Modulation of Matrix Metalloproteinase and TIMP-1 Expression by Cytokines in Human RPE Cells

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PURPOSE. The balance between matrix metalloproteinases (MMPs) and tissue inhibitors of MMPs (TIMPs) is crucial for homeostasis of ocular extracellular matrices. To assess altered MMP activity as a determinant in the migration of human retinal pigment epithelial (RPE) cells, expression characteristics of several MMPs and TIMP-1 in RPE cell cultures were investigated.

METHODS. Expression studies were performed with RT-PCR, ELISA, and immunofluorescence analysis. Secretion of MMP-2 was demonstrated by zymography. Migration of cytokine-stimulated RPE cells was evaluated with microporous membranes of permeable chambers.

RESULTS. MMP-1, -2, -3, and -9; MT2-MMP, and TIMP-1 were expressed in cultured RPE cells. MMP-2 was detected on the cell surface and in secreted inactive and active forms. TGF-β2, IL-β, and TNF-α enhanced secretion of MMP-1, -2, and -3. TGF-β2 also stimulated MT2-MMP cell surface expression and release of TIMP-1. The mRNA levels of MMP-1, -2, and -3 and TIMP-1 were markedly increased by TNF-α and TGF-β2. MMP-2 mRNA levels were also upregulated by PDGF-BB. Migration of RPE cells stimulated by TGF-β2 or PDGF-BB was inhibited in presence of a synthetic MMP inhibitor.

CONCLUSIONS. Proinflammatory cytokines and TGF-β2 play an important role in the upregulation of expression of MMP-1, -2, and -3 in RPE cells and account for a directional shift in the balance between MMPs and TIMPs. Facilitation of RPE cell migration stimulated by cytokines (i.e., TGF-β2 or PDGF-BB) in ocular diseases may be due to increased release of MMPs, in the presence of comparatively lower levels of their inhibitors. (Invest Ophthalmol Vis Sci. 2002;43:2767–2773)

Matrix metalloproteinases (MMPs) are a family of zinc-binding, calcium-dependent endopeptidases that are expressed by various tissues and cell types and function by degrading extracellular matrix (ECM) components. MMPs are involved in normal physiological and pathologic processes, such as degradation of basement membrane, remodeling of ECM, connective tissue turnover, angiogenesis, reproduction, wound repair, and tumor progression and metastasis. To date, 25 members of the MMP family, which are generally organized into several categories based on their substrate specificity, have been reported. MMPs can be divided into collagenases, stromelysins, gelatinases, and membrane type-MMPs (MT-MMPs). The functional effects of these enzymes are in part controlled by interactions with tissue inhibitors of metalloproteinases (TIMPs) acting as natural MMP inhibitors. A precise balance between MMP and TIMP activities may be important for the integrity of ECM components, including, among other, collagens, vitronectin, fibronectin, laminin, elastin, and proteoglycans.

Alteration of MMP expression and activity in several ocular disease processes, such as neovascularization and proliferative retinopathies, suggests that these enzymes may contribute significantly to the pathogenesis of ocular diseases. Disintegration of Bruch’s membrane and of the retinal pigment epithelium (RPE)-interphotoreceptor matrix are important pathomechanisms in such diseases. Altered MMP production by RPE cells and other ocular cells in choroidal neovascularization (CNV) may result in detachment of RPE and choroidal endothelial cells from their underlying basement membrane, resulting in migration and matrix invasion. Moreover, proliferative vitreoretinopathy (PVR) is associated with the production of inflammatory cytokines, ECM deposition, and release of MMPs from activated cells. RPE cells, being one cellular source of MMP release, proliferate and migrate toward the neural retina and contribute largely to the formation of fibrocellular retinal membranes.

Expression and activity of MMPs have been shown to be regulated by various cytokines and growth factors, including transforming growth factor (TGF)-β, tumor necrosis factor (TNF)-α, and interleukin (IL)-1 β. These cytokines modulate expression of MMP-1 (interstitial collagenase), MMP-2 (gelatinase A, type IV collagenase), MMP-3 (stromelysin-1), and MMP-9 (gelatinase B, type V collagenase) in several cell types. Changes in MMP levels associated with RPE cells, which are caused by altered expression, have been addressed only incompletely. Thus, we investigated whether cytokines modulate the expression of MMP-1, -2, -3, and -9, MT1- and MT2-MMP, and TIMP-1 in cultured human RPE cells. In particular, we studied the effects of proinflammatory cytokines and TGF-β2, which are implicated in several ocular disorders.

