Proc. Natl. Acad. Sci. USA
Vol. 84, pp. 2292–2296, April 1987
Cell Biology

Endothelial cell-derived basic fibroblast growth factor: Synthesis and deposition into subendothelial extracellular matrix

(heparin affinity/endothelial cell growth factors)

ISRAEL VLODAVSKY*, JUDAH FOLKMAN†‡, ROBERT SULLIVAN†, RAFAEL FRIDMAN*, RIVKA ISHAI-MICHAELI*, JOACHIM SASSE†, AND MICHAEL KLAGSBRUN†§¶

*Department of Radiation and Clinical Oncology, Hadassah University Hospital, Jerusalem, Israel 91120; and †Department of Surgery, Children's Hospital and Departments of ‡Anatomy and Cellular Biology and of §Biological Chemistry, Harvard Medical School, Boston, MA 02115

Communicated by Ruth Sager, December 8, 1986 (received for review, September 9, 1986)

ABSTRACT **Bovine aortic and corneal endothelial cells synthesize a growth factor that remains mostly cell-associated but can also be extracted from the subendothelial extracellular matrix (ECM) deposited by these cells. The endothelial cell-derived growth factors extracted from cell lysates and from the extracellular matrix appear to be structurally related to basic fibroblast growth factor by the criteria that they (*i*) bind to heparin-Sepharose and are eluted at 1.4–1.6 M NaCl, (*ii*) have a molecular weight of about 18,400, (*iii*) cross-react with anti-basic fibroblast growth factor antibodies when analyzed by electrophoretic blotting and immunoprecipitation, and (*iv*) are potent mitogens for bovine aortic and capillary endothelial cells. It is suggested that endothelium can store growth factors capable of autocrine growth promotion in two ways: by sequestering growth factor within the cell and by incorporating it into the underlying extracellular matrix.**

A role for cell–matrix interactions in the control of cell proliferation and morphogenesis has long been recognized by developmental biologists (1–3). Mammalian cells maintained under tissue culture conditions require, in order to proliferate and to express their normal phenotype, not only nutrients and growth factors but also an appropriate substratum upon which they can attach and spread (4–7). Cultured bovine endothelial cells lay down an extracellular matrix (ECM), which replaces the requirement that sparsely seeded endothelial cells have for fibroblast growth factor (FGF) to proliferate and express their differentiated functions (8–10). This supportive role of ECM on endothelial cell growth has been attributed to changes in cell shape dictated by structural components of the ECM (8–11). The possible involvement of ECM-bound growth factors in the induction of cell proliferation has been minimized because ECM treated to inactivate growth factors still supports endothelial cell growth (11–13). However, the possibility that ECM contains highly stable growth factors has not been fully ruled out. Recently, it has been shown that endothelial-cell growth factors have a strong affinity for heparin (14–16). These growth factors are also synergized (17) and stabilized (18, 19) by heparin. The presence of heparan sulfate as the major glycosaminoglycan in the subendothelial ECM (20) raises the possibility that ECM contains heparin-binding endothelial-cell growth factors that are tightly bound and possibly stabilized by the ECM heparan sulfate. In this report, we show that endothelial cells synthesize heparin-binding basic FGF, most of which remains cell-associated and some of which is deposited and sequestered in the subendothelial ECM. The matrix-derived growth factor may play a role in induction of cell proliferation and differentiation by ECM.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

MATERIALS AND METHODS

Cell Cultures. Cloned populations of bovine vascular aortic endothelial cells (21) and bovine eye-derived corneal endothelial cells (22) were cultured as described except that FGF was not included in the growth medium. Human hepatoma cells (SK-HEP-1) were grown as described (23).

Preparation of Dishes Coated with ECM. Bovine corneal or aortic endothelial cells were plated at an initial density of 10^6 cells per 10-cm tissue culture dish (Falcon). Six to 8 days after the cells reached confluency, the subendothelial ECM was exposed by dissolving the cell layer with 0.5% Triton X-100 and 20 mM NH_4OH in phosphate-buffered saline, followed by four washes in phosphate-buffered saline (8–10). Alternatively, the endothelial cells were removed intact by exposure (10–20 min) to 2 M urea in Dulbecco's modified Eagle's medium, a procedure that exposes the subendothelial ECM without lysing cells (11).

