Expression of Fibroblast Growth Factor-5 by Bovine Choroidal Endothelial Cells In Vitro

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Purpose. To demonstrate the expression of fibroblast growth factor-5 (FGF-5) by bovine choroidal microvascular endothelial (BCME) cells and to investigate its possible role as an autocrine mitogen in these cells.

Methods. Expression of FGF-5 by BCME cells was studied by a combination of Northern and Western blot analyses. Total RNA was isolated from BCME cultures at passages 5 through 8 and analyzed by Northern blot analysis for the presence of FGF-5 transcripts, using a 1-kb human complementary DNA. Slot-blot analysis was performed to determine possible cross-reactivity between this probe and acidic and basic FGFs of human and bovine species. A previously characterized antibody directed against the aminoterminus of the human FGF-5 sequence was used in Western blot analyses to identify immunoreactive proteins released by BCME cells into the medium. Finally, the mitogenic activity of human recombinant FGF-5 on a variety of cell types was evaluated, using a cellular proliferation assay.

Results. Northern blot analysis provided evidence for the expression of two major FGF-5 transcripts at 4 kb and 3 kb and two minor transcripts at 2.2 kb and 1.7 kb. A single immunoreactive protein with a molecular weight of 34 kDa was identified by Western blot analysis of conditioned media. In cellular proliferation assays, human recombinant FGF-5 was not mitogenic in BCME cells but exhibited an approximate ED50 of 1.8 to 3.7 nM in BALB/c3T3 fibroblasts. This ED50 was within the range reported by the manufacturer, using a thymidine incorporation assay and a similar embryonic fibroblast cell line. Fibroblast growth factor-5 also stimulated proliferation of human retinal pigment epithelial cells.

Conclusions. Bovine choroidal microvascular endothelial cells exhibit expression in vitro of FGF-5 at the messenger RNA and protein levels. Perivascular and endothelial cell staining for FGF-5 seen previously in choroidal neovascular membranes may therefore arise from expression by choroidal endothelial cells. Because nonglycosylated recombinant FGF-5 does not appear to be a mitogen in BCME cells in vitro, it is reasonable to question its role as an autocrine mitogen in vivo. Fibroblast growth factor-5 may instead be serving paracrine roles in the stimulation of fibroblasts and retinal pigment epithelial cells during the formation of choroidal neovascular membranes. Studies with fully glycosylated recombinant FGF-5 will be required, however, to assess the biologic activity of this member of the FGF gene family. Invest Ophthalmol Vis Sci. 1997;38:2073-2080.

Fibroblast growth factor-5 (FGF-5) is one of nine members of the fibroblast growth factor gene family, whose products display a wide variety of biologic activities. The best characterized members of this family include acidic (aFGF) and basic (bFGF) FGF, which are potent mitogens and trophic factors for many cell types. Fibroblast growth factor-5 was discovered as the product of a human oncogene in the laboratory of Dr. Mitchell Goldfarb in 1988. It has 267 amino acids in its full-length form and possesses 30% to 50% homology with other members of the FGF gene family (aFGF and bFGF). In contrast to bFGF, FGF-5 possesses a hydrophobic N-terminus, which suggests that it is secreted through conventional cellular pathways and which implies an extracellular role for this protein.

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most phases of mammalian embryogenesis and in the adult central nervous system.\textsuperscript{4-7} It is released from skeletal myocytes and acts as a trophic factor in cultured spinal motoneurons.\textsuperscript{8} It also displays mitogenic activity in fibroblasts in vitro.\textsuperscript{2,9,10} However, its in vivo functions are largely unknown.

Because of observations that FGF-5 is expressed and secreted in the adult central nervous system, it was suggested that it may play an important role as an extracellular mediator in the biology of the retina. Thus, the distribution of FGF-5 in the normal adult retina of the rhesus macaque was examined in our laboratory through the use of immunohistochemistry and in situ hybridization. This work demonstrated the expression of FGF-5 by retinal pigment epithelial (RPE) cells, photoreceptors (especially cones), and ganglion cells. However, there was no evidence for expression of FGF-5 in the choroid or from endothelial cells in the normal retinal vasculature.\textsuperscript{11}

Further studies in our laboratory have investigated a possible role for FGF-5 in the pathogenesis of proliferative diseases of the retina. Results of immunohistochemical studies of choroidal neovascular membranes from patients with age-related macular degeneration demonstrated the presence of FGF-5 in proliferating choroidal endothelial cells and in perivascular regions. This finding is in contrast to that in avascular subretinal membranes from patients with proliferative vitreoretinopathy, which display low levels of diffuse staining for FGF-5.\textsuperscript{12}

The studies presented here were performed to establish whether choroidal endothelial cells are capable of producing and secreting FGF-5 in vitro. In this study, the question of whether FGF-5 may act as an autocrine mitogen in choroidal endothelial cells was examined to assess one of the possible roles FGF-5 may play in the pathogenesis of choroidal neovascularization.

