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Purification of a factor from human placenta that stimulates capillary endothelial cell protease production, DNA synthesis, and migration

(angiogenic factor/plasminogen activator/collagenase/chemotaxis)

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ABSTRACT **A protein that stimulates the production of plasminogen activator and latent collagenase in cultured bovine capillary endothelial cells has been purified 10^6-fold from term human placenta by using a combination of heparin affinity chromatography, ion-exchange chromatography, and gel chromatography. The purified molecule has a molecular weight of 18,700 as determined by $NaDodSO_4$/PAGE under both reducing and nonreducing conditions. The purified molecule stimulates the production of plasminogen activator and latent collagenase in a dose-dependent manner between 0.1 and 10 ng of protein/ml. The purified protein also stimulates DNA synthesis and chemotaxis in capillary endothelial cells in the same concentration range. Thus, this molecule has all of the properties predicted for an angiogenic factor.**

During neovascularization of tumors, new capillaries arise as sprouts from existing microvessels in response to local release of angiogenic factors by the tumor cells (1). It has been proposed that the response of microvascular endothelial cells to angiogenic factors has three major components: an increase in endothelial cell protease production that allows the endothelial cells to penetrate the surrounding tissues, a stimulation of endothelial cell migration toward the source of the angiogenic factor, and an increase in the rate of endothelial cell proliferation (2). Possible correlates of each of these components have been identified in the response of cultured microvascular endothelial cells to angiogenic preparations—increased collagenase and plasminogen activator (PA) production (2), increased endothelial cell motility and chemotaxis (3, 4), and increased rate of multiplication (5, 6). However, it has not been demonstrated whether the stimulation of these different processes is due to a single molecule in the preparations or to several different molecules. Several groups have used heparin-Sepharose affinity chromatography to purify endothelial cell mitogens from various sources (7–12). We have investigated whether heparin-Sepharose affinity chromatography could also be used to purify the PA and collagenase-inducing activity from a preparation with known angiogenic activity and whether the purified protease-inducing molecule was also able to induce the other activities associated with angiogenesis—increased endothelial cell mitosis and motility. As a source of the protease-inducing activity, we have chosen term human placenta, which has been shown to contain an angiogenic activity (13) and to stimulate PA and collagenase production in cultured capillary endothelial cells (21).

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MATERIALS AND METHODS

Cell Culture. Bovine capillary endothelial (BCE) cells were isolated from the adrenal cortex of recently slaughtered yearling cattle by the method of Folkman *et al.* (14). Cells were grown to confluence in α minimal essential medium (αMEM) containing 10% (vol/vol) calf serum and supplemented with medium conditioned by mouse sarcoma 180 cells as described (15). When cultures reached confluence, the medium was changed to αMEM containing 5% (vol/vol) calf serum and no conditioning factors.

Assays for the Induction of PA and Collagenase. Confluent cultures of BCE cells that had been maintained for at least two days in αMEM containing 5% (vol/vol) calf serum were changed to fresh αMEM containing 5% (vol/vol) calf serum and the substance to be tested. After incubation at 37°C for 24 hr, the medium was collected from the cultures and was assayed for collagenase as described (16). All collagenase was in a latent form and was activated with trypsin to detect activity. The cell layers from these same cultures were washed twice with cold phosphate-buffered saline, pH 7.5, and were extracted with 0.5% Triton X-100 in 0.1 M sodium phosphate, pH 8.1, and the cell extracts were assayed for PA activity as described (15). Previous experiments have demonstrated that the amount of PA in cell extracts is proportional to the amount found in conditioned medium (15). One unit of protease-inducing activity was defined as the amount necessary to give half the maximal stimulation of PA and collagenase production.