Growth Factor Activity. Growth factor activity for 3T3 cells was measured as described (14, 16, 23). A unit of 3T3 cell-stimulatory activity was defined as the amount of growth factor required to yield half-maximal [^{3}H]thymidine incorporation into 3T3 cell DNA. Two methods were used to measure endothelial cell proliferation. (*i*) Cells were seeded sparsely (10,000 cells per 35-mm dish). Growth factor was added every other day; at various times after seeding, cells were dissociated with trypsin/EDTA and counted in triplicate on a Coulter Counter (12, 13). (*ii*) Cells were seeded at a clonal density (400 cells per 35-mm dish). Growth factor was added every other day; 8 and 14 days after seeding, the cultures were fixed with 3.7% formaldehyde, and cell colonies were stained with 0.1% crystal violet (21, 24).

Extraction of Growth Factor from Endothelial Cells and ECM. Growth factor was extracted from endothelial cells as described for SK-HEP-1 cells (23). Briefly, cells (5×10^8) were harvested from monolayer cultures by trypsinization, washed with phosphate-buffered saline, resuspended in 1 M NaCl/0.01 M Tris·HCl, pH 7.5 (10^7 cells per ml), and disrupted by three cycles of freezing and thawing followed by sonication and centrifugation at 25,000 × *g* for 30 min. Growth-promoting activity was extracted from the ECM either by scraping the ECM from plastic surfaces in 2 M NaCl or by incubating with collagenase (50 μg/ml, CLS II; Cooper Biomedical, Malvern, PA) or trypsin (50 μg/ml; Sigma) for 2 hr at 37°C in phosphate-buffered saline. Insoluble material was removed by centrifugation (5000 × *g*) for 20 min. Neither enzyme affected growth-factor activity or molecular weight under the conditions used. The amount of ECM protein

Abbreviations: FGF, fibroblast growth factor; ECM, extracellular matrix.

¶To whom reprint requests should be addressed at: Children's Hospital, Enders Bldg., Rm. 1029, 300 Longwood Avenue, Boston, MA 02115.

extracted with 2 M NaCl from a 10-cm dish was about 0.2 mg as compared to 1.1 mg of protein extracted with enzymes.

Column Chromatography. Heparin-Sepharose chromatography was performed as described (14–16, 23). NaCl extracts were diluted with 0.01 M Tris·HCl (pH 7.5) or were dialyzed against this buffer so as to lower the concentration of NaCl to 0.1 M. These preparations and enzyme extracts of ECM were applied directly to heparin-Sepharose (Pharmacia) columns (10 ml) equilibrated with 0.1 M NaCl/0.01 M Tris·HCl, pH 7.5. After a wash of about 5 column volumes, growth factor was eluted with a gradient (500 ml) of 0.1–3.0 M NaCl in 0.01 M Tris·HCl (pH 7.5) at a flow rate of 40 ml/hr at 4°C (14–16, 23).

Electrophoretic Protein Transfer Blot. Protein was electrophoresed on $NaDodSO_4$/polyacrylamide gels and transferred to nitrocellulose paper electrophoretically as described (23, 25). The nitrocellulose paper was either stained for protein with Aurodye colloidal gold reagent or was incubated with anti-basic FGF antiserum and visualized by successive incubations with biotinylated goat anti-rabbit antibodies, peroxidase-conjugated strepavidin, and 4-chloro-1-naphthol substrate (23, 25). Two anti-basic FGF antisera were prepared by immunizing rabbits with synthetic peptides corresponding to positions 1–12 (amino-terminal) and positions 33–43 (internal) of basic FGF (23, 25), according to the numbering system originally described for basic FGF (26).

Immunoprecipitation. Confluent cultures of aortic endothelial cells and SK-HEP-1 cells (about 10^7 cells per T 75 flask) were labeled with [^{35}S]methionine (Amersham; 680 Ci/mmol; 1 Ci = 37 GBq) at 50 μCi/ml for 20 hr in methionine-free Dulbecco's modified Eagle's medium containing 2% calf serum, extracted with 1 M NaCl/0.5% Triton X-100, and immunoprecipitated with preimmune sera and with antisera directed against the amino-terminal sequence of basic FGF as described (23, 25).

RESULTS

Endothelial Cell-Derived Growth Factors. Extracts of cultured bovine aortic endothelial cells were analyzed by heparin-Sepharose affinity chromatography for growth factor activity. A single growth factor peak was eluted from the column at about 1.5 M NaCl (Fig. 1*A*). Similar results were obtained with a cell lysate prepared from cultured corneal endothelial cells; 10^6 endothelial cells yielded 50–70 units of 3T3 growth factor activity. Heparin-affinity-purified endothelial cell-derived growth factor stimulated the proliferation of both bovine aortic and capillary endothelial cells at about 2 ng/ml.

