

学位論文

Extracellular matrix gene expression in human trabecular meshwork cells
following mechanical fluid flow stimulation

(ヒト線維柱帯細胞への水流機械刺激による細胞外マトリックス遺伝子発現に関する研究)

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Extracellular matrix gene expression in human trabecular meshwork cells following mechanical fluid flow stimulation

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Abstract

● **AIM:** To investigate changes in extracellular matrix (ECM) gene expression in human trabecular meshwork (HTM) cells in response to mechanical fluid flow stimulation.

● **METHODS:** HTM cells were grown on a glass plate coated with 0.02% type I collagen (COL) and exposed to shear stress (0, 0.2, 1.0 dyne/cm²) for 12h. Changes in genes related to the ECM were evaluated by real-time reverse transcriptase-polymerase chain reaction. Phosphorylation of Smad2 protein was investigated by Western blotting.

● **RESULTS:** After mechanical stimulation, COL type 4 alpha 2, COL type 6 alpha 1, and fibronectin-1 mRNA were significantly higher than the static control ($P < 0.05$, < 0.05 , and < 0.01 , respectively). The metalloproteinase-2 and plasminogen activator inhibitor-1 mRNA were significantly higher than the static control ($P < 0.05$ and < 0.01 , respectively), while the differences in the tissue inhibitors of metalloproteinases-2 mRNA were not significant. The phosphorylation of Smad2 levels was significantly higher compared to the static control cells.

● **CONCLUSION:** Changes in the expressions of genes associated ECM metabolism result in HTM cells after mechanical stimulation. The mechanical stimulation of the aqueous humor to the trabecular meshwork may promote ECM turnover and contribute to intraocular pressure homeostasis.

● **KEYWORDS:** trabecular meshwork; shear stress; aqueous humor; extracellular matrix; glaucoma; intraocular pressure

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INTRODUCTION

The intraocular pressure (IOP) is maintained *via* a function of the aqueous humor, which is produced by the ciliary body^[1]. About 70% to 95% of the aqueous humor discharge *via* the conventional outflow pathway^[2]. Increased aqueous outflow resistance in this outflow pathway is the reason of glaucoma accompanied by elevated IOP^[3]. Therefore, normal functioning of the outflow component is important to the IOP homeostasis and prevention of glaucoma^[4].

The trabecular meshwork (TM), juxtacanalicular (JCT) meshwork, Schlemm's canal (SC), collector channels, and episcleral veins comprise the conventional outflow pathway. Among those, the extracellular matrix (ECM) in the TM tissue, which is comprised of collagens (COL) or fibronectin (FN)^[5], is critical for the homeostatic maintenance of the normal outflow resistance^[6]. Of note, recent studies have revealed that ECM turnover is regulated by mechanical stress, at least in part^[7].

Experimentally, mechanical stretching of the TM cells up-regulates the matrix metalloproteinase-2 (MMP-2), the membrane type-1 MMP, and reciprocally down-regulates the tissue inhibitors of metalloproteinases (TIMP)-2^[8]. Transcription of MMPs and proMMP activation is regulated by tissue plasminogen activator^[9], which knockout mice expressed in outflow facility reduction and declined the expression of MMP-9 gene in angle tissues^[10]. Cyclic mechanical stress alters the contractility of the TM cells^[11]. Mechanistically, the mechanosensitive receptors on the TM cell surface reportedly sense the deformation of the ECM resulting from the IOP fluctuations *via* integrin-based cell-matrix contact^[12]. Collectively, these findings suggest that mechanical stress applied to the TM cells is important for the ECM turnover.

Clinically, it generally is considered that the TM function (e.g., ECM synthesis, phagocytosis, contractility) deteriorates after glaucoma filtration surgery. In addition, the aqueous outflow to the TM after filtration surgery decreases to about 10% of the preoperative level^[13]. These facts cause speculation that the mechanical fluid flow stimulation by aqueous outflow to the TM also plays an important role in maintaining the TM function. However, to our knowledge, no previous reports have noted the difference of mechanical fluid flow stimulation on TM cells and its effect on ECM turnover, although the effects of mechanical stimulation by stretching of the TM cells have been investigated previously^[14].

