Involvement of Plasminogen Activator Inhibitor-1 in the Pathogenesis of Atopic Cataracts

Kanji Hori,[1] *Akira Matsuda,*[1] *Nobuyuki Ebihara,*[1] *Kojiro Imai,*[2] *Kazuhiko Mori,*[2] *Toshinari Funaki,*[1] *Yasuo Watanabe,*[1] *Satoru Nakatani,*[1] *Kiyotaka Okada,*[3] *Osamu Matsuo,*[3] *and Akira Murakami*[1]

Purpose. Further to our previous report of a genetic association between interferon-gamma (IFN-γ) receptor 1 gene and atopic cataract, we investigated the roles of plasminogen activator inhibitor-1 (PAI-1), a fibrosis-related, IFN-γ downstream molecule, in the pathogenesis of atopic cataracts.

Methods. Cultured lens epithelial cells (LECs) were stimulated by IFN-γ and quantified by PAI-1 mRNA/protein expression. PAI-1 and TGF-β mRNA expression was quantified using cDNA samples obtained from the lens epithelium of atopic cataract patients ($n = 7$) and of senile cataract patients ($n = 8$). The anterior capsules obtained from atopic cataracts ($n = 9$) were immunostained with anti-PAI-1 and anti-alpha smooth muscle actin (α-SMA) antibodies. PAI-1 gene expression was knocked down by PAI-1 siRNA, and α-SMA expression was examined under TGF-β1 stimulation. Expression of α-SMA was examined as a pathological hallmark of anterior subcapsular cataracts, commonly observed in atopic cataracts.

Results. The IFN-γ stimulation induced PAI-1 mRNA/protein expression in the LECs from 24 to 48 hours after stimulation. The expression of PAI-1 mRNA and TGF-β1 mRNA was significantly higher in the cDNA samples obtained from the atopic cataracts than those obtained from the senile cataracts. PAI-1–positive immunostaining was observed at the fibrotic lesion of the atopic cataracts, and α-SMA–positive myofibroblasts were observed at the vicinity of the PAI-1–positive lesion in all nine samples examined. PAI-1 gene knockdown resulted in reduced α-SMA expression in the LECs.

Conclusions. The findings of this study suggest that the IFN-γ, PAI-1, and TGF-β1 are involved in the pathophysiology of atopic cataracts. (*Invest Ophthalmol Vis Sci.* 2012;53:1846–1851) DOI:10.1167/iovs.11-8380

From the [1]Department of Ophthalmology, Juntendo University School of Medicine, Tokyo, Japan; the [2]Department of Ophthalmology, Kyoto Prefectural University of Medicine, Kyoto, Japan; and the [3]Department of Physiology, Kinki University School of Medicine, Sayama, Japan.

Supported by Grant-in-Aids No. 19659454 (AMu) and No. 18604009 (AMa) from Ministry of Education, Culture, Sports, Science and Technology (MEXT) Japan.

Submitted for publication August 8, 2011; revised January 7, February 13, 2012, and February 15, 2012; accepted February 15, 2012.

Disclosure: **K. Hori**, None; **A. Matsuda**, None; **N. Ebihara**, None; **K. Imai**, None; **K. Mori**, None; **T. Funaki**, None; **Y. Watanabe**, None; **S. Nakatani**, None; **K. Okada**, None; **O. Matsuo**, None; **A. Murakami**, None

Corresponding author: Akira Matsuda, Department of Ophthalmology, Juntendo University School of Medicine, 2-1-1 Hongo, Bunkyo-Ku, Tokyo, 113-8431, Japan; akimatsu@juntendo.ac.jp.

Atopic cataracts are atopic-disease–related complications that typically affect individuals in their adolescent years.[1] Treatment of atopic cataracts usually requires cataract surgery that results in a loss of patient's natural power of accommodation. In some cases, a subsequent retinal detachment is also observed.[2] In a previous study, we reported an association between interferon-gamma (IFN-γ) receptor genetic polymorphisms and the occurrence of atopic cataracts.[3] Atopic cataracts often have the phenotype of anterior subcapsular cataracts, accompanied by abnormal fibrosis and epithelial-to-mesenchymal transition (EMT) of lens epithelial cells (LECs) into myofibroblasts.[4]

In the present study, we focused on several molecules associated with fibrosis and LEC transition. A recent study showed that the IFN-γ signal is essential for plasminogen activator inhibitor (PAI)-1–induced postoperative fibrosis in the abdomen, where PAI-1 expression is upregulated by IFN-γ and STAT1 signal cascades.[5] PAI-1 inhibits urokinase/tissue type plasminogen activator (uPA/tPA) and subsequent plasmin/plasmin-dependent matrix metalloprotease (MMP) activity, thus increasing PAI-1 expression that results in profibrotic collagen/matrix deposition by suppressing fibrinolysis.[6,7]

Reports from other lines of investigation have shown that the overexpression of TGF-β signals in LECs could induce an anterior subcapsular cataract in mice.[8] TGF-β is a typical cytokine related to fibrosis and EMT phenomena. In addition, TGF-β is known to induce PAI-1 expression,[7] and one previous study demonstrated that PAI-1 mediates TGF-β1+ epidermal growth factor–induced EMT.[9] In the present study, we investigated the roles of PAI-1 in association with TGF-β in the formation of atopic cataracts.