METHODS

All experimental procedures conformed to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and the tenets of the Declaration of Helsinki.

Complementary DNA Probes

The human FGF-5 complementary DNA (cDNA) probe was a 1-kb \textit{EcoRI} fragment from plasmid pLTR122E.\textsuperscript{2} The human \textit{aFGF} cDNA was a 0.475-kb \textit{Nol–EcoRI} fragment from plasmid pJC3-5.\textsuperscript{13} The human bFGF cDNA was an 0.8-kb \textit{EcoRI} fragment from plasmid pHFL1-7.\textsuperscript{14} The bovine \textit{aFGF} cDNA was a 1.989-kb \textit{BamHI–Ndel} fragment from plasmid pKB8, obtained as a generous gift from Y. Courtois (Paris, France). The bovine bFGF cDNA was a 1.4-kb \textit{EcoRI} in vitro fragment from plasmid p[||11-1, obtained as a generous gift from J. Abraham (Scios Nova, Mountain View, CA).

Tissue Culture

Bovine choroidal microvascular endothelial (BCME) cells were a generous gift from Dr. Margaret Burns. These cells were isolated using the technique of Su and Gillies,\textsuperscript{15} contained factor VIII-related antigen, and were able to phagocytize acetylated low-density lipoprotein. For all experiments, BCME cells were used at passages 4 through 8 and were cultured in media consisting of Dulbecco’s modified Eagle’s medium (DMEM; Gibco, Grand Island, NY) containing 10% fetal bovine serum (FBS; JRH Biosciences, Lenexa, KS). All flasks and plates used to culture BCME cells were coated with 50 μg/ml rat-tail collagen (Collaborative Biomedical Products, Bedford, MA) in 0.02 N acetic acid.

Bovine aortic endothelial cells were a generous gift from the lab of Dr. Martha O’Donnell and were cultured in media consisting of DMEM, containing 10% FBS. These cells were used at passages 6 through 8. Human umbilical vein endothelial cells were obtained from American Type Culture Collection (Rockville, MD) and were cultured in media consisting of F-12K Nutrient Mixture (Kaighn’s modification; Gibco) containing 100 μg/ml sodium heparin, 20% FBS, 100 μg/ml endothelial cell growth supplement (ECGS; Becton Dickinson, Bedford, MA), and 1 g/l additional sodium bicarbonate.

The primary culture and routine maintenance of human RPE cells were described previously.\textsuperscript{16} These cells were used at passages 14 through 16 and were cultured in media consisting of DMEM–Ham’s F-12 with L-glutamine and 15 mM Hepes buffer (Bio-Whittaker, Walkersville, MD), containing 10% FBS, 0.348% additional sodium bicarbonate, and 2 mM L-glutamine solution (Gibco). BALB/c3T3 murine fibroblasts were obtained from American Type Culture Collection and were cultured in DMEM with 10% calf serum (Gibco).

Cultures were incubated at 37°C in 10% CO\textsubscript{2}, and the medium was changed weekly for RPE cells and biweekly for BCME and BALB/c3T3 cells. Routine passage of cultures was accomplished by dissociation of the monolayers in 0.05% (wt/vol) trypsin and 0.02% (wt/vol) ethylenediaminetetraacetic acid in calcium–magnesium-free Hank’s balanced salt solution.

