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Basic Fibroblast Growth Factor Promotes Proliferation of Rat Glomerular Visceral Epithelial Cells *In Vitro*

Akihiko Takeuchi,* Nobuyuki Yoshizawa,† Mikio Yamamoto,‡ Yoshio Sawasaki,‡ Takashi Oda,† Akira Senoo,* Hirohumi Niwa,† and Yusuke Fuse*

From the Departments of Pathology, Medicine,† and Biochemistry,‡ National Defense Medical College, Saitama, Japan*

Glomerular visceral epithelial cells (vGEC) play an important role in the synthesis of the glomerular basement membrane (GBM), and together with glomerular endothelial cells and the GBM, in glomerular ultrafiltration. Therefore clarification of the properties of vGEC is essential to investigations of glomerular morphology and function in both physiologic and pathologic conditions. This article demonstrates that basic fibroblast growth factor (bFGF) is mitogenic to vGEC in vitro. ***Its effect was found at concentrations as low as 1.25 ng/ml, and was synergistic with epidermal growth factor (EGF). In contrast, EGF by itself had no demonstrable mitogenic effect at concentrations of 1.25–100 ng/ml. In addition, mRNA for bFGF was identified in cultured vGEC by the method of reverse transcriptase polymerase chain reaction and the immunoreactivity of bFGF was found in GEC of the Sprague-Dawley rat kidney. These results suggest that bFGF stimulates the proliferation of vGEC in an autocrine manner*** in vivo. ***A unique relationship similar to that observed in endothelial cells may also exist among bFGF, vGEC, and the extracellular matrix (ECM). In a word, bFGF may be produced by vGEC and stored in the ECM, that is the GBM, and may be one factor that stimulates vGEC to proliferate when vGEC are injured and lost*** in vivo. ***(Am J Pathol 1992, 141:107–116)***

Fibroblast growth factors (FGFs) were first isolated from bovine pituitary and brain as a potent growth-promoting activity on 3T3 fibroblasts.[1,2] FGFs are a family of seven structurally related proteins, that is, acidic FGF and basic FGF (bFGF), and the gene products int-2, hst/ks, FGF-5, FGF-6, and FGF-7/kGF (keratinocyte growth factor).[3] Among these proteins, the biochemical and biological properties of bFGF have been investigated the most. Originally, as indicated by the name, bFGF was believed to be simply mitogenic for fibroblasts. However, bFGF is known to distribute in many tissues of mesodermal and neuroectodermal origin and have wide-ranging effects on various cell types of this origin: proliferation, morphologic change, transformation, migration, differentiation, proteinase production, and senescence *in vitro;* angiogenesis, wound healing, fetal and embryonic development, and tumorigenesis *in vivo*.[3–12] The binding capacity of FGF to the extracellular matrix (ECM), heparan sulfate proteoglycan, is its hallmark, and the released FGF from the ECM is bioavailable to the target cells, as well as is FGF directly released from injured cells.[12–16]

The glomerulus consists of three cell types: epithelial (GEC), mesangial (MC), and endothelial (GENC). The GEC can be further subdivided into two types: parietal (pGEC) and visceral (vGEC) cells. vGEC, together with GENCs, play an important role in the metabolism of the glomerular basement membrane (GBM),[17,18] and is essential for the maintenance of normal ultrafiltration in company with the GBM and GENCs.[18] In fact, puromycin aminonucleoside (PAN), which causes GEC damage, can induce massive proteinuria resulting in the nephrotic syndrome.[19] Moreover, the degeneration of this cell, e.g., foot process fusion, vacuolization, and detachment, is frequently found both in human and experimental glomerular diseases, particularly at the proteinuric state.[18,20] On the other hand, GEC proliferation *in vivo* is observed in various glomerular diseases as crescents[21,22] and in focal segmental glomerulosclerosis (FGS).[23] As the formation of scar tissue after the GEC proliferation may lead to glomerular obsolescence, it is clinically important to clarify the pathogenic mechanism of GEC proliferation.

Cell cultures of GEC have been used to investigate the biological properties of this cell.[24–30] Conditioned medium taken from cultures of Swiss 3T3 fibroblasts are

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Address reprint requests to Dr. Nobuyuki Yoshizawa, Department of Medicine, National Defense Medical College, 3-2 Namiki, Tokorozawa, Saitama 359, Japan.

commonly used to grow the GEC *in vitro*.[24–26,28–30] On the other hand, the proliferation of 3T3 fibroblasts has been used as an indicator of FGF activity.[1,2] These facts permit us to link FGF to GEC. In addition, it has been shown that macrophage-conditioned medium is mitogenic to GEC,[31] and that macrophages probably secrete FGF.[32]

Although vGEC are reported to respond to several mitogenic substances, namely epidermal growth factor (EGF) and leukotriene C4 and D4 in addition to those mentioned earlier,[33,34] there have been no reports detailing the effect of bFGF on vGEC. This study determines whether bFGF can stimulate vGEC to proliferate and compares the mitogenic activity of bFGF and EGF.

