Contents lists available at ScienceDirect

Experimental Eye Research

journal homepage: www.elsevier.com

Topical administration of a ROCK inhibitor prevents anterior subcapsular cataract induced by UV-B irradiation

Toshiyasu Imaizumi[a], Daijiro Kurosaka[a, *], Umi Tanaka[a], Daisuke Sakai[a], Kazuhiro Fukuda[a], Atsushi Sanbe[b]

[a] Department of Ophthalmology, School of Medicine, Iwate Medical University, Morioka, Japan
[b] Department of Pharmacotherapeutics, School of Pharmacy, Iwate Medical University, Shiwa-gun, Japan

ARTICLE INFO

Keywords:
Lens epithelial cell
ROCK inhibitor
Epithelial-to-mesenchymal transition
Transforming growth factor-β
Type I collagen

ABSTRACT

The deposition of extracellular matrix (ECM)—which is mainly composed of type I collagen—in anterior subcapsular cataracts (ASCs) during epithelial-to-mesenchymal transition (EMT) of lens epithelial cells (LECs) decreases visual function. Transforming growth factor (TGF)-β is a key factor in the induction of EMT in LECs. Although Rho kinase (ROCK) plays an important role in EMT induced by TGF-β, it is unknown whether ROCK inhibition affects type I collagen expression in TGF-β-stimulated LECs and ASC formation. This was investigated in the present study both in vitro using human lens epithelium (HLE)-B3 cells and in vivo using mice with ultraviolet radiation (UVR)-B-induced cataracts. We found that TGF-β2 increased type I collagen mRNA expression in HLE-B3 cells; this was inhibited in a dose-dependent manner by treatment with the ROCK inhibitor Y-27632. UVR-B exposure caused ASC formation in mice. A histopathological examination revealed that LECs in the anterior subcapsular area were flattened and multi-layered, and had a spindle shape in cross section. Immunohistochemical analysis revealed the presence of α-smooth muscle actin and type I collagen around these flattened LECs; these opacities were reduced by topical instillation of Y-27632. These findings suggest that suppression of TGF-β signaling in LECs by topical application of a ROCK inhibitor can prevent the formation of ASCs.

1. Introduction

In anterior subcapsular cataracts (ASCs) (Korol et al., 2014; Lovicu et al., 2004) and posterior capsular opacification after cataract surgery (Kurosaka et al., 1999; Lovicu et al., 2016), the deposition of extracellular matrix (ECM) leads to decreased visual function (Johar et al., 2007; Kurosaka et al., 1999; Saika et al., 1998). This ECM is mainly composed of type I collagen and is produced by lens epithelial cells (LECs) (Johar et al., 2007; Saika et al., 1998; Sappino et al., 1990) that have undergone transdifferentiation via epithelial-to-mesenchymal transition (EMT) (Awasthi et al., 2009; Korol et al., 2014; Lovicu et al., 2016).

Transforming growth factor (TGF)-β is a key factor in the induction of EMT in LECs (Gupta et al., 2013; Korol et al., 2016; Kurosaka et al., 1995; Radeke et al., 2015; Zhu et al., 2013), mammary epithelial cells (O'Connor et al., 2013; 2015, 2016), and renal tubular epithelial (RTE) cells (Morita et al., 2007; Yang and Liu, 2001). TGF-β promotes the expression of type I collagen and α-smooth muscle actin (α-SMA)–a biomarker of myofibroblasts (Schulz et al., 1994)—in these cells (Casaroli-Marano et al., 1999; Chen et al., 2017; Darby et al., 1990; Fan et al., 2007; Grisanti and Guidry, 1995; Schulz et al., 1994; Thijssen et al., 2007). Rho kinase (ROCK) plays an important role in EMT. Pharmacological inhibition of ROCK by Y-27632 was shown to reduce α-SMA expression in mammary epithelial cells (O'Connor et al., 2013; 2015), RTE cells (Wei et al., 2013), and LECs (Korol et al., 2016) as well as type I collagen expression in RPE cells (Itoh et al., 2007), mammary epithelial cells (Brownfield et al., 2013), and RTE cells (Wei et al., 2013). However, it is unknown whether ROCK inhibition affects type I collagen expression in LECs and ASC formation.

Abbreviations: α-SMA, alpha smooth muscle actin; ASC, anterior subcapsular cataract; DMEM, Dulbecco's Modified Eagle's Medium; ECM, extracellular matrix; EMT, epithelial-to-mesenchymal transition; FBS, fetal bovine serum; HLE-B3, human lens epithelium B3; LEC, lens epithelial cells; MRTF-A, myocardin-related transcription factor A; qRT-PCR, quantitative reverse transcription polymerase chain reaction; ROCK, Rho kinase; RPE cell, retinal pigment epithelial cell; RTE cell, renal tubular epithelial cell; TGF-β, transforming growth factor beta; UVR-B, ultraviolet radiation B.

* Corresponding author.
Email address: kurosaka@iwate-med.ac.jp (D. Kurosaka)

https://doi.org/10.1016/j.exer.2019.01.016
Received 25 July 2018; Received in revised form 11 January 2019; Accepted 14 January 2019
Available online xxx

Table 1
Primer sets.

