Downregulation of MMP-2 and -9 by Proteasome Inhibition: A Possible Mechanism to Decrease LEC Migration and Prevent Posterior Capsular Opacification

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Purpose. The proliferation, epithelial–mesenchymal transition (EMT), and migration of residual lens epithelial cells (LECs) after cataract surgery leads to the development of posterior capsular opacification (PCO). The authors have shown that proteasome inhibition suppresses LEC proliferation and EMT. The present study investigates the prevention of LEC migration by proteasome inhibition through the suppression of matrix metalloproteinase (MMP) expression and activity.

Methods. HLE B-3 and primary human LEC migration assays were performed using polycarbonate membrane inserts and 20% fetal bovine serum (FBS) as chemoattractant. Cultured cells were treated with 1 ng TGF-β_2, with or without MG132 (proteasome inhibitor) or GM 6001 (MMP inhibitor). Capsular bags with intraocular lenses (IOLs) were prepared from human donor eyes and cultured in serum-free DMEM. The capsular bags were then treated with 1 or 10 ng/mL TGF-β_2, with or without MG132 (2.5 or 10 μM, respectively). The medium was sampled and replaced every 2 days and analyzed for MMP-2 and -9 activities by SDS-PAGE zymography. Protein and RNA expression were analyzed by Western blotting and RT-PCR, respectively.

Results. Proteasome inhibition blocks LEC migration in HLE B-3 and primary human LECs. To further evaluate the mechanism of decrease in LEC migration by proteasome inhibition, the authors measured MMP-2 mRNA and protein expression and MMP-2 and -9 activities. In HLE B-3 cells, TGF-β_2 increased MMP-2 mRNA and protein levels; these increases were inhibited by MG132 cotreatment. Medium from HLE B-3 cultures showed MMP-2 and -9 activities, which were induced by TGF-β_2 treatment and inhibited by MG132 co-treatment. TGF-β_2 treatment also increased MMP-2 and -9 activities in IOL capsular bag cultures; these were progressively decreased by proteasome inhibition.

Conclusions. Proteasome inhibition decreases LEC migration. This inhibition is correlated with decreased MMP-2 and -9 activities, observed both with and without TGF-β_2 treatment. These findings support proteasome inhibition as a therapeutic strategy to prevent PCO. (*Invest Ophthalmol Vis Sci.* 2008;49:1998–2003) DOI:10.1167/iovs.07-0624

Cataract is a common disease of the lens and is the leading cause of blindness worldwide. The incidence is expected to increase as the world population ages. Surgery, followed by implantation of a synthetic intraocular lens (IOL) in most populations, is the only treatment available. One of the common complications of modern cataract surgery is posterior capsular opacification (PCO)— clouding of the remaining lens capsular bag— causing secondary vision loss.[1] The incidence of PCO has been lowered by improvements in surgical techniques, but it is still a significant problem.[2,3] Published rates of PCO vary widely, and a meta-analysis reported an overall rate of 25% of patients who undergo cataract surgery experiencing significant PCO within 5 years of surgery.[4] The problems caused by PCO can usually be treated by Nd:YAG capsulotomy, but this procedure is estimated to cost the US Medicare program more than $250 million per annum. In addition, YAG laser treatment can lead to severe complications such as retinal detachment, macular edema, and iris hemorrhage. Therefore, because of the medical and significant economic implications of Nd:YAG capsulotomy, development of an alternative therapy for PCO prevention is important.[4]

PCO is a multifactorial disease caused by residual lens epithelial cells (LECs) at the anterior lens capsule after cataract surgery. In most cases, PCO does not become clinically relevant until months or years after surgery.[1] Long-term development of PCO is explained by the extended survival and growth of these leftover LECs for more than 100 days in protein-free media.[5] LEC proliferation, migration, epithelial–mesenchymal transition (EMT), collagen deposition, and lens fiber regeneration may all contribute to PCO. The resultant capsular changes may be associated with wrinkling, contraction, and matrix production, leading to significant visual impairment.[1,6,7]