Purification of Protease-Inducing Factor. Term human placentas were frozen at −20°C. The frozen placentas were broken into small pieces, ground with an electric food chopper (General Slicing, Walden, NY), and homogenized in a food processor. After homogenization, all subsequent steps were performed at 4°C. The homogenized placentas were added to cold 20 mM Tris·HCl (pH 7.5) and 3 mM EDTA and were sonicated for 10 min at 50 W (model 185 sonicator, Branson Sonic Power, Plainview, NY). Generally, 1 kg of frozen placenta yielded 2 liters of sonicate. The sonicate was brought to pH 4 with HCl, incubated at this pH for 2 min, and then neutralized with NaOH. NaCl was added to a final concentration of 0.5 M, and the sonicate was centrifuged at 10,000 × *g* for 60 min. The supernatant was loaded on an 85 × 153 mm column of heparin-Sepharose (Pharmacia) equilibrated with 0.5 M NaCl/3 mM EDTA/20 mM Tris·HCl (pH 7.5). The column was washed with the same buffer and was eluted with 2 M NaCl/3 mM EDTA/20 mM Tris·HCl (pH 7.5). The eluate was diluted with 3 mM EDTA/20 mM

Abbreviations: PA, plasminogen activator; BCE, bovine capillary endothelial; FPLC, fast protein liquid chromatography.

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Tris·HCl (pH 7.5) until the conductance was 24 mmho (mS) and loaded on a second heparin-Sepharose column (16 × 190 mm). The column was washed with 0.7 M NaCl/3 mM EDTA/20 mM Tris·HCl (pH 7.5) and was eluted with a 0.7 to 2 M NaCl gradient in the same buffer. Fractions were assayed for protease-inducing activity, and the active fractions were concentrated on a third heparin-Sepharose column (12 × 75 mm). This column was washed first with 0.8 M NaCl/3 mM EDTA/20 mM Tris·HCl (pH 7.5), then with 0.2 M NaCl/0.1 M sodium phosphate (pH 6.0), and was eluted with 2 M NaCl/0.1 M sodium phosphate (pH 6.0). The active fractions from the third heparin-Sepharose column were diluted with 20 vol of 0.1 M sodium phosphate (pH 6.0). The solution was clarified by centrifugation at 10,000 × *g* for 30 min and was loaded on a 9 × 72 mm column of CM-Sephadex C-50 (Pharmacia) equilibrated with the same buffer. The column was sequentially eluted with 0.15, 0.5, and 2 M NaCl each in 0.1 M sodium phosphate (pH 6.0), and the fractions were assayed for protease-inducing activity. The 0.5 M NaCl eluate of the CM-Sephadex column, which contained the protease-inducing activity, was concentrated on a 0.5-ml heparin-Sepharose column. This column was eluted with sequential 0.5-ml washes of 2 M NaCl/60 mM sodium phosphate (pH 6.0). All activity was eluted in the first 1 ml. This eluate was chromatographed on a fast protein liquid chromatography (FPLC) Superose-12 column (Pharmacia) in 2 M NaCl/60 mM sodium phosphate (pH 6.0) with a flow rate of 0.5 ml/min. The active fractions from this column contained a single band on $NaDodSO_4$/PAGE.

$NaDodSO_4$/PAGE. $NaDodSO_4$/polyacrylamide gels with 3% stacking gels and 10 to 18% gradient resolving gels were prepared and electrophoresed according to the procedure of Laemmli (17). Proteins were detected with the silver stain procedure (18).

Protein Determination. Protein concentrations were determined with the Bio-Rad protein assay (Bio-Rad) using bovine serum albumin as a standard.

^{125}I-Labeled Deoxyuridine Incorporation. Confluent cultures of BCE cells were maintained in αMEM with 5% (vol/vol) calf serum for 7 days. The medium was then replaced with fresh αMEM containing 5% (vol/vol) calf serum and various concentrations of purified placenta factor. After 20 hr, the medium was replaced with Dulbecco's modified Eagle's medium containing 5% (vol/vol) calf serum and 0.3 μCi/ml ^{125}I-labeled deoxyuridine (2000 Ci/mmol; 1 Ci = 37 GBq; New England Nuclear). After a 16-hr incubation in labeling medium, labeling was terminated by washing the cells with cold phosphate-buffered saline. Incorporation of ^{125}I-labeled deoxyuridine into acid-insoluble material was determined by incubating the cells in cold 5% (wt/vol) trichloroacetic acid (Cl_3CCOOH) for 30 min, washing twice with 5% (wt/vol) Cl_3CCOOH and distilled water. The Cl_3CCOOH-insoluble material was solubilized in 0.25 M NaOH, and radioactivity was measured in a Packard 5210 gamma scintillation counter.