Protein	Oligonucleotide	Expected PCR product size
human smooth muscle actin	Sense ATTGCCGACCGAATGCAGAAG Antisense AGAGGCCAGGATGGAGCCAC	120bp
human Collagen type 1A	Sense TGGACCAGCAGACTGGCAAC Antisense TCGTGCAGCCATCGACAGTGAC	120bp
GAPDH	Sense AGGTCATCCATGACAACTTTG Antisense TTCAGCTCAGGGATGACCTT	190bp

We investigated this in the present study by evaluating the effect of Y-27632 on type I collagen levels in human LECs stimulated with TGF-β2 and on the development of ASCs in mice with ultraviolet radiation (UVR)-B-induced cataracts.

2. Materials and methods

2.1. HLE-B3 cell culture and treatment

Human lens epithelium (HLE)-B3 cells were purchased from the American Type Culture Collection (Manassas, VA, USA) and cultured at 37 °C in Dulbecco's Modified Eagle's medium (DMEM) (Nacalai Tesque, Kyoto, Japan) containing 10% (v/v) fetal bovine serum (FBS) (GE Healthcare Life Sciences, South Logan, UT, USA) in a humidified atmosphere of 5% CO_2. When the cells reached confluence, the medium was changed to DMEM containing 0.5% FBS. For the treatments, the cells were cultured in medium containing TGF-β2 (12.5 ng/ml; Sigma-Aldrich, St. Louis, MO, USA) with or without Y-27632 (3, 10, and 30 μM) for 24 h.

2.2. RNA preparation and quantitative reverse transcription polymerase chain reaction (qRT-PCR)

Total RNA from HLE-B3 cells was prepared using ISOGEN reagent (Nippon Gene, Toyama, Japan). cDNA was prepared from 1 μg total RNA using PrimeScript Reverse Transcriptase (Takara Bio, Kyoto,

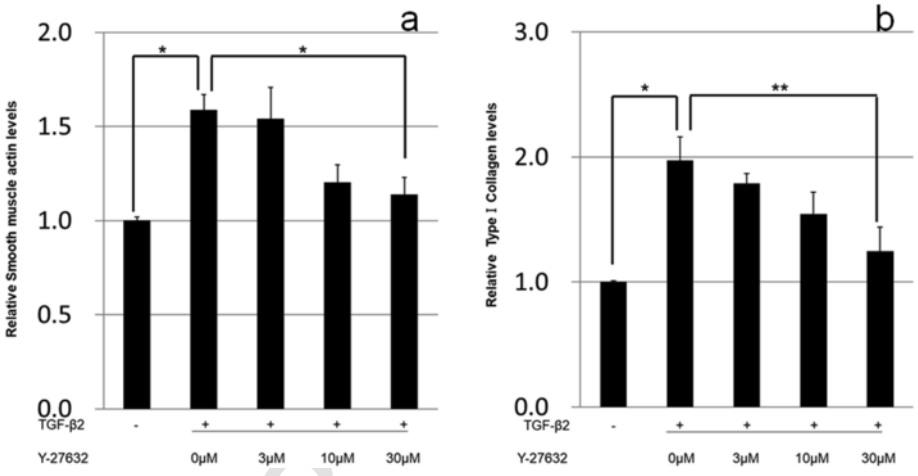

Fig. 1. Effect of Y-27632 on α-SMA and type I collagen expression in HLE-B3 cells stimulated with TGF-β2. Treatment with 12.5 ng/ml TGF-β2 increased α-SMA (a) and type I collagen (b) levels in the cells, as determined by qRT-PCR; Y-27632 reversed this effect in a dose-dependent manner. Data represent mean ± standard error of three independent experiments. *P < 0.001, **P < 0.05 (one-way ANOVA).

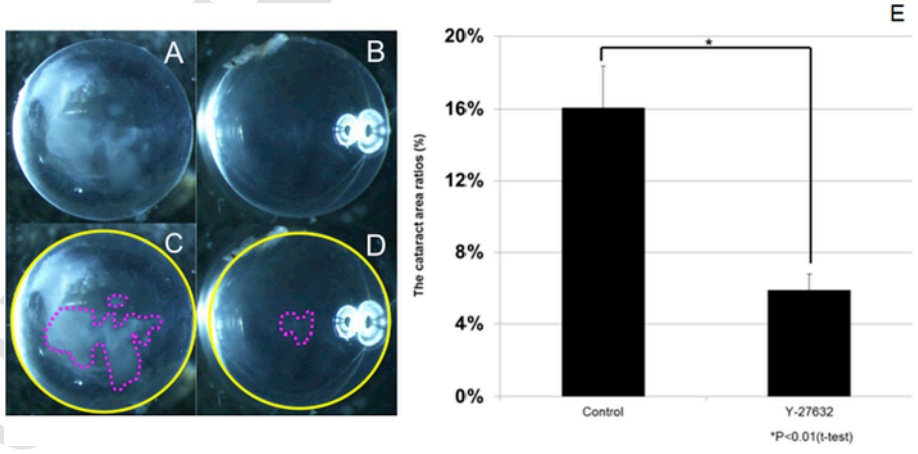

Fig. 2. Effect of Y-27632 on cataract formation in UVR-B-treated mice. (A–D) Stereo micrographs of lens opacities in mice 48 h after their final exposure to UVR-B without (A, C) and with (B, D) topical instillation of 2.5 mg/ml Y-27632. The area of opacity is delineated with a purple dotted line, and the anterior capsule is shown by a yellow line (C, D). (E) Lens opacity area ratio was 16.0% ± 2.2% and 5.8% ± 0.9% in mice without and with topical instillation of Y-27632, respectively. Values represent mean ± standard error of six lenses. *P < 0.01 vs. control (Student's *t*-test). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