Materials and Methods

Clinical Samples

Anterior capsules from 16 atopic-cataract and 8 senile-cataract patients were obtained at the time of surgery as previously described.[3] All the atopic cataracts patients were diagnosed by slit-lamp analysis by board-certificated ophthalmologists, and atopic dermatitis of these patients was diagnosed according to the criteria of Hanifin and Rajka.[10] Nine samples of atopic cataract were processed for immunofluorescence analyses and seven atopic cataract samples, along with senile cataracts, were used for isolation of RNA for real-time PCR experiments. Written informed consent was obtained from all patients prior to surgery. All procedures were approved by the Ethics Committees of Juntendo University School of Medicine and Kyoto Prefectural University of Medicine, and the study was conducted in accordance with the tenets of the Declaration of Helsinki.

Antibodies and Reagents

Goat anti-PAI-1 polyclonal antibody was purchased from R&D Systems, Inc. (Minneapolis, MN), mouse anti-human α–SMA monoclonal

antibody from Dako Japan (Kyoto, Japan), and rabbit anti-human α–SMA monoclonal antibody from Epitomics (Burlingame, CA). Recombinant human TGF-β1 and IFN-γ protein were obtained from PeproTech Ltd. (London, UK), and pEF6-V5-His plasmid from Life Technologies (Carlsbad, CA). All other reagents used in this study were analytical grade reagents.

LEC Culture with IFN-γ Stimulation, Anterior Lens Capsules Collection, and cDNA Preparation

Human immortalized LECs (SRA01/04) obtained from RIKEN cell bank (Tsukuba, Japan) were maintained with 10% fetal bovine serum (FBS) in MEM (Life Technologies).[11] Subconfluent LECs in 12-well cell culture dishes were stimulated with IFN-γ (20 ng/mL) for 24 and 48 hours.

Anterior lens capsules obtained during cataract surgery were immediately stored with RNA Later (Ambion, Austin, TX) to protect the RNA. Total RNA was extracted from the LECs with a NucleoSpin II RNA Isolation Kit (Macherey-Nagel GmbH, Duren, Germany), and from the anterior capsules by use of the Micro RNA Extraction Kit (Qiagen Japan, Tokyo, Japan). cDNAs were prepared using random primers and ReverTra Ace reverse transcriptase (both from Toyobo, Osaka, Japan) according to the manufacturer's protocol.

Real-Time PCR Analysis

Real-time PCR was carried out using human PAI-1 (Hs00167155_m1), TGF-β1 (Hs00998130_m1), TGF-β2 (Hs00234244_m1), and 18S-rRNA (4319413E) TaqMan Expression Assay (Assays-on-Demand gene expression products) with TaqMan Fast Master Mix. Expression of α-SMA was quantified using Fast SYBR Green Master Mix with a pair of primers (Forward 5′-CCCAGCCAAGCACTGTCA-3′; Reverse 5′-TCCAGAGTCCAGCACGATG-3′). Real-time PCR analysis was carried out on a PRISM 7500 Sequence Detection System (all from Life Technologies). The relative expression of PAI-1 in LECs was quantified by the standard curve method using full-length PAI-1 cDNA subcloned into the pEF6-V5-His plasmid as standard and 18S-rRNA expression in the same cDNA as the control. Relative expression of α-SMA, TGF-β1, and TGF-β2 was quantified by comparative Ct methods using 18S-rRNA expression in the same cDNA as the control.

Immunohistochemistry and Electron Microscopy

Lens capsules obtained at the time of cataract surgery were fixed in 4% paraformaldehyde/PBS and then embedded in paraffin, and 3-μm paraffin sections were then made and used for the immunohistologic analysis. For some experiments, the fixed lens capsules were immunostained as whole-mount samples. Nonspecific staining was blocked for 30 minutes with blocking buffer (10% normal donkey serum, and 1% BSA in PBS). Goat anti-PAI-1 polyclonal antibody (1:200 dilution) or mouse anti-α-SMA (1:100 dilution) was then applied and reacted overnight at 4°C. All the antibodies were diluted with the blocking buffer. After washing with PBS, the slides were incubated for 30 minutes with Alexa 488–conjugated donkey anti-mouse IgG or with Alexa 594–conjugated anti-goat IgG (both from Life Technologies). The slides were then inspected by the use of a confocal microscope (TCS SP5; Leica Microsystems, Tokyo, Japan). Ultrastructural analysis was carried out essentially as previously described.[12] Lens capsules obtained at the time of cataract surgery were immediately fixed with 2.5% glutaraldehyde and then postfixed with 2% osmic acid. The samples were embedded in epoxy resin and ultrathin sections (60–80 nm) were then made. The ultrathin sections were examined by use of a transmission electron microscope (7000-100; Hitachi High-Technologies, Inc., Tokyo, Japan).

PAI-1 Gene Knockdown in LECs

First, LECs in the 24-well cell dishes were stimulated with 20 ng/mL human TGF-β1 and chronological change of PAI-1/α-SMA mRNA was examined. A PAI-1 gene knockdown experiment was performed with MISSION esiRNA against PAI-1 (EHU016891), and esiRNA against Renilla luciferase (RLUC, negative control), using the N-TER Nanoparticle siRNA Transfection System (both from Sigma-Aldrich, St. Louis, MO) according to the manufacturer's protocol. In each well of a 24-well cell culture dish, 500 ng of siRNA was used with 1.6 μL N-TER reagents for transfection. The LECs in the 24-well cell dishes were then stimulated with 0.2 ng/mL human TGF-β1, immediately after the transfection procedures. cDNAs were prepared from total RNA, and PAI-1 mRNA and α-SMA mRNA expression was quantified by real-time PCR as described above.