MATERIALS AND METHODS

Isolation and Culture of Cells

Human RPE cells from different donors were isolated and cultured as previously described. Identity of cells was verified by immunocytochemical staining with the anti-cytokeratin monoclonal antibodies, AE1 and AE3 (Chemicon, Hofheim, Germany). The cells were cultured at 37°C in 5% CO2-95% air in DMEM-10% FCS, 1% penicillin-streptomycin (Life Technologies, Eggenstein, Germany). After trypsinization, an equal number of cells (5 × 10⁵) were recultured in six-well plates to near confluence. Cells of passages 2 to 6 were used in all experiments. The cells were incubated with or without cytokines for 24 hours in DMEM-10% FCS (before RNA isolation) or for 36 hours in serum-free DMEM-1% insulin-transferrin-sodium selenit (ITS; Sigma, Deisenhofen, Germany) before zymography, ELISA, or immunofluorescence analysis. Cytokines purchased from R&D Systems (Wiesbaden, Germany) were added as indicated in the legends to the figures. The number of RPE cells was assessed in each culture.

Gelatin Zymography

To detect gelatinase activity, culture-conditioned, serum-free media were collected, concentrated 10-fold using centrifugal filter units
(Ultrafree-4; Millipore, Bedford, MA), and analyzed by zymography. To distinguish latent from active forms of MMPs, samples were exposed to 1 mM 4-aminophenylmercuric acetate (APMA; Sigma), a mercurial agent that has been shown to convert MMPs from the latent to the active form. Samples were electrophoresed on SDS-10% polyacrylamide minigels containing gelatin (1 mg/mL). Medium from human fibrosarcoma HT-1080 cells was used as the control for the identification of comigrating MMP-9 and -2 bands. Gels were incubated in 2.5% Triton X-100 (30 minutes, 37°C) followed by overnight incubation in 50 mM Tris-HCl (pH 7.8), 5 mM CaCl$_2$, 0.02% NaN$_3$, 0.02% Brij 35. Gels were stained with 2.5% Coomassie brilliant blue in 50% methanol and 10% acetic acid to reveal zones of lysis within the gelatin matrix.

**Enzyme-linked Immunosorbent Assay**

MMP and TIMP-1 concentrations were determined with immunoassays performed according to the instructions of the manufacturers. ELISA systems were purchased from Calbiochem-Novabiochem, Bad Soden, Germany (MMP-2 and -9 ELISAs, sensitivity each 100 pg/mL; TIMP-1 ELISA, sensitivity 21 pg/mL). MMP-2 and -9 ELISAs were purchased from Calbiochem-Novabiochem, Bad Soden, Germany (MMP-2 and -9 ELISAs, sensitivity each 100 pg/mL; TIMP-1 ELISA, sensitivity 21 pg/mL). MMP-2 and -9 ELISAs, sensitivity each 100 pg/mL; TIMP-1 ELISA, sensitivity 21 pg/mL). Mean ± SD from several experiments were calculated, and the data were analyzed for significance by the paired Student’s t-test. P < 0.05 was considered to be statistically significant.

**Cell Surface Immunofluorescence Staining**

RPE cells were detached with HBSS-1 mM EDTA, washed, and resuspended in HBSS-1 mM Ca$^{2+}$, 1 mM Mg$^{2+}$, 0.05% NaN$_3$, 1% BSA (staining buffer). Cells were incubated for 60 minutes (4°C) with monoclonal antibodies (purchased from Calbiochem-Novabiochem) against MMP-2 (75-7F7), MT1-MMP2 (114-6G6), and MT2-MMP (162-226G5). Cells were washed with staining buffer and incubated with R-phycocyanin–conjugated goat anti-mouse immunoglobulin (Sigma). After washing, cells were fixed in PBS-1% formaldehyde and analyzed in a flow cytometer (FACSscan; BD Bioscience, San Jose, CA).

