Distribution and Putative Roles of Fibroblast Growth Factor-2 Isoforms in Corneal Endothelial Modulation

Xin Gu1 and EunDuck P. Kay1,2

PURPOSE. Corneal endothelial modulation factor (CEMF) released by inflammatory cells induces de novo synthesis of fibroblast growth factor (FGF)-2, which is a morphogen and a potent mitogen of corneal endothelial cells (CECs). Four isoforms of FGF-2 have been found in the nucleus, cytoplasm, or extracellular matrix (ECM) in different cell lines. In the present study, the profiles of the isoforms of FGF-2 that are induced by CEMF were investigated, and whether the differential localization of the isoforms of FGF-2 plays a role in CECs proliferation and subsequent modulation was examined.

METHODS. Nuclear, cytoplasmic, and ECM fractions of normal and modulated CECs were separated, and FGF-2 isoforms were further purified by heparin-Sepharose column. The molecular sizes of the isoforms were determined by immunoblot analysis, using a specific antibody directed against FGF-2. Cell proliferation was determined by cell counting. Cellular localization of FGF-2 was determined by immunofluorescence staining during different stages of cell growth.

RESULTS. To confirm that CEMF modulated CECs under the conditions used in this study, its effect on cell proliferation and cell shape was determined: CEMF-treated cells showed enhanced cell proliferation profiles and fibroblastlike morphology. In rapidly growing normal CECs, FGF-2 was predominantly present in the nucleus. As the cells reached confluence, the staining potential in the nucleus was markedly reduced. Cytoplasmic staining of FGF-2 was barely detectable, regardless of cell stages. In CEMF-modulated cells, the rapidly growing cells showed strong staining of FGF-2 in the nucleus, whereas cytoplasmic and ECM staining was weak. When modulated cells reached confluence, the staining of FGF-2 in the nuclei remained strong, whereas ECM staining was significantly increased. Immunoblot analysis of the subcellular fraction showed that the 24-kDa FGF-2 was predominantly present in the nucleus, whereas the 18-kDa form was the major molecule in cytoplasmic and ECM fractions in normal and modulated cells.

CONCLUSIONS. These findings indicate that 24-kDa nuclear FGF-2 may be involved in cell proliferation in growing CECs. The persistent nuclear localization and simultaneous ECM localization of FGF-2 are induced by CEMF, and these FGF-2 isoforms seem to play a role in cell proliferation and modulation. (Invest Ophthalmol Vis Sci. 1998;39:2252-2258)

Corneal endothelium is a monolayer of differentiated cells located in the posterior portion of the cornea. During wound healing, the regeneration capacities of corneal endothelial cells (CECs) vary among species. Human and primate CECs have shown limited regenerative ability in vivo.1,2 In response to certain pathologic conditions, CECs in vivo may respond by converting to fibroblastic-like cells. These morphologically modulated cells then assume their role in proliferation and start to produce fibrillar collagens, leading to the formation of a fibrillar extracellular matrix (ECM). One such clinical example is the development of retrocorneal fibrous membrane,3,4 the presence of which blocks vision, causing blindness. In our previous studies, corneal endothelium modulation factor (CEMF) secreted by polymorphonuclear leucocytes (PMNs), fibroblast growth factor (FGF)-2, or a combination of the two factors was found to modulate phenotypes of CECs, leading to a modulation similar to that observed in vivo (cell proliferation, cell shape changes, and collagen phenotype alteration).5,6 We further found that CEMF could induce de novo synthesis of FGF-2 and that the newly produced FGF-2 is the direct mediator for the modulation of CECs.7

FGF-2, a member of the fibroblast growth factor family, is a multifunctional regulator of cell development, differentiation, regeneration, senescence, proliferation, and migration.8-10 Although FGF-2 does not have the classic signal peptides needed for secretion, it is deposited in the ECM by an unknown mechanism.11,12 In normal cornea, FGF-2 is a component of Descemet’s membrane that may be necessary during wound repair.7,13,14 FGF-2 is produced in four isoforms with molecular masses of 18, 22, 22.5, and 24 kDa,15,16 all derived from a single mRNA. The translation of the 18-kDa low molecular mass form seems to originate from an AUG codon, whereas the three high molecular mass forms originate from three CUG codons located at 5’ to the AUG codon.15 The three high molecular mass FGF-2 isoforms are colinear N-terminal extensions of the 18-kDa form. These N-terminal extensions contain a nuclear translocation sequence17 that may enable the nuclear FGF-2 to react directly to stimulate cell proliferation.18

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One recent study has indicated that FGF-2 does not have to be secreted to stimulate the cell proliferation of fibroblast cells. Because CEMF induces de novo synthesis of FGF-2 in CECs, the present study was conducted to determine which isoforms of FGF-2 were induced by CEMF and whether their differential localization plays a role in corneal endothelial modulation.