In the current study, we applied mechanical fluid flow stimulation to the human trabecular meshwork (HTM) cells and investigated the gene changes related to the ECM and ECM remodeling.

MATERIALS AND METHODS

Ethical Approval The current study was conducted according to the tenets of the Declaration of Helsinki, and the study did not require ethical approval including those involving human-derived materials.

Materials Recombinant human transforming growth factor (TGF)- β 2 was bought from R&D Systems (Minneapolis, MN, USA). Dexamethasone (DEX) and Y-27632 were bought from Merck Millipore (Darmstadt, Germany). Polyclonal myocilin (MYOC) antibody (rabbit, ab41552, 1:1000) was bought from Abcam (Cambridge, UK); monoclonal β -actin antibodies (mouse, #3700, 1:3000) were bought from Cell Signaling Technology (Danvers, MA, USA); polyclonal FN antibodies (rabbit, F3648, 1:1000) and polyclonal COL IV antibodies (rabbit, SAB4500369, 1:1000) were bought from Sigma-Aldrich (St. Louis, MO, USA).

Cell Culture The primary HTM cells obtained from ScienCell (cat. no. 6590, lot no. 3423, Carlsbad, CA, USA) were maintained in Fibroblast Medium (ScienCell) containing 5% fetal bovine serum and fibroblast growth supplements and penicillin/streptomycin (FGS, P/S solution, ScienCell), in accordance with the manufacturer's protocol. The HTM cells were cultured at the bottom of poly-L-lysine-coated flasks (2 mg/cm²). The HTM cells were passaged with 0.05% trypsin 2 mmol/L ethylenediaminetetraacetic acid solution, seeding on glass plates (70×100×1.3 mm³; Matsunami Glass, Kishiwada, Japan) coated with 0.02% type I COL (Angio-Proteomie, Boston, MA, USA). The HTM cells in passages 5 to 7 were used for experiments.

Shear Stress Experiments As in previous shear stress experiments^[15], shear stress was applied to confluent HTM cells using a parallel plate-type flow chamber. Briefly, a flow chamber, a peristaltic pump (SJ1220, ATTO, Tokyo, Japan), and a medium reservoir compartment were all connected using

silicone tubes in this flow circuit. The culture medium without serum was circulated continually with a peristaltic pump in a carbon dioxide (CO₂) incubator at 37°C with 5% CO₂. In the current study, the HTM cells were cultured on glass plates and exposed to laminar shear stresses of the following magnitudes: 0 (static control, no fluid flow stimulation), 0.2, and 1.0 dyne/cm². The static cells were placed in an OmniTray (Thermo Fisher Scientific, Waltham, MA, USA) and cultured in the same amount of perfused medium. The shear stress within the flow chamber was calculated by the formula, $\tau = \mu \cdot 6Q/a^2b$, where τ is the shear stress (dyne/cm²), μ the viscosity of the perfused fluid (0.00796 poise), Q the volumetric flow rate (mL/s), a the flow channel height (0.04 cm), and b the flow channel width (5.5 cm) in the cross section. We used Dulbecco's Modified Eagle's medium (Wako Pure Chemical Industries, Ltd., Osaka, Japan) supplemented with 100 U/mL penicillin and 100 mg/mL streptomycin sulfate and GlutaMAX-I supplement (Life Technologies, Carlsbad, CA, USA) without serum to generate shear stress in a perfused medium. The perfused medium viscosities were measured using a viscometer (ViscoLab 4000, Japan Controls, Tokyo, Japan). The HTM cells were exposed to fluid flow for 12h, after which the cells cultured on glass plates were removed from the flow chamber and washed with phosphate buffered saline (PBS), and the collected HTM cells were used for the experiment.

Phagocytosis Assay HTM cells were plated into 48-well plates coated with poly-L-lysine. pHrodo green *Escherichia coli* bioparticles (Life Technologies) were suspended in a live cell imaging solution (Life Technologies) to a concentration of 1 mg/mL. The HTM cells were incubated at 37°C with pHrodo green bioparticles for 3h. The plates then were rinsed three times in live cell imaging solution and the HTM cells fixed for 10min at room temperature (RT) with 4% paraformaldehyde (Nacalai Tesque, Kyoto, Japan) and stained by Hoechst 33258 (Cosmo Bio, Tokyo, Japan) for 30min at RT. The HTM cells were photographed by a fluorescence microscope (BZ-X800, Keyence, Osaka, Japan).