Matrix metalloproteinases (MMPs) are key modulators of important biological processes during normal and pathophysiological events, including skeletal formation, angiogenesis, cellular migration, inflammation, wound healing, and cancer.[8] MMPs have been found in normal ocular tissues,[9] and their overexpression is associated with excessive scarring.[10] They are well known to cleave the protein components of the extracellular matrix (ECM) and thereby play a central role in tissue remodeling.[11] Changes in lens capsule structure during PCO development may include remodeling of the ECM by MMPs. The migration of residual LECs to the posterior capsule plays a crucial role in PCO development, which may involve capsular wrinkling, contraction, and matrix production. Less is known about the cell signaling mechanism of migration. A recent study[12] showed an upregulation of MMP-2 and -9 after sham cataract surgery, suggesting the involvement of MMP-2 and -9 in PCO development. In addition, MMP inhibitor has been shown to prevent human LEC migration and contraction of the lens capsule, suggesting that MMP inhibition may have a role in the therapeutic treatment of PCO.[13]

Transforming growth factor-β_2 (TGF-β_2) has been implicated as a strong inducer of transformation and pathologic

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fibrosis of epithelial cells by modulating components of the ECM.[14,15] The involvement of MMPs in ECM degradation and the induction of MMP-2 and MMP-9 secretion in lens epithelial cells by TGF-β_2[16–18] suggest the involvement of TGF-β_2 in LEC migration, leading to PCO development.

The proteasome is a 700-kDa multicatalytic cytoplasmic and nuclear complex that is responsible for most nonlysosomal protein degradation. Proteasomal protein degradation is critical for the regulation of cellular functions such as cell cycle control, signal transduction, transcriptional regulation, apoptosis, oncogenesis, migration, antigen presentation, and selective elimination of abnormal proteins.[19,20] This pathway also plays a role in lens cell development and differentiation.[21,22] The ubiquitin–proteasome pathway has been shown to regulate TGF-β_2 signaling: degradation of TGF-β_2 receptor and Smads turns off TGF-β_2 signaling,[23] whereas degradation of negative modulators such as Ski and SnoN maintains the signal.[24] Blocking PCO may require inhibition of the remaining LEC proliferation, migration, and EMT that occur after surgery. Previous studies in this laboratory have shown that proteasome inhibition blocks TGF-β_2 induced EMT markers of the LECs.[25] We have also demonstrated that proteasome inhibition suppresses LEC proliferation, alone or in the presence of other growth factors.[26] In a recent study, Src family kinase inhibitor (PP1) has been shown to prevent PCO by inhibiting LEC proliferation, migration, and EMT.[27] The present study investigates the effect of proteasome inhibition on LEC migration, which is one of the major causes of PCO. Additionally, we investigated the effect of proteasome inhibition on MMP-2 and -9 activities, which are likely part of the signaling mechanism that influences LEC migration.

Materials and Methods

Reagents

TGF-β_2 and MG132 were purchased from Sigma-Aldrich (St. Louis, MO). MMP inhibitor (Ilomastat), MMP-2 and -9 antibodies, and recombinant MMP-2 and MMP-9 were purchased from Chemicon International Inc. (Temecula, CA). Lactacystin was purchased from BioMol Research Laboratories (Plymouth Meeting, PA). Stock solutions of lactacystin were prepared in deionized water. Stock solutions of MG132 and MMP inhibitor (Ilomastat) were prepared in dimethyl sulfoxide (DMSO) at 5 mg/mL concentration. These stock solutions were diluted in cell growth media to give a 10 μM final concentration. TGF-β was suspended in 4 mM HCl containing 0.5% BSA to give a concentration of 10 μg/mL, which was diluted in cell growth media to 1 or 10 ng/mL final concentration.

Cell Culture

HLE B-3 cells were grown in minimum essential medium (MEM), containing 20% fetal bovine serum (FBS), 2 mM glutamine, and 50 μg/mL gentamicin at 37°C in a humidified 5% CO_2 atmosphere.

