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High Affinity Immunoreactive FGF Receptors in the Extracellular Matrix of Vascular Endothelial Cells—Implications for the Modulation of FGF-2

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Abstract. We recently characterized three FGF-binding proteins (FGF-BPs) which are soluble forms of the extracellular domains of the high affinity FGF receptors (Hanneken, A. M., W. Ying, N. Ling, and A. Baird. *Proc. Natl. Acad. Sci. USA*. 1994. 91:9170–9174). These proteins circulate in blood and have been proposed to modulate the biological activity of the FGF family of proteins. Immunohistochemical studies now demonstrate that these soluble, truncated FGF receptors are also present in the basement membranes of retinal vascular endothelial cells. These immunoreactive proteins can be detected with antibodies raised to the extracellular domain of FGFR-1 but not with antibodies raised to either the juxtamembrane domain or the cytoplasmic domain of FGFR-1. Western blotting of human retinal extracts with the antibody raised to the extracellular domain of FGFR-1 detects specific, low molecular mass proteins at 85 kD and 55 kD, corresponding in size to the FGF-BPs, which are not detected with antibodies against the cytoplasmic domain of the receptor.

The interaction of this receptor with the extracellular matrix is not dependent on the presence of FGF-2. Immunoreactive receptors are still detected in vascular basement membranes after the removal of FGF-2 with heparitinase. In addition, the recombinant extracellular domain of FGFR-1 continues to bind to corneal endothelial cell matrix after endogenous FGF-2 has been removed with 2 M NaCl. Acid treatment, which has been shown to disrupt protein interactions with the extracellular matrix, leads to a significant reduction in the presence of the matrix form of the FGF receptor. This loss can be restored with exogenous incubations of the recombinant extracellular domain of FGFR-1.

This report is the first demonstration that a truncated form of a high affinity growth factor receptor can be localized to the extracellular matrix. These findings add to the list of binding proteins associated with the extracellular matrix (IGFBP-5) and suggest a potentially new regulatory mechanism for controlling the biological availability of FGF, and other peptide growth factors, in the extracellular matrix.

BASIC fibroblast growth factor (FGF-2)¹ is a heparin-binding growth factor which is widely distributed in the basement membranes of many tissues (4, 6, 10). Although very low quantities of FGF-2 stimulate the proliferation of cells in vitro, the vast majority of cells which are surrounded by this FGF in vivo are quiescent. This highly controlled state suggests that the basement membrane stores of FGF-2 are relatively inert. Accordingly, several mechanisms have been proposed to explain this phenomenon: regulation by the number of high affinity cell surface FGF receptors, release of enzymes by target cells, and post-

translational modification of FGF (1). However, the issue remains largely unresolved.

We recently identified three circulating FGF-binding proteins (FGF-BPs) in human and bovine blood. These proteins, with molecular masses ranging from 55–85 kD, were detected by searching for proteins that bind to FGF-heparin Sepharose affinity columns and bind to FGF-2 by ligand blotting (12). Immunoblotting and protein sequencing data demonstrate that these proteins are forms of the extracellular domain of the high affinity FGF receptor, FGF receptor-1 (FGFR-1). Since the recombinant extracellular domain of FGFR-1 inhibits the biological properties of FGF-2 (18), these circulating FGF-BPs have the potential to modulate the biological activities of FGF-2, and other members of the FGF gene family, in vivo.

We show here that a truncated form of an immunoreactive high affinity FGF receptor is present in the extracellular matrix of vascular endothelial cells. The interaction of this receptor with the extracellular matrix is not affected by the removal of FGF-2 after heparitinase treatment but is reduced by treatment with 1N acetic acid. These data suggest that

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1. *Abbreviations used in this paper:* ACE, adrenal bovine capillary endothelial cell; BCE; bovine corneal endothelial cell; FGF-2, basic fibroblast growth factor; FGF-BP, FGF-binding protein; FGFR-1, FGF receptor-1.

FGF-2 may interact with both heparan sulfate and a truncated form of the FGF receptor in the basement membrane. Thus, an extracellular matrix-bound receptor may play a role in regulating the biological activities and availability of the FGFs in the basement membrane.

Materials and Methods

Antibodies

A number of different monoclonal antibodies to FGF receptors were used in this study. Ab6 is a mouse monoclonal antibody that was raised against the three Ig loop form of the extracellular domain (ECD) of human FGFR-1 expressed in insect cells (18). It was purified from ascites by ammonium sulfate precipitation and protein A purification (manuscript in preparation). It is a pan-specific FGF receptor antibody that recognizes recombinant FGFR-1, FGFR-2, and FGFR-3 and their extracellular domains by Western blotting. It recognizes the higher molecular weight FGF receptors on 3T3 cells and BHK cells, and the 55-kD and 85-kD truncated FGFR-1 receptors (FGF-BPs) which have been purified and sequenced from bovine calf serum (12). Antibody 50 is a mouse monoclonal antibody raised against a 16-amino acid peptide (EYELPEDPRWELPRDR) located in the juxtamembrane domain of FGFR-1. This sequence is well conserved among the different members of the FGF receptor gene family. Ab50 recognizes FGFR-1 and R-2 and was a generous gift from the laboratory of Dr. L. R. Williams (UCSF, San Francisco, CA) (3). Antibody 803 is an affinity-purified rabbit polyclonal antibody raised against a 17-amino acid peptide sequence (CLPRHPAQLANGGLKRR) located at the carboxyl terminus of FGFR-1. The immune serum was passed over a peptide affinity column prepared by conjugating the antigenic peptide to Affigel 102. Affinity-purified antibody was eluted from the column with 0.2M HCl-glycine and is specific for FGFR-1 (manuscript in preparation).

