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Increased capillary endothelial cell protease activity in response to angiogenic stimuli *in vitro*

(plasminogen activator/collagenase/aortic endothelial cells)

JANET L. GROSS*, DAVID MOSCATELLI, AND DANIEL B. RIFKIN

Department of Cell Biology, New York University Medical Center, 550 First Avenue, New York, New York 10016

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ABSTRACT **Bovine capillary endothelial (BCE) cells produce increased amounts of the proteases plasminogen activator (PA) and latent collagenase when cultured in the presence of the following preparations which are known to contain angiogenic activities: bovine retinal extract, mouse adipocyte conditioned medium, and human hepatoma cell lysate. These preparations stimulated both BCE cell PA and collagenase activities in a dose-dependent manner. Both activities were increased to about the same level by these preparations as by the tumor promoter 12-*O*-tetradecanoylphorbol 13-acetate. Mitogens that are not angiogenic, such as insulin, epidermal and fibroblast growth factors, and endothelial cell growth supplement, had no effect on BCE cell PA and collagenase activities. Two of the angiogenic preparations (retinal extract and mouse adipocyte-conditioned medium) had no effect on PA activity in endothelial cells derived from bovine aortae (BAE cells). The angiogenic preparations had little (human hepatoma cell lysate, mouse adipocyte-conditioned medium) or no (bovine retinal extract) effect on BAE cell collagenase activities. In the bovine system, the induction of high levels of both PA and collagenase activities by angiogenic preparations is limited to capillary endothelial cells.**

The formation of new blood vessels occurs only in the microvasculature and is marked by destruction of the capillary basal lamina followed by migration and proliferation of distinct endothelial cell populations in response to angiogenic factors (1). We have proposed that neovascularization requires the elaboration of proteases by capillary endothelial cells in order to degrade the proteins of the capillary basal lamina and the surrounding stroma (2, 3). Cultured bovine capillary endothelial (BCE) cells produce increased amounts of the proteases plasminogen activator (PA) and latent collagenase in response to nanomolar concentrations of the potent tumor promoter 12-*O*-tetradecanoylphorbol 13-acetate (TPA) (2, 3).

Preparations from several normal and diseased tissues have been reported to contain angiogenic factors. Three of these preparations have been partially characterized. (*i*) Extracts of bovine retinae stimulate angiogenesis in the chicken chorioallantoic membrane (4, 5), proliferation and thymidine incorporation by cultured bovine aortic endothelial (BAE) cells (4, 5), and migration of cultured BAE cells (6). (*ii*) When injected into athymic *nude* mice, undifferentiated 3T3-F442A cells differentiate to form a highly vascularized fat pad at the injection site (7), suggesting that the differentiated cells induce vascularization. Cultured 3T3-F442A cells which have been induced to differentiate into adipocytes secrete into the culture medium a potent growth-stimulating activity for both BAE and BCE cells (7). (*iii*) Extracts of tumors promote angiogenesis *in vivo* (8–10) and stimulate the proliferation and motility of cultured BCE cells (10–12). One of the most active angiogenic preparations is derived from lysates of a human hepatoma cell line (11, 12).

We have investigated whether the levels of PA and latent collagenase in BCE cells can also be stimulated by crude preparations known to contain angiogenic activity and have compared the response of BCE cells to these preparations to the response of bovine endothelial cells derived from aortae (BAE cells).

MATERIALS AND METHODS

Cell Culture. BCE cells were isolated from the adrenals of freshly slaughtered calves, as described by Folkman *et al.* (13). Large-vessel endothelial cells (BAE cells) were prepared from the aortae of the same animals (14). BCE and BAE cells were grown as described (3).

Endothelial cells were prepared for experimental use as described (3). After incubation (24 hr) with the agent to be tested [in Dulbecco modified Eagle medium with 5% (vol/vol) calf serum depleted of plasminogen (3)], cell monolayers from duplicate 35-mm cultures were washed three times with cold phosphate-buffered saline (P_i/NaCl), scraped into P_i/NaCl, and pelleted by centrifugation (400 × *g*, 10 min). The cell pellets were solubilized in 250 μl of 0.1 M Tris·HCl, pH 8.1/0.5% Triton X-100 and frozen at −20°C until total cell protein was measured (15).

PA Activity. Aliquots of Triton X-100-extracted cells (1.0 μg of cell protein) were assayed for PA as described (2, 3). Earlier studies (3) have shown that the cell-associated PA levels correlate with the amount of PA secreted into the medium. In addition, the PA activities of the various angiogenic extracts were measured.

Collagenase Assay. Latent collagenase in culture media was activated with trypsin (2, 3) and subsequently assayed by its ability to cleave ^{125}I-labeled collagen-Sepharose according to the method of Moscatelli *et al.* (16). The test substances, at the same concentrations applied to cells, were also assayed for latent collagenase activity by the same method.

Chemicals. TPA was obtained from Consolidated Midlands (Brewster, NY). Stock solutions of 0.1 and 1.0 mg/ml in ethanol were stored at −20°C. Epidermal growth factor (EGF), fibroblast growth factor (FGF), and endothelial cell growth supplement (ECGS) were obtained from Collaborative Research (Waltham, MA). A stock solution (2 mg/ml) of bovine insulin (Sigma) was made in 0.15 M NaCl/0.01 M HCl and stored at 4°C. Mitomycin C and 1-β-D-arabinofuranosylcytosine (cytosine arabinoside) were obtained from Sigma.