Migration Assay. Migration assays were performed in 200-μl blind wells (Nucleopore) according to published procedures (19, 20) by using 5-μm pore size polycarbonate PVP-free filters precoated with gelatin and fibronectin. Serial dilutions (1:10) of the purified protease-inducing factor in αMEM containing 0.5% fetal calf serum were placed in the bottom wells. The filters were then inserted and 5 × 10^4 BCE cells in 200 μl of αMEM with 0.5% fetal calf serum were added to the upper wells. After a 4-hr incubation at 37°C the medium in the upper wells was removed, and cells on the upper surfaces of the filters were gently scraped off with a cotton swab. Then the filters were removed, dried at room temperature, and stained with Wright–Giemsa stain (Baker Chemical). The total number of cells on the lower filter surface was counted under a light microscope (400× magnification).

RESULTS

We have previously demonstrated that several preparations with angiogenic activity, including term human placenta, are able to stimulate the production of both PA and latent collagenase in cultured bovine capillary endothelial (BCE) cells (2, 21). Since human placentas can be obtained in large quantities, a sonicate of homogenized human placenta was used as the starting material for the purification of the PA- and collagenase-inducing factor.

Preliminary experiments demonstrated that a brief acid treatment of the placenta sonicate resulted in an increase in the amount of activity recovered from the preparation. Longer exposure to pH 4 resulted in a loss of protease-stimulatory activity. Therefore, the placenta sonicate was initially adjusted to pH 4, incubated at this pH for 2 min, and then neutralized. This procedure gave a 90% increase in the amount of protease-inducing activity recovered (Table 1).

Since heparin affinity chromatography has been found to be a potent means of purifying endothelial cell mitogens (7–12), we have investigated if it would also aid the isolation of the factor that induces PA and collagenase in BCE cells. When acid-treated placenta sonicate was passed over a heparin-Sepharose column equilibrated with 0.5 M NaCl, all of the protease-inducing activity bound to the column and could be eluted with 2 M NaCl. Since only 0.14% of the protein in the sonicate bound and eluted under these conditions, this afforded a 1000-fold purification with little loss of activity (Table 1), but, further purification resulted in larger losses of activity (Table 1).

The protease-inducing activity was purified further on another heparin-Sepharose column and eluted with a 0.7 to 2 M NaCl gradient (Table 1). The active fractions from this column were concentrated by binding them to a small heparin-Sepharose column and eluting in a small volume. In addition to concentrating the protease-inducing activity, this step gave a further purification (Table 1). When the active fractions from the third heparin-Sepharose column were then diluted for binding to CM-Sephadex, a precipitate was

Table 1. Purification and recovery of protease-stimulating factor from human placenta

	Protein recovery, mg	Activity recovery		Specific activity, units/μg	Purification factor
		Units	%		
Sonicate	49,000	70,000	52	0.0014	1
After acid treatment	39,000	133,000	100	0.0033	2.4
Heparin-Sepharose 1	69.8	105,000	79	1.52	1,086
Heparin-Sepharose 2	9.7	41,100	31	4.35	3,107
Heparin-Sepharose 3	6.0	47,200	35	7.69	5,493
CM-Sephadex	0.15	15,000	11	100	71,429
FPLC	0.0046	7,000	5	1490	1,064,286

Values are based on 1 kg of human placenta.

obtained. The precipitate was removed by centrifugation and the supernatant, which contained the protease-inducing activity, was applied to the CM-Sephadex column. The column was washed with 0.15 M NaCl, and the protease-inducing activity was eluted with 0.45 M NaCl (Fig. 1*A*). Analysis of the eluate from this column by $NaDodSO_4$/PAGE showed the presence of a band with a molecular weight of 18,700 and variable amounts of a band with a molecular weight above 50,000. The protease-inducing activity could be shown to comigrate with the lower molecular weight protein by gel chromatography on a FPLC system (Fig. 1*B*). FPLC chromatography was used as the final step in purification. On $NaDodSO_4$/PAGE the purified molecule migrated as a single band with molecular weight 18,700 under both reducing and nonreducing conditions (Fig. 2). The purification procedure gave a 10^6-fold purification with 5% recovery of the activity in the original extract.