Materials and Methods

Cell Culture

A modification of a previously described model[24,25] was used to culture vGEC. Briefly, four- to five-week-old male Sprague-Dawley (SD) rats (Clea Japan, Inc., Tokyo, Japan) were anesthetized by a lethal dose of pentobarbital administered IP; before death both kidneys were excised under sterile conditions. After removing the capsule, the cortices were taken, finely minced, and suspended in Hanks' balanced salt solution (HBSS; GIBCO Laboratories, Grand Island, NY). They were then passed serially through sterile stainless steel meshes with 250-150-105-53 μm pore sizes, being washed with HBSS. Glomeruli were obtained by collecting materials retained on the 53-μm mesh. After centrifugation, glomeruli were resuspended with K1 (a defined epithelial growth mixture[35]) containing 10% heat-inactivated (56°C, 30 min) fetal calf serum (FCS; Cell Culture Laboratories, Cleveland, OH), and 10 ng/ml EGF (human recombinant, Wakunaga Pharm. Co., Ltd., Osaka, Japan), and were poured into four 60-mm plastic dishes without matrix coating. The purity of glomeruli was 70–90%, and 30–50% of them still had Bowman's capsules and/or arterioles. Tubular fragments and glomeruli with the Bowman's capsule were more frequently attached to the dish, and epithelial cells grew out from these tissue fragments more rapidly than they did from glomeruli without the Bowman's capsule. Moreover, it was difficult to distinguish among cells derived from glomeruli with and without the Bowman's capsule and tubular fragments after cell outgrowth. To eliminate contamination by tubular epithelial cells, pGEC and the other nonepithelial cells, tubular fragments, and glomeruli with the Bowman's capsule or arterioles were manually picked up and discarded using a thin caliber glass pipet under phase-contrast microscopy. Only glomeruli without Bowman's capsule and arterioles were incubated at 37°C in a humidified 5% CO_2 atmosphere. After 4 days of incubation, polygonal cells that grew out from about 100 glomeruli per dish with a cobblestone monolayer were trypsinized after washing once with HBSS, and were passed through a 20-μm nylon mesh being washed with HBSS. None of the glomeruli passed the mesh, and only outgrown polygonal cells from glomeruli were obtained. After centrifugation, the cell pellet was resuspended in K1-10% FCS with 10 ng/ml EGF and bFGF (human recombinant, Boehringer Mannheim GmbH, Mannheim, Germany) and were plated in a 35-mm dish coated with swine type I collagen coating (Cellmatrix Type I-P; Nitta Gelatin Inc., Osaka, Japan). Dishes coated with Cellmatrix Type I-P were used thereafter. Cultured cells were passaged twice a week, and third-passage cells were used in this study because the proliferative activity of cultured cells suddenly decreased in about 3 weeks.

Identification of Cultured Cells

The cells, which were believed to originate from decapsulated glomeruli, were characterized to be vGEC. Under phase-contrast microscopy, cells were homogeneous in appearance, polygonal, formed a cobblestonelike monolayer (Figure 1), and adhered tightly to each other at the edge of the growing monolayer; they displayed the formation of several domes when they became confluent and where they were densely packed, indicating that the cells were of epithelial origin. The cultured cells were sensitive to PAN. Some third-passage cultured cells were grown in Lab-Tek chamber slides (Miles Laboratories Inc., Naperville, IL), and these cells at a log phase were processed for immunohistochemical examination and electron microscopy. By immunohistochemical study, all the cells were positive for vimentin (Amersham International plc, Buckinghamshire, UK; Figure 2), but negative for factor VIII-related protein (Biomedicals AG, Rhein-

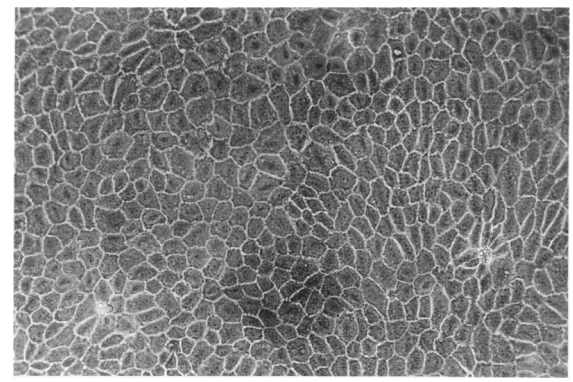

Figure 1. *Phase-contrast micrograph. Cultured cells look homogenous, show a polygonal shape and a clear cell margin, and form a monolayer of "cobblestone" appearance (×87.5).*