The use of human tissue in the study was in accordance with the provisions of the Declaration of Helsinki and was approved by the Institutional Review Board of the University of Medicine and Dentistry of New Jersey–New Jersey Medical School. For preparation of human LEC primary cultures, human fetal lenses of 17 to 20 weeks gestational age were dissected from eyes and transferred to Dulbecco modified Eagle medium (DMEM) supplemented with 20% FBS, 2 mM glutamine, and 50 μg/mL gentamicin. With fine forceps, the posterior lens capsule was torn and peeled from the fiber cell mass, which was discarded. The remaining lens capsule containing the adherent epithelial monolayer was cut into three pieces, which were individually incubated in six-well plates in DMEM with 20% FBS, 2 mM glutamine, and 50 μg/mL gentamicin at 37°C in a humidified 5% CO_2 atmosphere. All anterior capsule explants were 40% to 60% confluent monolayer cultures within 3 weeks of incubation. These primary cells were used for the cell migration assay. For the IOL capsular bag model, human donor eyes were obtained from eye banks throughout the country, placed through the National Disease Research Interchange (Philadelphia, PA). Twenty capsules with implanted IOLs from donors between 66 and 80 years of age were studied. Donor eyes, each with a capsular bag generated by cataract surgery and implanted with an IOL, were dissected free of zonules and cultured in 1 mL serum-free DMEM in 12-well dishes.[18] The capsular bags containing IOLs were cultured for 2 to 6 weeks to obtain constant MMP levels. These cultures were then treated as described. The medium was sampled and replaced every 2 days and was analyzed for MMP-2 and -9 activities by SDS-PAGE zymography.

Treatment Protocols

For the cell migration assay, HLE B-3 and primary human LECs were treated with 10 μM MMP inhibitor (Ilomastat), 10 μM MG132, or lactacystin and were incubated for 6 hours. MMP-2 mRNA and protein expression were observed in HLE B-3 cells treated with 10 ng/mL TGF-β_2 and 2.5 μM MG132, either alone or in combination for 24 hours. In HLE B-3 cells, MMP-2 and -9 activities were observed after 24-hour incubation with 1 ng/mL TGF-β_2 and 2.5 μM MG132, either alone or in combination. In IOL capsular bag cultures, MMP-2 and -9 activities were determined in vehicle, 1 ng/mL TGF-β_2, 2.5 μM MG132, 1 ng/mL TGF-β_2 + 10 μM MG132, and 10 ng/mL TGF-β_2 + 10 μM MG132.

Cell Migration Assay

Cell migration assay was performed with the use of an assay kit (CytoSelect Cell Migration; Cell Biolabs, Inc., San Diego, CA). Briefly, 300 μL serum-starved HLE B-3 (from 0.5×10^6 cells/mL suspension) or primary human LECs (from 0.12×10^6 cells/mL suspension) was added to the upper polycarbonate membrane insert (8-μm pore size) of the cell migration assay kit in a 24-well plate. In the lower well, 500 μL DMEM with 20% FBS was used as chemoattractant. After 6 hours of incubation, migratory cells were stained, photographed, and quantitated by absorption at 560 nm.

Gelatin Zymography

MMP-2 and -9 activities were determined by zymography by measuring gelatinolytic activity in culture media. Briefly, culture medium sampled after the desired incubation was concentrated approximately 25-fold (Amicon Centricon YM10; Millipore, Bedford, MA). Aliquots of 20 to 40 μL were applied to a 10% zymography gel (Criterion; Bio-Rad, Hercules, CA) and electrophoresed according to the manufacturer's directions. Gels were stained with Coomassie blue, and images were captured with a gel scanner. The clear zone on a dark background represented enzyme activity. Quantitation of bands was performed by densitometry.

Western Blot Analysis

Culture media of HLE B-3 cells were concentrated with Centricon filters, and equal amounts of protein were loaded per lane for each sample on 10% SDS-polyacrylamide gel (Bio-Rad, Hercules, CA). Protein bands were electrotransferred to nitrocellulose membranes, and the membranes were blocked at room temperature for 1 to 2 hours in TBS-T (10 mM Tris-HCl [pH 7.6], 150 mM NaCl, 0.05% Tween-20) containing 5% nonfat dry milk. The blots were incubated overnight at 4°C with primary antibodies: 1:500 monoclonal antibody for MMP-2 or 1:300 monoclonal antibody for MMP-9. These blots were then incubated for 1 hour at room temperature with horseradish peroxidase-conjugated secondary antibodies, and specific bands were detected using enhanced chemiluminescence reagent (Perkin Elmer Life Science, Boston, MA) on autoradiography film.