**RNA Preparation and Polymerase Chain Reaction Amplification of mRNA**

Total RNA of cells was prepared with a commercially purchased RNA isolation kit (InViTek, Berlin, Germany). Contaminating genomic DNA was eliminated with 1 U DNase I (Life Technologies). Single-stranded cDNA was synthesized from 1 μg total RNA in a 20-μL reaction with 200 U reverse transcriptase (Superscript II; Life Technologies), 500 μM dNTPs, and 0.5 μg oligo(dT)$_{15}$. PCR was performed in a 20-μL volume with 0.5 U DNA polymerase (InVitAQ; InViTek), 1 μL single-stranded cDNA, 100 μM dNTPs, 125 nM each of the specific primers (indicated in Table 1) in 50 mM Tris-HCl (pH 8.8), 16 mM (NH$_4$)$_2$SO$_4$, 2.5 mM MgCl$_2$, and 0.01% Triton X-100. For semi-quantitative analysis of mRNA expression, PCR amplification was performed under conditions that permit linear accumulation of PCR products, by using cDNA samples adjusted to equal glyceraldehyde-3-phosphate dehydrogenase (G3PDH) inputs. G3PDH-specific primers were used to amplify cDNA (566 bp) in the presence of a competitor (851 bp). A PCR cycle number known to remain within the limit of the exponential relationship between the PCR cycle number and amount of PCR product was chosen after amplification of cDNA derived from samples with the highest cDNA concentrations. Ethidium bromide–stained agarose gels were scanned with a gel documentation system (model 1000; Bio-Rad, Hercules, CA).

**Cell Migration Assay**

After treatment with cytokines, RPE cells were detached with HBBS-1 mM EDTA, and supernatants of cell cultures were preserved. The supernatants were supplemented with 0.5% BSA and used subsequently as the medium in migration assays. Briefly, migration was measured in duplicate using cell culture inserts with 8-μm pores (Becton Dickinson, Heidelberg, Germany), coated with 50 μg/mL fibronectin and 1 mg/mL gelatin on both sides. RPE cells were allowed to migrate in the presence or absence of the MMP inhibitor, BB-1101 (British Biotech, Oxford, UK). After incubation for 6 hours, the inserts were washed with PBS, fixed with Karnovsky’s reagent for 30 minutes, and stained with buffered azur II (1%)-methylene blue (1%). Nonmigrated cells were wiped off with a cotton tip, and migrated cells, attached to the lower surface, were counted with a microscope at × 200.

**RESULTS**

**MMP and TIMP-1 Expression in Human RPE Cells**

In the course of this study, expression in cultured RPE cells of MMPs and of one natural MMP inhibitor, TIMP-1, was investigated on the mRNA and protein levels. Expression of mRNAs was studied by means of RT-PCR, by using several primer combinations specific for different MMPs and TIMP-1 (Table 1).
Figure 1 demonstrates distinct amplification products for MMP-1, -2, -3, and -9 and TIMP-1, indicating mRNA expression of these molecules in RPE cells. Expression of pro-MMP-1, MMP-2 and -3, and TIMP-1 proteins was demonstrated with ELISA. Expression of MMP-2 was also confirmed by zymography, and the expression of membrane-associated MMP-2 and MT2-MMP on the RPE cell surface was indicated by immunofluorescence staining (described later).

**Expression of MMPs Associated with Gelatinase Activity in RPE Cells**

Because the enzymatic activity of several members of the MMP family is characterized by their capability of degrading gelatin, supernatants of RPE cells were first analyzed for gelatinolytic activity. Zymography showed that the most prominent bands in SDS gels were represented by two zones at approximately 74 and 69 kDa. (B) Quantitation of MMP-2 concentration in conditioned supernatants of RPE cells. Cells were incubated for 36 hours with TNF-α (10 ng/mL), IL-1β (10 ng/mL), TGF-β2 (10 ng/mL), IFN-γ (1000 U/mL), or PDGF-BB (10 ng/mL), and MMP-2 levels were determined by ELISA. Data are expressed as proportions of MMP-2 concentrations in relationship to the control culture without cytokines. Results representative of five experiments are summarized. Bars, mean ± SD (n = 5); *P < 0.05, **P < 0.01, analyzed by the paired Student’s t-test. (C) Increased number of MT2-MMP RPE cells elicited by treatment with TGF-β2. The increased number of MT2-MMP RPE cells after treatment with TNF-α is also shown. RPE cells were incubated for 36 hours, with or without cytokines; stained for membrane-anchored MMPs; and analyzed by flow cytometry. The figure shows MMP-2 and MT2-MMP expression (solid line) with background fluorescence (isotype-matched control antibody, broken line). The result is representative of five experiments.
Levels of MMPs and TIMP-1 Determined in Supernatants of Cultured Human RPE Cells