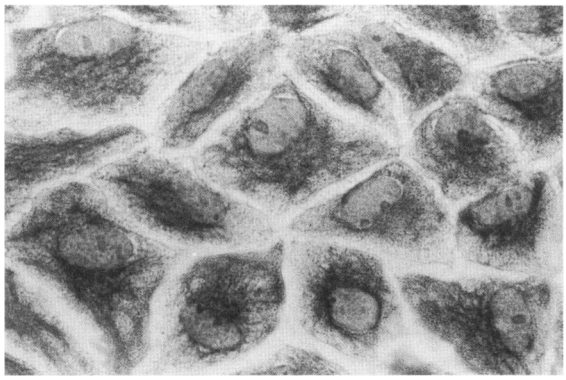

Figure 2. *Staining of cultured cells with antivimentin antibody. Filamentous components condensed in the perinuclear region radiate to the cell periphery (×560).*

strasse, Switzerland) and desmin (Dakopatts A/S, Glostrup, Denmark). These staining patterns were consistent with that of vGEC in frozen sections of the kidney of male SD rats with the same antibodies as described earlier; namely, vimentin stained positive in GEC; factor VIII-related protein was positive in vascular endothelial cells, including glomerular afferent and efferent arterioles, although it was obscure in GENCs; desmin was positive in mesangial pattern. Electron microscopic examination showed that the cultured cells had apparent cell junctions, many microvilli and barely discernible nuclear fibrous lamina, and formed lumenlike structures (Figure 3a, b). Cilia were also occasionally noted. These morphologic findings indicate strongly that the cultured cells were derived from vGEC.

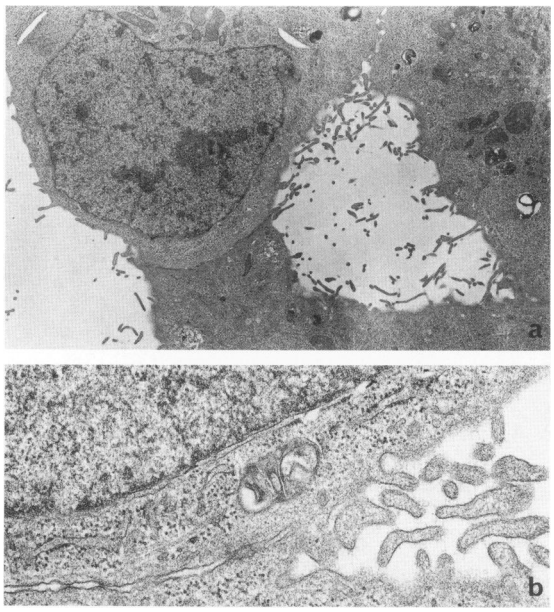

Figure 3. *Transmission electron micrographs of cultured cells. Polygonal cells with many microvilli form a lumenlike structure* (**a**, *×3,400). Apparent cell junctions and microvilli can be observed* (**b**, *×20,000).*

Effect of Growth Factors on vGEC Proliferation

Cultured vGEC resuspended in K1-10% FCS after trypsinization were plated on 24-well plates coated with Cellmatrix Type I-P at a density of 30 to 31,000/well. After about 6 h, when the cells had adhered (the percentage of adherence was 44–50%), the cells were washed twice with HBSS. Afterwards, in one experiment, K1-10% FCS containing various concentrations of bFGF or EGF was added to the wells. Four days after the addition of fresh medium, the cells were counted. In another experiment, K1-10% FCS containing 10 ng/ml bFGF, EGF, both, or neither growth factor was added to the wells. The cells were counted periodically after the medium change. Cell counting was done with an automatic hemocytometer (Toha Medical Electronics Corp., Hyogo, Japan).