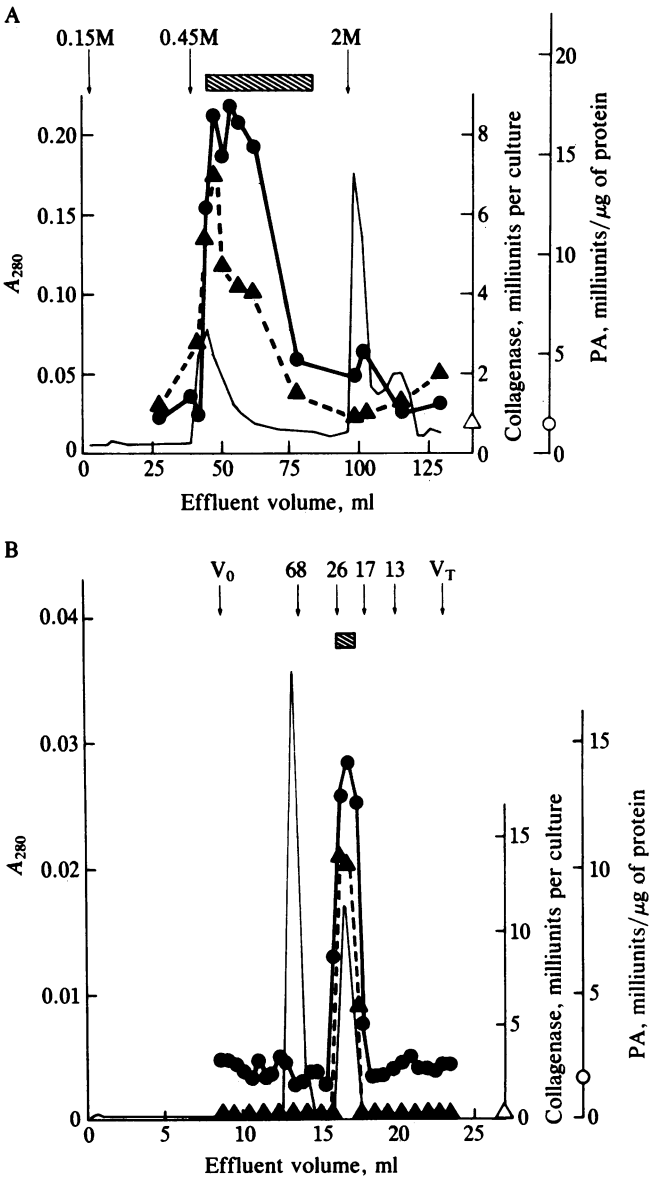

FIG. 1. (*A*) CM-Sephadex chromatography. The active fractions from the third heparin-Sepharose column were diluted, clarified by centrifugation, and loaded on a CM-Sephadex C-50 column equilibrated with 0.1 M sodium phosphate (pH 6.0). The column was eluted sequentially with 0.15, 0.45, and 2 M NaCl in the same buffer. Fractions from the elution were tested for their ability to stimulate PA (●) and latent collagenase (▲) production by BCE cells. The open symbols represent PA and collagenase production by untreated cells. The material pooled for subsequent purification is indicated by the horizontal hatched bar. (*B*) FPLC Superose chromatography. The active fractions from CM-Sephadex were concentrated on a small heparin-Sepharose column. The 2 M NaCl eluate from this column was chromatographed on a Superose 12 column on an FPLC system in 2 M NaCl/60 mM sodium phosphate (pH 6.0). Fractions were assayed as in *A*.

The purified molecule stimulated PA and collagenase in BCE cells in a dose-dependent manner (Fig. 3*A*). All collagenase was in a latent form and was activated with trypsin for detection. The production of PA and latent collagenase is stimulated in parallel. Half-maximal stimulation occurred with a concentration of protease-inducing factor of 1 ng/ml. The basal amount of PA and collagenase produced by untreated cells varied from experiment to experiment, and thus the extent of stimulation also varied. With very high concentrations of the protease-inducing factor, the stimulation of PA production was reduced (data not shown). The purified protease-inducing factor caused no increase in either PA or collagenase production when tested on bovine pericytes, smooth muscle cells, or skin fibroblasts but did cause an increase in the production of both enzymes when tested on human embryonic fibroblasts (data not shown).