<table>
<thead>
<tr>
<th>Level</th>
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<tr>
<td>Pro-MMP-1</td>
<td>0.76 ± 0.59 (n = 12)</td>
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<tr>
<td>MMP-2</td>
<td>11.33 ± 6.36 (n = 9)</td>
</tr>
<tr>
<td>MMP-3</td>
<td>0.90 ± 0.46 (n = 6)</td>
</tr>
<tr>
<td>TIMP-1</td>
<td>0.20 ± 0.02 (n = 6)</td>
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Data are the mean nanograms per 10⁶ cells ± SD. Human RPE cells of different donors were cultured for 36 hours, the number of cells was counted and supernatants were assayed for total MMP and TIMP-1 levels by ELISA.

74 and 69 kDa (Fig. 2A). This gelatinolytic doublet corresponded to MMP-2-gelatinase A activity, in that it was identified by Western blot analysis to be the latent and active form of this gelatinase (data not shown). Treatment of the samples with APMA resulted in a shift in the molecular mass of the 74-kDa proteolytic activity toward the 69-kDa zone, suggesting that the 69-kDa band represents active MMP-2. When supernatants of serum-free cell cultures from different donors were analyzed, active MMP-2 was apparently absent (Fig. 2A, lane 2) or was found in low amounts (lane 3). We did not detect bands corresponding to RPE cell-associated MMP-9--gelatinase B activity in the cell cultures.

In further experiments, we investigated whether exposure of RPE cells to cytokines results in altered MMP expression relating to release of MMP-2. First, MMP-2 expression itself was evaluated after stimulation by the cytokines, TGF-β₂, IFN-γ, PDGF-BB, TNF-α, and IL-1β, which have been partly described to be involved in regulation of MMP expression in ocular cells.10,16,22,23 Treatment of RPE cell cultures for 36 hours with these cytokines did not result in different cell numbers (data not shown). In the supernatants of unstimulated RPE cells of different donors (n = 9), MMP-2 release was detectable at mean 11.33 ± 6.36 (SD) ng/10⁶ cells (Table 2). When RPE cells were incubated with TGF-β₂, TNF-α, and IL-1β, elevated levels of MMP-2 were found in the supernatants. The MMP-2 concentration was most strongly increased by TGF-β₂ (3.1-fold) and to a lesser extent by TNF-α (1.5-fold) and IL-1β (1.4-fold). A slight increase in MMP-2 production was observed after stimulation with PDGF-BB, whereas a decrease in the release of MMP-2 to approximately 0.8% of the control cells was detected after treatment with IFN-γ. However, both effects were not significant (Fig. 2B). These findings demonstrated that mainly TGF-β₂ and proinflammatory cytokines may promote MMP-2 secretion of RPE cells and raise the possibility that IFN-γ plays an inhibitory role.

Remarkably, MMP-2 was found to be expressed on RPE cells as a membrane-anchored protein. Two subpopulations of MMP-2⁺ RPE cells were identified, one weakly positive population and a smaller fraction with higher staining intensity. TGF-β₂, as the cytokine with the strongest upregulating effect on MMP-2 release, also increased MMP-2 cell surface expression (Fig. 2C). In contrast, no significant effect of TNF-α was detected (not shown). Although the cell population exhibiting stronger expression of MMP-2 was barely affected by TGF-β₂, the number of the weakly MMP-2⁺ cells increased. Because MT-MMPs are responsible for activation of pro-MMP-2 on the surface of many cells, expression of MT1- and MT2-MMP on human RPE cells was also investigated. In these experiments, MT1-MMP was expressed on RPE cells at only very low levels (data not shown), whereas MT2-MMP was more strongly expressed. Expression of MT2-MMP, which is an activator of pro-MMP-2, was enhanced by TNF-α (Fig. 2C) but was not significantly altered by TGF-β₂ (not shown).