Detection of the mRNA for bFGF

The vGEC that reached subconfluency in K1-10% FCS containing 10 ng/ml bFGF and EGF were washed with HBSS, trypsinized, and centrifuged. Immediately after centrifugation, the cell pellet was frozen with liquid nitrogen and stored at −80°C until use. Total RNA was obtained by the method of acid guanidinium thiocyanate-phenol-chloroform extraction with some modifications.[36] Briefly, the cells were homogenized with 1 ml of solution D (4 mol/l guanidinium thiocyanate, 25 mmol/l sodium citrate [pH 7], 0.5% sarcosyl, 0.1 mol/l 2-mercaptoethanol [β-MSH]) in a glass Teflon homogenizer. After the homogenate was mixed well sequentially with sodium acetate (pH 4), phenol, and chloroform-isoamyl alcohol and cooled on ice for 15 minutes, the homogenate was centrifuged and the aqueous phase containing RNA was transferred to another tube. The precipitation was done by mixing with isopropanol at −20°C for at least 1 hour. After centrifugation, the supernant was discarded and the pellet was resuspended in solution D. RNA was reprecipitated with one volume of isopropanol at −20°C for at least 1 hour. Centrifugation was performed, and the pellet dissolved in 10 mmol/l Tris-HCl, 1 mmol/l EDTA-0.2% sodium dodecyl sulfate. Furthermore the purification of RNA was done by the phenol extraction of several times and the ethanol precipitation of two times. The mRNA for bFGF in the total RNA was amplified by the method of reverse transcriptase polymerase chain reaction (RT-PCR) with synthetic 20mer primers,[37] representing extremities of the mature bFGF of the rat.[38] The predicted size of amplified product was 438 bp in length. A primer extension hybridization was done with 1 μg of total

RNA in a volume of 10 μl containing 30 ng of 3′ (antisense) oligonucleotide primer, 50 mmol/l KCl, 20 mmol/l Tris (pH 8.3), and 0.2 mmol/l EDTA at 65°C for 1 hour, followed by gradual cooling to room temperature. Subsequently, a portion (5 μl) of this reaction was subjected to the reverse transcription reaction; the 15 μl reaction mixture, which contained 0.5 mmol/l deoxynucleoside triphosphates, 100 mmol/l Tris (pH 8.3), 35 mmol/l β-MSH, 10 mmol/l $MgCl_2$, 100 mmol/l KCl, was incubated in the presence or absence of avian myeloblastosis virus reverse transcriptase (Takara Shuzo Co., Ltd, Kyoto, Japan) at 37°C for 1 hour. The volume of 2.5 μl of this primer extension reaction was subjected to analysis by PCR. The PCR amplification contained 0.2 mmol/l deoxynucleoside triphosphates, 25 mmol/l Tris (pH 9.0), 50 mmol/l KCl, 2 mmol/l $MgCl_2$, 30 ng each of 5′ (sense) and 3′ (antisense) oligonucleotide primers and 1 U of Taq polymerase (Takara Shuzo Co., Ltd) in a total volume of 50 μl. Twenty-five rounds of amplification were done in the DNA Thermal Cycler (Perkin/Elmer Cetus, Norwalk, CT) with a 94°C denaturation step (1 min), 56°C annealing step (2 min), and 72°C extension step (3 min). The products were electrophoresed on a 4% polyacrylamide gel with size markers.

Detection of the Immunoreactivity of bFGF

Male SD rats were killed as described earlier. Immediately after removal of the kidneys, the tissue was immersed in OCT compound (Miles Laboratories Inc., Naperville, IL) and frozen in liquid nitrogen. Five-μm thick cryostat sections were fixed with 4% paraformaldehyde in phosphate-buffered saline (PBS). The endogenous peroxidase activity was then blocked by incubating the sections for 5 minutes in 3% hydrogen peroxide in distilled water. After the incubation with 3% bovine serum albumin (BSA) in PBS, the sections were incubated with rabbit anti-human bFGF (Biomedical Technologies Inc., Stoughton, MA). Subsequently, the sections were incubated with donkey anti-rabbit IgG peroxidase conjugate (Chemicon International Inc., Temecula, CA). Staining was performed in a solution of 0.02% 3,3′-diaminobenzidine and 0.005% hydrogen peroxide in 50 mmol/l Tris (pH 7.6). The sections were counterstained with Mayer's hematoxylin. All incubations were performed at room temperature in a moist chamber, and all steps were separated by PBS washing except between BSA and primary antibody. Specificity controls were carried out by omitting the primary antibodies or replacing the primary antibody by normal rabbit IgG. The specificity of primary antibody was confirmed by Western immunoblotting (data not shown).

Results

Effect of Growth Factors on Cultured vGEC Proliferation

bFGF promoted a dose-dependent proliferation of cultured vGEC in the range of 0 to 5 ng/ml; the maximum effect, approximately a fourfold increase as compared with no growth factors, was obtained at 5 ng/ml (Figure 4). In the dose range of 5 to 20 ng/ml, no further growth promotion was observed. At a dose concentration of 100 ng/ml the effect decreased somewhat, as has previously been found in other types of cultured cells.[39] EGF had no demonstrable mitogenic effect at any dose concentration used in this study.