We have characterized other biological properties of the purified protease-inducing factor. The most obvious was the striking effect of the factor on the morphology of cultured BCE cells. Incubation of BCE cells for 24 hr with concentrations of the protease-inducing factor in the range that induced PA and collagenase altered the morphology of the cells from their typical cobblestone appearance to a more elongated, spindle-shaped appearance, as described for another protease-inducing molecule (15).

Since the procedure used for the isolation of this factor was very similar to procedures used to isolate endothelial cell mitogens from other sources, we have investigated whether the protease-inducing factor also has mitogenic activity. Fig. 3*B* shows that addition of the protease-inducing factor to cultures of BCE cells stimulated the incorporation of ^{125}I-labeled deoxyuridine into DNA in a dose-dependent manner. Stimulation of ^{125}I-labeled deoxyuridine incorporation was achieved with the same concentrations of factor that were able to induce production of PA and collagenase. This factor was stimulatory at concentrations similar to those reported for molecules that

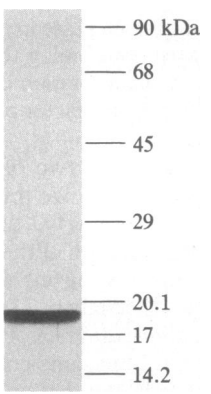

FIG. 2. $NaDodSO_4$/polyacrylamide gel electrophoresis of purified protease-inducing factor. About 120 ng of purified protease-inducing factor in a buffer containing 2.5% (wt/vol) $NaDodSO_4$/5% (vol/vol) 2-mercaptoethanol was heated in a boiling water bath for 2 min and electrophoresed on a 10 to 18% polyacrylamide gradient gel with a 3% stacking gel. Proteins were visualized with silver stain. Molecular size standards were β-galactosidase (130 kDa), phosphorylase *a* (90 kDa), bovine serum albumin (68 kDa), aldolase (45 kDa), carbonic anhydrase (29 kDa), soybean trypsin inhibitor (20 kDa), myoglobin (17 kDa), and lactalbumin (14.2 kDa).

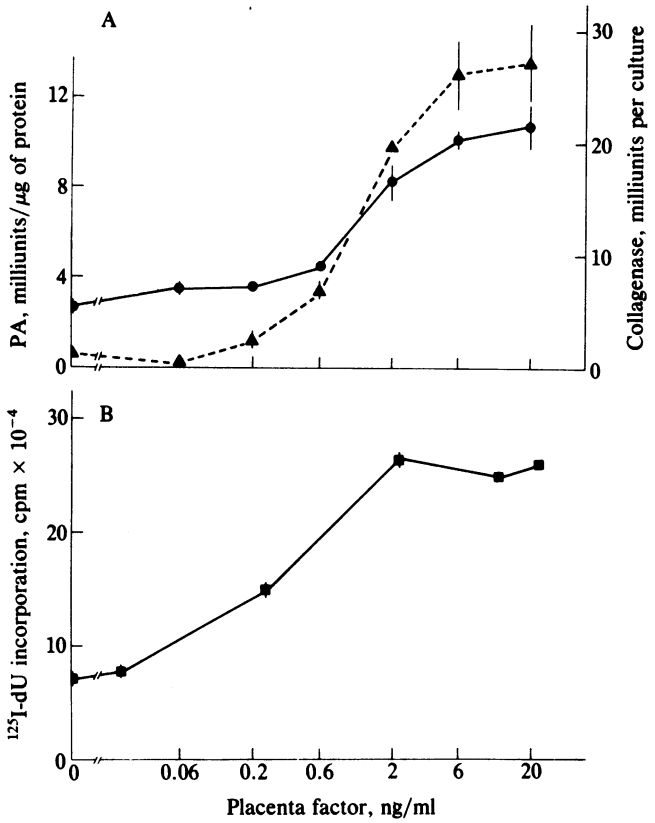

FIG. 3. Effect of purified protease-inducing factor on the production of PA and collagenase and the synthesis of DNA in BCE cells. Confluent cultures of BCE cells were given fresh medium containing the indicated concentrations of purified protease-inducing factor. (*A*) Some cells were incubated in these media for 24 hr at 37°C, then the medium was collected and assayed for latent collagenase (▲), and the cells were extracted and assayed for PA (●). (*B*) Other cells were incubated 20 hr and then were labeled for 16 hr with ^{125}I-labeled deoxyuridine (^{125}I-dU). The amount of ^{125}I-dU incorporated into Cl_3CCOOH-insoluble material was measured (■).

