Expression and Subcellular Distribution of Basic Fibroblast Growth Factor Are Regulated During Migration of Endothelial Cells

Sadatoshi Biro, Zu-Xi Yu, Ya-Min Fu, Georgeanne Smale, Joachim Sasse, Julian Sanchez, Victor J. Ferrans, Ward Casscells

Abstract Migration of endothelial cells is involved in normal and pathological angiogenesis and in reendothelialization after vascular injury or rupture of atherosclerotic plaques. Several types of endothelial cells are known to synthesize basic fibroblast growth factor (bFGF); in some of these, migration is increased by exogenous bFGF and inhibited by anti-bFGF antibodies. Using immunocytochemical techniques and RNase protection analysis, we studied endothelial cells from bovine coronary arteries and veins as well as from adrenal microvessels. We found that bFGF mRNA and peptide were present in confluent endothelial cells and were upregulated during migration stimulated by removal of some cells from the monolayer. During migration, extracellular matrix stores of bFGF were depleted, and bFGF immunoreactivity began to accumulate in the cytoplasm of endothelial cells between 2 and 6 hours. After migration had begun, but before the initiation of DNA synthesis, bFGF immunoreactivity increased in the nuclei and nucleoli. Exogenous bFGF stimulated endothelial migration, and antibodies to bFGF markedly inhibited migration, suggesting that an intracellular function of nuclear bFGF is not sufficient for cell migration. In all three types of endothelial cells studied, bFGF was identified as an endogenous regulator, but not as the sole regulator, of migration. Moreover, bFGF expression and subcellular localization were found to be regulated during endothelial cell migration. (Circ Res. 1994;74:485-494.)

Key Words • endothelial cells • reendothelialization • wound healing • immunocytochemistry • basic fibroblast growth factor

Endothelial cell migration is a feature of angiogenesis during embryogenesis, wound repair, and tumor growth and contributes to reendothelialization after angioplasty or rupture of atherosclerotic plaques. The migration of endothelial cells is not fully understood but is known to involve regulated attachment and detachment of cells, contraction of cytoplasmic filaments, changes in the plasticity of the cytoskeleton, and regulated cell-to-cell communication. These processes are regulated by a number of factors, the signals of which are transduced by ionic currents, cAMP, and protein kinases. Cell adhesion is thought to be controlled by the balance of the proteoglycans, collagens, and glycoproteins, especially laminin and fibronectin, in the extracellular matrix. Proteolysis of cell-substrate attachments, which is required for the cell to advance, is regulated by plasminogen activators, collagenases, and their inhibitors.

Extracellular signals for endothelial cell migration include physical forces and soluble regulators such as vasoactive hormones and polypeptide growth factors. Among the latter are several angiogenic factors: basic fibroblast growth factor (bFGF), acidic fibroblast growth factor, epidermal growth factor, transforming growth factor-α, platelet-derived endothelial cell growth factor, and vascular endothelial growth factor. The last two factors are specific for endothelial cells. bFGF is a broad-spectrum mitogen and chemoattractant that has been reported to enhance endothelial cell migration. However, some reports have disputed the ability of bFGF to stimulate endothelial cell migration. Other reports have noted the failure of neutralizing anti-bFGF antibodies to block angiogenesis in vivo. These discrepancies raise the possibility that bFGF, although active as an exogenous agent, is not an autocrine factor for endothelial cells. Alternatively, these disagreements may reflect differences either in the assay conditions or in the types of endothelial cells used in various studies. We chose endothelial cells from coronary arteries and veins, which are relevant to cardiovascular diseases, and from adrenal capillaries, which have been extensively studied and evaluated the role of bFGF in cell migration and reestablishment of the endothelial cell monolayer by use of an in vitro system of denudation of endothelial cells in culture. For this purpose, we have used cell counting and autoradiographic techniques, immunohistochemical methods for the detection of bFGF, and the technique of RNase protection analysis for the measurement of bFGF mRNA, and we have studied the effects of various pharmacologic agents on cell migration.

Materials and Methods

Three types of endothelial cells were used in the present study: (1) bovine adrenal capillary endothelial (BACE) cells, (2) bovine coronary sinus endothelial (BCSE) cells, and (3) bovine coronary artery endothelial (BCAE) cells. As described in “Results,” certain morphological differences were
observed among these cells when they were grown in culture. Except when specifically indicated, all three cell types were used in each experiment; however, the results obtained with each cell type were consistently similar, and for this reason, they are not described separately.