Two days after the addition of fresh medium, cells in the media containing bFGF and bFGF plus EGF began proliferating, which continued until the fifth day when the complete confluency was reached; cells in the media containing only EGF or no growth factor exhibited no increase in cell number (Figure 5). However, EGF enhanced remarkably the mitogenic activity of cells in combination with bFGF. The addition of EGF to bFGF was synergistic to cell proliferation. Detached cells were minimal, and the detachment had no influence on the results. Cells in the media containing no bFGF were somewhat large and looked inactive (Figure 6a, b). On the other hand, cells in the media containing bFGF and bFGF plus EGF were compact in shape and smaller at the periphery of colonies (Figure 6c, d), indicating active proliferation.

Detection of the mRNA for bFGF

Amplification of total RNA of cultured vGEC by the RT-PCR method resulted in a single band, the molecular size

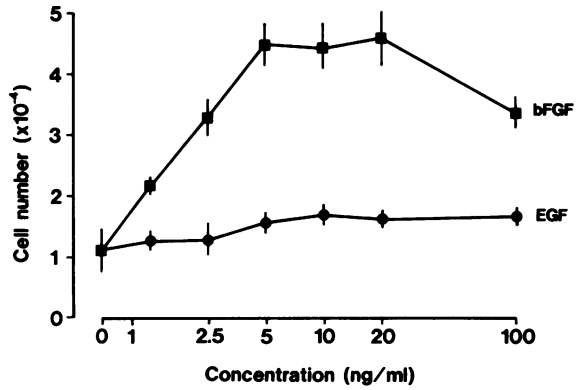

Figure 4. *Dose-response curves of vGEC for bFGF or EGF. Six hours after plating, K1-10% FCS was changed to a medium containing bFGF or EGF in various concentrations. Cells were counted in triplicate 4 days after the medium change. Values are means ±SD.*

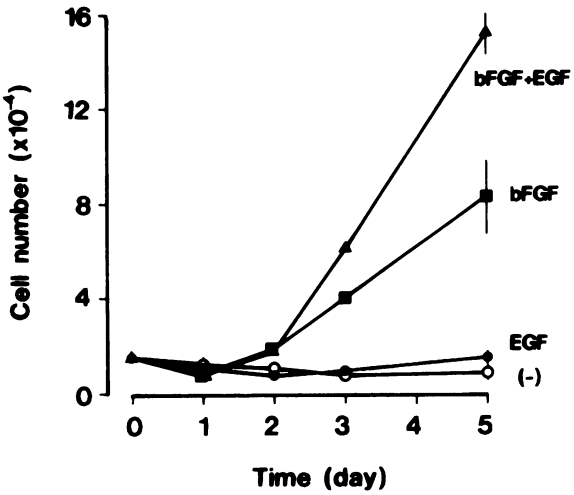

Figure 5. *Proliferation curves of vGEC. Six hours after plating, the K1-10% FCS medium was replaced with K1-10% FCS containing no growth factor or 10 ng/ml of bFGF, EGF, or bFGF + EGF. Thereafter, cells were counted in triplicate periodically. Values are means ± SD. SD are shown only when they exceed the size of the data points.*

of which was identical to that of the predicted size of mature bFGF mRNA lying between primers (Figure 7). The control reaction (minus reverse transcriptase, see Materials and Methods) generated no distinct band at the size of interest (data, not shown). Similarly, in total RNA of cultured MCs a single band was obtained (Fig. 7). This is consistent with an earlier report that cultured MCs proliferates upon stimulation by bFGF.[40]

Detection of the Immunoreactivity of bFGF

The immunoreactivity of bFGF was present in vGEC, pGECs, and the mesangial region, along the glomerular capillary walls, and in the arteriolar wall in the SD rat kidneys (Figure 8). It was also positive in the small arterial walls, and weakly positive in interstitial region and some tubular epithelial cells. This finding may indicate that an autocrine stimulatory pathway of bFGF exists in vGEC together with the demonstration of mRNA in cultured vGEC.