FN and COL Protein Expression in Response to TGF- β 2

To investigate the FN and COL IV synthesis by HTM cells in response to TGF- β 2 or Y-27632, a specific ROCK inhibitor, we conducted another experiment. The HTM cells were cultured into 10-cm plates and grown to a confluent state. The HTM cells with or without 10 μ mol/L Y-27632 pretreatment for 30min were stimulated with TGF- β 2 for 24h without washout of Y-27632. TGF- β 2 was dissolved in 4 mmol/L hydrochloric acid, and the final concentration of hydrochloric acid was 200 μ mol/L in culture medium. The cell lysates were collected and FN and COL IV proteins were quantified using Western blot analysis.

Table 1 Primary sequences for the analysis of gene expression by RT-PCR

Gene	Accession number	Forward primer	Reverse primer
<i>COL1A2</i>	NM_000089	GGTCTCGGTGGGAACCTTG	CCAGGTGGGCCTCTAGGT
<i>COL4A2</i>	NM_001846	CAAAAGGAAGAGCAGGCTTC	CGTCGCCTTCTGTACATCTG
<i>COL6A1</i>	NM_001848	GAAGAGAAGGCCCCGTTG	CGGTAGCCTTTAGGTCCGATA
<i>FN1</i>	NM_212482	GACGCATCACTTGCACTTCT	GCAGGTTTCCTCGATTATCCT
<i>MMP-2</i>	NM_004530	CCCCAAAACGGACAAAGAG	CTTCAGCACAAACAGGTTGC
<i>TIMP-2</i>	NM_003255	GAAGAGCCTGAACCACAGGT	CGGGGAGGAGATGTAGCAC
<i>PAI-1</i>	NM_000602	TCCTGGTTCTGCCAAAGTT	CCAGGTTCTCTAGGGGCTTC
<i>G6PDH</i>	NM_000402	CTGCAGATGCTGTGTCTGGT	TGCATTTCAACACCTTGACC

COL1A2: Collagen type 1 alpha 2; *COL4A2*: Collagen type 4 alpha 2; *COL6A1*: Collagen type 6 alpha 1; *FN1*: Fibronectin 1; *MMP-2*: Matrix metalloproteinase-2; *TIMP-2*: The tissue inhibitors of metalloproteinases-2; *PAI-1*: The plasminogen activator inhibitor; *G6PDH*: Glucose-6-phosphate dehydrogenase.

Western Blot Analysis The HTM cells were rinsed with PBS and lysed with RIPA lysis buffer (Merck Millipore, Germany), which containing phosphatase inhibitor cocktail tablets (Roche), protease inhibitor cocktail tablets (Roche, Basel, Switzerland), and phenylmethylsulfonyl fluoride. After that, the lysates were centrifuged at 13 000 rpm for 20min at 4°C. The total protein concentration was measured by a NanoDrop Fluorospectrometer (Thermo Fisher Scientific). Equal quantities of protein were loaded into each well and separated using sodium dodecyl sulfate polyacrylamide gel electrophoresis, and electroblotted onto nitrocellulose membranes. The membranes were blocked overnight at 4°C using PVDF blocking reagent (Toyobo, Osaka, Japan), then incubated for 1h with Can Get Signal (Toyobo) containing the primary antibodies and horseradish peroxidase-conjugated anti-rabbit or anti-mouse IgG secondary antibody (#7076 and #7074, 1:10000; Cell Signaling Technology). The membranes were incubated at RT with ECL Prime Western Blotting Detection Reagent (GE Healthcare, Piscataway, NJ, USA) and detected by an LAS-3000 Imager (Fujifilm, Tokyo, Japan).