Cell Cultures

BACE cells were prepared by a modification of the method of Folkman (Yu et al.13). BCSE cells and BCAE cells were obtained by gently rubbing the vascular intimal surfaces with a cotton-tipped applicator and further processed as described previously.21 Staining for acetylated low-density lipoprotein (LDL)22 was used as a positive control to confirm the identification of endothelial cells. BACE cells were grown on 1% gelatin-coated dishes in DMEM with low glucose (1 g/L), 10% calf serum (Hyclone, Inc, Logan, Utah), 2 mmol/L L-gluta-
mine, 100 U/mL penicillin, and 100 μg/mL streptomycin. BCSE and BCAE cells were grown in the same medium except that the DMEM contained high glucose (4.5 g/L). Endothelial cells were used between passages 4 and 14.

Wounding of Monolayers of Endothelial Cells and Wound Assays

Three different procedures were used for the detachment (wounding) of endothelial cells from confluent monolayers in cultures. To evaluate the effect of exogenous agents on in vitro wound healing, monolayers of cells grown in six-well plates were wounded by denudation with a cotton-tipped applicator using a cloning ring to create a large (8 mm in diameter) wound area. After wounding, the cells were washed three times with DMEM (without calf serum) and then incubated with agents in the presence of 10% calf serum as described below. Immediately after wounding and 3, 4, and 5 days later, the wounded areas were photographed, and planimetric measurements were made of the extent to which they had become reendothelialized.

The repopulation of large denuded areas is due not only to migration but also to proliferation of endothelial cells. Therefore, we next studied small wound areas (2 mm in diameter), which heal primarily by migration. For this purpose, monolayers of cells were grown in 24-well plates and wounded by aspiration with a glass pipette. Two different concentrations of calf serum (0.5% and 10%) were added to the culture medium used after wounding to study the effects of this variable on cell proliferation and migration. Measurements of the wounded areas were obtained as described above.

Because healing of small wounds is also a function of cell spreading, we counted the cells in the zones of reendothelialization. For these assays, monolayers of cells grown in six-well plates were wounded with a razor blade. The razor blade wounds were made in a standardized manner to create areas of denudation of 2×10 mm. Furthermore, the areas of the wounds were clearly outlined by the marks made by the razor blade in the plastic dish. The plates were washed three times with DMEM (without serum), and various agents were added to the wells as described below. After incubation for 24 hours, cells migrating from the wound edge were counted by phase-contrast microscopy.

Immunohistochemical Staining

For light microscopic studies, endothelial cells were washed gently with 0.1 mol/L phosphate-buffered saline (PBS, pH 7.4) in the dishes and fixed with freshly prepared 4% paraformaldehyde in PBS for 30 minutes at room temperature. After

Fig 1. Graph showing reendothelialization in vitro enhanced by basic fibroblast growth factor (bFGF) and inhibited by anti-bFGF antibodies and cycloheximide. As detailed in "Materials and Methods," a large in vitro "wound" was made by removal of cells from 2-day postconfluent monolayers of bovine adrenal capillary endothelial cells. The percentage of the denuded area that is relined by migrating and proliferating cells is shown on the y axis. The symbol ● indicates control; ○, 1 ng/mL bFGF; △, 0.1 μg/mL neutralizing anti-bFGF antibody MF-1; ○, 1 μg/mL MF-1; □, 10 μg/mL MF-1; and ○, 0.1 μg/mL cycloheximide in 10% calf serum. Each experiment was repeated three times with similar results. The error bars indicate 1 SD.

Fig 2. Bar graph showing sheet migration of bovine coronary artery endothelial (BACE) cells in 0.5% or 10% calf serum (CS) enhanced by basic fibroblast growth factor (bFGF) and inhibited by anti-bFGF antibodies. Migration was modestly enhanced by serum, inhibited by cycloheximide (CH), and not affected by arabinosylcytosine (Ara-C). The y axis indicates the percent of small area of denudation relined at 24 hours. The experiment was repeated once with similar results. Ctrl indicates control; MF1, neutralizing antibody MF-1. The error bars indicate ±1 SD. *P<.01 and **P<.001 vs 0.5% control; †P<.01 and ††P<.001 vs 10% control.
### Effect of Exogenous Agents on Migration of Bovine Coronary Artery Endothelial Cells Wounded by Razor Blade