Influence of Cytokines on MMP-1 and -3 and TIMP-1 Expression in Human RPE Cells

Upregulation of MMP-2 release by TGF-β₂, TNF-α, and IL-1β prompted us to investigate how these cytokines affect production of MMP-1 and -3 and TIMP-1 by RPE cells. Pro-MMP-1, MMP-3, and TIMP-1 were readily detectable in the medium of in vitro cultured RPE cells (the levels are indicated in Table 2). In contrast, a significant MMP-9 release was not found, suggesting that concentration of this MMP was probably too low to be detected by ELISA (data not shown). Further MMP-related effects mediated by TGF-β₂ were established through exposure of RPE cells to this cytokine. Although to a different extent, TGF-β₂ was found to stimulate pro-MMP-1, MMP-3, and TIMP-1 protein secretion. Although TGF-β₂ elicited only a marginal increase in pro-MMP-1 (1.74 ± 0.61-fold; n = 7, P = 0.02) and TIMP-1 levels (1.46 ± 0.38-fold; n = 7, P = 0.02), the impact of this cytokine on MMP-3 expression was relatively strong (3.7-fold increase; Fig. 3).

In experiments destined to assess further the effects of PDGF-BB on MMP-2 expression (as described earlier) we used the same conditioned media of RPE cells to study TIMP-1 protein secretion. As in the case of MMP-2 protein levels, the slight increase in TIMP-1 levels (1.2 ± 0.3-fold; n = 7) was not significant (P = 0.09). Notably, when RPE cells were cultured with TNF-α and IL-1β, by far the strongest increase in production of pro-MMP-1 and MMP-3 was recorded. In contrast, addition of TNF-α and IL-1β did not result in significantly increased TIMP-1 levels (Fig. 3). These results suggest that it is mainly the contact of the RPE with proinflammatory cytokines that elicits a shift in the MMP-TIMP ratio, favoring the predominance of MMP-1, -2, and -3.

As a mechanism for altered MMP release, regulation of MMP mRNA expression in RPE cells was addressed (Fig. 4). The data from semiquantitative PCR analyses were in agreement with a marked upregulation of MMP-2 after exposure to TGF-β₂, TNF-α, and PDGF-BB. TGF-β₂, apparently the most potent cytokine in inducing high MMP-2 protein levels (as mentioned earlier), elicited increased MMP-2 mRNA levels in a range of 0.5 to 10 ng/ml in a dose-dependent manner (data not shown). Similarly, TGF-β₂ treatment resulted in a significant upregulation of MMP-1 and -3 and TIMP-1 mRNAs. The data pointed also to upregulation of TIMP-1 mRNA levels by TNF-α (Fig. 4), whereas they were apparently not influenced by PDGF-BB (data not shown). When TNF-α was present during the culture of RPE cells, both MMP-1 and -3 mRNA levels were clearly elevated. These findings are consistent with the increased release of these MMPs after exposure to proinflammatory cytokines, as indicated earlier.

Effect of MMP Release on RPE Cell Migration through the Extracellular Matrix

It has been found that MMPs play a role in regulation of cell migration and invasion through basement-like matrices by degrading the ECM.12 In addition, it is known that cytokines are capable of modifying RPE cell behavior including proliferation, ECM synthesis, MMP production and contraction.24,25 To assess contribution of upregulated MMP expression to RPE cell migration during cytokine-mediated stimulation, permeable chamber assays (Transwell, Costar, Cambridge, MA) were performed in the presence or absence of the broad-spectrum hydroxamic acid-based MMP inhibitor BB-1101.26 In these experiments, RPE cells displayed increased transmigration through fibronectin-gelatin-coated filters after exposition for 36 hours to TGF-β₂ and PDGF-BB, respectively. Of note, transmigration induced by TGF-β₂ or PDGF-BB was suppressed by BB-1101 (Fig. 5). These findings suggest that upregulation of MMP mediated by these cytokines plays an important role in the migration of RPE cells.
DISCUSSION

Recent studies using ocular tissue or cell culture systems suggest that MMPs and their inhibitors play a key role in the homeostasis of extracellular matrices in the eye.\(^9\),\(^{23}\),\(^{27}\) Moreover, alterations in ECM (probably accompanied by local accumulation of a variety of substances that affect cellular functions) may modulate migration, proliferation, differentiation, and adhesion of cells in vitreoretinal disorders, such as CNV\(^28\) and PVR.\(^13\) Thus, the exudative form of age-related macular degeneration, which is associated with CNV, is characterized by disruption of homeostatic regulation of structural properties of Bruch’s membrane. Triggering signals for CNV, which are poorly defined and may comprise, for example, modified ECM components\(^28\) or advanced glycation end products,\(^25\) induce cellular damage and inflammatory reactions that participate also in MMP secretion by RPE cells.\(^{25,30}\) In PVR, activated RPE cells dissociate from Bruch’s membrane, migrate toward the surface of the neuroretina, and contribute to the formation of provisional matrices (i.e., periretinal membranes). These and other findings suggest that the RPE may have important regulatory functions in the expression of MMPs and that an imbalance in the activity of these proteinases could contribute to development of pathologic alterations in CNV or PVR.\(^9\),\(^{31,32}\)

Our experiments demonstrated the expression of MMP-1, -2, -3, and -9; MT2-MMP; and TIMP-1 in RPE cells and examined modulation of their relative levels in response to cytokines.