RT-PCR Assay

Total RNA was isolated from HLE B-3 cells (RNAzol; Tel-Test, Friendswood, TX) and reverse transcribed at 42°C for 60 minutes, followed by

heat denaturation at 95°C for 5 minutes and cooling at 4°C for 5 minutes. PCR was performed with an initial denaturation for 2 minutes at 95°C, followed by 25 or 30 amplification cycles, each for 1 minute at 94°C, 30 seconds at 55°C, and 1 minute at 72°C, followed by 7 minutes at 72°C and cooling at 4°C. Primer sequences used were as follows: β-actin forward, TTGGCCTTAGGGTTCAGGGGGG; β-actin reverse, CGTGGGGCGCCCCAGGCACCA; MMP-2 forward, CACCCATTTACACCTACACC; MMP-2 reverse, GTTTTTGCTCCAGTTAAAGG. PCR products were separated by 2% agarose gel electrophoresis and visualized with ethidium bromide. The bands were quantitated by densitometry and normalized with β-actin.

Statistical Analysis

Statistical significance was determined by the two-tailed Student's *t*-test; differences at $P < 0.05$ were considered statistically significant.

Results

Involvement of MMPs in LEC Migration

We investigated the effect of a broad-spectrum synthetic MMP inhibitor, MMP inhibitor (GM6001; Ilomastat), on LEC migration in 2 human LEC culture models to evaluate the involvement of MMPs on LEC migration. In HLE B-3 cell cultures, DMEM with 20% FBS was used as chemoattractant. The MMP inhibitor (Ilomastat; 10 μM) reduced cell migration by more than 35% after 6 hours of incubation (Fig. 1A). Similarly, 10 μM MMP inhibitor (Ilomastat) treatment for 6 hours inhibited the migration of primary human LECs by approximately 33% (Fig. 1B). This finding suggests a role for MMPs in human LEC migration.

Effect of Proteasome Inhibition on LEC Migration

To further evaluate proteasome inhibition as a therapeutic target to prevent PCO, we also tested the effect of the proteasome inhibitor MG132 (10 μM) on LEC migration, a major contributor to PCO development. In this experiment, DMEM containing 20% FBS was used as chemoattractant. MG132 treatment for 6 hours inhibited the migration of HLE B-3 cells by more than 50%, in comparison with vehicle-treated controls (Fig. 1C). Similarly, MG132 also inhibited the migration of primary human LECs by more than 40% (Fig. 1B). To confirm that the decrease in LEC migration by MG132 primarily resulted from proteasome inhibition, the migration assay was performed comparing inhibition by MG132 with that by lactacystin, a more proteasome-specific inhibitor.[28] In this experiment, MG132 (10 μM) and lactacystin (10 μM) inhibited serum-induced migration of LECs by 56% and 45%, respectively. The experiment was carried out in triplicate, and there was no statistically significant difference between the effects of the two inhibitors (Fig. 1D).

Effect of Proteasome Inhibition on MMP-2 mRNA and Protein Expression

To evaluate a possible mechanism for the proteasome inhibitor in reducing LEC migration, we observed the effect of MG132 on MMP-2 expression in the presence and absence of TGF-β_2. Using HLE B-3 cells, TGF-β_2 treatment (10 ng/mL for 24 hours) increased the expression of MMP-2 mRNA by approximately twofold. MG132 prevented the increase of MMP-2 mRNA caused by TGF-β_2, reducing the expression to control levels (Fig. 2A). A similar experiment comparing the effects of MG132 and the more proteasome-specific inhibitor, lactacystin, showed similar levels of inhibition, again demonstrating the proteasome as the primary target (data not shown). At the protein level, in concordance with their effects on mRNA expression, TGF-β_2 treatment (10 ng/mL for 24 hours) dramatically induced the protein levels of MMP-2, whereas MG132, either alone or in the presence of TGF-β_2, decreased the level of MMP-2 (Fig. 2B). These experiments suggested that decreases in MMP-2 expression may mediate the proteasome inhibitor-induced reduction in cell migration.