Western Blot Analysis

Western blotting analysis was carried out essentially as previously described.[13] In brief, LECs in the 12-well culture dish were washed twice with PBS. Cells in the amount of 4×10^4 were then solubilized in SDS-sample buffer (62.5 mM Tris-HCl, pH 6.8, 2% SDS, 20% glycerol, and 0.04% bromophenol blue). Next, 50 mM dithiothreitol (DTT) was added to the samples and incubated for 15 minutes at 95°C. Of each sample, 12 μL was loaded with 4% to 20% gradient Tris-glycine gel. The electrophoresed protein was transferred to a polyvinylidene fluoride (PVDF) membrane (Pall Japan, Tokyo, Japan), and the membrane was then incubated with the goat anti- PAI-1 polyclonal antibody (1:5000 dilution) or with the rabbit anti-α-SMA monoclonal antibody (1:3000 dilution) overnight at 4°C. After washing with Tris-buffered saline (10 mM Tris-HCl, pH 7.6, 150 mM NaCl) containing 0.05% Tween 20 (TBS-T), the membrane was incubated with a 1:10,000 dilution of horse radish peroxidase (HRP)-conjugated anti-rabbit IgG (GE Healthcare, Uppsala, Sweden) or HRP-conjugated anti-goat IgG (Jackson Immuno Research Lab Inc., West Grove, PA) for 1 hour and then visualized with ECL Plus Western blotting reagents (GE Healthcare). The density of PAI-1/α-SMA positive bands was quantified by ImageJ software (ImageJ software, http://rsbweb.nih.gov/ij/, NIH).

Results

IFN-γ Stimulation-Induced PAI-1 mRNA and Protein Up-Regulation

Recombinant IFN-γ stimulation (20 ng/mL) induced a 4.6-fold increase in PAI-1 mRNA expression in the cultured LECs at 48 hours after stimulation (Fig. 1A). Immunocytochemical staining of the LECs showed increased PAI-1 protein expression at 24 and 48 hours after stimulation (Fig. 1B). PAI-1 Western blot analysis of the IFN-γ (20 ng/mL) stimulated LECs showed increased PAI-1 expression at 24 and 48 hours after stimulation (Fig. 1C). A 6.3-fold increase in PAI-1 protein expression was observed in the LECs after 48 hours.

Increased PAI-1 mRNA Expression in the Lens Epithelial Cells Obtained from Atopic Cataracts

The relative expression of PAI-1 mRNA was significantly higher in the cDNA samples obtained from atopic cataracts compared with those from senile cataracts (Fig. 2A). TGF-β1 mRNA expression was also significantly higher in the atopic samples (Fig. 2B), whereas no significant difference was observed in regard to TGF-β2 expression (Fig. 2C). Statistical analysis was carried out by use of the Mann-Whitney *U* test.

PAI-1 Protein Was Deposited at the Subcapsular Fibrotic Region of Atopic Cataracts

PAI-1–positive immunostaining was observed at the subcapsular fibrotic lesion of the atopic cataracts (Fig. 3A, red color). A negative control section incubated with normal goat IgG did