![Figure 3](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/932916/)  
**Figure 3.** Quantitation of (top) pro-MMP-1 (n = 7), (middle) MMP-3 (n = 5), and (bottom) TIMP-1 (n = 7) levels in supernatants of stimulated human RPE cells. Cells were incubated for 36 hours with cytokines, as indicated in Figure 2B. Concentrations of secreted molecules in supernatants of nonstimulated cells were set to 1 and proportions compared with control cultures were calculated. Bars, mean \(\pm SD\); \(* P < 0.05, ** P < 0.01\), compared with the medium control and analyzed by a paired Student’s \(t\)-test.

![Figure 4](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/932916/)  
**Figure 4.** Increased MMP-1, -2, and -3 and TIMP-1 mRNA expression in human RPE cells after exposure to cytokines. Cells were incubated for 24 hours with TGF-\(\beta\)\(\_2\) (10 ng/mL), TNF-\(\alpha\) (10 ng/mL), or PDGF-BB (10 ng/mL). For analysis of mRNA expression, RNA was isolated from cultured RPE cells, reverse-transcribed into cDNA, and analyzed by semiquantitative PCR. Adjustment of sample cDNA amounts to the G3PDH housekeeping gene expression is also shown. One analysis of MMP-1, -2, and -3 and TIMP-1 mRNA expression in cells obtained from one donor out of three experiments with similar results is shown.
Although our culture conditions did not consider the contact of RPE cells with ECM components (as a condition more relevant to disorders such as PVR) our experiments provided insight into cytokine-mediated changes of MMP expression in RPE cells. The main conclusions are that (1) expression of MMP-1, -2, and -3 is modulated in a coordinated manner by particular cytokines (TNF-α, IL-1β, and TGF-β2), leading to increasing release of MMPs from RPE cells; (2) TIMP-1 protein levels are not subject to pronounced alterations induced by proinflammatory cytokines; and (3) changes in MMP expression apparently play a significant role in facilitating migration of RPE cells.

Our report extends results of previous studies that demonstrated MT1-MMP and MMP-1, -2, -3, and -9 in the choroid; our experiments provided evidence that active MMP-2 is secreted in only very small quantities, suggesting that activation of pro-MMP-2 did not play a significant role, at least not in nonstimulated RPE cells and under our culture conditions. Moreover, we could not find measurable levels of MMP-9 (in culture supernatants) and MT1-MMP (on the cell surface), not even after cytokine-mediated stimulation. Contrary to our results, increased MMP-9 levels have been demonstrated by others in supernatants of RPE cells stimulated with IL-1β or TNF-α. However, zymography analysis of the RPE cell culture supernatants revealed that active MMP-2 is secreted in only very small quantities, suggesting that activation of pro-MMP-2 did not play a significant role, at least not in nonstimulated RPE cells and under our culture conditions. Moreover, we could not find measurable levels of MMP-9 (in culture supernatants) and MT1-MMP (on the cell surface), not even after cytokine-mediated stimulation. Contrary to our results, increased MMP-9 levels have been demonstrated by others in supernatants of RPE cells stimulated with IL-1β or TNF-α. However, zymography analysis of the RPE cell culture supernatants revealed that active MMP-2 is secreted in only very small quantities, suggesting that activation of pro-MMP-2 did not play a significant role, at least not in nonstimulated RPE cells and under our culture conditions. Moreover, we could not find measurable levels of MMP-9 (in culture supernatants) and MT1-MMP (on the cell surface), not even after cytokine-mediated stimulation.

To summarize, we have reported cytokine-mediated upregulation of several MMPs in RPE cells and demonstrated that concomitantly increased MMP release may facilitate RPE cell migration. It is conceivable that targeting cytokine-mediated intracellular signaling pathways, in particular by agents with anti-inflammatory effects, is an appropriate strategy to treat the development of several sight-threatening disorders that are associated with excessive MMP activity and matrix degradation.

Acknowledgments

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References