**Materials and Methods**

**Cell Cultures**

Isolation and establishment in culture of rabbit CECs were performed as previously described. Chemicals were purchased from Sigma (St. Louis, MO) unless otherwise noted. Briefly, Descemet's membrane–corneal endothelium complex was treated with 0.2% collagenase and 0.05% hyaluronidase (both from Worthington Biochemical, Freehold, NJ) for 90 minutes at 37°C. Cultures were maintained in Dulbecco's modified Eagle's medium (DMEM; Life Technology, Gibco, Gaithersburg, MD) supplemented with 10% fetal bovine serum and 50 μg/ml gentamicin (DMEM-10) in a 5% CO₂ incubator, which has been shown to promote cell proliferation during the early phase of culture and to maintain the culture as a contact-inhibited monolayer when the cells reach confluence. First-passage CECs were used for all experiments. Modulated CECs were prepared by treating the cells with CEMF when plated and by feeding the cells with CEMF-containing DMEM-10 every other day. All animal experiments were performed in compliance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

**Preparation of CEMF**

Polymorphonuclear leukocytes were obtained from rabbits, as described previously. The purified PMNs were incubated in protein-free medium (UltraDOMA-PF; Biowhittaker, Walkersburg, MD) supplemented with 10 μg/ml bovine serum albumin, and maintained in a 5% CO₂ incubator for 16 hours. The PMN-conditioned medium was precipitated with ammonium sulfate to a final concentration of 80%. After dialysis against phosphate-buffered saline (PBS), the samples were applied to a heparin-Sepharose column equilibrated with PBS. After the samples were washed with five column volumes PBS, proteins were eluted with 0.6 M NaCl in PBS. This eluted fraction was dialyzed against PBS, made to a protein concentration of 250 μg/ml, and used to modulate the CECs to a concentration of 25 μg/ml. Partial characterization of CEMF has been made.

**Cell Proliferation Assay**

Normal and modulated CECs (3 × 10⁴) were plated in 24-well tissue culture plates, and cell numbers were determined 24, 48, 72, and 96 hours after plating. Triplicates of each sample were trypsinized with 0.2% trypsin and 5 mM EDTA and counted on a hemacytometer to determine the final cell density of the culture.

**Immunofluorescent Staining**

Normal and modulated CECs (3 × 10⁴/chamber) were seeded on four-chamber slides. Cells obtained 24, 48, and 72 hours after plating were washed with PBS and fixed with 3% paraformaldehyde in PBS. All washing and incubation were carried out in PBS at room temperature. Cells were then permeabilized with 0.5% Triton X-100 in PBS for 5 minutes and blocked with 2% bovine serum albumin. The chamber slides were incubated with 1:50 monoclonal antibody against FGF-2 (type II, catalog No. 05-118; Upstate Biotechnology, Lake Placid, NY) for 2 hours and then washed with PBS. Cells were then incubated with 1:50 biotinylated anti-mouse immunoglobulins (Vector Laboratories, Burlingame, CA) for 1 hour followed by incubation with 1:100 fluorescein conjugated to avidin (Vector) for 30 minutes. The slides were examined under a confocal microscope (Carl Zeiss, Oberkochen, Germany).