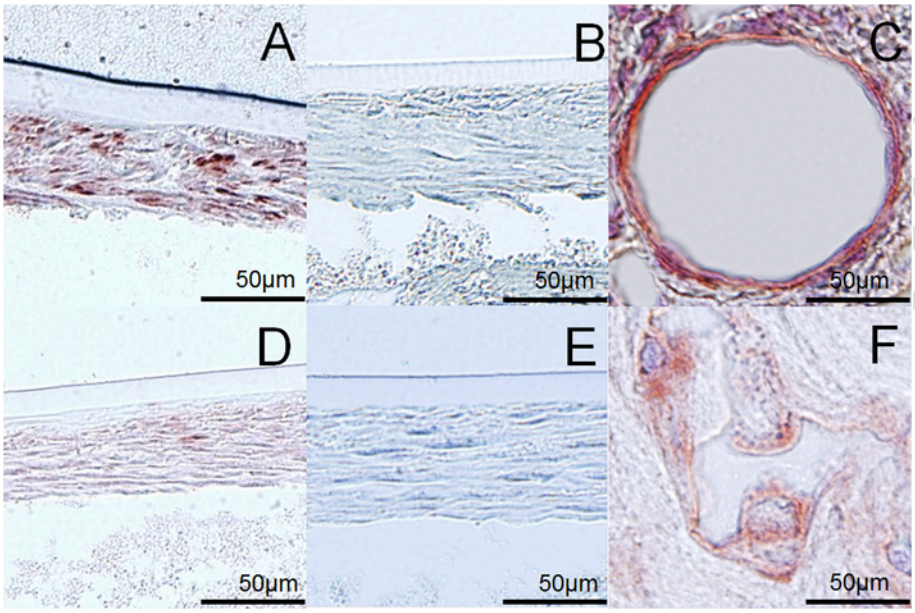

Fig. 3. Analysis of lens opacity induced by UVR-B exposure in mice. (A) α-SMA was expressed in flattened cells. (B) Negative control for α-SMA. (C) Positive control for α-SMA (mouse liver vessels). (D) type I collagen was expressed in flattened cells. (E) Negative control for type I collagen. (F) Positive control for type I collagen (mouse knee joint cartilage). Sections were stained with 3-amino-9-ethylcarbazole and counterstained with hematoxylin.

Japan) according to the manufacturer's instructions. PCR amplification was carried out using Ex Taq polymerase (Takara Bio) over 30 cycles of 94 °C for 0.5 min, 60 °C for 0.5 min, and 72 °C for 1 min on an Eco RT-PCR system (Illumina, San Diego, CA, USA) according to the manufacturer's protocol. Primers used for PCR are listed in Table 1.

2.3. Animal experiments

Female C57BL/6Cr Slc mice (12 weeks old; Japan SLC, Hamamatsu, Japan) used in this study were handled in accordance with the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research.

The experiments were approved by the Iwate Medical University School of Medicine animal ethics committee (approval number: 25–037).

UVR-B with a wavelength in the 302-nm region was generated with a transilluminator (TFML-20; UVP, Upland, CA, USA). UVR-B intensity in the corneal plane was 200 mW/cm^2, as measured with a radiometer (UV-340; Custom, Tokyo, Japan). The transilluminator was covered with aluminum foil except for a 5-mm hole. Each mouse was manually restrained (without anesthesia) and the right eye was irradiated through the hole. Prior to irradiation, all animals were examined with a slit lamp microscope (SL-15; Kowa, Nagoya, Japan) to exclude pre-existing cataracts, and 5 min before UVR-B exposure, 0.5% tropicamide and 0.5% phenylephrine sulfate hydrate were introduced into both eyes to induce mydriasis. Only the right eye of each mouse was exposed to UVR-B for 100s twice a week for 3 weeks, with an interval of 48 h from the last UVR-B exposure based on the previously reported time course of cataract progression after in vivo exposure to UVR-B (Meyer et al., 2005).

Y-27632 was diluted in purified water at a concentration of 2.5 mg/ml (7.4 mM). A 20-μl volume of this solution was topically instilled in the right eye of six mice twice daily starting from 3 days before UVR-B exposure until 2 days after the last exposure. Normal saline solution was applied as a control to the right eye of six other mice. Lens opacity was evaluated with a slit-lamp microscope before corneal opacities were induced by UVR-B exposure. After the last topical instillation, animals were euthanized by cervical dislocation. Immediately after removal from the eye, the lens was immersed in phosphate-buffered saline and cataract morphology was examined and recorded with a microscope and camera system. The lens was then fixed with Superfix (Kurabo, Osaka, Japan); the procedure was performed separately for each eye within 1 min. Digital images of the anterior capsule were captured and cataract area was measured using ImageJ software (National Institutes of Health, Bethesda, MD, USA). The ratio of cataract area to anterior capsule area was calculated as the cataract area ratio.