<table>
<thead>
<tr>
<th>Agent</th>
<th>Cells Counted, % of Control</th>
<th>No. of Samples</th>
<th>P*</th>
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<tbody>
<tr>
<td>Control</td>
<td>100±15</td>
<td>6</td>
<td></td>
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<tr>
<td>1 ng/mL bFGF</td>
<td>134±19</td>
<td>6</td>
<td>&lt;.01</td>
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<tr>
<td>DOG antibody</td>
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<tr>
<td>0.1 μg/mL</td>
<td>102±15</td>
<td>5</td>
<td>NS</td>
</tr>
<tr>
<td>1 μg/mL</td>
<td>85±10</td>
<td>5</td>
<td>NS</td>
</tr>
<tr>
<td>5 μg/mL</td>
<td>50±15</td>
<td>5</td>
<td>&lt;.05</td>
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<tr>
<td>10 μg/mL</td>
<td>23±10</td>
<td>5</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>10 μg/mL MF-1 antibody</td>
<td>61±8</td>
<td>6</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>0.1 μg/mL CH</td>
<td>60±6</td>
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bFGF indicates basic fibroblast growth factor; DOG, rabbit polyclonal IgG fraction raised against human recombinant bFGF; MF-1, mouse monoclonal antibody raised against bovine bFGF; and CH, cycloheximide. Values are mean±SD.

Agents were added in 10% calf serum immediately after wounding. Twenty-four hours later, migrating cells from the wound edge were counted. Values are normalized to those in control group.

*Results of t test for independent samples compared with control wells.

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**Fig 3.** Photomicrographs showing immunocytochemical staining for basic fibroblast growth factor (bFGF). A, Confluent bovine coronary artery endothelial (BCAE) cells stained with polyclonal antibody 773 show a reaction in the cytoplasm and nuclei. Bar=10 μm. B, Control preparation shows BCAE cells stained with polyclonal antibody 773, which had been preadsorbed with bFGF. No reactivity is evident. Bar=10 μm. C and D, Bovine adrenal capillary endothelial (BACE) cells are shown at 48 (C) and 72 (D) hours after wounding. Staining with monoclonal antibody 78 (C) shows a greater intensity of cytoplasmic, nuclear, and nucleolar bFGF immunoreactivity in migrating cells compared with confluent nonmigrating cells away from edge of wound (indicated by razor blade marks in the plastic dish). Similar results (D) were obtained at 72 hours. Bars=100 μm.
 Autoradiography were used. The wicz).

Antibodies

Two antibodies were used for the cytochemical localization of bFGF. The first, a polyclonal antibody (773), was raised in rabbits against the first 24 amino acids of the amino terminus of bovine bFGF (beginning at PAL...). This antibody, which detects bovine bFGF as a predominant band in Western blots of endothelial lysates, was kindly provided by Dr Andrew Baird, Whittier Institute, La Jolla, Calif. The second, a monoclonal antibody (78), which recognizes the epitope located at the first nine residues of HR-bFGF, was a kind gift from Takeda Pharmaceutical Co, Osaka, Japan. The characterization of antibodies has been described.2,3,13

To neutralize bFGF in culture media, two other antibodies were used. One antibody (DOG) was a rabbit polyclonal IgG fraction raised against HR-bFGF (gift of Dr D. Gospodarowicz). The other antibody (MF-1) was a mouse monoclonal antibody against bovine bFGF (Upstate Biotechnology Inc, Lake Placid, NY). These antibodies specifically detect bovine bFGF in Western blots, although DOG antibody also detects some non-bFGF bands. MF-1 antibody, like 78 antibody, is virtually monospecific. Normal rabbit or mouse IgG was used as a negative control for the experiments involving the addition of bFGF antisera to the cultures.

The following agents were added to the cell cultures to evaluate their effects on the healing of the in vitro wounds: HR-bFGF (gift from Drs Lawrence Cousins and Phil Barr, Chiron Corp, Emeryville, Calif), arabinosylcytosine (United States Biochemical Corp, Cleveland, Ohio), and cycloheximide (Sigma).

Autoradiography

Twenty-four hours after wounding, the cells were incubated with 3 µCi [3H]thymidine per milliliter (American Radiolabeled Chemicals, St Louis, Mo) for 1 hour. They were then washed, fixed, and stained for bFGF immunoreactivity as described above. Slides were then dipped in the dark in NTB2 photographic emulsion (Kodak) and developed 3 days later. The cells were mounted and examined with a light microscope for the simultaneous demonstration of thymidine uptake and bFGF immunoreactivity.