**Purification of the Subcellular Fraction**

Normal and modulated cells 24, 48, and 72 hours after plating were washed three times with PBS. The ECM FGF-2 fraction was initially extracted with 2 M NaCl in 20 mM HEPES (pH 7.6) and then with 2 M NaCl in 20 mM sodium acetate (pH 4.5) to extract FGF-2 bound to high- and low-affinity receptors. The cells were then suspended in buffer I (50 mM Tris-HCl [pH 7.4] 5 mM CaCl₂, and 25 mM sucrose) supplemented with protease inhibitors (100 μg/ml phenylmethylsulfonyl fluoride, 5 μg/ml aprotinin, and 5 μg/ml leupeptin). Cells were homogenized on ice, and the lysate was centrifuged at 1000g. The supernatant was collected as the cytoplasmic fraction, and the pellet was washed twice with buffer I and laid on top of 1 M sucrose. After spinning at 1000g for 15 minutes at 4°C, the pellet was dissolved in 50 mM Tris-HCl (pH 7.4) and 0.1% NP-40 containing protease inhibitors and was designated as the nuclear portion. The purity of the isolated fractions was evaluated by measuring the activity of plasma membrane 5'-nucleotidase and lysosomal acid phosphatase with assay kits (Sigma). For 5'-nucleotidase assay, 3.0 ml 5'-nucleotidase assay solution was placed in a cuvette, and the temperature was brought to 30°C, before the addition of 0.2 ml cell samples. The reaction mixture was maintained at 30°C for exactly 5 minutes, and absorbance was measured at 340 nm versus water as the reference; denoted A1. The reaction mixture was incubated in the water bath for exactly 5 minutes, and absorbance was again read and denoted A2. The observed difference between A2 and A1 was the relative enzymatic activity of the sample. For the acid phosphatase assay, 0.5 ml citrate buffer was added to 0.5 ml substrate solution. In tube 1, 0.1 ml water was added as the reagent blank, and 0.2 ml sample was added to another tube. The reaction mixture was maintained in a water bath at 37°C for 30 minutes, and the reaction was stopped with the addition of 5 ml 0.1 N NaOH. The absorbance was read at 410 nm. The difference between tube 1 and tube 2 was used as the relative enzymatic activity. Protein concentration of each fraction was determined using a protein assay (DC; Bio-Rad, Hercules, CA) kit. The nuclear, cytoplasmic, and ECM fractions were each applied to a heparin-Sepharose column, and the columns were washed with PBS. The bound protein was eluted with sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS–PAGE) sample buffer containing 1 M NaCl and boiled for 5 minutes. After a brief spin, the supernatant was subjected to SDS–PAGE. The gel's size was prestained standard protein (Bio–Rad) was also applied to the gel.

**SDS–PAGE and Immunoblot Analysis**

The conditions of electrophoresis were as described by Laemmli. FGF-2 was analyzed on a 15% gel in the nonreduced with 2% bovine serum albumin. The chamber slides were incubated with 1:50 monoclonal antibody against FGF-2 (type II, catalog No. 05-118; Upstate Biotechnology, Lake Placid, NY) for 2 hours and then washed with PBS. Cells were then incubated with 1:50 biotinylated anti-mouse immunoglobulins (Vector Laboratories, Burlingame, CA) for 1 hour followed by incubation with 1:100 fluorescein conjugated to avidin (Vector) for 30 minutes. The slides were examined under a confocal microscope (Carl Zeiss, Oberkochen, Germany).
condition using discontinued Tris-glycine buffer system (pH 8.3).

Proteins separated by SDS-PAGE were electrophoretically transferred to a polyvinylidene difluoride membrane at 0.22 A for 10 hours in a semidy transfer system (transfer buffer: 25 mM Tris [pH 8.3] 190 mM glycine, and 20% MeOH). Immunoblot analysis was performed as described previously using a commercial kit (ABC Vectastain; Vector). All washing and incubations were performed at room temperature in 0.9% NaCl, 100 mM Tris-HCl (pH 7.5), and 0.1% Tween 20. Briefly, the polyvinylidene difluoride membrane was immediately placed in the blocking buffer (5% nonfat milk containing 0.9% NaCl, 100 mM Tris-HCl [pH 7.5], and 0.1% Tween 20) for 1 hour. The membrane was incubated with 1:5000 primary antibody for 2 hours, with 1:2500 biotinylated secondary antibody for 1 hour, and with the ABC reagent for 30 minutes. The membrane was treated with enhanced chemiluminescence (ECL; Amersham Life Science (Buckinghamshire, UK) reagent for 1 minute and the ECL-treated membrane was exposed further to ECL film. The monoclonal antibody against the 18-kDa isoform of FGF-2 has previously been used in corneal endothelial cells, and this antibody interacts with all isoforms of FGF-2.