RT-PCR Analysis for Representative Genes Related to ECM The HTM cells were exposed to shear stress collected with a scraper, and the total RNA was isolated by a NucleoSpin RNA kit (Takara, Shiga, Japan). The total RNA (25 mg/mL) underwent reverse transcription using a Transcriptor First Strand cDNA Synthesis Kit (Roche), in accordance with the manufacturer's instructions, after which real-time PCR was subjected using the Universal ProbeLibrary and Light Cycler 480 (Roche). Specific primer pairs are listed in Table 1. The cycling conditions for all of the amplifications were as follows: an initial denaturation period for 5min at 95°C, followed by 45 cycles of 10s at 95°C, 30s at 60°C, and 1s at 72°C. The measurement of each gene expression signal was normalized to the glucose-6-phosphate dehydrogenase (G6PDH) gene signal. The relative fold changes in individual gene expression were determined using the $2^{-\Delta\Delta Ct}$ method.

Statistical Analysis The data were analyzed using GraphPad Prism version 5 (GraphPad Software, San Diego, CA, USA). Quantitative data were analyzed using the Dunn's nonparametric comparison for post-hoc testing following the Kruskal-Wallis test. The Mann-Whitney *U* test was used to analyze comparisons between two groups. $P < 0.05$ considered to be statistically significant. All experiment was performed with the same lot of cell lines.

RESULTS

Validation of the HTM Cell Phenotype To be sure that the HTM cells from lot number 3423 expressed the TM cell phenotype, we investigated whether they expressed MYOC in response to DEX, FN and COL IV in response to TGF- β 2 and presented phagocytic properties. MYOC induction in response to DEX is the most commonly used marker for TM cell phenotype^[16]. TM cells have phagocytic properties, which maintain the aqueous outflow pathway^[17]. The HTM cells used in the current study had significantly increased MYOC expression (1.6-fold, $P < 0.01$) induced by DEX (Figure 1A) and exhibited a phagocytotic function (Figure 1B). Further, the results of Western blot analysis for TGF- β 2-induced FN proteins in cell lysate were significantly higher than in the control (1.9-fold, $P < 0.05$; Figure 2A). Although COL IV proteins also increased with TGF- β 2 treatment, the differences were not significant (2.15-fold, $P = 0.53$; Figure 2B). The FN and COL IV protein levels did not differ significantly from the control when cells were treated with TGF- β 2 and Y-27632 concomitantly (1.59-fold and 1.87-fold, respectively).

Gene Expression of HTM Cells in Response to Shear Stress

The gene expression of the ECM components was analyzed by real-time RT-PCR. As a result, COL type 4 alpha 2 (*COL4A2*) mRNA, COL type 6 alpha 1 (*COL6A1*) mRNA, and FN1 mRNA after shear stress (1.0 dyne/cm²) were significantly higher than the static control (3.08-fold, $P < 0.05$; 1.43-fold, $P < 0.05$; and 1.47-fold, $P < 0.01$, respectively). Although COL type 1 alpha 2 (*COL1A2*) mRNA was higher than the static

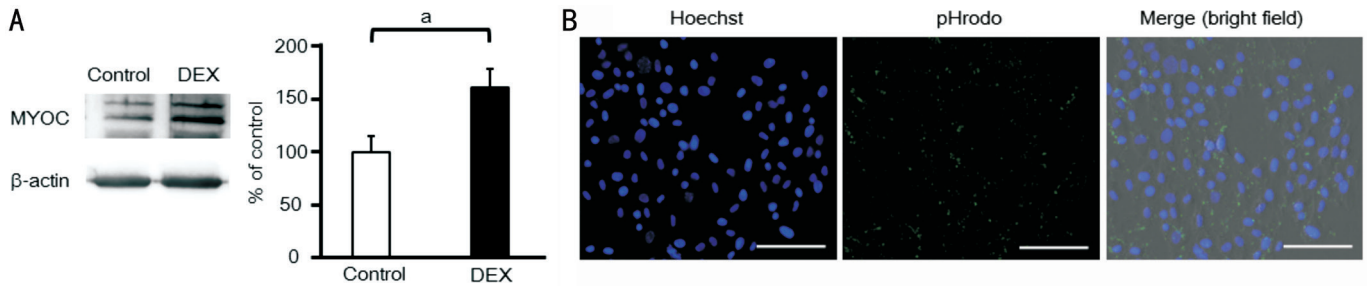


Figure 1 Characterization of the primary human trabecular meshwork (HTM) cells A: The HTM cells were treated with 500 nmol/L dexamethasone (DEX) for 7d. Cell lysates are subjected to Western blotting to detect polyclonal myocilin (MYOC) proteins. Quantitative assessment of the intensity of each band was performed by densitometry. The data indicate as the means±SDs ($n=5$). ^a $P<0.01$ using the Mann-Whitney U test. B: HTM cells are incubated with pHrodo *Escherichia coli* bioparticles for 3h (green). Cell nuclei are counterstained by Hoechst 33258 (blue) and observed by fluorescence microscope at 20× magnification ($n=4$). Scale bar: 100 μm.