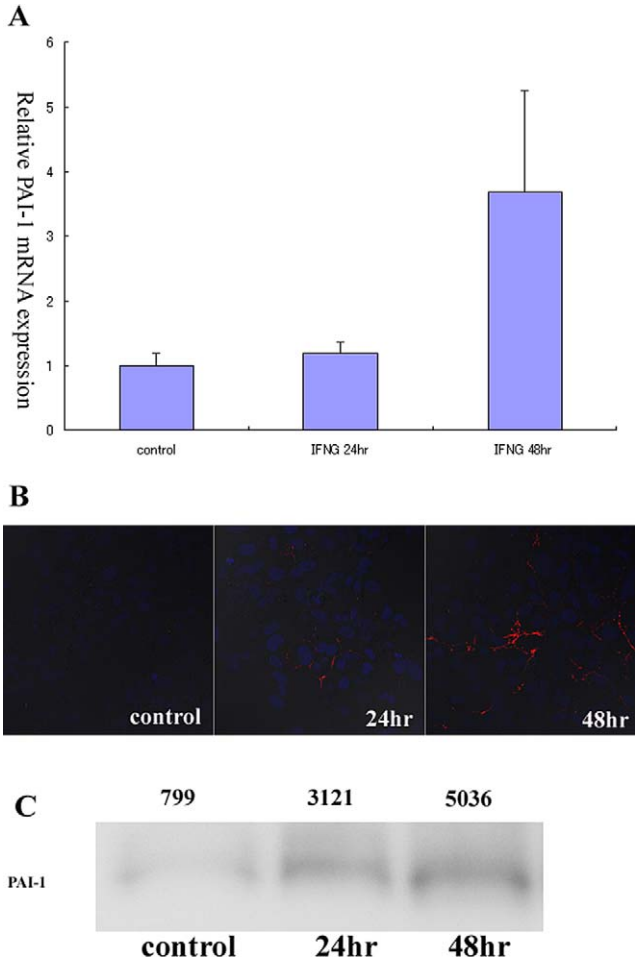

Figure 1. The effect of recombinant IFN-γ stimulation for PAI-1 mRNA and protein expression in LECs. (**A**) Real-time PCR analysis of PAI-1 mRNA expression. LECs were stimulated with 20 ng/mL IFN-γ for 24 and 48 hours. The results are shown as mean-fold ± SD compared with PAI-1 mRNA expression of the unstimulated sample. (**B**) Immunocytochemical staining of LECs with PAI-1 antibody (red). Note the positive staining between the junctions of the LECs at 24 and 48 hours after IFN-γ stimulation. The nuclei of the LECs were counterstained with DAPI. (**C**) Western blot analysis of PAI-1 protein expression. LECs were stimulated with 20 ng/mL IFN-γ for 24 and 48 hours. Western blot analysis was carried out using goat anti-PAI-1 antibody. The density of PAI-1–positive bands is shown on the top of each band.

not exhibit positive staining (Fig. 3B). α-SMA–positive myofibroblastic cells were observed in the vicinity of the PAI-1–positive deposits in the fibrotic region of the atopic cataracts. Whole-mount PAI-1 immunostaining showed positive immunostaining within and around LECs of the subcapsular cataract region (Fig. 3C); however, LECs at the non-cataractous clear region did not exhibit any PAI-1–positive staining (Fig. 3D). Ultrastructural analysis showed a structureless deposit between the irregular LECs and the basement membrane (Fig. 3E, asterisk). The clear region in the same lens capsule showed a monolayer of LECs (Fig. 3F). The results of PAI-1 immunostaining using nine anterior capsule samples obtained from the atopic cataract patients are summarized in Table 1. All the

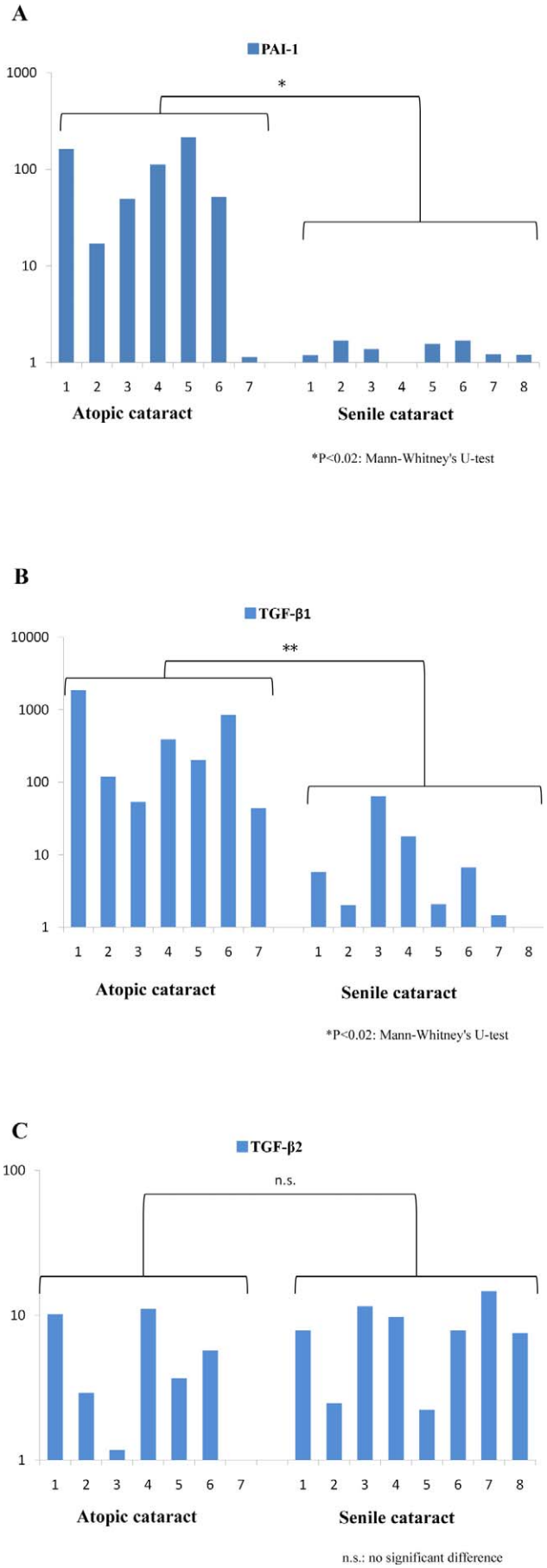

Figure 2. Real-time PCR analysis of (**A**) PAI-1, (**B**) TGF-β1, and (**C**) TGF-β2 mRNA expression in human anterior lens capsules. The amount of relative expression was normalized to that of 18S rRNA.