Discussion

This study demonstrated that cultured vGEC responded to bFGF (Figures 4, 5). Further, cultured vGEC was

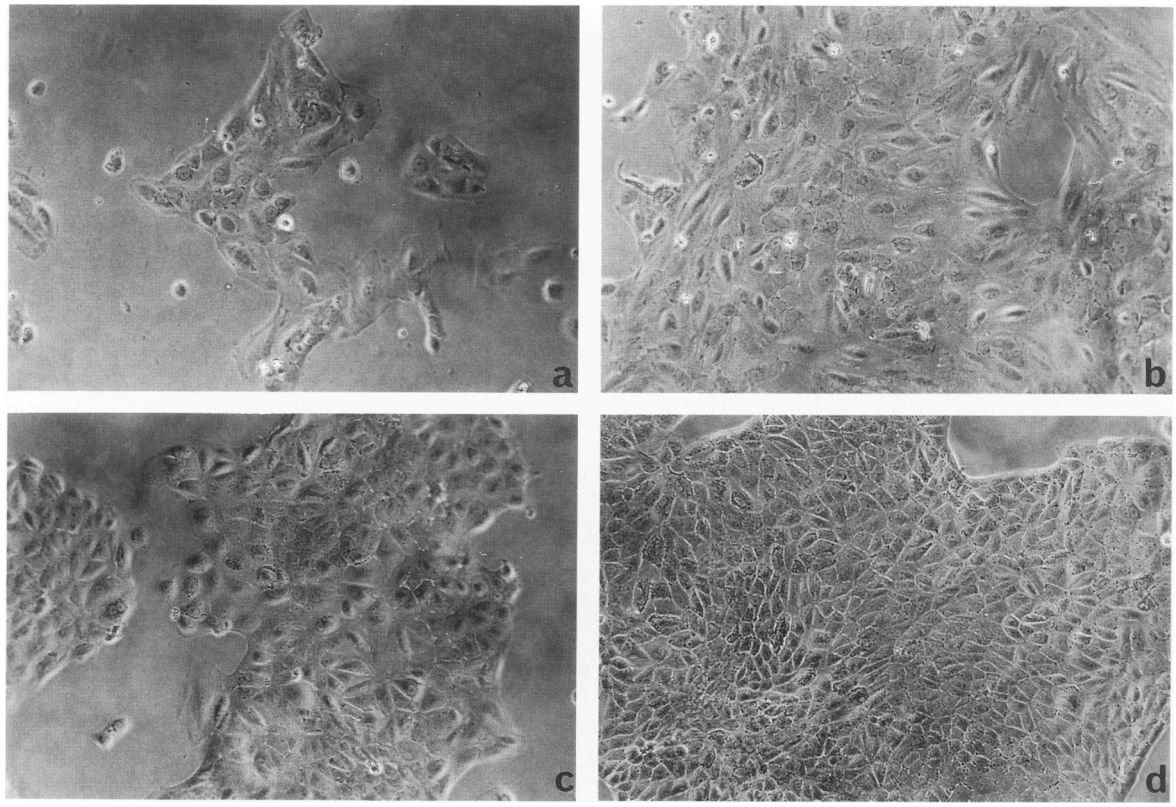

Figure 6. *Phase-contrast micrographs of cultured cells. Four days after replacement with medium containing each, both,or no growth factor. Cells cultured in the medium K1-10% FCS without growth factors* (a) *or with 10 ng/ml EGF* (b)*are rather large and appear inactive; cells in the medium with 10 ng/ml bFGF are compact in shape and proliferating* (c)*; the addition of both of bFGF and EGF is the most mitogenic* (d). *Magnification, ×87.5.*

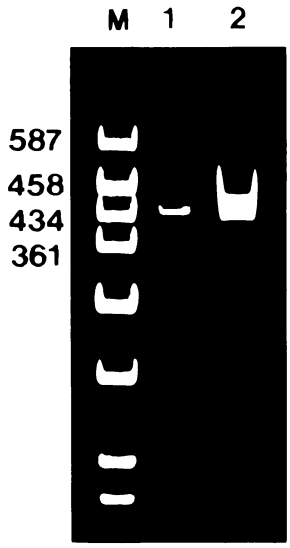

Figure 7. *Detection of mRNA for bFGF with RT-PCR. A single band is seen in both lanes (1, vGEC; 2, MC), which has an identical molecular weight (438 bp) with the predicted size of the fragment given by the primers specific to the mRNA for bFGF. The total RNA of MCs was obtained by the same method with vGEC as described in Materials and Methods. The glomeruli that were retained on the top of 20-μm nylon mesh when getting vGEC in Materials and Methods were sequentially cultured in Dulbecco's modified eagle media containing 10% FCS and 10 ng/ml bFGF. The spindle-shaped cells grown out from those glomeruli were positive for desmin and negative for vimentin, and considered to be MCs. Third passage cells were used for mRNA analysis. Molecular weight markers are* HaeIII *digest of PUC119.*