Antibodies to heparan sulfate proteoglycan, type IV collagen, and laminin were purchased from Chemicon (Temecula, CA), antibodies to Factor VIII related antigen were purchased from Dako Corporation (Carpinteria, CA).

Reagents

A nonglycosylated, three Ig loop form of the extracellular domain of FGFR-1 was prepared in *E. coli*. The sequence encoding the extracellular domain of human FGFR-1 was amplified from a plasmid containing the entire coding sequence of FGFR-1 (15) using specific primers and the polymerase chain reaction. The product was subcloned into the pMAL-p2 vector (New England Biolabs., Boston, MA) and the recombinant plasmid used to transform *E. coli*. The extracellular domain was expressed and purified according to the manufacturer's instructions. A glycosylated form of the three Ig loop form of the extracellular domain of FGFR-1 was expressed in insect cells and was a generous gift from Drs. P. Barr and M. Kiefer (Chiron Corp., Emeryville, CA) (18).

Cells and Media

Confluent adrenal bovine capillary endothelial (ACE) were grown in HDMEM (Gibco Life Technologies, Inc., Grand Island, NY) with 10% calf serum (Hyclone Labs, Logan, UT) and 100 U/ml penicillin, and 100 µg/ml streptomycin. Bovine corneal endothelial (BCE) cells were a generous gift of Dr. Sandy Feldman (UCSD, San Diego, CA). The cells were maintained in DMEM: Nutrient Mixture F-12 1:1 (DMEM/F12; Gibco Life Technologies, Inc.) supplemented with 10% FCS (Hyclone Laboratories) and 15 mM Hepes buffer, 100 U/ml penicillin, and 100 µg/ml streptomycin.

Immunohistochemistry

Tissue sections (12 µm) were cut on a cryostat at -20°C and placed on gelatin-coated slides. Sections were fixed in 2% paraformaldehyde for 5 min, treated with 3% H₂O₂, blocked with 1% BSA for 20 min, and processed with the use of a streptavidin-peroxidase technique previously described (11). FGF receptor antibodies (antibodies 6, 50, 803) were used at a concentration of 14 µg/ml and incubated with the sections for 90 min at 37°C. Controls for the monoclonal antibodies consisted of identical incubations with an unrelated hybridoma mouse IgG₁ (Jackson ImmunoResearch Labs., West Grove, PA). Controls for the polyclonal antibody consisted of incubations with the unretained fractions (flowthrough) of the peptide affinity purification column.

Tissue Extractions and Western Blot Analyses

Human retinas were obtained from autopsy eyes within the first 4–24 h post-mortem. Retinas were homogenized at 4°C in 0.32 M sucrose, 10 mM Tris-HCl (pH 7.4), 1 mM magnesium chloride (MgCl₂), containing 1 mg/ml each leupeptin, aprotinin, and pepstatin A, and 1 mM PMSF. The homogenate was centrifuged at 1,000 g for 5 min to remove nuclei and cell debris. The supernatant was spun at 17,500 g for 15 min. The pellet was then resuspended in homogenization buffer, sonicated, solubilized in Laemmli's sample buffer, boiled at 100°C for 5 min, and the proteins separated on a 7.5% SDS-polyacrylamide gel. The proteins were transferred to nitrocellulose (Schleicher and Schuell, Keene, NH) and immunoblotted with 5 µg/ml of Ab6 or Ab50 in Tris-buffered saline (TBS), pH 7.4, with 5% nonfat milk (NFM). After an overnight incubation, the nitrocellulose was washed three times with TBS, treated with 1 µg/ml of affinity-purified rabbit anti-mouse IgG (Jackson ImmunoResearch Labs.) in TBS, 5% NFM for 2 h, washed three times with TBS, and incubated with 2 µCi of ¹²⁵I-Protein A (ICN Radiochemicals, Irvine, CA) in 5% NFM for 2 h. After washing three times in TBS, the blots were dried in a 60°C oven, and exposed to XAR5 autoradiographic film (Eastman Kodak, Rochester, NY) at -70°C as indicated.

Confluent adrenal capillary endothelial cells were washed in cold PBS (×2) and solubilized with Laemmli's sample buffer. The proteins were separated on a 7.5% SDS-polyacrylamide gel and Western blots were performed as described above.