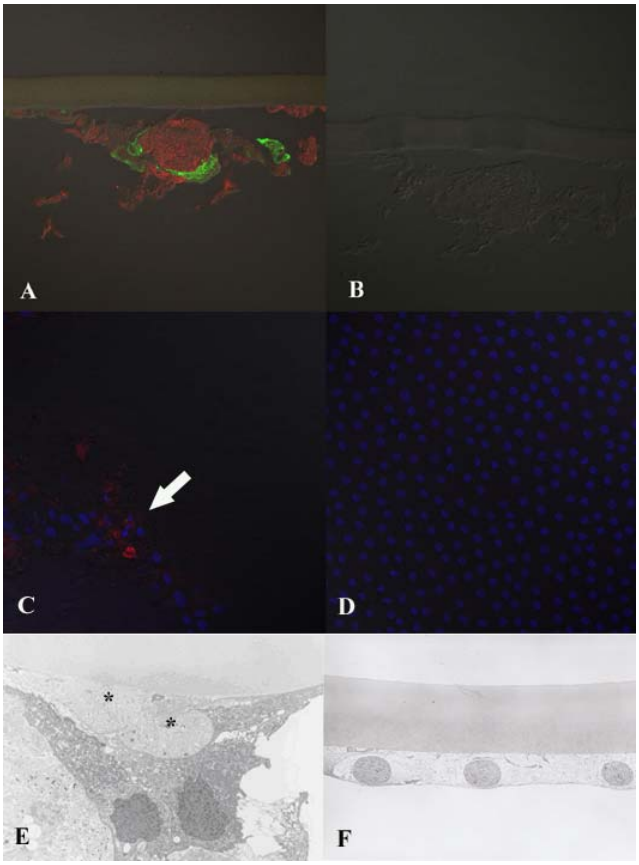

Figure 3. Immunohistochemical staining of human anterior lens capsules with the anti-PAI-1 antibody. Paraffin sections of the lens capsule were immunostained with (**A**) goat anti-PAI-1 antibody and mouse anti-α-SMA antibody or with (**B**) normal goat IgG and normal mouse IgG1. Alexa 594–conjugated anti-goat IgG (red) and Alexa 488–conjugated anti-mouse IgG (green) were used for the secondary antibody. Whole-mount PAI-1 immunostaining of (**C**) the fibrotic plaque or (**D**) the clear part of the same anterior lens capsule of an atopic cataract patient. Positive PAI-1 immunostaining (red) was observed around the fibrotic-plaque (**C**). (**A–D**) Original magnification ×600. Ultrastructural analysis of LECs in (**E**) the fibrotic plaque and in (**F**) the clear part of the anterior lens capsule of an atopic cataract patient. An acellular deposit (asterisk) was observed beneath the epithelial cells with fibrotic morphology (**E**).

anterior capsules showed positive PAI-1 immunostaining, and the staining was classified as focal, diffuse, and diffuse + focal, as shown in Fig. 4. α-SMA–positive myofibroblastic cells were observed in all samples near the PAI-1–positive fibrotic lesions.

PAI-1 Expression Knockdown Could Suppress TGF-β1–Dependent α-SMA mRNA Expression in the LECs

First, chronological changes of PAI-1 mRNA and α-SMA mRNA in response to TGF-β1 (20 ng/mL) stimulation were evaluated. TGF-β1 stimulation induced PAI-1 mRNA that at first peaked at 8 hours after simulation, followed by α-SMA mRNA being induced from 8 hours after stimulation and peaking at 24 hours after stimulation (Fig. 5). PAI-1 siRNA transfection using unstimulated (without TGF-β1) LECs resulted in 77% knockdown efficiency at 12 hours and 71% knockdown efficiency of PAI-1 mRNA expression at 24 hours after transfection (data not shown). PAI-1 siRNA transfection resulted in 32% knockdown efficiency at 12 hours and 73% knockdown efficiency at 24 hours after siRNA transfection under simultaneous TGF-β1 (0.2 ng/mL) stimulation (Fig. 6A). PAI-1 siRNA transfection suppressed α-SMA expression compared with the control siRNA at 12 hours (60% reduction) to 24 hours (42% reduction) after TGF-β1 stimulation/siRNA transfection (Fig. 6A). Western blot analysis of TGF-β1 (0.2 ng/mL)–stimulated/siRNA transfected LECs showed a 43% reduction of PAI-1 protein expression and a 40% reduction of α-SMA protein expression compared with the RLUC siRNA-transfected control sample (Fig. 6B) at 24 hours after the stimulation/transfection procedures.

Table 1. Summary of PAI-1 Immunostaining

Patients	Age	Sex	Affected eye(s)	PAI-1 immunostaining
NO.1	23	F	R	Focal
NO.2	32	M	R	Diffuse
NO.3	31	F	L	Diffuse+Focal
NO.4	28	M	R, L	Diffuse
NO.5	40	M	L	Diffuse+Focal
NO.6	43	M	R, L	Focal
NO.7	18	M	R, L	Diffuse
NO.8	32	M	R, L	Diffuse
NO.9	40	M	R	Diffuse+Focal

Discussion

In our previous study, we showed increased IFN-γ receptor 1 mRNA expression and major histocompatibility complex (MHC) class-II immunostaining in the anterior capsules of atopic cataracts, indicating the relevance of IFN-γ signals for atopic cataract formation.[3] In this present study, we first showed that IFN-γ treatment could up-regulate PAI-1 mRNA/protein expression in LECs (Fig. 1). Considering the previous reports, which showed that STAT1 activation leads to PAI-1 gene promoter up-regulation,[5,14] the effect of IFN-γ treatment is proposed to be indirect and dependent on STAT1 activation. Therefore, a relatively long duration of time (24–48 hours) is required before PAI-1 mRNA/protein induction by IFN-γ (Fig. 1). The roles of the PAI-1 molecule as a profibrotic molecule have been reported in many disease-related systems (e.g., glomerulonephritis,[15] bleomycin-induced pulmonary fibrosis,[16] keloid formation in the skin[17]).