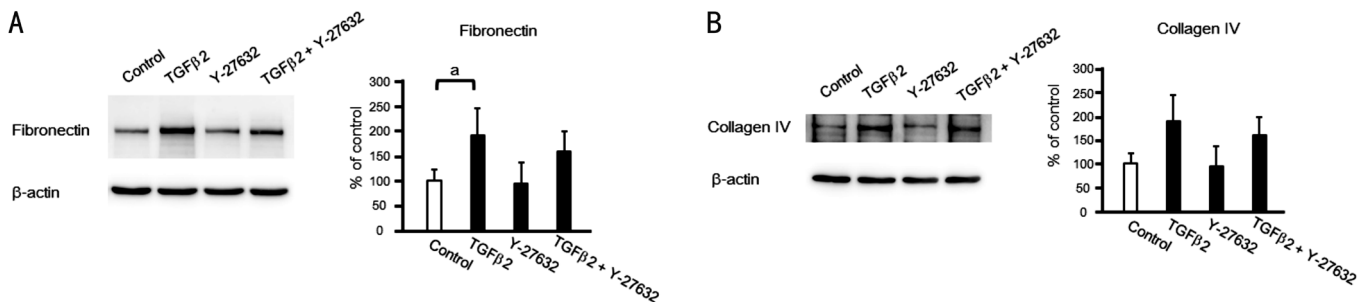


Figure 2 Effect of transforming growth factor (TGF)-β2 on fibronectin (FN) and collagen (COL) IV protein expression in human trabecular meshwork (HTM) cells The HTM cells treated with 5 ng/mL TGF-β2 for 24h and/or 10 μmol/L Y-27632 pretreatment for 30min are stimulated with TGF-β2. Representative images of Western blot analysis of FN (A) and COL IV (B) expression. Quantitative assessment of the intensity of each band was performed by densitometry. The data indicate as the means±SDs ($n=5$). ^a $P<0.05$.

control, the difference was not significant ($P=0.16$; Figure 3). The MMP2 mRNA was significantly higher (0.2 dyne/cm², 2.09-fold vs static control, $P<0.05$ and 1.0 dyne/cm², 2.39-fold vs static control, $P<0.05$; Figure 4A), while the differences in the TIMP2 mRNA was not significant (0.2 dyne/cm², 1.22-fold vs static control, and 1.0 dyne/cm², 1.25-fold vs static control, $P=0.98$; Figure 4B). The plasminogen activator inhibitor-1 (PAI-1) mRNA was significantly higher (1.0 dyne/cm², 15.49-fold vs static control, $P<0.01$; Figure 4C).

Phosphorylation of Smad2 in Response to Shear Stress TGF-β is the fibrogenic cytokine in TM cells, enhancing ECM and PAI-1 gene expression^[18]. The canonical TGF-β2 signal transduction is mediated through phosphorylation of Smad2 protein. We examined the effect of shear stress on the phosphorylation of Smad2 in HTM cells. The phosphorylation of Smad2 was significantly higher in HTM cells exposed to shear stress after 12h than in the static control (1.0 dyne/cm², 10.79-fold, $P<0.05$; Figure 5).

DISCUSSION

The IOP is regulated by the quantitative balance between the secretion and drainage of aqueous humor; when the aqueous outflow decreases, the pressure increases and it is the main risk factor for primary openangle glaucoma^[19]. There is evidence that progressive accumulation of misfolded proteins and