RESULTS

Effect of CEMF on Cell Proliferation and Cell Shape Modulation of Corneal Endothelial Cells

Our previous study shows that CEMF in low serum (1% fetal bovine serum) conditions has no stimulatory effect on cell proliferation of quiescent CECs. Because the experiments in this study were performed in growth-supporting medium (DMEM-10), the effect of CEMF on cell proliferation of CECs was reexamined and compared with that of cells grown in DMEM-10. First-passage cells were plated under conditions in which the cells reached confluence in 4 days. This plating condition was used throughout the study. The growth profile of CECs in the absence and presence of CEMF is shown in Figure 1. The control cells grew rapidly during the first 3 days, after which cell division seemed to decrease markedly, creating a monolayer of cells. The cells treated with CEMF show a much enhanced growth potential throughout the growth stages and confirm our previous findings that PMNs stimulate cell proliferation of CECs. Another phenotypic switch induced by CEMF was change in cell shape. Polygonal endothelial cells became elongated and assumed a fibroblastlike morphology. Because our previous studies were performed under different induction conditions, the effect of CEMF on cell shape was also reexamined under the conditions used in this study (Fig. 2), for which cells were simultaneously treated with 25 μg/ml CEMF (partially purified fraction) at the time of plating. Cells treated with CEMF for 24 hours formed sparse cultures but showed normal morphology. Those treated for 48 hours grew rapidly and became elongated. Cells treated for 72 hours reached confluence, seemed to lose the characteristic polygonal cobblestone monolayer, and showed an elongated morphology, whereas the control monolayer cells maintained polygonal morphology. These findings confirmed our previous results regarding corneal endothelial modulation by CEMF, providing a foundation for the subsequent experiments, in which CEMF was used to induce de novo synthesis of FGF-2.

Subcellular Localization of FGF-2

Recent studies have shown that FGF-2 is translocated to the nucleus. It has been proposed that some biologic activities of FGF-2 may be mediated by the nuclear FGF-2. To examine the exact subcellular location of recently synthesized molecules and their subsequent translocation, cells grown in DMEM-10 and cells treated with CEMF were examined for the subcellular location of FGF-2 as a function of the growing stage (Fig. 3). In control cultures, the growth of which are maintained by DMEM-10, prominent nuclear staining with FGF-2 antibody was observed in the rapidly growing cells. After 48 hours in culture, some cells appeared to lose nuclear staining, and at 72 hours the confluence culture showed a loss of FGF-2 staining in the nucleus. Cytoplasmic staining was negligible, regardless of the cell stages. In contrast, CECs treated with CEMF showed differential staining profiles of FGF-2 distribution when compared with those of control cells (Fig. 4). The rapidly growing cells of the 24-hour culture showed strong nuclear staining and weaker but still prominent staining of FGF-2 in the cytoplasm. The 48-hour culture, which assumed an elongated morphology, showed strong nuclear staining and enhanced cytoplasmic and ECM staining. When cells reached confluence in the 72-hour culture, nuclei and ECM showed strong staining profiles of FGF-2, whereas cytoplasm appeared to lose staining potential. These observations suggest that newly synthesized FGF-2, induced by fetal calf serum or CEMF, is predominantly present in the nucleus during proliferation. However, CEMF maintains the nuclear FGF-2 throughout the growth stages and further promotes the distribution of FGF-2 in the ECM.

FGF-2 Isoforms: Molecular Sizes of Nuclear, Cytoplasmic, and ECM Forms

Multiple forms of FGF-2 exist, differing only in the length of their NH₂-terminal extensions. Because these isoforms of

FIGURE 1. Cell proliferation assay of corneal endothelial cells (CECs). Cells were plated in a 24-well tissue culture dish in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum and 50 μg/ml gentamicin. Modulated cells were induced as described in the Methods section. The cells were treated with trypsin-EDTA solution. Cell numbers were determined 24, 48, 72, and 96 hours after plating. The data represent the two experiments performed in triplicate. Normal cells (▲), modulated cells (●).
FGF-2 have been reported to have unique subcellular distributions, the respective isoforms induced by CEMF were purified from nucleus, cytoplasm, and ECM and compared with those of control cells. To confirm the purity of these subcellular fractions, each fraction was examined by phase-contrast microscopy and by measuring the activity of 5'-nucleotidase (plasma membrane marker) and acid phosphatase (lysosomal marker, data not shown). When the nuclear fraction was checked under phase-contrast microscopy, it showed little contamination with cytoplasmic membrane and organelles. The biochemical assays showed that the nuclear fraction and the ECM fraction each contained approximately 10% of the total cellular activity of 5'-nucleotidase. For acid phosphatase, the nuclear fraction contained 2% and the ECM portion 3% of the total activity. These observations are similar to those in the previous study. To compare the relative expression of isoforms among cell samples, the same amount of protein was purified on a heparin-Sepharose column before immunoblot analysis. When nuclear fractions of normal and CEMF-treated cells were examined 24, 48, and 72 hours after plating, normal cells showed a major band of 24-kDa and a minor band of 18 kDa during the early stage of growth (Fig. 5). As the cells reached confluence, the 18-kDa band disappeared. The cells treated with CEMF for 24 hours showed a profile similar to that of the control counterpart during the early stages of growth, with a major 24-kDa band and a minor 18-kDa band. The proportion of the 18-kDa band markedly decreased as cells reached confluence, at which time the higher molecular forms showed a doublet of approximately 22-kDa and 24 kDa. The amount of 24-kDa isoform in the nucleus of the treated cells was much higher than that of control cultures, confirming the immunofluorescent staining profiles (Figs. 3, 4).