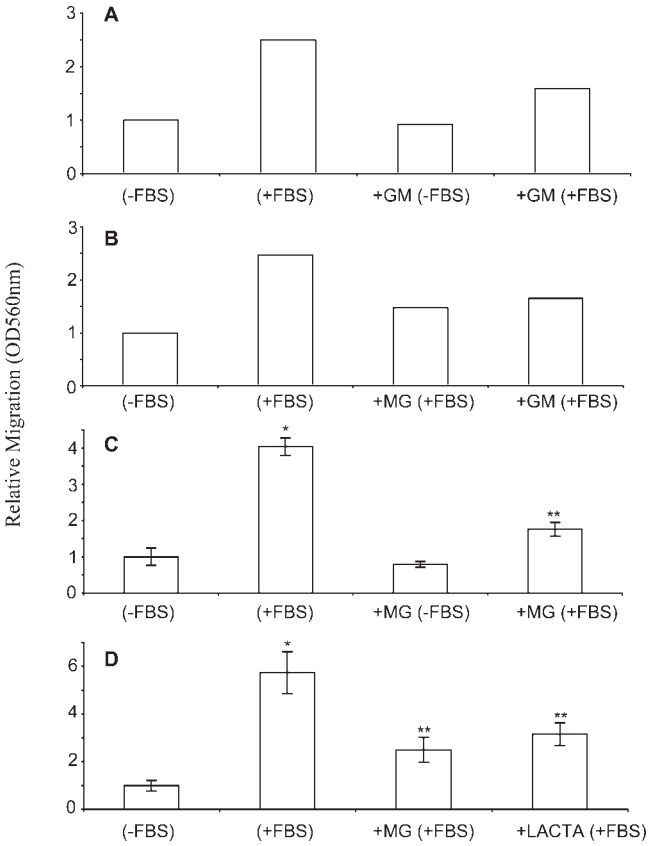

Figure 1. MMP inhibitor and proteasome inhibitors (MG132 and lactacystin) inhibit LEC migration. Serum-starved cells (300 μL) were added in upper polycarbonate membrane inserts in 24-well plates. Lower wells contained 500 μL DMEM with or without 20% FBS added as chemoattractant. Migratory cells were stained and quantitated by absorption at 560 nm after 6 hours of incubation. (**A**) HLE B-3 cells (1.5×10^5 cells/well) were treated with 10 μM MMP inhibitor (GM). (**B**) Primary human LECs (3.6×10^4 cells/well) were treated with 10 μM MMP inhibitor or 10 μM MG132 (MG). (**A**, **B**) Data are the average of duplicate determinations from one experiment, representative of at least two separate experiments with similar results. (**C**, **D**) HLE B-3 cells (1.5×10^5 cells/well) were treated with 10 μM MG132 and 10 μM lactacystin (LACTA). Data are from one experiment, representative of at least three independent experiments with similar results, and are the mean ± SD of triplicate determinations. *Significant difference compared with −FBS. **Significant difference compared with +FBS.

Effect of Proteasome Inhibition on MMP-2 and MMP-9 Activities

We also investigated the activities of MMP-2 and -9 in HLE B-3 cells and the cultured IOL capsular bag model by SDS-PAGE zymography. In HLE B-3 cells, TGF-β_2 treatment (1 ng/mL) induced MMP-2 and -9 activities after 24 hours of incubation in low-serum media (1% FBS) compared with vehicle-treated controls. MG132 treatment (2.5 μM) alone only slightly decreased MMP-2 and -9 activities compared with controls. When the cells were cotreated with TGF-β_2 (1 ng/mL) and MG132 (2.5 μM), MG132 blocked the TGF-β_2–induced MMP-2 and -9 activities, and the activities of MMP-2 and -9 were comparable to those of vehicle-treated controls (Figs. 3A, 3B).