RNase Protection Analysis

Confluent monolayers of cells grown in large dishes (570 cm²) were wounded with a cotton-tipped applicator, creating parallel and perpendicular denuded stripes totaling ~50% of the area. Twenty-four hours later, the cells were harvested, and RNA was isolated by a modified guanidinium/CsCl centrifugation method.24 RNase protection analysis was performed according to the method of Gilman.35 To produce an antisense bFGF probe, the 625-bp EcoRI/Acc I fragment of the bovine bFGF cDNA25 was subcloned into the pBluescript II skt (Stratagene, La Jolla, Calif). Transcription was initiated from the pBluescript T3 promoter to produce a 32P-labeled 732-bp probe. The probe was annealed to total RNA samples, and 625 bp was protected from subsequent RNase digestion by hybridization to bFGF transcripts. The protected probe was recovered and analyzed by electrophoresis on a denaturing acrylamide gel. The gel was exposed to preflashed x-ray film for 24 hours in the presence of an intensifying screen.

Statistical Analysis

Data were analyzed by unpaired t test, with two-tailed

Results

Morphology of Confluent Endothelial Cells

Endothelial cells were identified on the basis of their characteristic morphology in culture and by their reactivity for acetylated LDL. BACE cells had a swirling appearance, whereas BCSE cells and BCAE cells formed cobblestone-like patterns. When confluent,
BCSE cells were less tightly apposed than BACE cells and BCAE cells.

**Wound Healing and Migration Assays**

Large wounds in monolayers of endothelial cells became completely reendothelialized within 5 days. BCAE cells and BCSE cells migrated in sheets, whereas BACE cells migrated both in sheets and as isolated individual cells. HR-bFGF (1 ng/mL) modestly stimulated wound healing (Fig 1). In BACE cells, HR-bFGF was more effective than in the other two cell types. The neutralizing anti-bFGF antibodies substantially inhibited wound healing in a dose-dependent fashion (Fig 1). No effects were observed when normal mouse IgG was added instead of the anti-bFGF monoclonal antibody. Cycloheximide (0.1 μg/mL, once a day) markedly inhibited wound healing (Fig 1).

Small areas denuded by aspiration with a pipette became completely reendothelialized by 48 hours when the cells were grown in the presence of either 0.5% or 10% calf serum. However, at 24 hours the percentages of the wound areas relined by endothelial cells were 83% and 70%, respectively, for cells grown in 10% and in 0.5% calf serum (P<.01, Fig 2). HR-bFGF significantly stimulated wound healing in both 0.5% (n=8, P<.01) and 10% (n=11, P<.001) calf serum (Fig 2). In contrast, anti-bFGF antibody MF-1 inhibited wound healing significantly and dose-dependently (Fig 2). Addition of normal mouse IgG instead of MF-1 monoclonal antibody had no effect. To further distinguish migration from proliferation, arabinosylcytosine (4×10^{-8} mol/L) was used to inhibit DNA synthesis. Arabinosylcytosine (5×10^{-8} mol/L) has been shown to inhibit the synthesis of DNA by fetal bovine aortic endothelial cells. Addition of arabinosylcytosine did not influence wound healing in cultures grown either with 0.5% or with 10% calf serum (Fig 2). Cycloheximide markedly inhibited wound healing in 0.5% (n=3, P<.001) and 10% (n=5, P<.001) calf serum (Fig 2).
Counts of cells migrating from the edges of 24-hour wounds produced by razor blade gave results comparable to those obtained by measuring surface areas (Table). Control procedures involving the addition of normal mouse or rabbit IgG (instead of monoclonal antibody MF-1 and DOG polyclonal antibody, respectively) gave negative results. Thus, the effects of bFGF, anti-bFGF antibody, 0.5% versus 10% calf serum, and cycloheximide were not due to their influences on cell spreading.