found to have mRNA for bFGF by the RT-PCR method (Figure 7), and GEC *in vivo* showed the immunoreactivity of bFGF (Figure 8). This finding means that bFGF is also synthesized by vGEC probably in addition to other residual glomerular cells, MC and GENC, and may stimulate vGEC in an autocrine manner, although the secretion mechanism of bFGF is still unclear since FGF has no classical signal peptide.[41] The finding that bFGF could stimulate the vGEC to proliferate was unexpected. Furthermore, it was surprising that EGF alone showed no demonstrable mitogenic effect for vGEC even in the 10% FCS condition in this study. This is not in accordance with a previous report.[33] On the contrary, this finding is consistent with the data that primary kidney cultured cells have shown no response to the same recombinant EGF,[42] and with the absence of the immunoreactivity of EGF and EGF receptor on GEC *in vivo*, even in glomerular crescents.[43–45] *In situ* hybridization of prepro-EGF mRNA is also negative in glomeruli.[45] However, EGF had a synergistic effect when combined with bFGF. Interestingly, bFGF is considered to be a candidate of synergistic molecules for the mitogenic activity of EGF or transforming growth factor-α.[46] The reason for the difference between our result and a previous one[33] is unclear. However, one possible reason for the difference might be the purity of EGF; we used human recombinant EGF, whereas they used receptor grade EGF from mouse submaxillary glands. Species difference is probably of no concern.[42]

bFGF is a cationic protein (isoelectric point = 9.6), binds matrix molecules, i.e., heparan sulfate proteoglycan,[6] and is stored in the ECM.[12–14,47,48] Heparinase, heparitinase, and plasmin can release FGF as an active form of the bFGF-heparan sulfate complex by the proteolytic degradation of the ECM,[13,14,49] and bFGF release may be regulated by a balance among factors affecting the pericellular proteolytic activity,[49] while FGF by itself stimulates endothelial cells (ENCs) to produce urokinase-type plasminogen activator (u-PA).[6] There may be three mechanisms by which bFGF produced by ENCs and stored in the ECM becomes bioavailable to the target cells: 1) increased expression of high affinity receptors for bFGF on the target cell; 2) some post-translational mod-

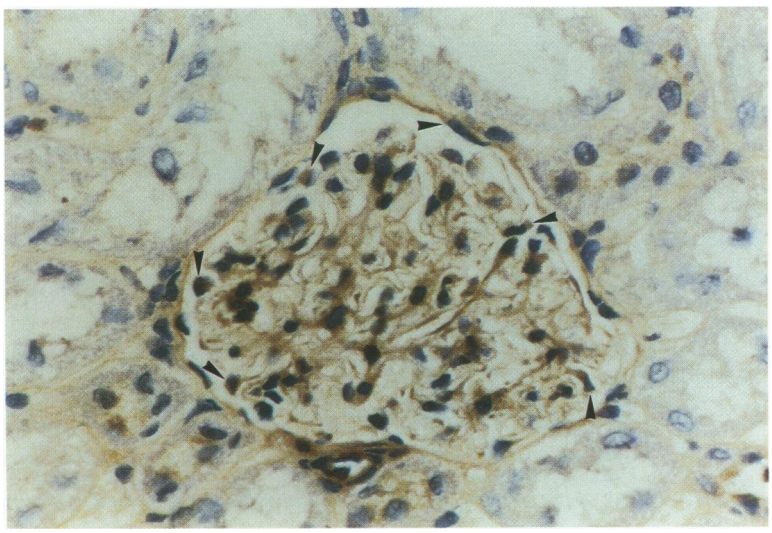

Figure 8. *Staining of kidney tissue of SD rats with antihuman bFGF antibody. The immunoreactivity of bFGF is apparently positive in vGEC, pGECs (arrowheads) in addition to the mesangial region, glomerular capillary walls, and the arteriolar wall (×590).*

ification of bFGF that would alter its affinity for the ECM; and 3) damage to the ECM by physical or biochemical insult as mentioned earlier.[12] The only mechanism for the direct release of bFGF from cells is reported to be cell death or injury[15,16] as the defect of the classical signal peptide indicates.[41]