We next showed that significantly higher PAI-1 mRNA (Fig. 2A) and TGF-β1 mRNA (Fig. 2B) expression in atopic cataracts compared with senile cataracts. Suzuki et al. recently reported a significantly higher PAI-1 protein concentration in the aqueous humor of atopic cataracts compared with senile cataracts, which is consistent with the findings of this study.[18] Lee and Joo reported increased TGF-β1 mRNA expression in anterior polar cataracts compared with nuclear cataracts.[19] Although there was no description in that report as to the atopic status of the anterior polar cataracts, our results are in agreement with the finding of that study when considering the common phenotype of the cataracts.[19] It should be noted that atopic cataracts are not necessarily anterior subcapsular cataracts. Nonetheless, the anterior subcapsular cataract is one of the common and specific atopic cataract phenotypes.[20,21]

Immunohistologic analysis showed PAI-1 protein deposition (Fig. 3A, 3C red color) at the fibrotic lesion of the anterior lens capsules in atopic cataracts. Our findings also revealed α-SMA–positive myofibroblastic cells in the lesion of the anterior subcapsular cataracts (Fig. 3A, green color). Furthermore, PAI-1–positive immunostaining and nearby α-SMA–positive myofi-

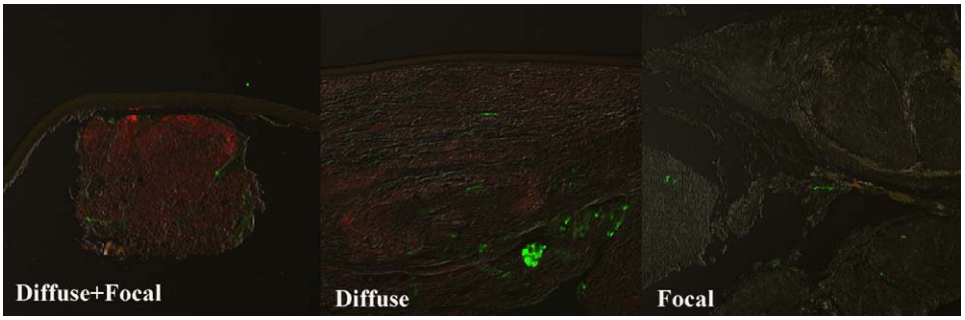

FIGURE 4. Immunohistochemical staining of human anterior lens capsules with the anti-PAI-1 antibody. Paraffin sections of the lens capsule were immunostained with goat anti-PAI-1 (red) antibody and mouse anti-α-SMA (green) antibody. Arrows indicate the focal PAI-1 staining. Staining patterns were classified as diffuse + focal staining, diffuse staining, or focal staining as shown in the Figure. Original magnification ×200.

broblastic cells were found in all nine anterior capsule samples obtained from the atopic cataract patients (Table 1; Fig. 4). These findings are consistent with those of a previous study, which reported that myofibroblast is the predominant PAI-1 expression cell type in human breast carcinomas.[22] Ultrastructural analysis of the atopic cataracts capsule showed myofibroblastic cells at the fibrotic lesion and an abnormal acellular deposit beneath the myofibroblastic cells (Fig. 3E). These results suggest the possibility that PAI-1 protein is deposited around the myofibroblasts of atopic cataracts and may play some role as a scaffold for the EMT phenomenon, as suggested in keratinocyte experimental models.[9] Further immuno-electron microscopic studies are ongoing to show the precise localization of PAI-1 protein in relation to myofibroblastic cells in atopic cataracts.

The results of the PAI-1 gene knockdown experiments using LECs (Fig. 5, 6) also support the roles of PAI-1 for the induction of α-SMA, a pathological hallmark of anterior subcapsular cataracts and EMT.[4] First, we evaluated the chronological changes of PAI-1 mRNA and α-SMA mRNA in response to TGF-β1 stimulation. TGF-β1 stimulation induced PAI-1 mRNA that first peaked at 8 hours after simulation, followed by α-SMA mRNA being induced from 8 hours after stimulation and peaking at 24 hours after stimulation (Fig. 5). In our preliminary experiment, we utilized a relatively high dose of TGF-β1 (20 ng/mL) for the LECs stimulation and found that owing to the very strong effect of TGF-β1 for PAI-1 mRNA induction, knockdown efficiency of PAI-1 siRNA was not sufficient under TGF-β1 stimulation compared with control samples without TGF-β1 stimulation. Thus, we optimized the dose of TGF-β1 stimulation to 0.2 ng/mL. We then evaluated the chronological effect of siRNA inhibition of PAI-1 gene expression and found that PAI-1 siRNA could knock down PAI-1 gene expression at 32% (12 hours) to 73% (24 hours) efficiency post transfection (Fig. 6A). We simultaneously evaluated the effect of PAI-1 gene knockdown for TGF-β1–induced α-SMA up-regulation in the LECs and showed a 42% reduction of α-SMA mRNA and a 40% reduction of α-SMA