have been purified as endothelial cell mitogens (7–12). We have previously determined that, with crude placenta sonicate, increased incorpration of ^{125}I-labeled deoxyuridine into DNA correlates with other measurements of mitogenesis (21). Indeed, treatment of cultures of BCE cells with purified protease-inducing factor resulted in an increase in total cell protein in the cultures (data not shown), confirming that the stimulation of ^{125}I-labeled deoxyuridine incorporation correlated with an increase in cell number. Thus, this factor behaves as a *bona fide* endothelial cell mitogen.

Since the purified factor had two of the three proposed properties of angiogenic factors, we investigated whether it also possessed the third property, the ability to induce endothelial cell motility. Addition of the factor at concentrations between 0.001 and 0.1 ng/ml stimulated BCE cell chemotaxis in blind well chambers (Table 2). With higher concentrations stimulation of chemotaxis did not occur. Performing this assay with the same concentration of factor in both chambers demonstrated that only 25% of cell movement from the upper chamber to the lower chamber could be attributed to chemokinesis. Increased cell movement from the upper chamber to the lower chamber was maximal when the lower chamber contained a higher concentration of factor than the upper chamber (data not shown), demonstrating that true chemotaxis was occurring. Thus, a single purified molecule seems to have the ability to induce PA and collagenase synthesis in BCE cells, to stimulate their replication, and to stimulate their motility.

Table 2. Stimulation of chemotaxis in BCE cells by purified placenta factor

Placenta factor, ng/ml	Cells per filter
0	43 ± 7
0.001	110 ± 20
0.01	100 ± 15
0.1	380 ± 35
1	43 ± 9
10	63 ± 28

Chemotaxis studies were performed in blind well chambers. Medium containing 0.5% fetal calf serum and the indicated concentrations of purified placenta factor were placed in the lower chambers. BCE cells (5×10^4) in medium containing 0.5% fetal calf serum were placed in the upper chambers. After a 4 hr incubation, the number of cells on the lower surface of the filter separating the two chambers was counted.

DISCUSSION

By assaying cultured capillary endothelial cells for the induction of two proteases, PA and latent collagenase, we have purified to homogeneity a molecule that also stimulates chemotaxis and DNA synthesis in these cells. This demonstrates that a single molecule has all three of the *in vitro* properties predicted for an angiogenic factor. It is not clear how this molecule is related to other endothelial cell mitogens that have been isolated. It has been proposed that the known endothelial cell mitgens fall into two classes: molecules related to endothelial cell growth factor, which are eluted from heparin-Sepharose with 1 M NaCl and have an acidic pI (8–10), and molecules related to fibroblast growth factor, which bind more strongly to heparin-Sepharose and have a basic pI (7, 10, 12). Molecules from each of these classes have been shown to be angiogenic *in vivo* (22, 23). Based on its binding to heparin-Sepharose, the placenta factor seems to fall into the fibroblast growth factor family. Indeed, the placenta factor cross-reacts with an antiserum prepared by us as well as an antiserum prepared earlier (24), both raised against the amino-terminal decapeptide of bovine basic fibroblast growth factor, in an ELISA assay (data not shown) and in an immunoblot assay (A. Baird, personal communication). These results indicate a similarity to basic fibroblast growth factor, although certain quantitative differences exist indicating that the two molecules are not identical. A precise comparison of the placenta factor with fibroblast growth factor awaits further structural and immunological characterization.

In addition to its effects on cell proliferation, the factor from placenta also stimulates protease production and cell motility. At concentrations in the range of 0.1 to 10 ng/ml, the molecule stimulates the production of PA and latent collagenase in BCE cells. *In vivo*, the PA can convert the zymogen plasminogen to active plasmin, a protease of wide specificity. The plasmin can also convert latent collagenase to active collagenase. Thus, under the influence of low concentrations of this factor, capillary endothelial cells can generate two proteases that are able to degrade most of the proteins in the surrounding tissues, which would allow the cells to penetrate the tissues. Of the above mentioned endothelial cell mitogens, none have been tested for effects on protease production.

