton PB, Gonzalez P. Stress response of the trabecular meshwork. *Journal of glaucoma*. 2008;17(5):378-85.
18. Hirt J, Liton PB. Autophagy and mechanotransduction in outflow pathway cells. *Experimental eye research*. 2017;158:146-53.
19. Porter KM, Jeyabalan N, Liton PB. MTOR-independent induction of autophagy in trabecular meshwork cells subjected to biaxial stretch. *Biochimica et biophysica acta*. 2014;1843(6):1054-62.
20. Bradley JM, Kelley MJ, Rose A, Acott TS. Signaling pathways used in trabecular matrix metalloproteinase response to mechanical stretch. *Investigative ophthalmology & visual science*. 2003;44(12):5174-81.

21. Keller KE, Kelley MJ, Acott TS. Extracellular matrix gene alternative splicing by trabecular meshwork cells in response to mechanical stretching. *Investigative ophthalmology & visual science*. 2007;48(3):1164-72.
22. Vittal V, Rose A, Gregory KE, Kelley MJ, Acott TS. Changes in gene expression by trabecular meshwork cells in response to mechanical stretching. *Investigative ophthalmology & visual science*. 2005;46(8):2857-68.
23. Mitton KP, Tumminia SJ, Arora J, Zelenka P, Epstein DL, Russell P. Transient loss of alphaB-crystallin: an early cellular response to mechanical stretch. *Biochemical and biophysical research communications*. 1997;235(1):69-73.
24. Liton PB, Luna C, Bodman M, Hong A, Epstein DL, Gonzalez P. Induction of IL-6 expression by mechanical stress in the trabecular meshwork. *Biochemical and biophysical research communications*. 2005;337(4):1229-36.
25. Luna C, Li G, Liton PB, Epstein DL, Gonzalez P. Alterations in gene expression induced by cyclic mechanical stress in trabecular meshwork cells. *Molecular vision*. 2009;15:534-44.
26. Stamer WD, Seftor RE, Williams SK, Samaha HA, Snyder RW. Isolation and culture of human trabecular meshwork cells by extracellular matrix digestion. *Current eye research*. 1995;14(7):611-7.
27. Keller KE, Bhattacharya SK, Borrás T, Brunner TM, Chansangpetch S, Clark AF, et al. Consensus recommendations for trabecular meshwork cell isolation, characterization and culture. *Experimental eye research*. 2018.
28. Thomas PD, Campbell MJ, Kejariwal A, Mi H, Karlak B, Daverman R, et al. PANTHER: a library of protein families and subfamilies indexed by function. *Genome research*. 2003;13(9):2129-41.
29. Wang J, Duncan D, Shi Z, Zhang B. WEB-based GENE SeT Analysis Toolkit (WebGestalt): update 2013. *Nucleic acids research*. 2013;41(Web Server issue):W77-83.
30. Hauser MA, Aboobakar IF, Liu YT, Miura S, Whigham BT, Challa P, et al. Genetic variants and cellular stressors associated with exfoliation syndrome modulate promoter activity of a lncRNA within the LOXL1 locus. *Human molecular genetics*. 2015;24(22):6552-63.
31. Drewry MD, Challa P, Kuchtey JG, Navarro I, Helwa I, Hu Y, et al. Differentially expressed microRNAs in the aqueous humor of patients with exfoliation glaucoma or primary open-angle glaucoma. *Human molecular genetics*. 2018;27(7):1263-75.
32. Robinson MD, Oshlack A. A scaling normalization method for differential expression analysis of RNA-seq data. *Genome Biol*. 2010;11(3):R25.
33. Drewry M, Helwa I, Allingham RR, Hauser MA, Liu Y. miRNA Profile in Three Different Normal Human Ocular Tissues by miRNA-Seq. *Investigative ophthalmology & visual science*. 2016;57(8):3731-9.
34. Agarwal V, Bell GW, Nam JW, Bartel DP. Predicting effective microRNA target sites in mammalian mRNAs. *Elife*. 2015;4.
35. Vlachos IS, Paraskevopoulou MD, Karagkouni D, Georgakilas G, Vergoulis T, Kanellos I, et al. DIANA-TarBase v7.0: indexing more than half a million experimentally supported miRNA:mRNA interactions. *Nucleic acids research*. 2015;43(Database issue):D153-9.
36. Xiao F, Zuo Z, Cai G, Kang S, Gao X, Li T. miRecords: an integrated resource for microRNA-target interactions. *Nucleic acids research*. 2009;37(Database issue):D105-10.
37. Gonzalez P, Li G, Qiu J, Wu J, Luna C. Role of microRNAs in the trabecular meshwork. *Journal of ocular pharmacology and therapeutics : the official journal of the Association for Ocular Pharmacology and Therapeutics*. 2014;30(2-3):128-37.
38. Spyropoulou A, Karamesinis K, Basdra EK. Mechanotransduction pathways in bone pathobiology. *Biochimica et biophysica acta*. 2015;1852(9):1700-8.
39. Robinson JA, Chatterjee-Kishore M, Yaworsky PJ, Cullen DM, Zhao W, Li C, et al. Wnt/beta-catenin signaling is a normal physiological response to mechanical loading in bone. *The Journal of biological chemistry*. 2006;281(42):31720-8.