RNA Isolation and Northern Blot Analysis

Total RNA was isolated according to the method of Chomczynski.\textsuperscript{17} Essentially, monolayers of cells grown to near confluence were lysed directly in culture flasks by the addition of Tri Reagent LS (Leedo, Houston, TX), which includes phenol and guanidine thiocya-
nate; the resulting homogenate was extracted with chloroform, precipitated in isopropanol, and washed with 75% ethanol. The final RNA pellets were resuspended in Molecular Biology Grade Water (5Prime 5Prime, Boulder, CO) and the concentration of RNA was determined by spectrophotometric analysis. Total RNA samples were electrophoresed in formaldehyde–agarose gels and transferred to 0.45 μm Hybond-N membrane (Amersham, Arlington Heights, IL) according to standard procedures. After washing, the blots were UV cross-linked on a Stratalinker UV cross-linker (Stratagene, La Jolla, CA). Northern blots were probed with human FGF-5 cDNA probe, which was radiolabeled with a random priming labeling kit (GibcoBRL, Gaithersburg, MD). Blots were prehybridized for 2 hours at 55°C in hybridization buffer without formamide (5 × SSC, 5 × Denhardt’s solution, 100 μg/ml salmon sperm DNA, 0.1% sodium dodecyl sulfate [SDS]). Radiolabeled cDNA probe (4 ng/ml with specific activity of 6 × 10⁸ cpm/mg) was heated to 100°C for 10 minutes and added to the hybridization buffer. The blot was hybridized at 55°C for 15 hours and then washed for 2 hours (three changes of buffer) in 0.5 × SSC–0.1% SDS at 55°C. Blots were exposed and developed according to standard procedures.

**Slot-Blot Analysis**

A nylon Hybond-N membrane (Amersham) was immersed in water and placed in the slot-blot manifold. The cDNAs were pipetted directly onto the nylon membrane, using a dilution series ranging from 30 ng to 0.1 ng for each lane, and vacuum was applied to draw fluid through the membrane. The membrane was removed and placed on 3MM chromatography paper (Whatman, Fairfield, NJ) and soaked in 0.5 N NaOH for 1 minute to denature the cDNA; the procedure was repeated twice. The membrane was placed on 3MM paper soaked in 1 M Tris-HCl, pH 7.5, and in 2 × SSC. After drying, the blot was cross-linked, hybridized, exposed, and developed as described in the methods section for Northern blot analysis.

**Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis and Western Blot Analysis**

Bovine choroid microvascular endothelial cells grown to confluence in T75 culture flasks were used to condition DMEM culture media with 5% FBS, 10 μM N⁶,⁴⁰-Dibutyryl-cAMP and 5 μg/ml porcine sodium heparin (Sigma) for 24 hours. Conditioned media were mixed overnight at 4°C with 1.5 μl heparin–Sepharose (Pharmacia, Piscataway, NJ) per milliliter of media. The heparin–Sepharose was collected by centrifugation, washed twice with 15 mM Tris and 0.2 M NaCl and twice with 10 mM Tris-HCl, pH 7.6; proteins were eluted by boiling in 2 × sample buffer for 4 minutes. Positive control lanes consisted of human recombinant FGF-5 (R & D Systems, Minneapolis, MN) with 0.1% bovine serum albumin and 1 μg/ml sodium heparin. Identical conditioning media without exposure to BCME cells was treated similarly with heparin–Sepharose as above and was used as a negative control. Biotinylated, broad-range molecular weight markers (Bio-Rad Laboratories, Richmond, CA) were added at 3 μl (diluted to final volume with 1 × sample buffer) per lane. Samples of 24 μl each were electrophoresed on a 12% SDS polyacrylamide gel and transferred to 0.45 μm nitrocellulose membrane (Schleicher & Schuell, Keene, NH); and blots were probed and visualized, as previously described, with the exception of a different Tris-buffered saline–high salt (TBS–HS) solution containing 20 mM Tris-HCl with 0.4 M NaCl. We used a previously described affinity-purified rabbit polyclonal antibody (#4539), which was generated against the aminoterminus (CFKQSEQPELSFTVTVPEK) of the human FGF-5 sequence.

**Mitogenesis Assay**

Cells were plated in 24-well tissue culture plates (Costar, Cambridge, MA). After 24 hours the media were removed, the wells were rinsed with media without serum, and media were replaced with 1.5 ml/well of the appropriate low-serum medium with 5 μg/ml of sodium–heparin, containing sequential dilutions of the appropriate mitogen. Human recombinant bFGF was a generous gift from Dr. J. Abraham (CalBio, Mountain View, CA). The cultures were then incubated under standard conditions for several days, at which time the cells were trypsinized and counted, using a Coulter counter (Coulter Electronics, Hialeah, FL). Data are expressed as a percentage of average experimental net cell counts to average control net cell counts.