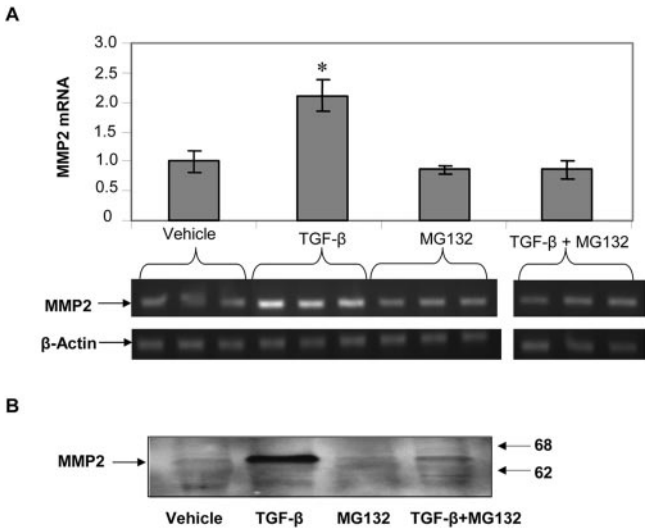

FIGURE 2. Proteasome inhibition decreases MMP-2 mRNA and protein expression. (**A**) RT-PCR analysis of MMP-2 and β-actin mRNA. HLE B-3 cells were treated for 24 hours with TGF-β_2 (10 ng/mL) and MG132 (2.5 μM), either alone or in combination. The relative expression of mRNA was normalized to β-actin. Data are from one experiment, representative of at least three independent experiments with similar results, and are the mean ± SD of triplicate determinations. *Significant difference compared with vehicle control. (**B**) Western blot analysis of MMP-2 protein in HLE B-3 cell culture medium after 24-hour incubation with TGF-β_2, MG132 (2.5 μM), or both. Culture media were concentrated, and equal amounts of protein were loaded for each sample; proteins were separated by SDS-PAGE, and gel was immunoprobed. Data are representative of at least three independent experiments.

IOL capsular bags were cultured for 2 to 6 weeks in serum-free DMEM to obtain constant levels of MMP-2 and -9. Vehicle treatment of these IOL capsular bag cultures for 10 days induced variable levels of MMP-2 and -9 activities (Fig. 4A). These IOL capsular bags were then treated with 1 ng/mL TGF-β_2 and incubated for 10 days. TGF-β_2 treatment caused an increase in MMP-2 and -9 activities (Fig. 4B). These IOL capsular bag cultures were then treated with 2.5 μM MG132 and incubated for 10 days. MG132 treatment (2.5 μM) alone gradually decreased the MMP-2 and -9 activities (Fig. 4C). We also cotreated IOL capsular bag cultures with TGF-β_2 (1 ng/mL) + MG132 (10 μM) or TGF-β_2 (10 ng/mL) + MG132 (10 μM) after vehicle treatment. At concentrations of 1 ng/mL TGF-β_2 and 10 μM MG132, MMP-9 was undetectable at day 2. By day 4, MMP-2 activity was also eliminated. At 10 ng/mL TGF-β_2 + 10 μM MG132, there was induction of MMP-2 and -9 activities, but by day 6, both MMP activities had decreased (Fig. 4D). These experiments suggest that in HLE B-3 cells and IOL capsular bag cultures, TGF-β_2 induces MMP-2 and -9 activities, and this induction is blocked by MG132.