40. Brunt LH, Begg K, Kague E, Cross S, Hammond CL. Wnt signalling controls the response to mechanical loading during zebrafish joint development. *Development*. 2017;144(15):2798-809.
41. Holguin N, Brodt MD, Silva MJ. Activation of Wnt Signaling by Mechanical Loading Is Impaired in the Bone of Old Mice. *J Bone Miner Res*. 2016;31(12):2215-26.
42. Morgan JT, Raghunathan VK, Chang YR, Murphy CJ, Russell P. Wnt inhibition induces persistent increases in intrinsic stiffness of human trabecular meshwork cells. *Experimental eye research*. 2015;132:174-8.
43. Wang WH, McNatt LG, Pang IH, Millar JC, Hellberg PE, Hellberg MH, et al. Increased expression of the WNT antagonist sFRP-1 in glaucoma elevates intraocular pressure. *The Journal of clinical investigation*. 2008;118(3):1056-64.
44. He L, Thomson JM, Hemann MT, Hernando-Monge E, Mu D, Goodson S, et al. A microRNA polycistron as a potential human oncogene. *Nature*. 2005;435(7043):828-33.
45. Kim NH, Kim HS, Kim NG, Lee I, Choi HS, Li XY, et al. p53 and microRNA-34 are suppressors of canonical Wnt signaling. *Science signaling*. 2011;4(197):ra71.
46. Onyido EK, Sweeney E, Nateri AS. Wnt-signalling pathways and microRNAs network in carcinogenesis: experimental and bioinformatics approaches. *Mol Cancer*. 2016;15(1):56.
47. Cheng WP, Wang BW, Lo HM, Shyu KG. Mechanical Stretch Induces Apoptosis Regulator TRB3 in Cultured Cardiomyocytes and Volume-Overloaded Heart. *PloS one*. 2015;10(4):e0123235.
48. Jia LX, Zhang WM, Zhang HJ, Li TT, Wang YL, Qin YW, et al. Mechanical stretch-induced endoplasmic reticulum stress, apoptosis and inflammation contribute to thoracic aortic aneurysm and dissection. *The Journal of pathology*. 2015;236(3):373-83.
49. Zode GS, Kuehn MH, Nishimura DY, Searby CC, Mohan K, Grozdanic SD, et al. Reduction of ER stress via a chemical chaperone prevents disease phenotypes in a mouse model of primary open angle glaucoma. *The Journal of clinical investigation*. 2011;121(9):3542-53.
50. Anholt RR, Carbone MA. A molecular mechanism for glaucoma: endoplasmic reticulum stress and the unfolded protein response. *Trends Mol Med*. 2013;19(10):586-93.
51. Zhou T, Souzeau E, Sharma S, Landers J, Mills R, Goldberg I, et al. Whole exome sequencing implicates eye development, the unfolded protein response and plasma membrane homeostasis in primary open-angle glaucoma. *PloS one*. 2017;12(3):e0172427.
52. McMahon M, Samali A, Chevet E. Regulation of the unfolded protein response by noncoding RNA. *Am J Physiol Cell Physiol*. 2017;313(3):C243-C54.
53. Duan Q, Chen C, Yang L, Li N, Gong W, Li S, et al. MicroRNA regulation of unfolded protein response transcription factor XBP1 in the progression of cardiac hypertrophy and heart failure in vivo. *Journal of translational medicine*. 2015;13:363.
54. Fuchshofer R, Tamm ER. The role of TGF-beta in the pathogenesis of primary open-angle glaucoma. *Cell and tissue research*. 2012;347(1):279-90.
55. Duscher D, Maan ZN, Wong VW, Rennert RC, Januszyk M, Rodrigues M, et al. Mechanotransduction and fibrosis. *J Biomech*. 2014;47(9):1997-2005.
56. Papachroni KK, Karatzas DN, Papavassiliou KA, Basdra EK, Papavassiliou AG. Mechanotransduction in osteoblast regulation and bone disease. *Trends Mol Med*. 2009;15(5):208-16.
57. Danford ID, Verkuil LD, Choi DJ, Collins DW, Gudiseva HV, Uyhazi KE, et al. Characterizing the "POAGome": A bioinformatics-driven approach to primary open-angle glaucoma. *Progress in retinal and eye research*. 2017;58:89-114.
58. Wang J, Harris A, Prendes MA, Alshawa L, Gross JC, Wentz SM, et al. Targeting Transforming Growth Factor-beta Signaling in Primary Open-Angle Glaucoma. *Journal of glaucoma*. 2017;26(4):390-5.
59. Wordinger RJ, Sharma T, Clark AF. The role of TGF-beta2 and bone morphogenetic proteins in the trabecular meshwork and glaucoma. *Journal of ocular pharmacology and therapeutics : the official journal of the Association for Ocular Pharmacology and Therapeutics*. 2014;30(2-3):154-62.