Immunohistochemical Observations

The immunohistochemical reactivity for bFGF was similar in all three types of endothelial cells. More than 95% of confluent cells showed diffuse cytoplasmic staining. The perinuclear area frequently contained small reactive vesicles. Nuclear and nucleolar staining was observed in ~30% of the cells (Fig 3A). Immunohistochemical control procedures gave negative results (Fig 3B). All three types of migrating endothelial cells exhibited increased immunoreactivity for bFGF in comparison with the corresponding confluent cells in non-denuded areas (Fig 3C and 3D). As shown in Fig 3D, at 72 hours after wounding, the cells that were migrating and dividing at the edges of the wounds were intensely immunoreactive for bFGF, whereas cells in adjacent areas had normal reactivity. To determine whether this increase in immunoreactivity was due to binding of bFGF released by wounded cells, the cultured cells were washed with heparin (10 μg/mL) immediately after wounding. This procedure prevents the binding of bFGF to heparan sulfate on the cell surface (which accounts for 90% of the total binding). Such washing did not prevent the increase in bFGF immunoreactivity observed at the edges of the wounds (Fig 4A and 4B). The addition of cycloheximide, which prevented wound healing (Figs 1 and 2 and the Table), blocked the increase in immunoreactivity for bFGF (Fig 4C). In a study of the time course of the change in immunoreactivity for bFGF, an increase in cytoplasmic staining was noted as early as 2 hours after wounding (Fig 5), before any cells had migrated or entered into the S phase. This early increase in cytoplasmic reactivity was followed by a later increase, first noted at 12 hours, in nuclear and nucleolar staining (Fig 5). This was found in virtually all cells, and these cells began their migration at the same time, indicating that, once density-arrested, they were synchronized in terms of the cell cycle. DNA synthesiz-
ing and rounded (M-phase) cells were noted between 12 and 24 hours. Together with the results of double-labeling experiments using [3H]thymidine autoradiography and bFGF staining, these data indicate that nuclear bFGF is found late in G1 and also in M phase and G2 (Fig 6A and 6B).

We considered the possibility that this increase in immunoreactivity was an artifact caused by the rounding and thickening of cells in the M phase. However, study with Hoffman optics showed that many of the intensely immunoreactive cells were flatter than the confluent cells in nondenuded areas (Fig 7). The increase in immunoreactivity for bFGF was also evident in cross sections of cells studied by electron microscopy (Fig 8), thus showing that such changes were not a function of cell thickness. Observation on cross sections of cells also indicated that the increase in reactivity did not take place in the extracellular matrix beneath the cells but in the cytoplasm and nuclei. The cells in nonwounded confluent monolayers had a thick layer of bFGF-rich subcellular matrix; however, there was little matrix beneath the migrating cells (Fig 8).

RNase Protection Analysis

RNase protection analysis revealed more bFGF mRNA after monolayer wounding (Fig 9). The twofold increase in signal could underestimate the increase in abundance of bFGF mRNA, since by 24 hours only \( \approx 30\% \) of the cells have begun to migrate. Thus, on a per-cell basis, the increase in bFGF mRNA in the migrating cells could be even greater.

Discussion

We have studied the migration of arterial, venous, and microvascular endothelial cells. We have found that, when a confluent monolayer of endothelial cells is partially denuded, the migration of the remaining cells is accompanied by an upregulation of bFGF gene expression, with the increased peptide appearing in the cytoplasm and nucleus before DNA synthesis. This suggests an intracrine function for bFGF. However, extracellular bFGF is also important, because migration is markedly inhibited by neutralizing antibodies to bFGF. Some of the extracellular bFGF used at the onset of migration probably derives from the extracellular matrix, which in the confluent cells is abundant and immunoreactive for bFGF. In contrast, the migrating cells have little subcellular matrix, yet their migration is inhibited by anti-bFGF antibodies, suggesting the possibility that bFGF is released by migrating endothelial cells.
Despite the upregulation of bFGF, some further stimulation of migration by the addition of exogenous bFGF was observed. The stimulus to increased bFGF expression presumably relates to the loss of contact inhibition and is not due to release of bFGF by the wounding procedure.

The migration of microvascular endothelial cells is known to be critical for angiogenesis. The sheet migration of arterial endothelial cells is important in reendothelializing denuded arteries after rupture of atherosclerotic plaques or after balloon angioplasty. The distinction between migration and proliferation is important in that (1) migration precedes proliferation and is sustained despite inhibition of DNA synthesis, as noted here and in previous studies, (2) several factors are known to be chemotactic or chemokinetic but are not mitogenic for endothelial cells, and (3) recent reports suggest that bFGF's mitogenic and chemotactic signals may be transduced by distinct mechanisms, at least in fetal bovine aortic endothelial cells.