Enzyme Digestions

Fresh frozen tissue sections were fixed in 2% paraformaldehyde for 5 min and washed three times over 15 min. Heparitinase digestions were performed for 30–60 min at 37°C using 0.01 IU/slide of heparitinase (ICN ImmunoBiologicals, Costa Mesa, CA) prepared in 50 mM Tris-HCl, 20 mM Ca-Acetate, pH 7.4 (13). Collagenase digestions were performed using 0.05 mg/ml of type IV collagenase (Sigma Chem. Co., St. Louis, MO) prepared in PBS. Chondroitinase digestions were performed using 20 U/slide Chondroitinase ABC (Sigma Chem. Co.) prepared in PBS with 1 mM CaCl₂, 1 mM MgCl₂. The slides were next treated with 3% H₂O₂ and incubated with Ab6, or stained with antibodies to FGF-2 as described (9). Control sections were treated with buffer alone.

Acid Dissociation and Exogenous Binding of the ECD to Matrix

Fresh frozen or 2% paraformaldehyde fixed sucrose embedded sections were cut on a cryostat and air-dried. The slides were treated with 1 N acetic acid for 30 min at room temperature, and then washed with PBS. After acid treatment, the sections were fixed in 2% paraformaldehyde and staining was performed according to the protocol outlined above. In some cases, after a 20-min block in 1% BSA, tissue sections were incubated with 10 µg/ml nonglycosylated extracellular domain in 1% BSA (to prevent nonspecific binding). After five washes with PBS, the sections were incubated with Ab6 (14 µg/ml).

Matrix-binding Studies

Bovine corneal endothelial cell matrix was prepared by incubating a confluent plate of BCE cells with 20 mM NH₄OH in H₂O according to published techniques (8). The plates were washed with PBS four times to remove cell debris. To evaluate the ability of the extracellular domain to bind to basement membrane, bovine corneal endothelial cell matrix was incubated with the insect or *E. coli* expressed extracellular domain of FGFR-1 for 30 min at 37°C at a concentration of 0.5 µg/ml in 1% BSA (to prevent nonspecific binding). In some cases, matrix was first treated with 2M NaCl at 37°C for 30 min to remove FGF-2, and then incubated with the recombinant extracellular domain of FGFR-1 in 1% BSA. After the incubations, the matrix was washed extensively, solubilized with 2.5 × sample buffer, and the proteins were separated on a 7.5% SDS-polyacrylamide gel, transferred to nitrocellulose, and blotted with Ab6 as described above.

Ligand Blotting

Ligand blotting was performed by solubilizing 150–300 ng of recombinant ECD (prepared in insect cells) in 1× Laemmli's sample buffer under non-reducing conditions. The proteins were separated on an 8% SDS-polyacrylamide gel, and transferred to nitrocellulose in a Tris/glycine transfer buffer (25 mM Tris base, 190 mM glycine). The nitrocellulose was blocked with 5% nonfat milk in TBS (20 mM Tris-HCl/150 mM NaCl, pH