2.4. Immunohistochemistry

After fixation, the extracted lenses were dehydrated through a graded series of alcohol and embedded in paraffin. Sections cut at a thickness of 5 μm were incubated with mouse monoclonal anti-α-SMA (clone1A4; Dako, Glostrup, Denmark) and rabbit polyclonal anti-type I collagen (ab34710; Abcam, Cambridge, UK) antibodies diluted 1:500. As a negative control, isotype mouse IgG (ab 18413; Abcam, Cambridge, UK) and isotype rabbit IgG (ab 37415; Abcam, Cambridge, UK) antibodies were used. Immunoreactivity was detected with the Vectastain ABC Standard kit (Vector Laboratories, Burlingame, CA, USA) according to the manufacturer's protocol.

2.5. Statistical analysis

Data are expressed as mean ± standard error. Differences between group means were evaluated with the unpaired Student's *t*-test or by one-way analysis of variance followed by a post-hoc Tukey's multiple comparisons test. Statistical analyses were performed using Kaleida Graph software v.4.1 (Synergy Software, Reading, PA, USA).

3. Results

3.1. Y-27632 suppresses TGF-β2-induced type I collagen expression in lens cells

To determine the effect of ROCK inhibition on type I collagen and α-SMA expression in LECs, HLE-B3 cells were cultured with 12.5 ng/ml TGF-β2 in the presence or absence of Y-27632 (3, 10, and 30 μM). TGF-

β2 increased α-SMA mRNA expression relative to the control group (1.59 ± 0.08 vs. 1.00 ± 0.02); however, Y-27632 treatment reversed this effect in a dose-dependent manner (1.54 ± 0.17, 1.20 ± 0.09, and 1.14 ± 0.09 at 3, 10, and 30 μM Y-27632, respectively; all $P < 0.001$) (Fig. 1A). These results are consistent with previous findings in rat lens epithelial explants (Korol et al., 2016). Similar trends were observed for type I collagen: the level was increased in cells treated with TGF-β2 relative to control cells (1.97 ± 0.19 vs. 1.00 ± 0.01), but was dose-dependently reduced in the presence of 3, 10, and 30 μM Y-27632 (1.79 ± 0.19, 1.54 ± 0.08, and 1.25 ± 0.19, respectively; $P < 0.05$ and $P < 0.001$) (Fig. 1B).

3.2. Y-27632 suppresses ASC formation in vivo

To examine the effect of ROCK inhibition on ASC formation in vivo, mice exposed to UVR-B to induce cataract formation were topically instilled with Y-27632 or with saline as a control. In this model, ASC develops upon UVR-B exposure, and the degree of cataract formation can be evaluated quantitatively (Ishikawa et al., 2012). Slit lamp examination revealed no lens opacity at 1 and 2 weeks (images not shown). At 3 weeks, lens opacity could not be accurately assessed due to corneal damage caused by UV irradiation. However, a microscope and camera system revealed that at 48 h after the last UVR-B exposure, the cataract area in control lenses (Fig. 2A, C) was larger than that in lenses treated with Y-27632 (Fig. 2B, D). Thus, the mean cataract area ratio of lenses treated with Y-27632 (5.8% ± 0.9%) was lower than that of control lenses (16.0% ± 2.2%; $P < 0.01$) (Fig. 2E).

A histopathological examination revealed that LECs in the anterior subcapsular area of ASC model mice were flattened and multilayered and had a spindle shape in cross section. α-SMA (Fig. 3A) and type I collagen (Fig. 3D) were detected around the flattened cells by immunohistochemistry.

4. Discussion

In this study, TGF-β2 enhanced the mRNA expression of type I collagen in HLE-B3 cells, but this was suppressed by the ROCK inhibitor Y-27632. UVR-B exposure induced the formation of lens opacities around the anterior subcapsule of mice and surrounding LECs expressed α-SMA and type I collagen; these effects were reversed by topical instillation of Y-27632. Thus, ROCK inhibition can prevent ASC formation.

It is not known whether ROCK plays an important role in collagen expression in TGF-β-induced EMT. However, Y-27632 inhibited TGF-β-induced type I collagen expression in RPE cells (Itoh et al., 2016) and altered the activity of myocardin-related transcription factor (MRTF)-A in LECs (Korol et al., 2016). After translocating to the nucleus from the cytoplasm, MRTF-A cooperates with serum response factor to induce α-SMA expression (Luchsinger et al., 2011; Small et al., 2010). The subcellular localization of MRTF-A is controlled by binding to monomeric (G-)actin, which results in its retention in the cytoplasm (Guettler et al., 2008; Parreno et al., 2014). Y-27632 prevents F-actin stress fiber formation via Rho activation, which in turn induces G-actin expression (Korol et al., 2016). Thus, Y-27632 blocks the nuclear translocation of MRTF-A in LECs (Korol et al., 2016). The interaction of MRTF-A with serum response factor can also induce type I collagen in lung fibroblasts and trabecular meshwork cells (Luchsinger et al., 2011; Pattabiraman et al., 2014). These findings suggest that the suppression of TGF-β-dependent type I collagen expression by Y-27632 is related to a block in nuclear MRTF-A transport.