When the isoforms of the cytoplasmic fraction were analyzed (Fig. 6), the rapidly growing cells produced the 18-kDa isoform, whereas the confluent cultures produced a higher molecular weight isoform as a minor component in addition to the major band of 18 kDa. Similarly, the CEMF-treated cells produced the 18-kDa isoform as a predominant species with a barely detectable production of the higher molecular weight form as cells reached confluence. The amount of the 18-kDa isoform in the cytoplasm of the treated cells is much greater than that in the control cells, regardless of cell stage. When ECM fractions obtained from the normal and CEMF-treated cells were analyzed, the 18-kDa form was the only isoform found in both normal and modulated cells, and the amount of the 18-kDa isoform in ECM of the treated cells was also much higher than that in the normal cells (Fig. 7). Unlike the observation obtained with immunofluorescent staining (Fig. 3), the control cells in the immunoblot analysis showed the presence of the 18-kDa isoform in ECM. This is probably because of the much larger amount of protein used for the immunoblot analysis. Of interest, the 18-kDa ECM form was not observed by immunoblot analysis in the rapidly growing (24-hour culture) normal and modulated cells; it is likely that the 18-kDa ECM form requires time for secretion and deposition in the ECM (data not shown).

DISCUSSION

Our previous study showed that FGF-2, a component of Descemet's membrane, can modulate CECs into fibroblast-
FIGURE 3. Immunofluorescent staining of fibroblast growth factor (FGF)-2 in normal corneal endothelial cells (CECs). First-passage CECs in chamber slides were examined at (A) 24 hours, (B) 48 hours, and (C) 72 hours (confluent cells) after plating. After fixing with paraformaldehyde, the cells were stained with anti-FGF-2 monoclonal antibody, as described in the Methods section. (D) Cells after 24-hour culture stained without anti-FGF-2 antibody. Magnification, ×840.

FIGURE 4. Immunofluorescent staining of fibroblast growth factor (FGF)-2 in corneal endothelial modulation factor (CEMF)-modulated corneal endothelial cells (CECs). First-passage CECs in chamber slides were treated with 25 μg/ml CEMF immediately after subculture. Cells were fixed with paraformaldehyde and stained with anti-FGF-2 monoclonal antibody as described in the Methods section. (A) Cells after 24-hour culture, (B) 48-hour culture, and (C) 72-hour culture. (D) Cells after 24-hour culture without anti-FGF-2 antibody staining. Magnification, ×840.

FIGURE 5. Immunoblot analysis of fibroblast growth factor (FGF-2) in the nuclear fraction of normal and modulated corneal endothelial cells (CECs). First-passage CECs were treated with corneal endothelial modulation factor immediately after subculture; normal cells were grown in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum and 50 μg/ml gentamicin. Immunoblot analysis was performed as described in the Methods section. Lane 1, normal cells collected at 24 hours; lane 2, modulated cells collected at 24 hours; lane 3, normal cells collected at 48 hours; lane 4, modulated cells collected at 48 hours; lane 5, normal cells collected at 72 hours; and lane 6, modulated cells collected at 72 hours.

FIGURE 6. Immunoblot analysis of fibroblast growth factor (FGF-2) in the cytoplasmic fraction of normal and modulated corneal endothelial cells (CECs). First-passage CECs were treated with corneal endothelial modulation factor immediately after plating; normal cells were grown in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum and 50 μg/ml gentamicin. Immunoblot analysis was performed as described in the Methods section. Lane 1, normal cells collected at 24 hours; lane 2, modulated cells collected at 24 hours; lane 3, normal cells collected at 48 hours; lane 4, modulated cells collected at 48 hours; lane 5, normal cells collected at 72 hours; and lane 6, modulated cells collected at 72 hours.