Endothelial cell-derived growth factor activity was further characterized by comparing its heparin-affinity-column elution profile with that of hepatoma cell extracts (Fig. 1*B*), bovine brain (Fig. 1*C*), and bovine serum (Fig. 1*D*). It has been shown that hepatoma cells contain a species of basic FGF eluted at 1.5 M NaCl; that brain has a similar basic FGF species and, in addition, has a species of acidic FGF eluted at about 1 M NaCl; and that serum contains no species of FGF, but rather a growth factor eluted at 0.5 M NaCl that is platelet-derived growth factor (15, 16, 18, 23). The endothelial cell-derived growth factor species eluted at 1.5 M NaCl comigrated with hepatoma and brain basic FGF, thus providing circumstantial evidence that it was structurally related to basic FGF. There appeared to be no evidence for acidic FGF or platelet-derived growth factor-like activities in the endothelial cell extracts. The marked difference in the growth factor elution profiles between extracts of bovine endothelial cells (Fig. 1*A*) and bovine serum (Fig. 1*D*) suggested that the endothelial cell-derived growth factor was not an artifact of absorption of growth factor from serum.

Biochemical Characterization of Endothelial Cell-Derived Growth Factor. When heparin-Sepharose column-purified endothelial cell-derived growth factor was analyzed by HPLC TSK size-exclusion chromatography, a single peak of growth-factor activity with a molecular weight between 16,000 and 18,000 was found (results not shown), consistent with the molecular weight of basic FGF, which has been shown to be between 16,500 (26) and 18,500 (23). More conclusive evidence that endothelial cell-derived growth factor was a form of basic FGF was obtained by using specific anti-FGF antibodies in electrophoretic transfer blots and in immunoprecipitation (Fig. 2). Antibodies to synthetic peptides corresponding to both the amino-terminal (Fig. 2 *Left*, lane 1) and internal regions (Fig. 2 *Left*, lane 2) of pituitary and brain basic FGF cross-reacted with a M_r 18,400 polypeptide doublet purified from aortic endothelial cells by heparin-Sepharose affinity chromatography. Biosynthesis of basic FGF was demonstrated by metabolic labeling of cultured aortic endothelial cells, followed by immunoprecipitation with anti-basic FGF antiserum, $NaDodSO_4$/PAGE, and fluorography (Fig. 2 *Right*). The anti-FGF antiserum (Fig. 2 *Right*, lane 3) but not preimmune serum (lane 2) immunoprecipitated a M_r 18,400 polypeptide doublet. In a parallel experiment, the same anti-FGF antiserum (Fig. 2 *Right*, lane 5) but not preimmune serum (lane 4) immunoprecipitated a similar polypeptide doublet from SK-HEP-1 cells, previously shown to be a hepatoma cell-derived basic FGF (23).