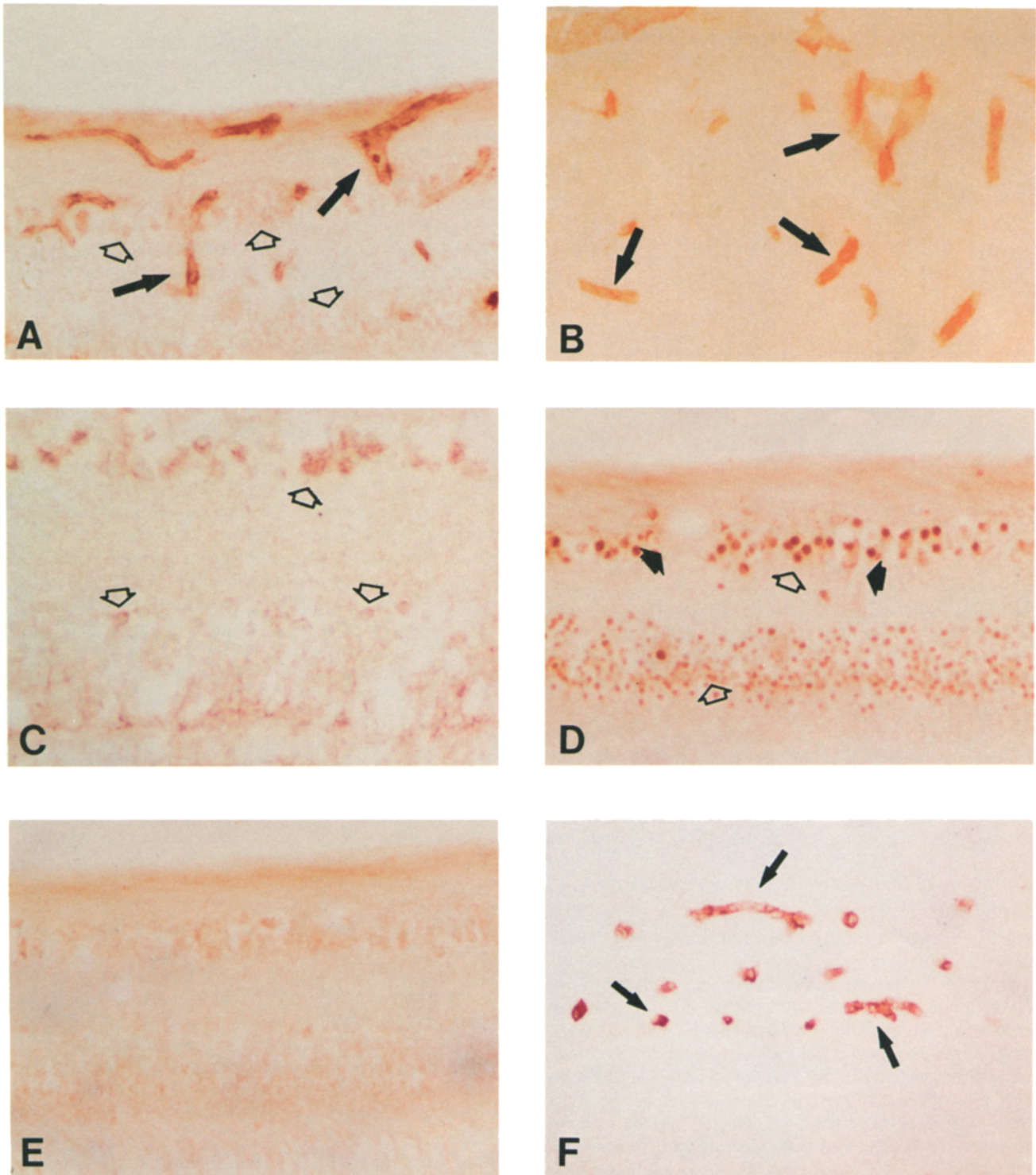


Figure 1. Vascular basement membrane localization of FGF receptors in human retina. Twelve micron cryostat sections of human retina were fixed with 2% paraformaldehyde, incubated with 3% H₂O₂, blocked with 1% BSA, and processed using a streptavidin peroxidase technique as described in Materials and Methods. Immunohistochemical localization with antibodies to (A) the extracellular domain of FGFR-1, (Ab6); (B) FGF-2 (9); (C) the juxtamembrane domain of FGFR-1, (Ab50); (D) the carboxyl terminus of FGFR-1, (Ab803); (E) control hybridoma mouse IgG₁; and (F) heparan sulfate proteoglycan. Solid black arrows highlight vascular basement membranes and open arrows highlight neuronal staining. Note the red reaction product in basement membranes in A-B-F and the absence of basement membrane staining in C-D.

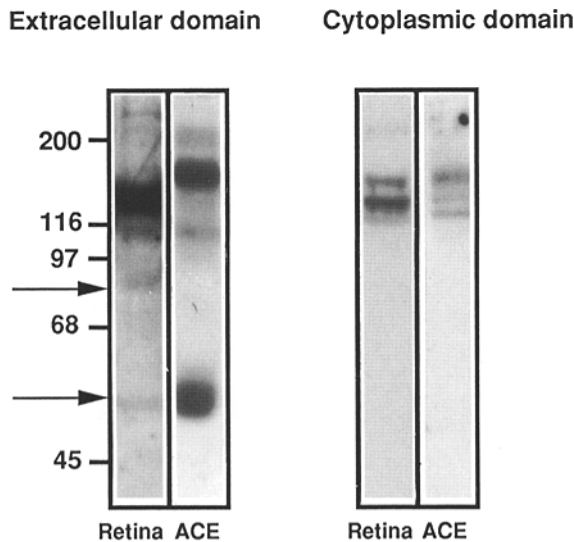


Figure 2. Western blot analysis of FGF receptors in human retina and adrenal capillary endothelial (ACE) cells. Tissue and cell extracts were prepared as described in Materials and Methods and immunoblotted using antibodies to the extracellular domain of FGFR-1 (Ab6, *left panel*) or antibodies to the juxtamembrane region of FGFR-1 (Ab50, *right panel*). Both antibodies recognize high molecular mass bands between 125–140 kD but only Ab6 recognizes 85 kD and 55 kD proteins in human retina and a 55–60-kD protein in ACE cells.

7.4) for 30 min and incubated overnight with 500,000 cpm of ^{125}I -FGF-2 per ml in the presence or absence of 50 μg of unlabeled recombinant FGF-2. The nitrocellulose was then washed five times with TBS and exposed to Kodak XAR5 film overnight.

Results

Localization of FGF Receptor Immunoreactivity to Basement Membranes

While examining the distribution of high affinity FGF receptors in the human retina, we observed a consistent pattern of staining that was unexpectedly associated with microvascular basement membranes. To confirm that this staining represented the extracellular domain of a high affinity FGF receptor, immunohistochemical studies were performed with three distinct antibodies to FGFR-1. Using an antibody which recognizes the extracellular domains of the high affinity FGF receptors (Ab6), we detected immunoreactive FGF receptors in neuronal cells throughout the inner and outer retina (Fig. 1 *A*, *open arrows*). Remarkably, we also detected immunoreactive FGF receptors in the basement membranes of retinal capillary endothelial cells (Fig. 1 *A*, *closed arrows*). This matrix staining was very similar to the patterns observed with antibodies to Factor VIII and antibodies to several different basement membrane proteins, including type IV collagen, laminin, and heparan sulfate proteoglycan (Fig. 1 *F*). Surprisingly, this basement membrane staining of FGFR was also identical to the basement membrane staining of FGF-2 (9), indicating that FGF-2 and high affinity FGF receptor immunoreactivity colocalize to the extracellular matrix (Fig. 1 *B*).