60. Prendes MA, Harris A, Wirostko BM, Gerber AL, Siesky B. The role of transforming growth factor beta in glaucoma and the therapeutic implications. *The British journal of ophthalmology*. 2013;97(6):680-6.
61. Guo L, Zhang Y, Zhang L, Huang F, Li J, Wang S. MicroRNAs, TGF-beta signaling, and the inflammatory microenvironment in cancer. *Tumour Biol*. 2016;37(1):115-25.
62. Villarreal G, Jr., Oh DJ, Kang MH, Rhee DJ. Coordinated regulation of extracellular matrix synthesis by the microRNA-29 family in the trabecular meshwork. *Investigative ophthalmology & visual science*. 2011;52(6):3391-7.
63. Luna C, Li G, Qiu J, Epstein DL, Gonzalez P. MicroRNA-24 regulates the processing of latent TGFbeta1 during cyclic mechanical stress in human trabecular meshwork cells through direct targeting of *FURIN*. *J Cell Physiol*. 2011;226(5):1407-14.
64. Liu X, Li J, Qin F, Dai S. miR-152 as a tumor suppressor microRNA: Target recognition and regulation in cancer. *Oncol Lett*. 2016;11(6):3911-6.
65. Wang J, Gao Y, Ma M, Li M, Zou D, Yang J, et al. Effect of miR-21 on renal fibrosis by regulating MMP-9 and TIMP1 in kk-ay diabetic nephropathy mice. *Cell Biochem Biophys*. 2013;67(2):537-46.
66. De Groef L, Van Hove I, Dekeyster E, Stalmans I, Moons L. MMPs in the trabecular meshwork: promising targets for future glaucoma therapies? *Investigative ophthalmology & visual science*. 2013;54(12):7756-63.
67. Chen Y, Lin Y, Vithana EN, Jia L, Zuo X, Wong TY, et al. Common variants near *ABCA1* and in *PMM2* are associated with primary open-angle glaucoma. *Nature genetics*. 2014;46(10):1115-9.
68. Gharahkhani P, Burdon KP, Fogarty R, Sharma S, Hewitt AW, Martin S, et al. Common variants near *ABCA1*, *AFAP1* and *GMDS* confer risk of primary open-angle glaucoma. *Nature genetics*. 2014;46(10):1120-5.
69. Hysi PG, Cheng CY, Springelkamp H, Macgregor S, Bailey JNC, Wojciechowski R, et al. Genome-wide analysis of multi-ancestry cohorts identifies new loci influencing intraocular pressure and susceptibility to glaucoma. *Nature genetics*. 2014;46(10):1126-30.
70. Li G, Luna C, Qiu J, Epstein DL, Gonzalez P. Alterations in microRNA expression in stress-induced cellular senescence. *Mech Ageing Dev*. 2009;130(11-12):731-41.
71. Wang Y, Li F, Wang S. MicroRNA93 is overexpressed and induces apoptosis in glaucoma trabecular meshwork cells. *Mol Med Rep*. 2016;14(6):5746-50.

Figure Legends

Figure 1. Expression level of cyclic mechanic stretch-responsive miRNAs in non-glaucoma human TM tissue (n=6). Error bars represent SEM.

Figure 2. Gene network of cyclic mechanic stretch-responsive miRNAs, denoted in red, and their validated target genes. The miRNAs within an oval have significant expression in non-glaucoma human TM tissues (n=8), with ≥ 10 normalized sequencing reads.