α-SMA and type I collagen were expressed around flattened LECs following exposure to UVR-B, suggesting that the induction of EMT in these cells leads to cataract formation. Type I collagen and TGF-β2 have been detected in human ASC samples (Ishida et al., 2005), while adenoviral injection of active TGF-β1 into the anterior chamber led to ASC formation (Korol et al., 2014). Injury-induced EMT in the lens in vivo depends on Mothers against decapentaplegic homolog 3, a downstream effector of TGF-β signaling (Saika et al., 2004). At 4 and 8 h after UVR-B exposure, TGF-β1 transcript level was upregulated in human keratinocytes (Lee et al., 1997); expression was also increased by whole-body exposure to UVR-B in humans (Ciążyńska et al., 2017). Thus, TGF-β plays an important role in EMT of LECs induced by UVR-B exposure. However, we did not measure TGF-β concentrations in the aqueous humor of mice before and after UVR-B exposure. Various factors such as connective tissue growth factor, fibroblast growth factor 2, and gremlin are associated with EMT in LECs (Ma et al., 2014), and it is possible that these contribute to ASC formation in lens exposed to UVR-B.

In this study, local instillation of 2.5 mg/ml (7.39 mM) Y-27632 suppressed ASC formation in mice. Topical application of 10 mM Y-27632 to rat eyes was reported to decrease intraocular pressure (Pattabiraman et al., 2015). Although the trabecular meshwork in rats constitutively expressing active RhoA showed an upregulation of α-SMA and type I collagen, the levels were reduced by topical application of 10 mM Y-27632 (Pattabiraman et al., 2015). Topically instilled radiolabeled ROCK inhibitor can be detected in the lens up to 4 h later (Isobe et al., 2014). Thus, the 2.5 mg/ml Y-27632 administered in this study was likely sufficient to affect LECs.

Ripasudil, another ROCK inhibitor applied as eye drops, was approved in Japan in 2014 for the treatment of glaucoma and ocular hypertension (Garnock-Jones, 2014; Terao et al., 2017). Adverse events associated with ripasudil in clinical trials included conjunctival hyperemia, conjunctival hemorrhage, ocular irritation, and abnormal sensation in the eye (Tanihara et al., 2013a, 2013b, 2015a, 2015b), although there were no severe side effects. The Rho signaling pathway is related to early lens development (Borges et al., 2011), and disruption of Rho GTPase function in developing mouse lens leads to cataract formation (Maddala et al., 2004; Rao et al., 2002). Y-27632, which destabilizes stress fibers, induces lens cell differentiation in vitro (Weber and Menko, 2006). These findings imply that Y-27632 may affect the structure of normal lens. Therefore, further investigation is required to determine its safety as a prophylactic drug against ASCs.

5. Conclusions

The results of this study demonstrate that ROCK inhibition suppresses TGF-β2-dependent induction of type I collagen expression in LECs and ASC formation induced by UVR-B exposure in mice. These findings suggest that ROCK inhibitors are candidate drugs for preventing ASCs.

Declaration of interest

None.

Funding

This work was supported by a Japan Society for the Promotion of Science KAKENHI grant (no. 17K11465).

Acknowledgments

We thank Ms. Rieko Higashio for technical assistance with the real-time PCR analysis.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.exer.2019.01.016.

Uncited refrence

Hales et al., 1994

References

Awasthi, N., Guo, S., Wagner, B.J., 2009. Posterior capsular opacification: a problem reduced but not yet eradicated. Arch. Ophthalmol. 127, 555–562 https://doi.org/10.1001/archophthalmol.2009.3.

Borges, R.M., Lamers, M.L., Forti, F.L., Santos, M.F., Yan, C.Y., 2011. Rho signaling pathway and apical constriction in the early lens placode. Genesis 49, 368–379 https://doi.org/10.1002/dvg.20723.

Brownfield, D.G., Venugopalan, G., Lo, A., Mori, H., Tanner, K., Fletcher, D.A., Bissell, M.J., 2013. Patterned collagen fibers orient branching mammary epithelium through distinct signaling modules. Curr. Biol. 23, 703–709 https://doi.org/10.1016/j.cub.2013.03.032.

Casaroli-Marano, R.P., Pagan, R., Vilaró, S., 1999. Epithelial-mesenchymal transition in proliferative vitreoretinopathy: intermediate filament protein expression in retinal pigment epithelial cells. Invest. Ophthalmol. Vis. Sci. 40, 2062–2072.

Chen, Q., Yang, W., Wang, X., Li, X., Qi, S., Zhang, Y., Gao, M.Q., 2017. TGF-β1 induces EMT in bovine mammary epithelial cells through the TGFβ1/Smad signaling pathway. Cell. Physiol. Biochem. 43, 82–93 https://doi.org/10.1159/000480321.