To determine whether the form of the FGF receptor im-

munoreactivity in the basement membrane consisted only of the extracellular domain of the receptor, we performed immunohistochemical studies using FGF receptor antibodies raised to two different regions of the cytoplasmic domain. Antibodies raised to the juxtamembrane region (Ab50) and the carboxyl terminus (Ab803) of the high affinity FGF receptors localize immunoreactive FGF receptors to neuronal cells (*open arrows*, Fig. 1, *C–D*), consistent with the cell staining obtained with Ab6. However, no staining was observed in vascular basement membranes with these antibodies. Thus, vascular basement membranes appear to contain a form of FGF receptor immunoreactivity which lacks the cytoplasmic domain. Mouse IgG (Fig. 1 *E*) and the flow through from the polyclonal affinity column (not shown) were both used as negative controls and showed only faint background staining.

Western Blot Analysis with FGF Receptor Antibodies

The immunohistochemical detection of the extracellular domain of a high affinity FGF receptor in microvascular basement membranes predicts that a low molecular mass form of an FGF receptor (50–90 kD) should be detected in extracts of retina and capillary endothelial cells. The immunohistochemical studies also predict that the low molecular weight FGF receptor will be detected by Ab6, but not by Ab50, because Ab50 does not recognize the extracellular domain of the FGF receptor. As expected, immunoblotting of capillary endothelial cells and human retinal extracts with Ab6 and Ab50 reveals high molecular mass bands between 116–140 kD which correspond to the expected sizes of the full length, transmembrane high affinity FGF receptors (Fig. 2). Only Ab6 detects additional low molecular mass proteins at 85 kD and 55 kD in human retinal extracts and 55–60 kD proteins in capillary endothelial cell extracts. These proteins are similar in size to the FGF-binding proteins which have been isolated from blood and shown to be portions of the extracellular domain of FGFR-1 (12).

A 200+ kD band, which is higher than any of the expected molecular masses for high affinity FGF receptors, is also present in the immunoblots with Ab6 and Ab50. The intensity of this band varies as a function of the preparation of the cells and is thought to represent a high molecular weight aggregate. A 116-kD band, which is detected by immunoblotting with Ab6 but not with Ab50, is most likely a form of FGFR-3 since Ab6 is pan-specific and recognizes FGFR-1, FGFR-2, and FGFR-3. In contrast, Ab50 recognizes only the cytoplasmic domains of FGFR-1 and FGFR-2.

Matrix Binding of FGFR-1 Is Independent of FGF

A high degree of cooperative binding appears to exist between FGF, heparan sulfate, and the FGF receptor (20, 29). To determine whether the removal of FGF-2 from basement membranes affects the localization of high affinity FGF receptors in basement membranes, we incubated tissue sections with heparitinase (which is known to release FGF-2 from heparan sulfate proteoglycans [10, 13]) before staining with Ab6. Heparitinase treatment abolished the staining of FGF-2 in the basement membrane (Fig. 3, *A–B*), but did not reduce the staining of the FGF receptor (Fig. 3 *C*). In an unexpected result, we found heparitinase treatment significantly increased the basement membrane staining. Similar