The phenomena described earlier have been vigorously studied in ENCs. A unique relationship similar to that demonstrated in ENCs may also exist between bFGF, vGEC, and ECM. Macrophages, T lymphocytes, neutrophils, platelets, and coagulation-fibrinolysis system are considered to contribute to both human and experimental glomerular diseases.[50–52] On the other hand, macrophages seem to be able to synthesize FGF,[32] and macrophages, T lymphocytes, neutrophils, and platelets can release heparitinase.[53–55] Macrophages and neutrophils also produce u-PA.[56] The results obtained in this study suggest that bFGF is also a factor that might be involved in glomerular diseases, along with many other factors.[52] This admitted speculation is drawn upon the following: when the glomerular capillary wall is injured and vGEC is detached and lost, bFGF is directly released from injured vGEC, and concomitantly injured residual glomerular cells, i.e., GENCs and MCs and probably infiltrating macrophages. bFGF-heparan sulfate complex is also released from the injured ECM, the GBM, and the mesangial matrix through the degradation by plasmin and/or heparitinase. Furthermore, since the bulk flow across the GBM occurs in the region of the injured capillary wall[57] and bFGF-heparin and/or heparan sulfate complexes are able to diffuse freely,[58] local FGF activity subsequently may increase around the injured vGEC. Finally, vGEC neighboring to lost vGEC may proliferate and recover the naked area of the GBM. Although in this study the functional effect of FGF on GEC was not investigated, physiologically and pathologically FGF might be concerned with the remodeling of the GBM in view of its functional effect on cells to induce u-PA, which activates matrix-degrading proteinases such as plasminogen and latent collagenase.[56]

The GEC proliferation *in vivo* is observed in various glomerular diseases as crescents[21,22] and in FGS.[23] The contribution of macrophages to the crescent formation[21,22] suggests the possibility of FGF participation. Although an epithelial component of crescents is considered to be pGEC but not vGEC,[21,22,59] pGEC is also supposed to respond to bFGF because the immunoreactivity of bFGF was found in pGECs as well as in vGEC in this study and bFGF is reported to be mitogenic to tubular epithelial cells.[60] In FGS, vGEC injury has been implicated in pathogenesis.[23,61–63] Detachment from the GBM and necrosis of vGEC are microscopically observed.[61,63] In contrast, GEC proliferation and hypertrophy have been demonstrated[23,61] and precede the formation of segmental sclerosis.[23] In addition, adhesion between glomerular tufts and the Bowman's capsule is also frequently seen in FGS, which may be associated with the segmental lesion.[63,64] Accordingly, when the extent of the injury is too large for vGEC to promptly restore the injured GBM area, pGEC may restore the area to cover the naked GBM, since the mitogenic activity of pGEC may be higher than vGEC as is conjectured from the fact that the epithelial component in glomerular crescents is derived from pGEC.[21,22,59] Subsequently, this restoration may lead to the formation of the adhesion, and finally to that of the segmental sclerosis which characterizes FGS.

In nephrology, FGF has not been investigated as a main theme. We suggest that FGF may explain several previously reported phenomena in the nephrologic field. An *in vitro* study using a cell line of bovine aortic ENC has shown that heparin and heparan sulfate reduce the amount of ECM-bound bFGF released into the medium.[13] These data suggest that FGF may be attributed to the following phenomena *in vitro:* heparin and heparinlike species secreted by GENCs and GEC inhibit the proliferation of MCs;[65,66] heparin inhibits vGEC growth;[67] heparinlike glycosaminoglycans prevent MC migration.[68] In detail, heparin and heparan sulfate have a suppressive effect on the bFGF release; however, chondroitin sulfate has no effect on it.[13] Similarly the former two substances inhibit the GEC growth, and the latter does not.[67] This consistency suggests more that FGF is involved in the several phenomena described earlier. However, heparin exerts its antiproliferative effect through multiple targets,[69] and also competes with decorin, which is reported to be related to cell proliferation,[70] in the binding to its receptors.[71] Protamine sulfate markedly inhibits FGF binding to its receptor in vascular ENCs *in vitro*.[72] On the other hand, protamine sulfate also increases the severity of PAN-induced nephrosis in rats by coadministration,[73] whereas PAN is well known to damage vGEC leading to the nephrotic syndrome.[19] The coadministration of protamine sulfate in PAN nephrosis may inhibit the regenerative response of vGEC by FGF after PAN-induced vGEC death.

In summary, bFGF has a mitogenic activity for cultured vGEC and is also synthesized by vGEC. A unique relationship similar to that observed in ENCs might also exist between FGF, vGEC, and the ECM. bFGF might play an important role in the maintenance of the GBM in both pathologic and physiologic conditions. Furthermore, it might be related to GEC proliferation in various glomerular diseases as crescents and in FGS. Active investigation of FGF in the nephrologic field should provide new insights into the biology of the kidney.

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