Effect of bFGF and Anti-bFGF Antibodies on Endothelial Migration

In the present investigation, we studied the migration of three types of adult bovine endothelial cells under a variety of conditions. In all cases, the migration of all three types of cells was modestly enhanced by bFGF. This is consistent with most previous reports using the Boyden chamber assay. Sato and Rifkin found that bFGF stimulated the sheet migration of bovine aortic and adrenal microvascular endothelial cells after wounding of the in vitro monolayer. bFGF is well known to stimulate angiogenesis (see References 1 and 18 for review). Moreover, Lindner et al. reported that intravenous administration of bFGF enhanced reendothelialization after balloon denudation of the rat carotid artery. The results of these in vivo studies, however, are likely to reflect both migration and proliferation and, in the case of microvascular angiogenesis, invasion as well. Because these are distinct functions and because in some types of endothelial cells migration is not enhanced by added bFGF, we note that the present investigation is the first to describe the role of bFGF in coronary arterial and coronary sinus endothelial cells. In this respect, bFGF plays a similar role in BACE cells and in BCAE and BCSE cells, although there are morphological differences among these cell types.

Expression of bFGF by Endothelial Cells

Aortic, adrenal capillary, and umbilical vein endothelial cells are known to synthesize bFGF but the physiological factors that regulate bFGF expression are poorly understood. In the present study, we found that the migration of BACE, BCAE, and BCSE cells is preceded and accompanied by an increase in bFGF expression, as indicated by an increase in bFGF cytoplasmic immunoreactivity seen within 2 hours of disruption of the monolayer. The increase in bFGF immunoreactivity was blocked by inhibiting protein synthesis with cycloheximide. In addition, an increase in bFGF mRNA was noted by RNase protection assay.

Recognizing that increased immunoreactivity, even if reproducible in repeated assays, does not necessarily mean an increased concentration of bFGF, we took several precautions. We rigidly controlled the assay conditions and used intermediate antibody concentrations so that increases or decreases could be detected. Since the same dish contained both the migrating and the confluent populations, the assays were internally controlled. We recently quantified, as an external control, the amounts of bFGF in pure populations of endothelial and smooth muscle cells, both in vivo and in vitro, by heparin-Sepharose chromatography, mitogen assays, radioimmunoassay, immunoblotting, and immunoneutralization. We found that these results correlated with bFGF immunoreactivity by immunocytochemistry. Thus, it is highly likely that the increase in bFGF immunoreactivity in migrating cells, seen repeatedly and with more than one antibody, indicates an increase in bFGF concentration. The nature of the stimulus to bFGF expression is unknown but presumably involves the loss of contact inhibition. Although several peptide growth factors have been reported to stimulate their own expression, this is unlikely to be the case because of the arguments noted above. It should be noted that, even though bFGF expression was increased by disrupting the confluent monolayer, even the nonwounded confluent monolayer contained some bFGF mRNA, despite the near absence of DNA synthesis. This suggests that bFGF could have a nonmitogenic function, as implied by the effects of exogenous bFGF on endothelial cell longevity and differentiation and expression of angiotensin-converting enzyme.

Nuclear bFGF

We noted a marked increase in nuclear bFGF immunoreactivity 12 hours after wounding of the monolayer, several hours before the initiation of DNA synthesis.
This finding was obtained with two different monospecific antibodies, which were raised against different domains of bFGF, and was confirmed by the immunohistochemical control procedures. Moreover, in non-wounded monolayers of endothelial cells, we have confirmed and extended these results by cell fractionation and purification of bFGF by heparin-Sepharose chromatography, with identification by immunoblotting and mitogen assays. Several reports (see Yu et al11 for review) have suggested that the nuclei of endothelial cells contain 24- and 26-kD forms of bFGF, in contrast to the 18-kD form usually found in the cytoplasm. The role of these high molecular weight forms of bFGF is not known.

It is likely that some newly synthesized bFGF is transported directly from the cytoplasm to the nucleus, since suramin does not block the nuclear accumulation. Whatever the intracrine role, at least some bFGF released from the cell is critical for endothelial migration, since neutralizing antibodies to bFGF markedly inhibited migration. This is in agreement with the results of Sato and Rifkin. The residual migration could reflect unblocked extracellular bFGF or other factors, such as acidic fibroblast growth factor, fibronectin, released from the cell is critical for endothelial migration.52 Whatever the intracrine role, at least some bFGF transport directly from the nucleus. Whatever the intracrine role, at least some bFGF transport directly from the nucleus. It is likely that some newly synthesized bFGF is

Acknowledgments
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