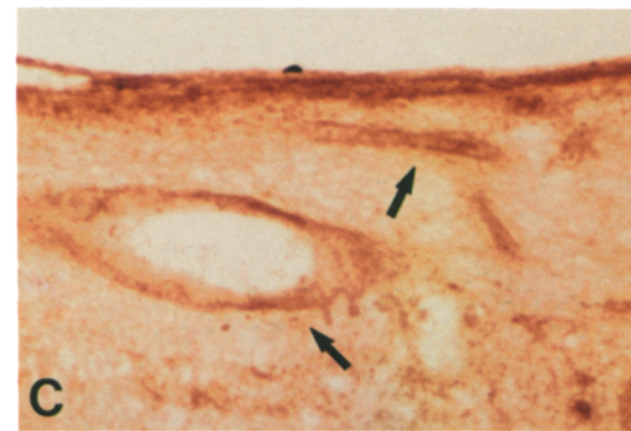
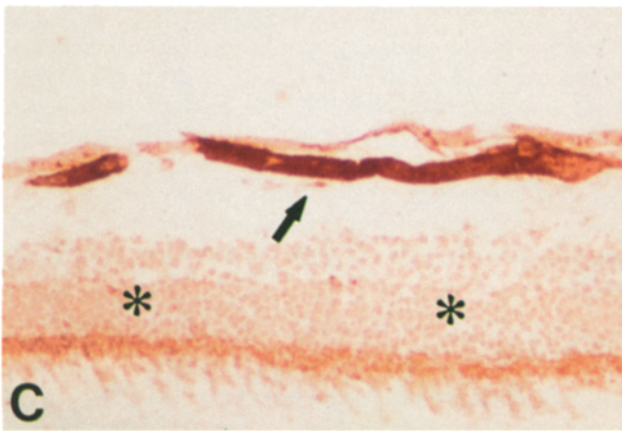
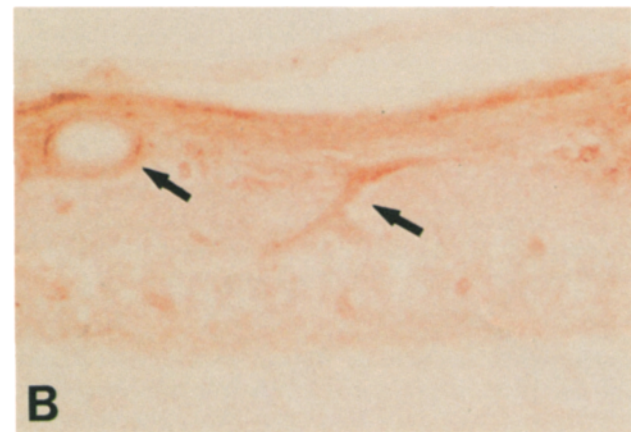
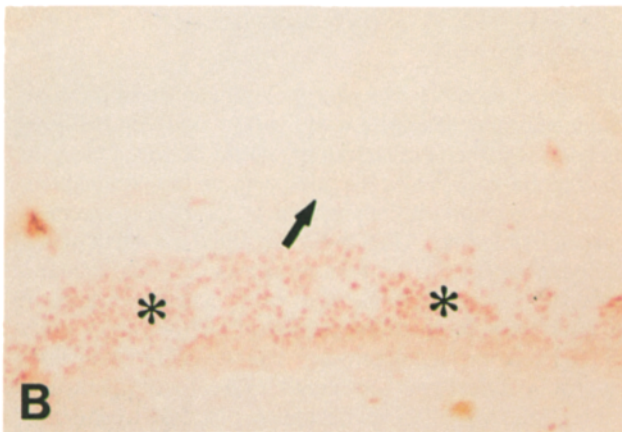
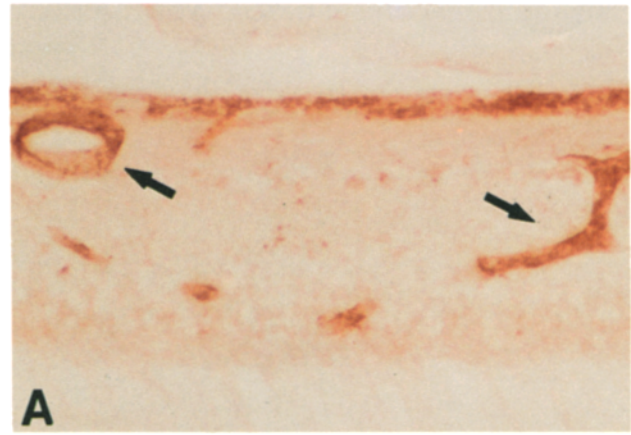
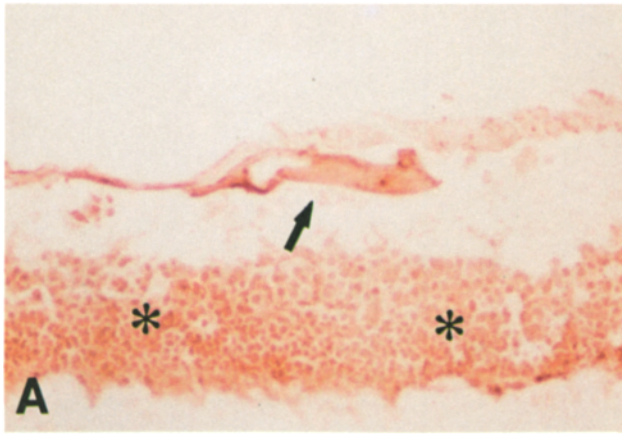


Figure 3. Immunolocalization of FGF receptors after removal of extracellular FGF-2. Fresh frozen tissue sections were fixed in 2% paraformaldehyde for 5 min, washed three times over 15 min, and (A) incubated with PBS or (B and C) digested with heparitinase for 45 min at 37°C as described in Materials and Methods. The slides were next treated with 3% H₂O₂ and (A and C) incubated with antibodies to the extracellular domain of FGFR-1 (Ab6), or (B) stained with antibodies to FGF-2, as described in Materials and Methods. Solid dark arrows indicate vascular basement membranes. Asterisks indicate FGF receptor localization to neuronal cells. Note the increase in FGF receptor immunoreactivity in vascular basement membranes after heparitinase digestion (C) even though the intensity of the neuronal staining is similar. The loss of basement membrane FGF-2 immunoreactivity is demonstrated in Fig. 3 (B).