Ciążyńska, M., Bednarski, I.A., Wódz, K., Narbutt, J., Sobjanek, M., Woźniacka, A., Lesiak, A., 2017. Impact of ultraviolet radiation on expression of transforming growth factor β, Smad2, metalloproteinases-1, -3, -8, -9, cathepsin K and progerin. Photochem. Photobiol. 94, 362–369 https://doi.org/10.1111/php.12866.

Darby, I., Skalli, O., Gabbiani, G., 1990. Alpha-smooth muscle actin is transiently expressed by myofibroblasts during experimental wound healing. Lab. Invest. 63, 21–29.

Fan, L., Sebe, A., Péterfi, Z., Masszi, A., Thirone, A.C., Rotstein, O.D., Nakano, H., McCulloch, C.A., Szászi, K., Mucsi, I., 2007. Cell contact-dependent regulation of epithelial-myofibroblast transition via the rho-rho kinase-phospho-myosin pathway. Mol. Biol. Cell 18, 1083–1097 https://doi.org/10.1091/mbc.e06-07-0602.

Garnock-Jones, K.P., 2014. Ripasudil: first global approval. Drugs 74, 2211–2215 https://doi: 10.1007/s40265-014-0333-2.

Grisanti, S., Guidry, C., 1995. Transdifferentiation of retinal pigment epithelial cells from epithelial to mesenchymal phenotype. Invest. Ophthalmol. Vis. Sci. 36, 391–405.

Guettler, S., Vartiainen, M.K., Miralles, F., Larijani, B., Treisman, R., 2008. RPEL motifs link the serum response factor cofactor MAL but not myocardin to Rho signaling via actin binding. Mol. Cell Biol. 28, 732–742 https://doi.org/10.1128/MCB.01623-07.

Gupta, M., Korol, A., West-Mays, J.A., 2013. Nuclear translocation of myocardin-related transcription factor-A during transforming growth factor beta-induced epithelial to mesenchymal transition of lens epithelial cells. Mol. Vis. 19, 1017–1028.

Hales, A.M., Schulz, M.W., Chamberlain, C.G., McAvoy, J.W., 1994. TGF-beta 1 induces lens cells to accumulate alpha-smooth muscle actin, a marker for subcapsular cataracts. Curr. Eye Res. 13, 885–890 https://doi.org/10.3109/02713689409015091.

Ishida, I., Saika, S., Okada, Y., Ohnishi, Y., 2005. Growth factor deposition in anterior subcapsular cataract. J. Cataract Refract. Surg. 31, 1219–1225 https://doi.org/10.1016/j.jcrs.2004.11.039.

Ishikawa, Y., Hashizume, K., Kishimoto, S., Tezuka, Y., Nishigori, H., Yamamoto, N., Kondo, Y., Maruyama, N., Ishigami, A., Kurosaka, D., 2012. Effect of vitamin C depletion on UVR-B induced cataract in SMP30/GNL knockout mice. Exp. Eye Res. 94, 85–89 https://doi.org/10.1016/j.exer.2011.11.010.

Isobe, T., Mizuno, K., Kaneko, Y., Ohta, M., Koide, T., Tanabe, S., 2014. Effects of K-115, a rho-kinase inhibitor, on aqueous humor dynamics in rabbits. Curr. Eye Res. 39, 813–822 https://doi.org/10.3109/02713683.2013.874444.

Itoh, Y., Kimoto, K., Imaizumi, M., Nakatsuka, K., 2007. Inhibition of RhoA/Rho-kinase pathway suppresses the expression of type I collagen induced by TGF-beta2 in human retinal pigment epithelial cells. Exp. Eye Res. 84, 464–472 https://doi.org/10.1016/j.exer.2006.10.017.

Johar, K., Vasavada, A.R., Tatsumi, K., Dholakia, S., Nihalani, B., Rao, S.S., 2007. Anterior capsular plaque in congenital cataract: occurrence, morphology, immunofluorescence, and ultrastructure. Invest. Ophthalmol. Vis. Sci. 48, 4209–4214 https://doi.org/10.1167/iovs.07-0312.

Korol, A., Pino, G., Dwivedi, D., Robertson, J.V., Deschamps, P.A., West-Mays, J.A., 2014. Matrix metalloproteinase-9-null mice are resistant to TGF-β-induced anterior subcapsular cataract formation. Am. J. Pathol. 184, 2001–2012 https://doi.org/10.1016/j.ajpath.2014.03.013.

Korol, A., Taiyab, A., West-Mays, J.A., 2016. RhoA/ROCK signaling regulates TGFβ-induced epithelial-mesenchymal transition of lens epithelial cells through MRTF-A. Mol. Med. 22, 713–723 https://doi.org/10.2119/molmed.2016.00041.

Kurosaka, D., Ando, I., Kato, K., Oshima, T., Kurosaka, H., Yoshino, M., Nagamoto, T., Ando, N., 1999. Fibrous membrane formation at the capsular margin in capsule contraction syndrome. J. Cataract Refract. Surg. 25, 930–935 https://doi.org/10.1016/S0886-3350(99)00078-4.