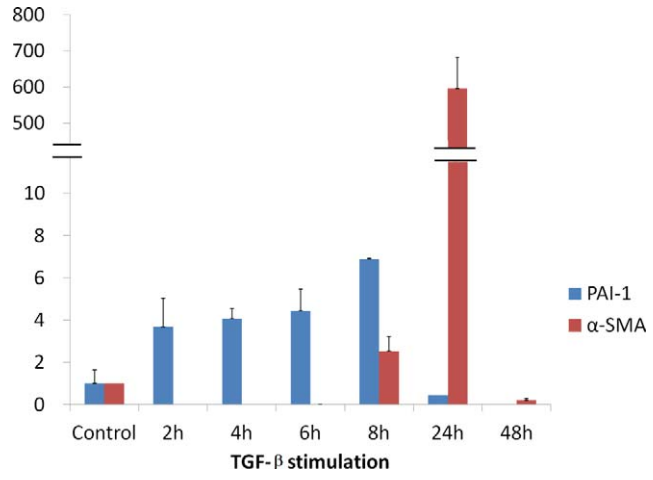

FIGURE 5. Chronological change of PAI-1 and α-SMA mRNA expression in LECs. TGF-β1 (20 ng/mL) treatment induced PAI-1 mRNA up-regulation from 8 hours after stimulation and then triggered α-SMA up-regulation that peaked at 24 hours after stimulation. All results are shown as mean-fold ± SD compared with that of the PAI-1/α-SMA mRNA expression of the untreated sample.

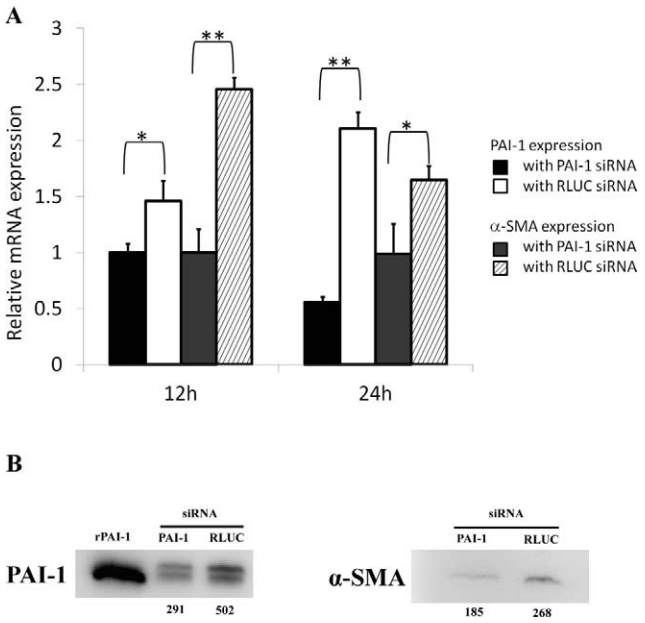

FIGURE 6. PAI-1 gene knockdown suppressed TGF-β1 (0.2 ng/mL)–induced α-SMA expression in the LECs. (**A**) PAI-1 gene knockdown suppressed TGF-β1–induced PAI-1 expression from 12 to 24 hours, compared with the RLUC gene knockdown control, and PAI-1 gene knockdown also inhibited TGF-β1–induced α-SMA expression at 24 hours after stimulation. All results are shown as mean-fold ± SD compared with PAI-1/α-SMA mRNA expression of the PAI-1 siRNA–treated samples at 12 hours after stimulation/transfection (* $P < 0.05$, ** $P < 0.01$, Student's *t*-test). (**B**) Western blot analysis of LECs with PAI-1 gene knockdown under TGF-β1(0.2 ng/mL) stimulation. PAI-1 and α-SMA Western blot analysis was carried out. Recombinant rPAI-1 (5 ng/mL) was loaded as a control. The density of PAI-1/α-SMA-positive bands is shown at the bottom of each band.

protein expression at 24 hours (Fig. 6A). These results support our hypothesis that PAI-1 may play a role for scaffold protein for α-SMA expression and subsequent EMT in LECs.

In summary, our findings demonstrated that IFN-γ could induce PAI-1 expression in LECs, PAI-1 protein expression at the vicinity of α-SMA–positive myofibroblasts in atopic cataracts, and the essential roles of PAI-1 for α-SMA expression of LECs under TGF-β1 stimulation. These results suggest that IFN-γ, PAI-1, and TGF-β1 all play significant roles in the pathophysiology of atopic cataracts. Previous studies have shown several roles of the PAI-1 molecule in atopy-related disorders. Oh et al. reported that PAI-1 promotes extracellular matrix deposition in the airway of mouse asthma models.[23] Sejima et al. reported the role of PAI-1 in an ovalbumin (OVA)-induced nasal allergy model, showing decreased eosinophil infiltration, goblet cell hyperplasia, and Th2-skew immune responses in the PAI-1 knockout mouse.[24] These results are consistent to our results showing increased PAI-1 expression and essential roles of PAI-1 in the pathophysiology of atopic cataracts. Further investigations are currently being conducted to elucidate methods for preventing the development of atopic cataracts by intervening in the PAI-1–induced fibrotic cascades.

Acknowledgments

The authors thank Julian M. Hopkin and Shigeru Kinoshita for their invaluable continuous support.