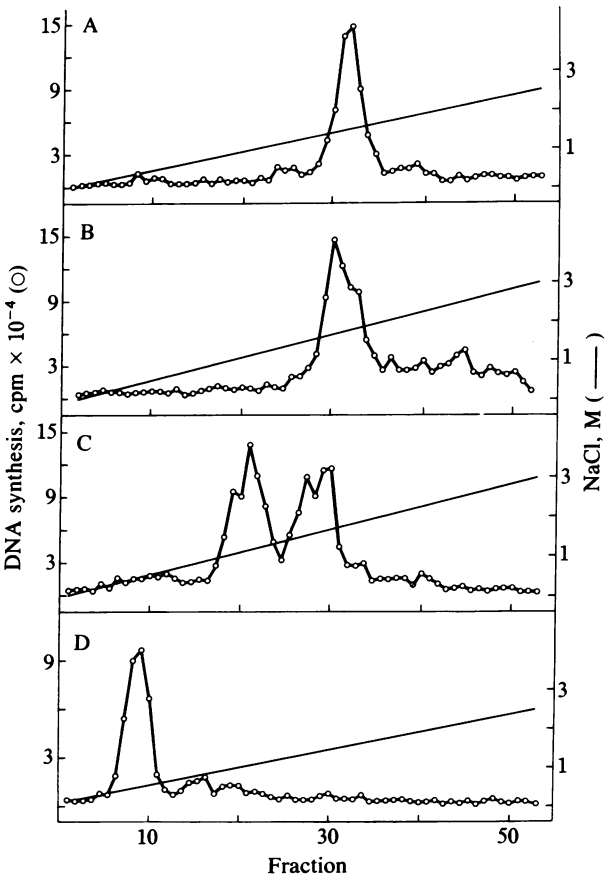

FIG. 1. Heparin-Sepharose chromatography. (*A–C*) Bovine aortic endothelial cells (5×10^8) (*A*), human SK-HEP-1 hepatoma cells (1×10^9) (*B*), and bovine brain (250 g) (*C*) were extracted in 1 M NaCl/0.01 M Tris·HCl, pH 7.5, and analyzed on individual heparin-Sepharose columns after lowering the salt concentration to 0.1 M NaCl. (*D*) Calf serum (40 ml) was applied directly to the heparin-Sepharose column. Growth factors were eluted with a gradient of 0.1–3 M NaCl, and their mitogenic activity for 3T3 cells was measured.

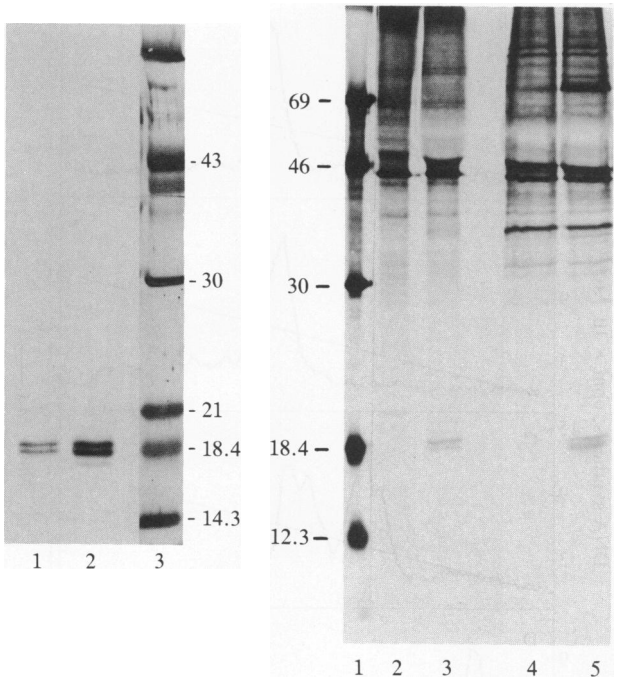

FIG. 2. Immunodetection of endothelial cell-derived growth factors by antisera directed against basic FGF. (*Left*) Electrophoretic protein transfer blot. The active fractions (29–32, 8000 units) from the heparin-Sepharose column of endothelial cell lysates (Fig. 1*A*) were pooled, dialyzed against distilled water, lyophilized, and electrophoresed on $NaDodSO_4$/PAGE. The proteins were transferred electrophoretically to nitrocellulose paper and stained separately with site-specific antisera directed against synthetic peptides corresponding to amino-terminal (lane 1) and internal (lane 2) sequences of basic FGF. Molecular weight (shown $\times 10^{-3}$) markers stained with Aurodye colloidal gold are seen in lane 3. (*Right*) Immunoprecipitation: Confluent cultures of bovine aortic endothelial cells and SK-HEP-1 cells were incubated with [^{35}S]methionine, extracted, immunoprecipitated, and analyzed by $NaDodSO_4$/PAGE and fluorography. Lanes: 1, ^{14}C-labeled molecular weight (shown $\times 10^{-3}$) standards; 2, aortic endothelial cell extract incubated with preimmune serum; 3, aortic endothelial-cell extract incubated with antiserum against the amino-terminal sequence of basic FGF; 4, SK-HEP-1 cell extract incubated with preimmune serum; 5, SK-HEP-1 extract incubated with antiserum to the amino-terminal sequence of basic FGF.

Extraction of Matrix-Derived Growth Factors from the Subendothelial ECM. Cultured vascular and corneal endothelial cells secrete an ECM in a polar fashion exclusively underneath the cell layer (8–13). Unlike tissue culture plastic (Fig. 3*A*), subendothelial ECM supports the growth of aortic endothelial cells seeded at clonal cell densities (Fig. 3*D*). To test whether growth factors might be involved in ECM induction of cell proliferation, ECM was extracted with 2 M NaCl, and the extracts were tested for growth-factor activity. Since ECM produced by vascular endothelial cells is thinner and tends to detach from tissue culture plastic, corneal endothelial cell ECM, which is similar to vascular ECM in chemical composition and biological activity but which remains free of cellular debris and firmly bound to tissue culture plastic even after extensive washing, was used for the extraction. Extracts of ECM were able to support the proliferation of endothelial cells seeded on tissue culture plastic at clonal densities in a dose-dependent manner (Fig. 3 *B* and *C*) and to the same extent as did extracts of cell lysates (not shown).