Recently discovered FGF-2 isoforms and their unique subcellular location have suggested that there may be differential biologic activities among these isoforms. For example, FGF-2, which is normally detected in the nuclei of neurons of the rodent brain, can be found in the cytoplasm after a central nervous system lesion.8 Regardless of whether these changes reflect the translocation of existing protein to the cytoplasm, the differential location of like cells; however, the mere presence of FGF-2 in Descemet’s membrane is not sufficient for enhancing cell proliferation or for modulation of phenotypes in CECs (cell shape change and collagen phenotype switches). We have also reported that CEMF released by inflammatory cells can induce FGF-2, which acts as a direct modulator in the corneal endothelium.7 Recent discoveries of FGF-2 isoforms and their unique subcellular location have suggested that there may be differential biologic activities among these isoforms. For example, FGF-2, which is normally detected in the nuclei of neurons of the rodent brain, can be found in the cytoplasm after a central nervous system lesion.8 Regardless of whether these changes reflect the translocation of existing protein to the cytoplasm, the differential location of newly synthesized FGF-2 remains unclear. Because CEMF can induce de novo synthesis of FGF-2 in CECs, the present study was conducted to determine which isoforms of FGF-2 were induced by CEMF and whether their differential localization plays a role in corneal endothelial modulation.

Our present study shows that higher molecular mass isoforms are predominantly present in the nucleus, regardless of the presence of CEMF-induction. However, the amount of the high molecular mass 24-kDa nuclear form in the CEMF-treated cells is much higher than that in control cells. When cells reach confluence, the amount of the 24-kDa form is significantly reduced in normal cells, compared with the CEMF-induced nuclear isoform, the high level of which is continuously maintained in the nucleus. Of interest, the 18-kDa form is also present in the nuclei, although at a low concentration, and the 18-kDa form disappears from the nuclei as the cells reach confluence. It has been shown that exogenously added FGF-2 (the 18-kDa form) is internalized and translocated to the nuclei of endothelial cells, fibroblasts, and myoblasts.25 Although these reports support our observation on the presence of the 18-kDa isoform in the nuclei, it is unclear whether the nuclear 18-kDa form is translocated directly from cytoplasm after synthesis to the nuclei or is secreted first and internalized later. The low molecular mass 18-kDa form is the major species in cytoplasm and ECM regardless of the presence of CEMF-mediated induction. However, CEMF greatly enhanced the expression level of these cytoplasmic and ECM 18-kDa forms throughout the growth stages. The discrepancy observed in the immunostaining of FGF-2 and immunoblot analysis in normal cells is probably caused by the experimental conditions in which much higher protein amounts of affinity-purified FGF-2 were used for the immunoblot analysis, which subsequently enhanced the resolution signals. Together, these findings suggest that the higher molecular isoforms (22–24 kDa) are located predominantly in the nuclei, whereas the 18-kDa isoform is predominantly present in the cytoplasm and ECM, regardless of cell stages in
growth. Although CEMF does not significantly alter the subcellular distribution of FGF-2 isoforms, it markedly enhances the production of all the FGF-2 isoforms and deposition of the ECM isoform, and it induces the persistent presence of nuclear isoforms. From these findings, it is probable that the 24-kDa form is initially responsible for cell proliferation and that the 18-kDa cytoplasmic isoform later joins the action of FGF-2 until confluence is reached in normal cells. These isoforms cannot stimulate cell proliferation further in the confluent culture of normal corneal endothelial cells. Conversely, the higher molecular mass nuclear forms are responsible for cell proliferation in the CEMF-treated cells, and their persistent presence is responsible for continuous cell proliferation. The 18-kDa ECM form may be responsible for modulating cell shape, with or without a partnership with 24-kDa isoforms, because time required for modulation of cell shape change coincides with that required for accumulation of the 18-kDa isoform in the ECM. It has been suggested that nuclear binding of FGF-2 may mediate the long-term effect (e.g., changes in chromatin structure to allow transcription and replication of genes). Currently, we are continuing our investigation into whether the induced and retained nuclear isoform or the ECM isoform is responsible for differential phenotypic changes such as cell shape changes and collagen phenotype switches during corneal endothelial modulation using specific antibodies against nuclear forms or the ECM form.

References