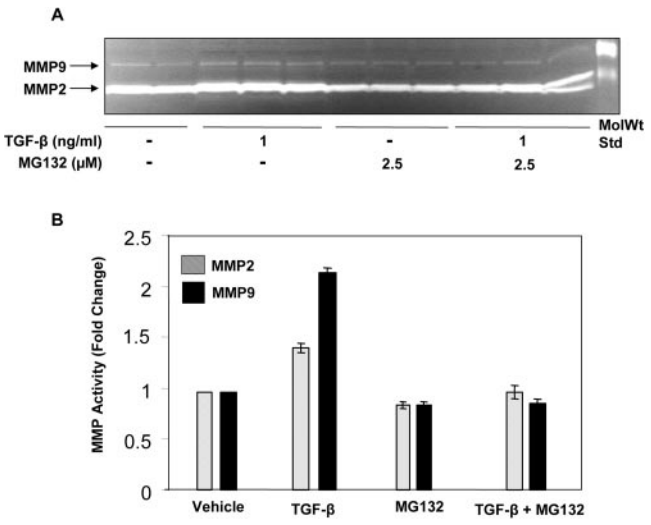

FIGURE 3. MG132 decreases TGF-β_2–induced MMP-2 and -9 activities in HLE B-3 cells. (**A**) HLE B-3 cells were treated with 1 ng/mL TGF-β_2, 2.5 μM MG132, or both, and were incubated in 1% serum containing MEM for 24 hours. After incubation, culture media were concentrated, and equal amounts were applied on a 10% SDS-polyacrylamide gel containing 2.25 mg/mL denatured gelatin. The clear zone on a dark background represented MMP activities. (**B**) The intensity of bands was quantitated by densitometry. Data shown, expressed as the mean ± SD of triplicate determinations, are from 1 of 3 independent experiments with similar results. Standards were recombinant MMP-9 and MMP-2.

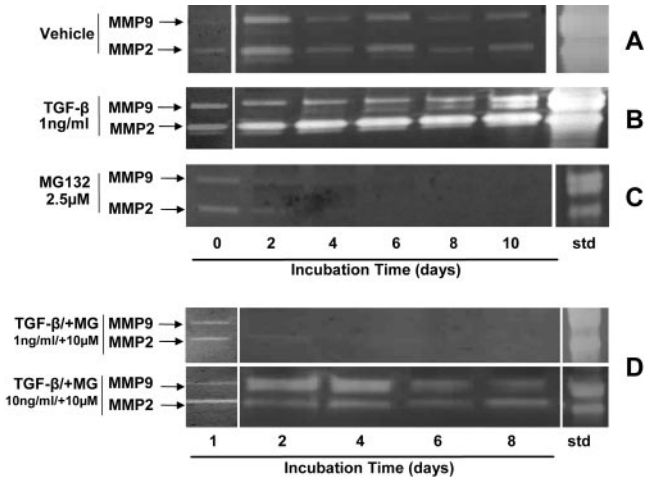

FIGURE 4. Proteasome inhibition blocks TGF-β_2–induced MMP-2 and -9 activities in IOL capsular bag cultures. These capsular bag cultures were sequentially treated for 10 days with (**A**) vehicle, (**B**) TGF-β_2 (1 ng/mL), (**C**) MG132 (2.5 μM), and (**D**) TGF-β_2 (1 ng/mL) + MG132 (10 μM) and TGF-β_2 (10 ng/mL) + MG132 (10 μM). Culture media sampled after the desired incubation were concentrated, and equal amounts were used for measuring gelatinolytic activity for zymography. Standards were recombinant MMP-9 and MMP-2.

DISCUSSION

PCO is the main complication of cataract surgery; no medical treatment is available for its prevention or amelioration. PCO arises from the proliferation, migration, and EMT of residual LECs after cataract surgery. The levels of several cytokines and growth factors, including TGF-β_2, FGF-2, HGF, IL-6, and EGF, increase in the aqueous humor after cataract surgery, and they influence LEC proliferation, migration, and EMT.[29,30] Among these, TGF-β_2 has been shown to play a key role in the etiology of PCO. TGF-β_2 initiates cellular and molecular changes in LECs that are associated with PCO development, including myofibroblast formation, wrinkling of the lens capsule, identification of fibroblast markers, and deposition of ECM.[18,31–33] TGF-β_2 has also been shown to inhibit LEC proliferation,[34,35] but this suppressive effect of TGF-β_2 on LEC proliferation is cancelled by other growth factors such as FGF-2,[36] leading to normal LEC proliferation and PCO development. FGF-2 plays a role in PCO development by inducing LEC proliferation, ECM production, multilayering, and plaque formation.[33] HGF has been shown to induce LEC proliferation leading to PCO development.[37] Therefore, because of the differential effect of cytokines and

growth factors available to LECs after cataract surgery, we suggest that simultaneous blocking of LEC proliferation, migration, and EMT would be a good strategy to prevent PCO.