Table 1. List of differentially expressed genes in primary human TM cells in response to cyclic mechanical stretch (15%, 1 cycle/s, 6 hrs).

Gene Symbol	Accession #	Fold Change	FDR P-value
<i>DHRS9</i>	NM_005771	1086.39	0.02
<i>ESM1</i>	NM_007036	33.41	0.02
<i>SCML4</i>	AK074125	29.74	0.02
<i>HOXC13</i>	NM_017410	25.02	0.04
<i>BMP2</i>	NM_001200	20.06	0.02
<i>NP25</i>	NM_013259	19.73	0.02
<i>PTHLH</i>	NM_002820	16.73	0.03
<i>EVA1A</i>	NM_032181	12.27	0.01
<i>PTX3</i>	NM_002852	9.77	0.02
<i>MMP1</i>	NM_002421	8.85	0.02
<i>GPAT3</i>	NM_032717	8.32	0.02
<i>HSPA1B</i>	NM_005346	8.12	0.02
<i>KCNC4</i>	NM_004978	7.97	0.02
<i>MFSD2A</i>	AK093223	7.67	0.04
<i>PNP</i>	NM_000270	7.61	0.01
<i>PPP1R15A</i>	NM_014330	7.11	0.02
<i>RGS17</i>	NM_012419	6.96	0.04
<i>CHST11</i>	NM_018413	6.80	0.03
<i>ARL7</i>	NM_005737	6.47	0.02
<i>DKK1</i>	NM_012242	6.46	0.01
<i>BTEB1</i>	NM_001206	-13.88	0.03
<i>CNTN3</i>	AB040929	-13.41	0.04
<i>NR4A2</i>	NM_006186	-13.28	0.02
<i>NR4A1</i>	NM_173158	-12.59	0.03
<i>NR4A1</i>	NM_173158	-10.82	0.02
<i>FAM83D</i>	NM_030919	-10.39	0.02
<i>DLX2</i>	NM_004405	-9.82	0.01
<i>EGR3</i>	NM_004430	-8.32	0.02
<i>C6orf111</i>	NM_032870	-7.02	0.03
<i>TNFSF10</i>	NM_003810	-6.62	0.02
<i>FBXO32</i>	NM_058229	-6.56	0.02
<i>SLC40A1</i>	NM_014585	-6.41	0.02
<i>PDE5A</i>	NM_001083	-6.26	0.03
<i>ZFP36L2</i>	NM_006887	-5.68	0.02
<i>CEMIP</i>	AB033025	-5.37	0.05
<i>TIEG</i>	NM_005655	-5.25	0.02
<i>RASL11B</i>	NM_023940	-4.97	0.03
<i>ATF3</i>	NM_004024	-4.89	0.02
<i>ZNF331</i>	NM_018555	-4.83	0.03
<i>GUCY1A3</i>	NM_000856	-4.74	0.04

Table 2. List of differentially expressed miRNAs in primary human TM cells in response to the cyclic mechanical stretch (15%, 1 cycle/s, 24 hrs)