Extraction of ECM from a 10-cm dish with 2 M NaCl yielded 20–30 units of growth-factor activity compared to the 300–400 units of growth-factor activity found in lysates of the 6×10^6 cells recovered from the same dish. The ability of 2 M NaCl-extracted ECM to be still mitogenic for endothelial cells suggested that more growth factor might be present within the ECM that could not be extracted with 2 M NaCl. Accordingly, the ECM was extracted with either trypsin or collagenase, and the yield of ECM-derived growth factor extracted from a 10-cm dish was found to be increased to about 120 units or about 30% of that found in the endothelial cell lysate. To verify that the matrix-derived growth factor was not an artifact of intracellular growth-factor release occurring when cells were lysed by the Triton/NH_4OH treatment, endothelial cells were removed intact from culture dishes with 2 M urea, a procedure that exposes subendothelial ECM with little or no cell lysis (11). Extraction of ECM after urea treatment yielded about 100 units of matrix-derived growth factor per 10-cm dish—an amount similar to that obtained after the Triton/NH_4OH treatment. These results suggested that most of the matrix-derived growth factor represented growth factor deposited by viable endothelial cells into ECM rather than by lysed cells.

Biochemical Characterization of Matrix-Derived Growth Factor. Extracts of ECM were analyzed by heparin-Sepharose affinity chromatography (Fig. 4). One single peak of growth-factor activity was eluted at about 1.5 M NaCl, regardless of whether the ECM was produced by corneal (Fig. 4 *Left*) or vascular (not shown) endothelial cells. When a sample from this peak was analyzed by electrophoretic transfer blot, anti-FGF antiserum cross-reacted with a polypeptide doublet of about 18,400 (Fig. 4 *Right*, lane 1) suggesting that the matrix-derived growth factor was a form of basic FGF. Heparin affinity-purified matrix-derived

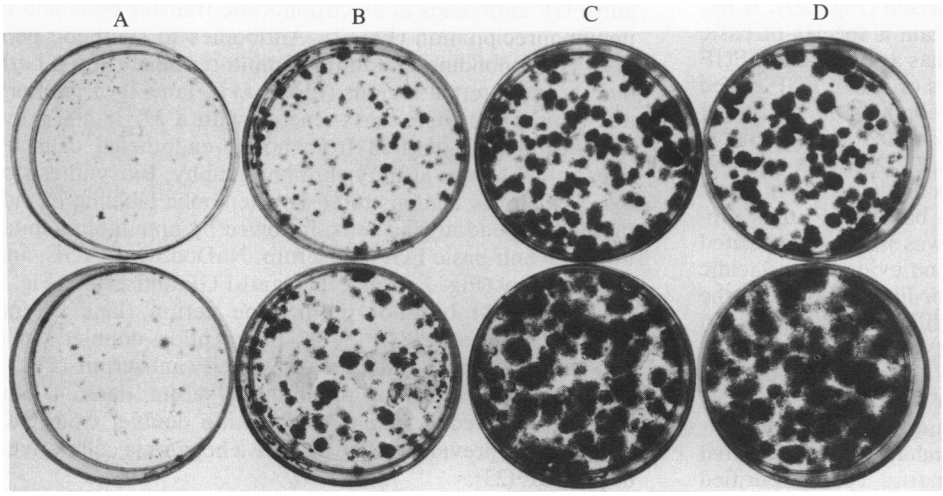

FIG. 3. Growth-factor activity of crude ECM extract. Bovine aortic endothelial cells were seeded at a clonal density (400 cells per 35-mm dish) into regular tissue culture plastic dishes (*A–C*) and into dishes coated with ECM (*D*). Aliquots of 10 μl containing 2 μg of protein (*B*) and of 100 μl containing 20 μg of protein (*C*) of a dialyzed 2 M NaCl extract of ECM were added to dishes 24 hr after seeding and every other day thereafter. Eight (*A–D Upper*) and 14 days (*A–D Lower*) after seeding, cultures were fixed, and cell colonies were stained with 0.1% crystal violet.