Figure 5. Acid dissociation and exogenous binding of a recombinant form of the extracellular domain of FGFR-1 to tissues. 12- μ m fresh frozen or minimally fixed 2% paraformaldehyde-treated sections were treated for 30 min at room temperature with (A) PBS or (B and C) 1N acetic acid. The sections were treated with 3% H₂O₂, blocked with 1% BSA, and (A and B) stained with Ab6 as described in Materials and Methods or (C) incubated with a 10 μ g/ml dilution of the recombinant extracellular domain of FGFR-1 as described in Materials and Methods, extensively washed with PBS, and then stained with Ab6.

Extracellular matrix binding of the rECD

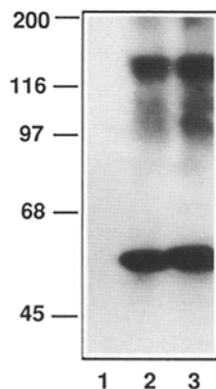


Figure 4. Binding of the extracellular domain of FGFR-1 to bovine corneal endothelial (BCE) cell matrix. BCE matrix was immunoblotted with the antibody to the extracellular domain of FGFR-1 (Ab6) as described in Materials and Methods. The effect of FGF-2 removal on the binding of FGFR-1 was examined by incubating recombinant extracellular domain of FGFR-1 with BCE matrix which had been treated with PBS (lane 2) or 2M NaCl (lane 3). Recombinant extracellular domain bound to BCE matrix in the presence or the absence of FGF-2. Lane 1 demonstrates the low levels of endogenous FGF receptor in BCE matrix.

results were obtained in five separate experiments. To show that nonspecific degradation of the basement membrane was not responsible for this increase in FGF receptor immunoreactivity, we performed enzymatic digestions with chondroitinase and collagenase. Neither enzyme changed the intensity of FGF receptor staining in the extracellular matrix (results not shown).

We next examined whether the removal of FGF-2 from bovine corneal endothelial cell (BCE) matrix would alter the binding of the recombinant extracellular domain to the extracellular matrix. Although the endogenous level of FGF receptors in BCE cell matrix is very low (Fig. 4, lane 1), after incubating BCE matrix with 500 ng/ml of recombinant extracellular domain (in the presence of 1% BSA to eliminate nonspecific binding), an intense 55-kD band is detected in BCE matrix extracts with Ab6 (Fig. 4, lane 2). High molecular weight aggregates of the recombinant extracellular domain are also present. The signal is not diminished if endogenous FGF-2 is removed from the matrix with 2M NaCl before the addition of the extracellular domain (Fig. 4, lane 3), indicating that FGF-2 is not a requirement for FGF receptor binding to the extracellular matrix. These results are independent of whether the recombinant extracellular domain was produced either in *E. coli* (non-glycosylated) or SF9 cells (glycosylated, not shown).

Dissociation of the FGF-2/FGF Receptor Complex in Extracellular Matrix

Because acid treatment is known to disrupt both the high affinity FGF-2/FGF receptor complex and other ionic interactions with extracellular matrix (19), we examined whether acid could disrupt the interaction of high affinity FGF receptors with the extracellular matrix. We found that FGF recep-

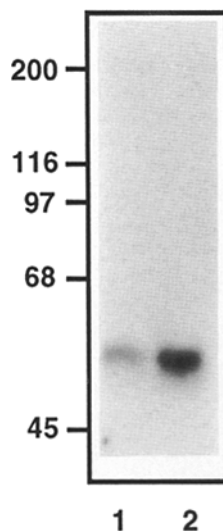


Figure 6. Ligand blotting of ^{125}I -FGF-2 to the recombinant extracellular domain of FGFR-1. 150 μg (lane 1) and 300 μg (lane 2) of the extracellular domain was separated on an 8% SDS-polyacrylamide gel under nonreducing conditions and transferred to nitrocellulose as described in Materials and Methods. 500,000 cpm of ^{125}I -FGF-2 per ml was added to the nitrocellulose overnight.

tor immunoreactivity was nearly eliminated from the basement membranes after acid washing (Fig. 5, A–B). However, this basement membrane staining could be restored by incubating the acid-treated sections with the recombinant extracellular domain (in the presence of 1% BSA to eliminate nonspecific binding). (Fig. 5 C) We found the effect of acid treatment was specific for FGF receptors, since the immunohistochemical localization of all other antigens present in vascular basement membranes (Factor VIII, Type IV collagen, and heparan sulfate proteoglycan) was completely unaffected by acid treatment (not shown). The structural integrity of the tissues was also unaffected by this acid treatment.

Ligand Blotting of the Recombinant Extracellular Domain of FGFR-1

The above studies demonstrate that the recombinant extracellular domain is capable of binding to extracellular matrix of vascular endothelial cells. To demonstrate that the recombinant extracellular domain of FGFR-1 is capable of binding FGF-2, we performed ligand blotting. We found that ^{125}I -FGF-2 binds to the recombinant extracellular domain in a dose-dependent manner (Fig. 6), and this binding could be eliminated in the presence of excess nonradioactive FGF-2 (not shown).