**RESULTS**

**Expression of Fibroblast Growth Factor-5 by Bovine Choroidal Microvascular Endothelial Cells**

We first sought to demonstrate expression of FGF-5 by BCME cells at the level of mRNA, using the method of Northern blot analysis. Total RNA for Northern blot analysis was isolated from near-confluent cultures of BCME cells. As shown in Figure 1, this experiment provides evidence for the expression of FGF-5 mRNA by BCME cells. There are two major (4-keb and 3-keb) and two minor (2.2-keb and 1.7-keb) transcripts seen. The lane of total RNA from human RPE cells was used as positive control because RPE cells have been shown previously to express FGF-5. The expected major
BCME hRPE

FIGURE 1. Northern blot analysis of FGF-5 expression in BCME cells. The BCME and human RPE cells were grown to confluence in complete medium, and messenger RNA was isolated from each culture. Samples of BCME messenger RNA (25 μg), lane 1, and human RPE messenger RNA (5 μg), lane 2 (as positive control), were run on formaldehyde-agarose gels and blotted onto nylon membranes. The blot was probed with a 32P-labeled human FGF-5 complementary DNA probe, followed by autoradiography. The locations of the 28s and 18s ribosomal RNA bands are shown. BCME = bovine choroidal microvascular endothelium; FGF = fibroblast growth factor; RPE = retinal pigment epithelium.

transcripts at 4 kb and 2.1 kb from human RPE total RNA were detected.

A slot-blot analysis was performed to be certain that our human FGF-5 cDNA probe would not cross-react with human or bovine aFGF and bFGF DNA sequences. As shown in Figure 2, there was no hybridization between the human FGF-5 cDNA probe and the human and bovine aFGF and bFGF cDNA probes when using the same hybridization conditions employed in the Northern blot analysis. There was a strong hybridization signal, which diminished appropriately according to the dilution series, between the human FGF-5 cDNA probe and itself as a positive control.

Next, Western blot analysis was performed to demonstrate release of FGF-5 protein in conditioned media isolated from BCME cells. Heparin-binding proteins from conditioned media were electrophoresed on a 12% polyacrylamide gel, alternating with lanes of human recombinant FGF-5 as positive control. An affinity-purified antibody directed against the amino-terminus of the human FGF-5 sequence was used as a probe at a dilution of 1:200. In Figure 3, lane 2 shows a band at 34 kDa, representing positive control for FGF-5, with lane 3 of BCME-conditioned media also showing a distinct band at 34 kDa. To be certain that this latter band indeed represents FGF-5, a blocking control was performed in lanes 5 and 6 by absorbing the antibody onto a column of human recombinant FGF-5, coupled to AffiGel-10 (Biorad, Richmond, CA), demonstrating the disappearance of the specific band at 34 kDa. No bands were observed in lane 4, consisting of serum-containing media that had not been conditioned by BCME cells. Lanes 7 and 8 show nonspecific bands when probed with equivalent amounts of nonimmune rabbit immunoglobulin G as another negative control. Results of previous studies have demonstrated that this class of antibodies does not cross-react with aFGF or bFGF.

Mitogenic Effects of Fibroblast Growth Factor-5

Mitogenesis assays were performed to determine the possible role of FGF-5 as an autocrine mitogen in choroidal endothelial cells. The positive control for this assay was bFGF, which is known to be a potent mitogen in endothelial cells. The data in Figure 4 show that bFGF is mitogenic in BCME cells. In our experiments, the ED50 was approximately 20 pM, with a 2.3-fold maximum population increase over control cell counts in a 4-day period. We were surprised to find that no concentration of human recombinant FGF-5 tested, ranging from 40 nM to 0.4 pM, provoked a mitogenic response in BCME cells. In fact, all concentrations demonstrated inhibition of cell growth. The maximum inhibitory effect (60% of control cell counts) was observed at the highest concentration tested (40 nM; data not shown).
Fibroblast Growth Factor-5 Expression by Choroidal Endothelial Cells

Molecular Weight (kDa)

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Choroidal neovascular membranes. They have also been reported to express FGF-5 in vivo and in vitro. As demonstrated in Figure 6, human recombinant FGF-5 is mitogenic in human RPE cells. Although a true maximum response has not been determined by this assay, the approximate ED₅₀ of 4 nM is consistent with that seen in fibroblasts. Basic FGF was also mitogenic for RPE cells, a finding that has been demonstrated previously.²⁰