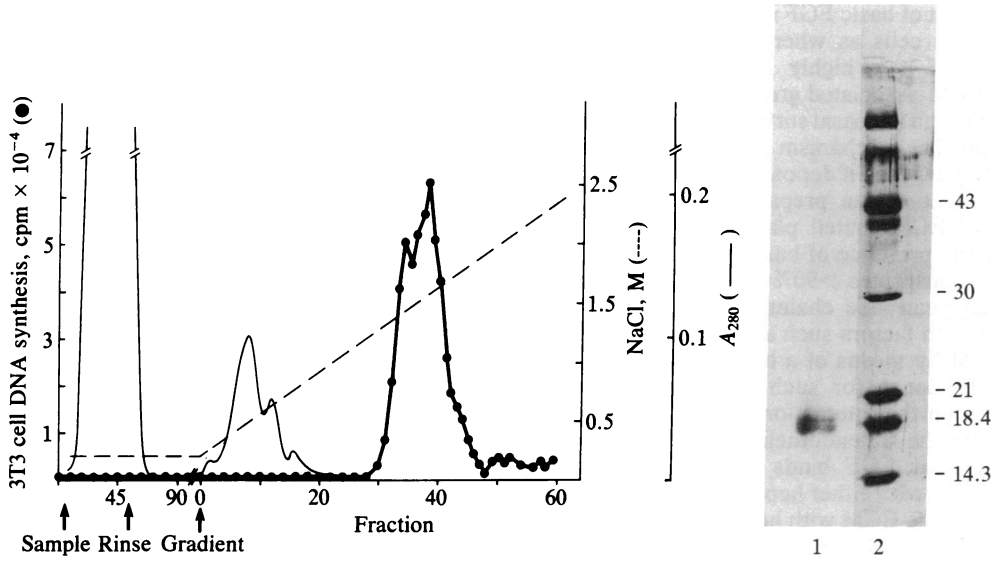

FIG. 4. Heparin-Sepharose chromatography and electrophoretic transfer blot of matrix-derived growth factor. (*Left*) Heparin-Sepharose chromatography. ECM-coated dishes (200 10-cm dishes) were digested with collagenase; after centrifugation the supernatant was applied directly to a column of heparin-Sepharose. A gradient of 0.1–2.5 M NaCl was applied, and fractions were collected and measured for 3T3 cell growth factor activity, absorbance at 280 nm, and conductivity. (*Right*) Electrophoretic transfer blot. Active fractions 33–40 (8000 units) were dialyzed against distilled water, lyophilized, and electrophoresed by $NaDodSO_4$/PAGE. Lanes: 1, proteins were transferred electrophoretically to nitrocellulose paper and stained with antiserum directed against a synthetic peptide representing an internal sequence of basic FGF (a similar result not shown was obtained with an antiserum directed against the amino-terminal portion of basic FGF); 2, molecular weight (shown × 10^{-3}) markers stained with Aurodye colloidal gold.

growth factor stimulated endothelial-cell proliferation about 20-fold over controls in 9 days, as did heparin-affinity-purified endothelial cell-derived growth factor and brain basic FGF (Fig. 5). It was estimated that matrix-derived growth factor stimulated the proliferation of both aortic and capillary endothelial cells at a concentration of 0.2–1.0 ng/ml. There was no evidence for the presence of acidic FGF or platelet-derived growth factor-like activity in the ECM.

DISCUSSION

Vascular endothelial cells are stimulated to proliferate by addition of soluble heparin-binding polypeptide growth factors (14–16, 18, 23). Proliferation of these cells in the absence of exogeneous growth factors can be supported by plating them on the subendothelial ECM produced by cultured endothelial cells (10, 12, 13, 24). The proliferative effect of the ECM has been attributed to structural constituents that induce a permissive change in the shape of cells, allowing them to respond more readily to physiologically occurring hormones and growth factors present in serum and plasma (7–13). The present study suggests that the proliferative effect of the ECM could also be due to growth factors that are firmly sequestered in the ECM. We can demonstrate that endothelial cells synthesize a heparin-binding growth factor, most of which remains cell-associated but up to 30% of which is deposited and sequestered by the subendothelial ECM. The endothelial cell-derived growth factors (both cell-associated and ECM-associated) appear to be structurally related to basic FGF by the criteria of (*i*) having M_r values of ≈18,400; (*ii*) tight binding to heparin-Sepharose and elution at 1.4–1.7 M NaCl; (*iii*) cross-reactivity with antibodies directed against basic FGF; and (*iv*) potent mitogenic activities for aortic and capillary endothelial cells. To date the structural evidence suggests that the same form of basic FGF is associated with endothelial cells and with their subendothelial ECM. Endothelial cells do not appear to secrete basic FGF into their medium (27).