Previous findings in our laboratory have shown that a reversible peptide aldehyde inhibitor of the proteasome, MG132, blocks TGF-β_2–induced EMT[25] and proliferation in the presence of TGF-β_2, FGF-2, and HGF in LECs.[26] The present investigation was performed to evaluate the effect of MG132 on LEC migration and its mechanism of action. Our results show that LEC migration is influenced by MMP activities and MG132 strongly blocks LEC migration. We also observed that TGF-β_2 induced MMP-2 and -9 activities and MG132 prevented this increase, suggesting that the proteasome inhibitor's effect on LEC migration is mediated by attenuating the activities of MMP-2 and -9. There are reports in the literature showing the downregulation of MMP-2 or -9 by inhibition of the proteasome in different cell types. The proposed mechanism of action is through NF-κB transcriptional regulation of several MMPs and proteasomal pathway control of NF-κB activation. This occurs because I-κB, an inhibitor of NF-κB, is a substrate of the proteasome. Inhibition of the proteasome, therefore, causes inhibition of the NF-κB pathway, resulting in decreased activity of MMPs.[38–40]

Matrix contraction plays a key role in PCO development. Cell migration is intrinsically involved in matrix contraction, and previous reports have shown a reduction in cell migration and matrix contraction by the inhibition of MMP activities.[41,42] The MMPs constitute a family of at least 23 members and are widely expressed in different tissues, including various tissues of the eye.[43,44] Among these members, the activities of MMP-2 and -9 have been detected in normal lenses.[45] Other studies reported the presence of MMP-2 and -9 only after culture, cataract surgery, or the addition of stress factors.[16–18,46] MMP-2 and -9 activities have been shown to be induced by TGF-β_2, which plays an important role in PCO development.[16–18] In our studies, we have observed that TGF-β_2 treatment causes an increase in MMP-2 mRNA and protein expression in HLE B-3 cells and an increase in MMP-2 and -9 activities in HLE B-3 cells and IOL capsular bag cultures, suggesting the involvement of TGF-β_2 in MMP-2 and -9–induced cell migration and PCO development. In contrast, MG132 treatment strongly decreases MMP-2 mRNA and protein expression in HLE B-3 cells and suppresses MMP-2 and MMP-9 enzyme activities in HLE B-3 cells and IOL capsular bag cultures.

Several attempts have been made to find an appropriate therapeutic target to prevent PCO, but none has proven effective either because of its toxic effect on other ocular tissues or because of only partial or differential effect on the major causes of PCO, such as LEC proliferation, migration, and transdifferentiation. Therefore, a good strategy to prevent PCO is simultaneous blockage of most of the PCO-causing pathways, with less toxic effect on other ocular tissues. The ubiquitin proteasome pathway has been shown to regulate TGF-β signaling, which plays a key role in the etiology of PCO by inducing EMT and MMP expression. In the present study, we used MG132, a reversible and cell-permeable peptide aldehyde inhibitor of the proteasome, as an agent to prevent the changes associated with PCO development. Because MG132 inhibits the proteasome reversibly, we hypothesize that normal cells can recover from its effect and that MG132 will significantly inhibit proliferation, migration, and EMT of residual LECs after cataract surgery, preventing PCO development. This speculation is supported by recent approval of proteasome inhibitors for the treatment of cancer based on their ability to selectively kill cancer cells.[46]

In conclusion, the results of this study indicate that proteasome inhibition decreases LEC migration by downregulating MMP-2 and -9 activities as part of the mechanism. Because proteasome inhibition also decreases other possible causative factors of PCO, such as LEC proliferation and EMT, we propose proteasome inhibition as therapy for blocking PCO development.

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