Lee, H.S., Kooshesh, F., Sauder, D.N., Kondo, S., 1997. Modulation of TGF-beta 1 production from human keratinocytes by UVB. Exp. Dermatol. 6, 105–110 https://doi.org/10.1111/j.1600-0625.1997.tb00155.x.

Lovicu, F.J., Steven, P., Saika, S., McAvoy, J.W., 2004. Aberrant lens fiber differentiation in anterior subcapsular cataract formation: a process dependent on reduced levels of Pax6. Invest. Ophthalmol. Vis. Sci. 45, 1946–1953 https://doi.org/10.1167/iovs.03-1206.

Lovicu, F.J., Shin, E.H., McAvoy, J.W., 2016. Fibrosis in the lens. Sprouty regulation of TGFβ-signaling prevents lens EMT leading to cataract. Exp. Eye Res. 142, 92–101 https://doi.org/10.1016/j.exer.2015.02.004.

Luchsinger, L.L., Patenaude, C.A., Smith, B.D., Layne, M.D., 2011. Myocardin-related transcription factor-A complexes activate type I collagen expression in lung fibroblasts. J. Biol. Chem. 286, 44116–44125 https://doi.org/10.1074/jbc.M111.276931.

Ma, B., Kang, Q., Qin, L., Cui, L., Pei, C., 2014. TGF-β2 induces transdifferentiation and fibrosis in human lens epithelial cells via regulating gremlin and CTGF. Biochem. Biophys. Res. Commun. 447, 689–695 https://doi.org/10.1016/j.bbrc.2014.04.068.

Maddala, R., Deng, P.F., Costello, J.M., Wawrousek, E.F., Zigler, J.S., Rao, V.P., 2004. Impaired cytoskeletal organization and membrane integrity in lens fibers of a Rho GTPase functional knockout transgenic mouse. Lab. Invest. 84, 679–692 https://doi.org/10.1038/labinvest.3700105.

Meyer, L.M., Söderberg, P., Dong, X., Wegener, A., 2005. UVR-B induced cataract development in C57 mice. Exp. Eye Res. 81, 389–394 https://10.1016/j.exer.2005.02.009.

Morita, T., Mayanagi, T., Sobue, K., 2007. Dual roles of myocardin-related transcription factors in epithelial mesenchymal transition via slug induction and actin remodeling. J. Cell Biol. 179, 1027–1042 https://doi.org/10.1083/jcb.200708174.

O'Connor, J.W., Gomez, E.W., 2013. Cell adhesion and shape regulate TGF-beta1-induced epithelial-myofibroblast transition via MRTF-A signaling. PLoS One 8, e83188 https://doi.org/10.1371/journal.pone.0083188.

O'Connor, J.W., Mistry, K., Detweiler, D., Wang, C., Gomez, E.W., 2016. Cell-cell contact and matrix adhesion promote αSMA expression during TGFβ1-induced epithelial-myofibroblast transition via Notch and MRTF-A. Sci. Rep. 6, 26226 https://doi.org/10.1038/srep26226.

O'Connor, J.W., Riley, P.N., Nalluri, S.M., Ashar, P.K., Gomez, E.W., 2015. Matrix rigidity mediates TGFβ1-induced epithelial-myofibroblast transition by controlling cytoskeletal organization and MRTF-A localization. J. Cell. Physiol. 230, 1829–1839 https://doi.org/10.1002/jcp.24895.

Parreno, J., Raju, S., Niaki, M.N., Andrejevic, K., Jiang, A., Delve, E., Kandel, R., 2014. Expression of type I collagen and tenascin C is regulated by actin polymerization through MRTF in dedifferentiated chondrocytes. FEBS Lett. 588, 3677–3684 https://doi.org/10.1016/j.febslet.2014.08.012.

Pattabiraman, P.P., Maddala, R., Rao, P.V., 2014. Regulation of plasticity and fibrogenic activity of trabecular meshwork cells by Rho GTPase signaling. J. Cell. Physiol. 229, 927–942 https://doi.org/10.1002/jcp.24524.

Pattabiraman, P.P., Rinkoski, T., Poeschla, E., Proia, A., Challa, P., Rao, P.V., 2015. RhoA GTPase-induced ocular hypertension in a rodent model is associated with increased fibrogenic activity in the trabecular meshwork. Am. J. Pathol. 185, 496–512 https://doi.org/10.1016/j.ajpath.2014.10.023.

Radeke, M.J., Radeke, C.M., Shih, Y.H., Hu, J., Bok, D., Johnson, L.V., Coffey, P.J., 2015. Restoration of mesenchymal retinal pigmented epithelial cells by TGFβ pathway inhibitors: implications for age-related macular degeneration. Genome Med. 7, 58 https://doi.org/10.1186/s13073-015-0183-x.

Rao, V., Wawrousek, E., Tamm, E.R., Zigler Jr., S., 2002. Rho GTPase inactivation impairs lens growth and integrity. Lab. Invest. 82, 231–239 https://doi.org/10.1038/labinvest.3780415.