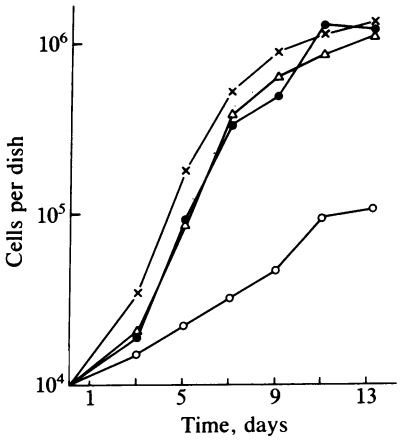

FIG. 5. Growth-factor activity of heparin-affinity-purified growth factors derived from endothelial cell lysates and ECM. Bovine aortic endothelial cells were seeded (10^4 cells per 35-mm dish) and incubated with heparin-affinity-purified growth factors (about 10 units per 2 ml per dish) derived from endothelial cell lysate (●), subendothelial ECM (△), and bovine brain basic FGF (×). Control cultures were maintained in the absence of added growth factors (○). Cells were counted in triplicate. The standard deviation in different determinations did not exceed 15% of the mean.

There is always the possibility that the sequestering of basic FGF in ECM occurs when the endothelial cell layer is solubilized by treatment with Triton/NH_4OH. However, several pieces of evidence suggest that most of the ECM-bound growth factor activity does not originate from cell lysis when the ECM is prepared. First, extraction of ECM with 2 M NaCl releases only 20–25% of the basic FGF that is obtained when ECM is totally solubilized by trypsin or collagenase, suggesting that basic FGF is firmly embedded within the subendothelial ECM. Second, it is possible to prepare ECM by incubation with 2 M urea without lysing endothelial cells (11). About as much basic FGF can be extracted from ECM prepared in this manner as from ECM prepared by lysis of cells with Triton/NH_4OH. In addition, when the endothelial cell layer is trypsinized so as to dissociate the cells intact while digesting the underlying

ECM, the same amount of basic FGF is extracted from ECM relative to endothelial cells as when cells are lysed with Triton/NH_4OH. Thus, it is highly probable that a large percentage of the ECM-associated growth factor is secreted in a polar fashion through the basal surface during the process of matrix deposition. The mechanism of this mode of release is not known. However, even if deposition of basic FGF into ECM were an artifact of the preparation technique, the inductive effects of ECM-coated plates could still be explained in part by the presence of basic FGF.

Heparan sulfate constitutes >90% of the subendothelial ECM glycosaminoglycan side chains (20), suggesting that heparin-binding growth factors such as basic FGF might be sequestered in ECM by means of a high-affinity binding to heparan sulfate. Evidence for such binding comes from preliminary studies on the interaction of hepatoma-derived basic FGF (23) with the subendothelial ECM. Exogeneous hepatoma-derived basic FGF binds to ECM and can be released by incubation with either heparan sulfate or heparin as well as by treating the ECM with heparanase but not with chondroitinase ABC (28). Heparin has been shown to stabilize the activity of both basic (19) and acidic FGF (18). Thus, heparan sulfate in the ECM may serve as a sink to concentrate and stabilize heparin-binding growth factors in the vicinity of cell surface receptors. Stabilization of heparin-binding growth factors by interaction with heparan sulfate proteoglycan could explain the survival of the mitogenic effects of endothelial cell ECM even after harsh treatments that inactivate soluble heparin-binding growth factors (11–13).

Storage of basic FGF in endothelial cells and subendothelial ECM may play a role in angiogenesis. After injury or mild perturbation of endothelial cells, basic FGF might be released from storage in cells or basement membranes. The released basic FGF could stimulate the autocrine proliferation of adjacent endothelial cells. Tumor angiogenesis may in part be mediated by the action of tumor-derived heparan sulfate-degrading enzymes (20, 29), which would release basic FGF stored in capillary basement membrane. We propose that release of intracellular and extracellular stores of basic FGF may be a mechanism for the rapid mobilization of angiogenesis factors.