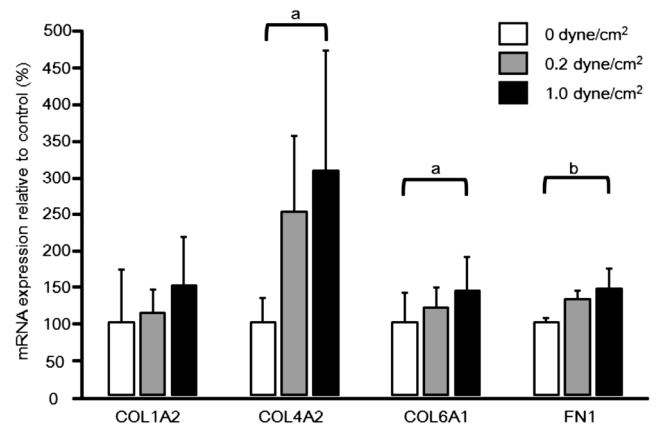


Figure 3 Real-time reverse transcriptase polymerase chain reaction (RT-PCR) analysis of collagen (COL) 1A2, COL4A2, COL6A1, and fibronectin (FN) 1 mRNA expression in human trabecular meshwork (HTM) cells COL4A2, COL6A1, and FN1 mRNA levels in the HTM cells exposed to shear stress (0.2 and 1 dyne/cm²) for 12h. The data indicate as the means±SDs ($n=5$). ^a $P<0.05$, ^b $P<0.01$.

malfunctioning of TM cells why may the aqueous outflow decreases^[20]. In the current study, different intensities of fluid shear stress were applied to HTM cells to investigate the changes in gene and protein expression related to ECM. Shear stress induced phosphorylation of Smad2 (Figure 5)

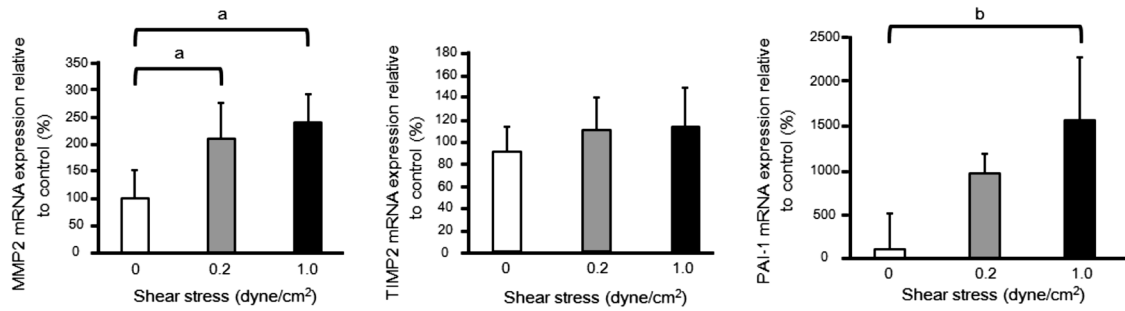


Figure 4 Real-time reverse transcriptase polymerase chain reaction (RT-PCR) analysis of matrix metalloproteinase (MMP)-2 mRNA, tissue inhibitors of metalloproteinases (TIMP)-2, and plasminogen activator inhibitor (PAI)-1 mRNA expression in human trabecular meshwork (HTM) cells. MMP2, TIMP2, and PAI-1 mRNA levels in the HTM cells exposed to shear stress (0.2 and 1 dyne/cm²) for 12h. The data indicate as the means±SDs (n=5). ^aP<0.05, ^bP<0.01.

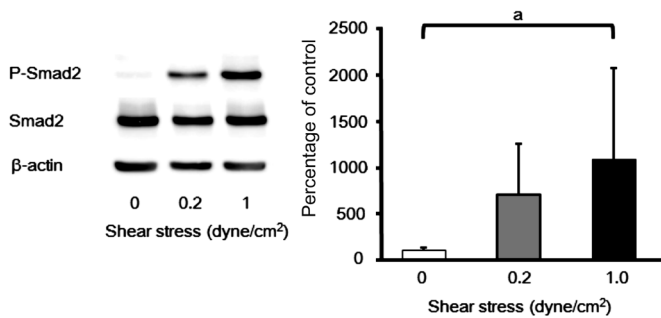


Figure 5 Phosphorylation of Smad2 in human trabecular meshwork (HTM) cells exposed to shear stress. After 12h of exposure to shear stress (0.2 and 1 dyne/cm²), the phosphorylation of Smad2 expression was examined by Western blotting. Quantitative assessment of the intensity of each band was performed by densitometry. The blots were stripped and re-blotting with anti-Smad2 antibody to demonstrate similar loading of all samples. The data indicate as the means±SDs (n=4). ^aP<0.05.

and promotes COL4A2, COL6A1, FN1, and PAI-1 mRNA (Figures 3, 4). A previous report that the ECM gene, MMP-2, and PAI-1 gene expression were induced by TGF-β signaling^[21]; therefore, HTM cells were thought to promote ECM gene up-regulation in the presence of shear stress. However, we could not detect the TGF-β1 and TGF-β2 protein in perfused cell culture medium, because these proteins were below the detection limit of enzyme-linked immunosorbent assay.