Discussion

This study demonstrates that a form of the high affinity FGF receptor is present in the extracellular matrix of vascular endothelial cells. This finding is supported by the facts that (a) the matrix staining localizes to capillary-like structures throughout the inner retina, consistent with the anatomical distribution of the retinal vasculature, (b) the localization matches the pattern obtained with Factor VIII-related antigen, as well as antibodies to the basement membrane components type IV collagen, heparan sulfate proteoglycan, and laminin, and (c) the pattern is identical to the matrix localization of FGF-2 (9). Of course, unequivocal confirmation of the matrix localization of FGFR-1 awaits the results of EM localization.

Our data also suggest that the immunoreactive, high affinity FGF receptor that is present in the matrix lacks the

cytoplasmic domain of the receptor. These data are based on the immunohistochemical-staining patterns obtained with three distinct FGF receptor antibodies (Ab6, Ab50, and Ab803) that recognize different epitopes in either the extracellular or cytoplasmic domains of the FGF receptor. All three antibodies recognize neuronal cell-associated FGF receptors in human retina. The two antibodies which immunoblot recognize high molecular protein bands with sizes characteristic of full-length, high affinity FGF receptors (16, 27). Only the antibody to an epitope in the extracellular domain shows matrix staining and detects 85-kD and 55-kD proteins in retinal and endothelial cell extracts. These proteins are similar in size to two low molecular weight, high affinity FGF receptors (FGF-BPs) which have recently been identified in bovine and human blood. Peptide sequencing of the amino terminus of the 55-kD and 85-kD FGF-BP show they are identical to the amino terminus of the two Ig loop form of FGFR-1 (12). Thus, the FGF receptors detected in matrix and identified in blood are both presumably the truncated forms of the high affinity FGF receptor. A formal confirmation of the identity of these proteins awaits the results of ongoing extracellular matrix purification studies.

Although the cellular source of the matrix-associated immunoreactivity is not known, the intense 55-kD band in the Western blots of capillary endothelial cells (Fig. 2) suggests that the vascular endothelial cells may synthesize this protein and deposit it into their basement membranes. Vascular endothelial cells may also be the source of the soluble FGF receptors which have been detected in blood. In support of this hypothesis, an mRNA encoding a secreted form of FGFR-1 (16) was detected in extracts of human umbilical vein endothelial cells (3). It remains to be determined whether these matrix receptors are specific, secreted forms of the receptor or are generated by cleavage of the full-length transmembrane receptor.

The heparitinase studies indicate that the binding of FGF receptors to the extracellular matrix is, at least partially, independent of the presence of FGF-2 and heparan sulfate glycosaminoglycans. Surprisingly, instead of a marked decrease in matrix receptor immunoreactivity after the release of FGF-2, we detected a striking increase in the matrix signal. This effect is highly reproducible, is specific for heparitinase, and cannot be reproduced with other enzyme treatments. Because this effect is not seen in neuronal cells, and is only detected in the matrix, it is unlikely to be an artifact due to the consequences of enzymatic treatment of the tissue sections. Presumably, this effect is due to improved accessibility of the FGF receptor antibody for its antigen after the specific removal of FGF-2 and glycosaminoglycans from the basement membrane. Once the specific isoform of the matrix FGF receptor has been identified from the numerous potential isoforms (14), it will be possible to examine the binding of this receptor to specific components of the extracellular matrix and to elucidate the nature of this interaction.

Although low affinity receptors for FGF-2 and TGF- β and binding proteins for other growth factors have been identified in basement membranes (2, 4, 17, 22, 26, 28), this is the first demonstration of a high affinity cell surface growth factor receptor in the extracellular matrix. The role that it plays in FGF function is not known, but it is interesting to speculate that it modulates the interaction between FGF-2 and signal-transducing cell surface receptors. This may help

explain why the proliferation of FGF target cells is so low in vivo despite the presence of FGF-2 in the adjacent basement membranes. We now suggest that the matrix stores of FGF-2 are sequestered by both low affinity heparan sulfate proteoglycans and truncated high affinity FGF receptors. In order for matrix stores of FGF-2 to bind to the FGF receptors on adjacent cells, they must be released from these high affinity receptors in the matrix. The finding that acid treatment of matrix eliminates the matrix receptor staining supports this hypothesis. For example, in the acidic environment of wound fluids, the presence of endogenous FGF-2 will be revealed to the target cell by its dissociation from the receptor in the matrix. Protected from degradation by its interaction with heparin (23, 24), FGF would then be free to promote angiogenesis and wound healing.

Many cytokines, including IL-7, GM-CSF, IL-5, G-CSF, and NGF, have truncated receptors which are capable of blocking cell surface binding to high affinity receptors (5, 7, 21, 25, 30). However, none of these receptors has been detected in the extracellular matrix. The presence of high affinity FGF receptors in the extracellular matrix suggests a potential regulatory mechanism to restrict the potent biological activities of the FGF family.

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