References

1. Uehara M, Amemiya T, Arai M. Atopic cataracts in a Japanese population. With special reference to factors possibly relevant to cataract formation. *Dermatologica*. 1985;170:180–184.
2. Hida T, Tano Y, Okinami S, Ogino N, Inoue M. Multicenter retrospective study of retinal detachment associated with atopic dermatitis. *Jpn J Ophthalmol*. 2000;44:407–418.
3. Matsuda A, Ebihara N, Kumagai N, et al. Genetic polymorphisms in the promoter of the interferon gamma receptor 1 gene are associated with atopic cataracts. *Invest Ophthalmol Vis Sci*. 2007;48:583–589.
4. Schmitt-Graff A, Pau H, Spahr R, Piper HM, Skalli O, Gabbiani G. Appearance of alpha-smooth muscle actin in human eye lens cells of anterior capsular cataract and in cultured bovine lens-forming cells. *Differentiation*. 1990;43:115–122.
5. Kosaka H, Yoshimoto T, Yoshimoto T, Fujimoto J, Nakanishi K. Interferon-gamma is a therapeutic target molecule for prevention of postoperative adhesion formation. *Nat Med*. 2008;14:437–441.
6. Eddy AA, Fogo AB. Plasminogen activator inhibitor-1 in chronic kidney disease: evidence and mechanisms of action. *J Am Soc Nephrol*. 2006;17:2999–3012.
7. Ghosh AK, Vaughan DE. PAI-1 in tissue fibrosis. *J Cell Physiol*. 2011;227:493–507.
8. Lovicu FJ, Schulz MW, Hales AM, et al. TGFbeta induces morphological and molecular changes similar to human anterior subcapsular cataract. *Br J Ophthalmol*. 2002;86:220–226.
9. Freytag J, Wilkins-Port CE, Higgins CE, Higgins SP, Samarakoon R, Higgins PJ. PAI-1 mediates the TGF-beta1+EGF-induced "scatter response in transformed human keratinocytes." *J Invest Dermatol*. 2011;130:2179–2190.
10. Hanifin JM, Rajka RG. Diagnostic features of atopic dermatitis. *Acta Derm Venereol*. 1980;92(suppl);44–47.
11. Ibaraki N, Chen SC, Lin LR, Okamoto H, Pipas JM, Reddy VN. Human lens epithelial cell line. *Exp Eye Res*. 1998;67:577–585.
12. Matsuda A, Ebihara N, Yokoi N, et al. Basophils in the giant papillae of chronic allergic keratoconjunctivitis. *Br J Ophthalmol*. 2010;94:513–518.
13. Matsuda A, Okayama Y, Terai N, et al. The role of interleukin-33 in chronic allergic conjunctivitis. *Invest Ophthalmol Vis Sci*. 2009;50:4646–4652.
14. Kasza A, Kiss DL, Gopalan S, et al. Mechanism of plasminogen activator inhibitor-1 regulation by oncostatin M and interleukin-1 in human astrocytes. *J Neurochem*. 2002;83:696–703.
15. Kitching AR, Holdsworth SR, Ploplis VA, et al. Plasminogen and plasminogen activators protect against renal injury in crescentic glomerulonephritis. *J Exp Med*. 1997;185:963–968.
16. Eitzman DT, McCoy RD, Zheng X, et al. Bleomycin-induced pulmonary fibrosis in transgenic mice that either lack or overexpress the murine plasminogen activator inhibitor-1 gene. *J Clin Invest*. 1996;97:232–237.
17. Tuan TL, Hwu P, Ho W, et al. Adenoviral overexpression and small interfering RNA suppression demonstrate that plasminogen activator inhibitor-1 produces elevated collagen accumulation in normal and keloid fibroblasts. *Am J Pathol*. 2008;173:1311–1325.
18. Suzuki S, Sagara H, Senoo T. Developmental Factors of Fibrous Opacification in the Atopic Cataract Lens Capsule. *Ophthalmic Res*. 2011;45:216–220.
19. Lee EH, Joo CK. Role of transforming growth factor-beta in transdifferentiation and fibrosis of lens epithelial cells. *Invest Ophthalmol Vis Sci*. 1999;40:2025–2032.
20. Bair B, Dodd J, Heidelberg K, Krach K. Cataracts in atopic dermatitis: a case presentation and review of the literature. *Arch Dermatol*. 2011;147:585–588.
21. Fagerholm P, Palmquist BM, Philipson B. Atopic cataract: changes in the lens epithelium and subcapsular cortex. *Graefes Arch Clin Exp Ophthalmol*. 1984;221:149–152.
22. Offersen BV, Nielsen BS, Hoyer-Hansen G, et al. The myofibroblast is the predominant plasminogen activator inhibitor-1-expressing cell type in human breast carcinomas. *Am J Pathol*. 2003;163:1887–1899.
23. Oh CK, Ariue B, Alban RF, Shaw B, Cho SH. PAI-1 promotes extracellular matrix deposition in the airways of a murine asthma model. *Biochem Biophys Res Commun*. 2002;294:1155–1160.
24. Sejima T, Madoiwa S, Mimuro J, et al. Protection of plasminogen activator inhibitor-1-deficient mice from nasal allergy. *J Immunol*. 2005;174:8135–8143.