The placenta factor stimulated motility at concentrations lower by a factor of 10 to 100 than the concentrations necessary for stimulation of protease production or mitosis. Similarly, several crude angiogenic preparations have been shown to stimulate motility in BCE cells at concentrations lower by a factor of 10 to 100 than the concentrations necessary to stimulate protease production (4). The signifi-

cance of these findings is unclear; they may reflect either differences in the sensitivities of the assays or actual differences in the response to angiogenic factors. In either case, it is clear that the purified factor from the placenta stimulates motility in BCE cells. Only one of the above endothelial cell mitogens has been shown to also be chemotactic for endothelial cells (25). The fact that this single molecule stimulates endothelial cell protease production, multiplication, and chemotaxis strongly suggests that it is an angiogenic factor.

Note Added in Proof. In collaboration with Liliana Ossowski of The Rockefeller University and John J. Castellot of Harvard Medical School, we have found that 5–50 ng of the purified protease-inducing factor inoculated on the chorioallantoic membrane of the chicken embryo induces angiogenesis, as predicted from its *in vitro* properties.

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1. Folkman, J. & Cotran, R. (1976) *Int. Rev. Exp. Pathol.* **16,** 207–248.
2. Gross, J. L., Moscatelli, D. & Rifkin, D. B. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 2623–2627.
3. Zetter, B. R. (1980) *Nature (London)* **285,** 41–43.
4. Mullins, D. E. & Rifkin, D. B. (1984) *J. Cell. Physiol.* **119,** 247–254.
5. D'Amore, P. A., Glaser, B. M., Brunson, S. K. & Fenselau, A. H. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 3068–3072.
6. Schor, A., Schor, S. L., Weiss, J. B., Brown, R. A., Kumar, S. & Phillips, P. (1980) *Br. J. Cancer* **41,** 790–799.
7. Shing, Y., Folkman, J., Sullivan, R., Butterfield, C., Murray, J. & Klagsbrun, M. (1984) *Science* **223,** 1296–1299.
8. Maciag, T., Mehlman, T., Friesel, R. & Schreiber, A. B. (1984) *Science* **225,** 932–935.
9. D'Amore, P. A. & Klagsbrun, M. (1984) *J. Cell Biol.* **99,** 1545–1549.
10. Lobb, R. R. & Fett, J. W. (1984) *Biochemistry* **23,** 6295–6299.
11. Conn, G. & Hatcher, V. B. (1984) *Biochem. Biophys. Res. Commun.* **124,** 262–268.
12. Gospodarowicz, D., Cheng, J., Lui, G.-M., Baird, A. & Bohlen, P. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 6963–6967.
13. Burgos, H. (1983) *Eur. J. Clin. Invest.* **13,** 289–296.
14. Folkman, J., Haudenschild, C. C. & Zetter, B. R. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 5217–5221.
15. Gross, J. L., Moscatelli, D., Jaffe, E. A. & Rifkin, D. B. (1982) *J. Cell Biol.* **95,** 974–981.
16. Moscatelli, D., Jaffe, E. A. & Rifkin, D. B. (1980) *Cell* **20,** 343–351.
17. Laemmli, U. K. (1970) *Nature (London)* **277,** 680–685.
18. Wray, W., Boulikas, T., Wray, V. P. & Hancock, R. (1981) *Anal. Biochem.* **118,** 197–203.
19. Castellot, J. J., Jr., Karnovsky, M. J. & Spiegelman, B. M. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 5597–5601.
20. Grotendorst, G. R., Seppa, H. E. J., Kleinman, H. K. & Martin, G. R. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 3669–3672.
21. Presta, M., Mignatti, P., Mullins, D. E. & Moscatelli, D. A. (1985) *Bioscience Rep.* **5,** 783–788.
22. Thomas, K. A., Rios-Candelore, M., Gimenez-Gallego, G., DiSalvo, J., Bennett, C., Rodkey, J. & Fitzpatrick, S. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 6409–6413.
23. 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.
24. Bohlen, P., Baird, A., Esch, F., Ling, N. & Gospodarowicz, D. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 5364–5368.
25. Terranova, V. P., DiFlorio, R., Lyall, R. M., Hic, S., Friesel, R. & Maciag, T. (1985) *J. Cell Biol.* **101,** 2330–2334.