**FIGURE 3.** Western blot analysis of media conditioned by BCME cells probed with an anti-FGF-5 antibody. The BCME cells were passaged at a split ratio of 1:6 in T75 flasks and grown to confluence (5 days). Media were then replaced with conditioned media with 5% FBS, 10 μM cyclic adenosine monophosphate, and 5 μg/ml heparin and were collected 24 hours later. Heparin-binding proteins were concentrated on heparin–agarose overnight, eluted in sample buffer, electrophoresed on a 12% SDS–PAGE gel, and transferred onto a nitrocellulose membrane. Lane 1 contains biotinylated molecular weight standards. Lanes 2, 5, and 7 each contain 25 ng of human recombinant FGF-5. Lanes 3, 6, and 8 each contain heparin-binding proteins from 17.5 ml of conditioned media concentrated on 26 μl of heparin–agarose. Lane 4 contains identical conditioning media without exposure to cells. Lanes 2, 3, and 4 were probed with a 1:200 dilution of an affinity-purified anti-FGF-5 antibody (SC4539). Lanes 5 and 6 were probed with the same antibody, which had been absorbed onto a recombinant FGF-5 coupled to AffiGel-10. Lanes 7 and 8 were probed with an equivalent concentration of nonimmune rabbit immunoglobulin G (1 μg/ml). Bands were visualized by indirect alkaline–phosphatase immunohistochemistry. BCME = bovine choroidal microvascular endothelium; FBS = fetal bovine serum; FGF = fibroblast growth factor; SDS–PAGE = sodium dodecyl sulfate–polyacrylamide gel electrophoresis.

To provide a positive control for the mitogenic activity of human recombinant FGF-5, we examined BALB/c3T3 cells, fetal murine fibroblasts that have previously been shown to be stimulated by recombinant FGF-5.¹⁰ This assay demonstrates that our preparation of human recombinant FGF-5, obtained commercially, is mitogenic in BALB/c3T3 fibroblasts (Fig. 5). Although our assays do not yield a true maximum response, the range of activity (ED₅₀ = 0.05 to 0.1 μg/ml, which is equivalent to 1.8 to 3.7 nM) is consistent with that reported by the manufacturer, using a thymidine incorporation assay and a similar fibroblast cell line of fetal origin. Basic FGF was mitogenic in these cells, although with a peak mitogenic effect at a lower concentration than in BCME cells with maximum activity at approximately 6 pM.

We evaluated the mitogenic activity of FGF-5 in RPE cells, because they are a prominent cell type in choroidal neovascular membranes. They have also been reported to express FGF-5 in vivo and in vitro. As demonstrated in Figure 6, human recombinant FGF-5 is mitogenic in human RPE cells. Although a true maximum response has not been determined by this assay, the approximate ED₅₀ of 4 nM is consistent with that seen in fibroblasts. Basic FGF was also mitogenic for RPE cells, a finding that has been demonstrated previously.²⁰

**DISCUSSION**

High-stringency hybridization and washing conditions yielded no detectable signal on a blot of total RNA from BCME cells probed with the human FGF-5 sequence. Only by significantly lowering the hybridization and washing stringency were we able to detect clearly BCME mRNA specific for FGF-5. We found that the major FGF-5 transcripts of 4 kb and 3 kb, observed in the BCME total RNA, were similar in size to the

**FIGURE 4.** Mitogenic activity of FGF-5 and basic FGF in BCME cells. The BCME cells were plated (20,000 cells/well) in 24-well, collagen-coated plates in complete medium. After 24 hours, the medium was changed to assay medium (DMEM with 0.5% calf serum and 5 μg/ml of heparin) containing concentrations of the growth factor to be evaluated (□ = basic FGF, 0 = FGF-5). Each concentration was evaluated in triplicate wells. Cells were counted 4 days after adding the growth factor. Data are expressed as the percentage of control cell counts, with standard error bars shown when they exceed 5% of the calculated mean. BCME = bovine choroidal microvascular endothelium; DMEM = Dulbecco’s modified Eagle’s medium; FGF = fibroblast growth factor.
FIGURE 5. Mitogenic activity of FGF-5 and basic FGF in BALB/c3T3 fibroblasts. Cells were plated (10,000 cells/well) in 24-well plates in complete medium. After 24 hours, the medium was changed to assay medium (DMEM with 0.5% calf serum and 5 μg/ml of heparin) containing concentrations of the growth factor to be evaluated (□ = basic FGF, ○ = FGF-5). Each concentration was evaluated in triplicate wells. Cells were counted 4 days after adding the growth factor. Data are expressed as the percentage of control cell counts, with standard error bars shown when they exceed 5% of the calculated mean. DMEM = Dulbecco's modified Eagle's medium; FGF = fibroblast growth factor.