Saika, S., Kawashima, Y., Miyamoto, T., Okada, Y., Tanaka, S.I., Ohmi, S., Minamide, A., Yamanaka, O., Ohnishi, Y., Ooshima, A., 1998. Immunolocalization of prolyl 4-hydroxylase subunits, alpha-smooth muscle actin, and extracellular matrix components in human lens capsules with lens implants. Exp. Eye Res. 66, 283–294 https://doi.org/10.1006/exer.1997.0434.

Saika, S., Kono-Saika, S., Ohnishi, Y., Sato, M., Muragaki, Y., Ooshima, A., Flanders, K.C., Yoo, J., Anzano, M., Liu, C.Y., 2004. Smad3 signaling is required for epithelial-mesenchymal transition of lens epithelium after injury. Am. J. Pathol. 164, 651–663 https://doi.org/10.1016/S0002-9440(10)63153-7.

Sappino, A.P., Schürch, W., Gabbiani, G., 1990. Differentiation repertoire of fibroblastic cells: expression of cytoskeletal proteins as marker of phenotypic modulations. Lab. Invest. 63, 144–161.

Small, E.M., Thatcher, J.E., Sutherland, L.B., Kinoshita, H., Gerard, R.D., Richardson, J.A., Dimaio, J.M., Sadek, H., Kuwahara, K., Olson, E.N., 2010. Myocardin-related transcription factor-a controls myofibroblast activation and fibrosis in response to myocardial infarction. Circ. Res. 107, 294–304 https://doi.org/10.1161/CIRCRESAHA.110.223172.

Tanihara, H., Inoue, T., Yamamoto, T., Kuwayama, Y., Abe, H., Araie, M., K-115 Clinical Study Group, 2013a. Phase 1 clinical trials of a selective Rho kinase inhibitor, K-115. JAMA Ophthalmol 131, 1288–1295 https://doi.org/10.1001/jamaophthalmol.2013.323.

Tanihara, H., Inoue, T., Yamamoto, T., Kuwayama, Y., Abe, H., Araie, M., K-115 Clinical Study Group, 2013b. Phase 2 randomized clinical study of a Rho kinase inhibitor, K-115, in primary open-angle glaucoma and ocular hypertension. Am. J. Ophthalmol. 156, 731–736 https://doi.org/10.1016/j.ajo.2013.05.016.

Tanihara, H., Inoue, T., Yamamoto, T., Kuwayama, Y., Abe, H., Suganami, H., Araie, M., K-115 Clinical Study Group, 2015a. Intra-ocular pressure-lowering effects of a Rho kinase inhibitor, ripasudil (K-115), over 24 hours in primary open-angle glaucoma and ocular hypertension: a randomized, open-label, crossover study. Acta Ophthalmol. 93, e254–e260 https://doi.org/10.1111/aos.12599.

Tanihara, H., Inoue, T., Yamamoto, T., Kuwayama, Y., Abe, H., Suganami, H., Araie, M., K-115 Clinical Study Group, 2015b. Additive intraocular pressure-lowering effects of the Rho kinase inhibitor ripasudil (K-115) combined with timolol or latanoprost: a report of 2 randomized clinical trials. JAMA Ophthalmol 133, 755–761 https://doi.org/10.1001/jamaophthalmol.2015.0525.

Terao, E., Nakakura, S., Fujisawa, Y., Fujio, Y., Matsuya, K., Kobayashi, Y., Tabuchi, H., Yoneda, T., Fukushima, A., Kiuchi, Y., 2017. Time course of conjunctival hyperemia induced by a Rho-kinase inhibitor anti-glaucoma eye drop: ripasudil 0.4%. Curr. Eye Res. 42, 738–742 https://doi.org/10.1080/02713683.2016.1250276.

Thijssen, S., Lambrichts, I., Maringwa, J., Van Kerkhove, E., 2007. Changes in expression of fibrotic markers and histopathological alterations in kidneys of mice chronically exposed to low and high Cd doses. Toxicology 238, 200–210 https://doi.org/10.1016/j.tox.2007.06.087.

Weber, G.F., Menko, A.S., 2006. Actin filament organization regulates the induction of lens cell differentiation and survival. Dev. Biol. 295, 714–729 https://doi.org/10.1016/j.ydbio.2006.03.056.

Wei, J., Li, Z., Ma, C., Zhan, F., Wu, W., Han, H., Huang, Y., Li, W., Chen, D., Peng, Y., 2013. Rho kinase pathway is likely responsible for the profibrotic actions of aldosterone in renal epithelial cells via inducing epithelial-mesenchymal transition and extracellular matrix excretion. Cell Biol. Int. 37, 725–730 https://doi.org/10.1002/cbin.10082.

Yang, J., Liu, Y., 2001. Dissection of key events in tubular epithelial to myofibroblast transition and its implications in renal interstitial fibrosis. Am. J. Pathol. 159, 1465–1475 https://doi.org/10.1016/S0002-9440(10)62533-3.

Zhu, J., Nguyen, D., Ouyang, H., Zhang, X.H., Chen, X.M., Zhang, K., 2013. Inhibition of RhoA/Rho-kinase pathway suppresses the expression of extracellular matrix induced by CTGF or TGF-β in ARPE-19. Int. J. Ophthalmol. 6, 8–14 https://10.3980/j.issn.2222-3959.2013.01.02.