Keller *et al*^[22] reported that when the TM cells detected the increased IOP, they released numerous activated proteinases, such as gelatinase A, which degraded the existing ECM and concurrently synthesized new proteins to replace the degraded matrix. Considering that flow stimulation corresponds to increased IOP in this study, it is considered that an increase in MMP leads to degradation of the ECM.

Furthermore, considering the results of Bradley *et al*^[8], *i.e.*, that mechanical stretching of the TM cells up-regulates the MMP-2, we suggested that HTM cells under the mechanical fluid flow stimulation contributes to ECM turnover.

In addition, clinically, the current results implied that the

aqueous humor outflow through the TM may be necessary to maintain the physiologic IOP. According to Dan *et al*^[23], the PAI-1 concentration in the aqueous humor contributes to the development of glaucoma, and we considered that shear stress in the TM may in part elevate the IOP.

In the current study, although the HTM cells were stimulated by steady fluid flow using our shear stress experimental system, it is presumed that the fluid flow in the TM of a normal eye is probably not a laminar or steady flow. A previous report that simulated the wall shear stress in each component of the conventional outflow pathway reported that the shear stress on the inner wall of SC was about 0.01 dyne/cm²^[24]. Sherwood *et al*^[25] reported that the normal physiologic value of the TM/SC resistance is 0.4 dyne/cm². Therefore, in the JCT, which is the primary tissue of the ECM metabolism in the TM, the shear stress might be equal to or less than that in the inner wall of SC. Considering these facts, our experiment should have been performed under weaker stress to approximate the reaction *in vivo*; however, we could not create shear stress that was less than 0.2 dyne/cm² due to the limitation of our experimental system. Furthermore, the TM tissue pulsates in conjunction with the heart rate^[26]. Taken together, considering this histologic feature of the meshwork, the aqueous outflow in the TM may be turbulent. Further examination is necessary to clarify if the results of the current shear stress experiment also occur *in vivo*.

In conclusion, gene expression changes related to the ECM/ECM remodeling were observed as a result of continuous fluid shear stress in HTM cells *in vitro*, and up-regulation of FN and COL IV mRNA may be mediated by the phosphorylation of Smad2.