These investigators have stated that the unmodified FGF-5 precursor is a 29.5-kDa protein, as is predicted from the coding sequence. In the current study, the finding of a larger single band for the BCME-conditioned media probably represents a secreted and thus presumably glycosylated form of FGF-5. The band represents a bovine protein whose coding sequence is unknown and could be a different length than that of the human FGF-5 protein. There may also be differing glycosylation processes of endothelial cells in contrast to that of other cells studied previously. Finally, there may be differences in truncation of the sequence through posttranslational modification processes by these cells. These speculations may explain the unusual observation that the fully processed and secreted form of bovine FGF-5 runs at the same position on an SDS gel as does the recombinant bacterial form, which presumably has a molecular weight of 29 kDa. In any case, our SDS gel did not correctly estimate the molecular weight of the bacterial form.

Although FGF-5 has repeatedly been hypothesized to be a mitogen in endothelial cells, only one known

FIGURE 6. Mitogenic activity of FGF-5 and basic FGF in RPE cells. The RPE cells were plated (5000 cells/well) in 24-well plates in complete medium. After 24 hours, the medium was changed to assay medium (DMEM–F-12 with 0.5% FBS and 5 μg/ml of heparin) containing concentrations of the growth factor to be evaluated (□ = basic FGF, ○ = FGF-5). Each concentration was evaluated in quadruplicate. Cells were counted 7 days after adding the growth factor. Data are expressed as the percentage of control cell counts, with standard error bars shown when they exceed 5% of the calculated mean. DMEM = Dulbecco's modified Eagle's medium; FGF = fibroblast growth factor; RPE = retinal pigment epithelium.
report in the literature supports this claim. Zhan et al in 1988 used conditioned media from FGF-5-transformed NIH 3T3 cells to stimulate the proliferation of fetal bovine heart endothelial cells. Our results demonstrate that bacterial recombinant FGF-5 inhibits growth of BCME cells at all concentrations tested. Trivial explanations for this discrepancy can be ruled out by our control experiments. Bovine choroidal microvascular endothelial cells exhibit dose-dependent growth in response to bFGF. This experiment serves as a positive control for the assay conditions and for the FGF response of BCME cells. We also controlled for the bioactivity of our preparation of bacterial recombinant FGF-5 by demonstrating a dose-dependent proliferative response of BALB/c3T3 fibroblasts. This confirms previous observations on BALB/c3T3 cells, using conditioned media and recombinant FGF-5. An additional explanation for these observations may be that RPE cells and BCME cells express and secrete FGF-5, which may already maximally activate receptors to which bacterial recombinant FGF-5 is added during the mitogenesis assay. This intriguing possibility would require a precipitating antibody for future exploration, which we unfortunately lack at the present time.

It is possible that there are significant differences in activity between recombinant FGF-5 produced by prokaryotes and glycosylated forms of FGF-5 produced in higher eukaryotic cells. In this study, we used recombinant FGF-5 expressed in *Escherichia coli* whose stability in vitro and ability to bind to receptors may be significantly lessened compared with that of the more fully processed, glycosylated forms of FGF-5 present in vivo. Our results concerning the mitogenic activity of FGF-5 on endothelial cells and RPE cells must therefore be taken with caution. Future studies will examine directly the activity of pure preparations of glycosylated human FGF-5 on endothelial and RPE cells.

Taken together, these data pose two large questions concerning FGF-5. First, why is FGF-5 not expressed at constitutive levels in vivo in endothelial cells in the choroid, when it is expressed by choroidal endothelial cells in vitro and in vivo in choroidal neovascularization? The data in the current study do not evaluate the regulation of FGF-5 gene expression by endothelial cells. Secondly, what is the biologic function of the FGF-5 expressed during choroidal neovascularization? The data from this study suggest two possibilities. Fibroblast growth factor-5 may function as a paracrine mitogen in nonendothelial cell types, including fibroblasts and RPE cells, in the process of choroidal neovascularization. The second possibility is that FGF-5 may be acting as an autocrine inhibitor of endothelial cell growth. Both of these possibilities are speculations; our studies do not directly evaluate roles in vivo for FGF-5. Resolution of these questions will require several additional studies, including our planned analysis of the mitogenic activity of glycosylated human FGF-5.

**Key Words**
cell proliferation, choroid, endothelium, fibroblast growth factor, neovascularization

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