This work was supported by National Cancer Institute Grants CA 30289 to I.V. and CA 37392 to M.K.; by the Fund for Basic Research administered by the Israel Academy of Sciences; and by a grant to Harvard University from the Monsanto Company. I.V. is a Leukemia Society of America Scholar.

1. Hay, E. D., ed. (1981) *Cell Biology of Extracellular Matrix* (Plenum, New York), pp. 115–334.
2. Grobstein, C. (1967) *Natl. Cancer Inst. Monogr.* **26,** 279–299.
3. Wessels, N. K. (1964) *Proc. Natl. Acad. Sci. USA* **52,** 252–256.
4. Kleinman, H. K., Klebe, R. J. & Martin, G. R. (1981) *J. Cell Biol.* **88,** 473–485.
5. Folkman, J. & Moscona, A. (1978) *Nature (London)* **273,** 345–349.
6. Salomon, D. S., Liotta, L. A. & Kidwell, W. R. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 383–386.
7. Gospodarowicz, D., Greenburg, G. & Birdwell, C. R. (1978) *Cancer Res.* **38,** 4155–4171.
8. Vlodavsky, I., Lui, G. M. & Gospodarowicz, D. (1980) *Cell* **19,** 607–616.
9. Gospodarowicz, D., Delgado, D. & Vlodavsky, I. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 4094–4098.
10. Gospodarowicz, D. & Ill, C. R. (1980) *J. Clin. Invest.* **65,** 1351–1364.
11. Gospodarowicz, D., Gonzalez, R. & Fujii, D. K. (1983) *J. Cell. Physiol.* **114,** 191–202.
12. Fridman, R., Ovadia, H., Fuks, Z. & Vlodavsky, I. (1985) *Exp. Cell Res.* **157,** 181–194.
13. Greenburg, G. & Gospodarowicz, D. (1982) *Exp. Cell Res.* **140,** 1–14.
14. Shing, Y., Folkman, J., Sullivan, R., Butterfield, C., Murray, J. & Klagsbrun, M. (1984) *Science* **223,** 1296–1298.
15. Lobb, R., Sasse, J., Shing, Y., D'Amore, P., Sullivan, R., Jacobs, J. & Klagsbrun, M. (1986) *J. Biol. Chem.* **261,** 1924–1928.
16. Klagsbrun, M. & Shing, Y. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 805–809.
17. Thornton, S. C., Mueller, S. N. & Levine, E. M. (1983) *Science* **222,** 623–625.
18. Schreiber, A. B., Kenney, J., Kowalski, W. J., Friesel, R., Mehlman, T. & Maciag, T. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 6138–6142.
19. Gospodarowicz, D. & Cheng, J. (1986) *J. Cell. Physiol.* **128,** 475–484.
20. Kramer, R. H., Vogel, K. G. & Nicolson, G. L. (1982) *J. Biol. Chem.* **257,** 2678–2686.
21. Gospodarowicz, D., Moran, J., Braun, D. & Birdwell, C. (1976) *Proc. Natl. Acad. Sci. USA* **73,** 4120–4124.
22. Gospodarowicz, D., Mescher, A. L. & Birdwell, C. R. (1977) *Exp. Eye Res.* **25,** 75–89.
23. Klagsbrun, M., Sasse, J., Sullivan, R. & Smith, J. A. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 2448–2452.
24. Fridman, R., Alon, Y., Doljanski, F., Fuks, Z. & Vlodavsky, I. (1985) *Exp. Cell Res.* **158,** 461–476.
25. Wadzinski, M., Folkman, J., Sasse, J., Ingber, D., Devey, K. & Klagsbrun, M. (1987) *Clin. Physiol. Biochem.*, in press.
26. Esch, F., Baird, A., Ling, N., Ueno, N., Hill, F., Denoroy, L., Klepper, R., Gospodarowicz, D., Bohlen, P. & Guillemin, R. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 6507–6511.
27. Vlodavsky, I., Fridman, R., Sullivan, R., Sasse, J. & Klagsbrun, M. (1987) *J. Cell. Physiol.*, in press.
28. Vlodavsky, I., Sullivan, R., Fridman, R., Sasse, J., Folkman, J. & Klagsbrun, M. (1986) *J. Cell Biol.* **103,** 98a.
29. Vlodavsky, I., Fuks, Z., Bar-Ner, M., Ariav, Y. & Schirrmacher, V. (1983) *Cancer Res.* **43,** 2704–2711.