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REFERENCES

- 1 Huang AS, Francis BA, Weinreb RN. Structural and functional imaging of aqueous humour outflow: a review. *Clin Exp Ophthalmol* 2018;46(2):158-168.
- 2 Tamm ER. The trabecular meshwork outflow pathways: structural and functional aspects. *Exp Eye Res* 2009;88(4):648-655.
- 3 Johnson M. What controls aqueous humour outflow resistance? *Exp Eye Res* 2006;82(4):545-557.
- 4 Costagliola C, dell'Omo R, Agnifili L, Bartollino S, Fea AM, Uva MG, Zeppa L, Mastropasqua L. How many aqueous humor outflow pathways are there? *Surv Ophthalmol* 2020;65(2):144-170.
- 5 Ueda J, Wentz-Hunter K, Yue BYJT. Distribution of myocilin and extracellular matrix components in the juxtacanalicular tissue of human eyes. *Invest Ophthalmol Vis Sci* 2002;43(4):1068-1076.
- 6 O'Callaghan J, Cassidy PS, Humphries P. Open-angle glaucoma: therapeutically targeting the extracellular matrix of the conventional outflow pathway. *Expert Opin Ther Targets* 2017;21(11):1037-1050.
- 7 Hirt J, Liton PB. Autophagy and mechanotransduction in outflow pathway cells. *Exp Eye Res* 2017;158:146-153.
- 8 Bradley JM, Kelley MJ, Zhu X, Anderssohn AM, Alexander JP, Acott TS. Effects of mechanical stretching on trabecular matrix metalloproteinases. *Invest Ophthalmol Vis Sci* 2001;42(7):1505-1513.
- 9 Hu KB, Yang JW, Tanaka S, Gonias SL, Mars WM, Liu YH. Tissue-type plasminogen activator acts as a cytokine that triggers intracellular signal transduction and induces matrix metalloproteinase-9 gene expression. *J Biol Chem* 2006;281(4):2120-2127.
- 10 Hu Y, Barron AO, Gindina S, Kumar S, Chintala S, Nayyar A, Danias J. Investigations on the role of the fibrinolytic pathway on outflow facility regulation. *Invest Ophthalmol Vis Sci* 2019;60(5):1571-1580.
- 11 Duffy L, O'Reilly S. Functional implications of cross-linked actin networks in trabecular meshwork cells. *Cell Physiol Biochem* 2018;45(2):783-794.
- 12 Acott TS, Kelley MJ. Extracellular matrix in the trabecular meshwork. *Exp Eye Res* 2008;86(4):543-561.
- 13 Rouhiainen HJ, Terasvirta ME, Tuovinen EJ. Peripheral anterior synechiae formation after trabeculoplasty. *Arch Ophthalmol* 1988;106(2):189-191.
- 14 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(3):364-371.
- 15 Ishibazawa A, Nagaoka T, Takahashi T, Yamamoto K, Kamiya A, Ando J, Yoshida A. Effects of shear stress on the gene expressions of endothelial nitric oxide synthase, endothelin-1, and thrombomodulin in human retinal microvascular endothelial cells. *Invest Ophthalmol Vis Sci* 2011;52(11):8496-8504.
- 16 Keller KE, Bhattacharya SK, Borrás T, et al. Consensus recommendations for trabecular meshwork cell isolation, characterization and culture. *Exp Eye Res* 2018;171:164-173.
- 17 Stamer WD, Clark AF. The many faces of the trabecular meshwork cell. *Exp Eye Res* 2017;158:112-123.
- 18 Wordinger RJ, Sharma T, Clark AF. The role of TGF-beta2 and bone morphogenetic proteins in the trabecular meshwork and glaucoma. *J Ocul Pharmacol Ther* 2014;30(2-3):154-162.
- 19 Jonas JB, Aung T, Bourne RR, Bron AM, Ritch R, Panda-Jonas S. Glaucoma. *Lancet* 2017;390(10108):2183-2193.
- 20 Rozpedek-Kamińska W, Wojtczak R, Szafflik JP, Szafflik J, Majsterek I. The genetic and endoplasmic reticulum-mediated molecular mechanisms of primary open-angle glaucoma. *Int J Mol Sci* 2020;21(11):4171.
- 21 Lee EH, Park KI, Kim KY, Lee JH, Jang EJ, Ku SK, Kim SC, Suk HY, Park JY, Baek SY, Kim YW. Liquiritigenin inhibits hepatic fibrogenesis and TGF-beta1/smad with hippo/YAP signal. *Phytomedicine* 2019;62:152780.
- 22 Keller KE, Aga MN, Bradley JM, Kelley MJ, Acott TS. Extracellular matrix turnover and outflow resistance. *Exp Eye Res* 2009;88(4):676-682.
- 23 Dan J, Belyea D, Gertner G, Leshem I, Lusky M, Miskin R. Plasminogen activator inhibitor-1 in the aqueous humor of patients with and without glaucoma. *Arch Ophthalmol* 2005;123(2):220-224.
- 24 Villamarin A, Roy S, Hasballa R, Vardoulis O, Reymond P, Stergiopoulos N. 3D simulation of the aqueous flow in the human eye. *Med Eng Phys* 2012;34(10):1462-1470.
- 25 Sherwood JM, Stamer WD, Overby DR. A model of the oscillatory mechanical forces in the conventional outflow pathway. *J R Soc Interface* 2019;16(150):20180652.
- 26 Madekurozwa M, Stamer WD, Reina-Torres E, Sherwood JM, Overby DR. The ocular pulse decreases aqueous humor outflow resistance by stimulating nitric oxide production. *Am J Physiol Cell Physiol* 2021;320(4):C652-C665.