miRNA ID	Fold Change	P-value	miRNA ID	Fold Change	P-value
miR-32-5p	2.38	1.2E-05	miR-137	1.59	1.3E-02
miR-4286	2.42	1.3E-05	miR-31-5p	1.59	1.4E-02
miR-135b-5p	2.03	4.6E-05	miR-455-5p	1.57	1.4E-02
miR-136-5p	2.11	7.0E-05	miR-152-3p	1.54	1.6E-02
miR-19b-3p	1.98	3.1E-04	miR-24-3p	1.67	1.6E-02
miR-93-5p	2.07	2.8E-04	miR-30a-5p	1.68	1.5E-02
miR-27a-3p	1.68	9.1E-04	miR-3151-5p	-1.50	1.6E-02
miR-362-3p	1.68	1.3E-03	miR-411-5p	1.50	1.6E-02
miR-151a-3p	1.78	1.6E-03	miR-26b-5p	1.57	1.7E-02
miR-25-3p	1.94	1.7E-03	miR-100-5p	1.82	1.7E-02
miR-337-5p	1.72	1.8E-03	miR-221-5p	1.50	1.8E-02
miR-140-5p	1.82	2.6E-03	miR-628-3p	1.49	1.9E-02
miR-497-5p	1.57	2.9E-03	miR-1290	-1.43	2.0E-02
miR-20a/b-5p	1.70	3.3E-03	miR-376c-3p	1.49	2.1E-02
miR-106a-5p/17-5p	1.66	4.3E-03	miR-642a-5p	1.45	2.1E-02
miR-15a-5p	1.85	4.7E-03	miR-185-5p	1.45	2.2E-02
miR-27b-3p	1.92	4.0E-03	miR-28-5p	1.54	2.6E-02
miR-374a-5p	1.93	4.5E-03	let-7i-5p	1.72	2.8E-02
miR-188-5p	-1.52	5.1E-03	miR-16-5p	1.64	2.8E-02
miR-29a-3p	1.96	5.9E-03	miR-29c-3p	1.69	2.8E-02
miR-21-5p	1.98	6.4E-03	miR-301a-3p	1.47	2.9E-02
miR-127-3p	1.67	9.9E-03	miR-542-3p	1.43	3.0E-02
miR-1275	-1.54	1.1E-02	miR-1206	1.44	3.3E-02
miR-187-3p	-1.52	1.0E-02	miR-574-3p	1.59	3.3E-02
miR-190a-5p	1.52	8.7E-03	miR-590-5p	1.40	3.4E-02
miR-1972	-1.50	8.0E-03	miR-409-3p	1.42	3.6E-02
miR-199a-5p	1.73	9.0E-03	miR-505-3p	1.41	3.7E-02
miR-2117	1.49	9.4E-03	miR-34a-5p	1.60	4.0E-02
miR-218-5p	1.63	9.1E-03	miR-1827	1.42	4.0E-02
miR-33a-5p	1.48	1.1E-02	miR-520f-3p	-1.40	4.2E-02
miR-376a-3p	1.63	1.1E-02	let-7f-5p	1.49	4.2E-02
miR-494-3p	1.77	1.0E-02	miR-639	1.42	4.3E-02
miR-495-3p	1.53	1.1E-02	miR-125a-5p	1.62	4.5E-02
miR-642a-3p	1.49	7.4E-03	miR-566	-1.30	4.7E-02
miR-98-5p	1.70	9.0E-03	miR-147b	-1.39	4.7E-02
miR-1323	-1.47	1.2E-02	miR-323a-3p	1.38	4.9E-02
miR-19a-3p	1.55	1.2E-02	miR-340-5p	1.40	4.9E-02

Table 3. List of the interacting miRNAs and mRNA target genes in response to the cyclic mechanical stress in primary human TM cells

miRNA ID	Fold Change	Gene Symbol	Fold Change	Function
miR-125a-5p*	1.62	<i>ID1</i>	-3.2	Transcription regulator
miR-125a-5p*	1.62	<i>ID2</i>	-2.19	Transcription regulator
miR-125a-5p*	1.62	<i>PPT2</i>	-2.17	Lysosomal enzyme
miR-15a-5p	1.85	<i>NT5DC1</i>	-2.36	Enzyme
miR-15a-5p	1.85	<i>OGT</i>	-2.83	Enzyme
miR-15a-5p	1.85	<i>OSGEPL1</i>	-2.27	Enzyme
miR-15a-5p	1.85	<i>RHOT1</i>	-3.13	GTPase
miR-15a-5p	1.85	<i>SLC35A1</i>	-2.28	Transporter
miR-15a-5p	1.85	<i>WEE1</i>	-3.42	Kinase
miR-93-5p*	2.07	<i>BAMBI</i>	-3.89	Receptor
miR-93-5p*	2.07	<i>HBPI</i>	-3.26	Transcription regulator
miR-93-5p*	2.07	<i>MAP3K12</i>	-2.74	Kinase
miR-93-5p*	2.07	<i>MYLIP</i>	-3.23	Ubiquitin ligase
miR-19b-3p*	1.98	<i>MYLIP</i>	-3.23	Ubiquitin ligase
miR-19b-3p*	1.98	<i>NR4A2</i>	-13.28	Receptor
miR-27b-3p*	1.92	<i>WEE1</i>	-3.42	Kinase
miR-29a-3p*	1.96	<i>DUSP2</i>	-2.17	Phosphatase
miR-30a-5p*	1.68	<i>ATRX</i>	-2.6	Transcription regulator
miR-30a-5p*	1.68	<i>IDH1</i>	-2.07	Enzyme
miR-32-5p	2.38	<i>CDKN1C</i>	-2.48	Kinase inhibitor
miR-32-5p	2.38	<i>MYLIP</i>	-3.23	Ubiquitin ligase

* indicates miRNAs with significant expression in non-glaucoma human TM tissue (n=8), defined as a normalized number of sequencing reads ≥ 10 .