Extracts. Retinal extract was prepared from bovine retinae by the procedure of Glaser *et al.* (4), sterilized by filtration, and

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Abbreviations: BCE, bovine capillary endothelial; BAE, bovine aortic endothelial; PA, plasminogen activator; TPA, 12-*O*-tetradecanoylphorbol 13-acetate; P_i/NaCl, phosphate-buffered saline.

* Present address: Dept. of Physiological Chemistry, The Johns Hopkins Univ. School of Medicine, Baltimore, MD 21205.

stored at −20°C. Adipocyte-conditioned medium was kindly supplied by J. Castellot, Jr. (Harvard Medical School). The medium was collected after 24 hr from confluent predifferentiated or differentiated 3T3-F442A fibroblasts maintained in Dulbecco modified Eagle medium and 0.5% fetal calf serum (Reheis), sterilized by filtration, and stored at −20°C.

Human hepatoma cell lysate was prepared from cultured human hepatoma cells (SK-HEP-1 cells obtained from J. Fogh, Sloan–Kettering Institute for Cancer Research, Rye, NY) grown in Eagle minimal essential medium with 15% fetal calf serum and supplemented with nonessential amino acids (Flow Laboratories). Cells from confluent monolayers were scraped into cold P_i/NaCl and pelleted by centrifugation (400 × *g*, 10 min). The cell pellet was suspended in 10 vol of P_i/NaCl and sonicated at 4°C for 3 min. The extract was centrifuged (10,000 × *g*, 30 min), and the lysate was sterilized by filtration through a Swinnex 0.45-μm filter (Millipore) and stored at −70°C.

Primary cultures of rat hepatocytes were prepared according to the procedure of Diamond *et al.* (17). When these cells reached confluence, extracts were prepared by the procedure used for SK-HEP-1 cells.

RESULTS

We examined the effects of angiogenic preparations on protease levels in cultured BCE and BAE cells. Confluent endothelial cell cultures were incubated for 24 hr with varying concentrations of bovine retinal extract (0–167 μg/ml), conditioned medium from differentiated 3T3-F442A adipocytes (0–500 μl/ml of medium), or human hepatoma cell lysate (0–570 μg/ml). The culture medium was then collected and assayed for latent collagenase and the cell layers were extracted with Triton X-100 and assayed for PA. Addition of extract of bovine retinae to the medium resulted in a concentration-dependent increase in latent collagenase and cell-associated PA activities in BCE cells (Fig. 1). Addition of conditioned medium from differentiated 3T3-F442A adipocytes also resulted in a coordinate stimulation of PA and collagenase activities of cultured BCE cells (Fig. 2). Conditioned medium from predifferentiated 3T3-F442A cells had no effect on PA or collagenase levels of these cells (data not shown). A lysate of cultured human hepatoma cells also coordinately stimulated both PA and collagenase activities in BCE cells (Fig. 3). The concentrations necessary for maximal stimulation of BCE cell PA (3–10 times basal levels) and collagenase levels (6–55 times basal levels) were 100–133 μg/ml for bovine retinal extract (Fig. 1), 150 μl/ml for adipocyte-conditioned medium (Fig. 2), and 267 μg/ml for human hepatoma cell lysate (Fig. 3). These concentrations are in accord with those described by others to affect BAE or BCE cell mitosis or migration as well as to induce angiogenesis (4, 7, 11).

In contrast, cell-associated PA activities of BAE cells were unaffected by any concentration of bovine retinal extract (Fig. 1) or adipocyte-conditioned medium (Fig. 2). There was a concentration-dependent enhancement of BAE cell PA activity (up to 4 times basal levels) with increasing amounts of human hepatoma cell lysate (Fig. 3). Because hepatoma cell lysate also augmented PA activity of bovine embryo skin fibroblasts (3.6 times basal PA levels at 570 μg/ml) (data not shown), it is likely that this PA promotion is not endothelial cell specific. There was no increase of latent collagenase levels in BAE cell cultures with any concentration of retinal extract tested (Fig. 1). There

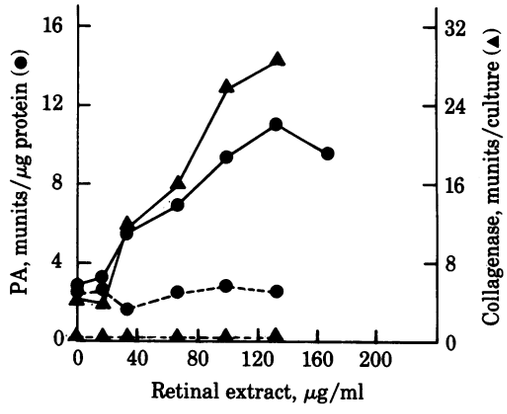

FIG. 1. Effect of bovine retinal extract on BCE (——) and BAE (---) cell PA (●) and collagenase (▲) activities. Confluent BCE and BAE cell cultures were maintained in 5% calf serum (depleted of plasminogen) in Dulbecco modified Eagle medium with various concentrations of bovine retinal extract. After 24 hr the media (1.5 ml per culture) from duplicate 35-mm cultures were combined, activated with trypsin, and assayed for collagenase activity. The cell layers from the same cultures were extracted with Triton X-100 and assayed for PA. The data represent the mean of duplicate measurements.

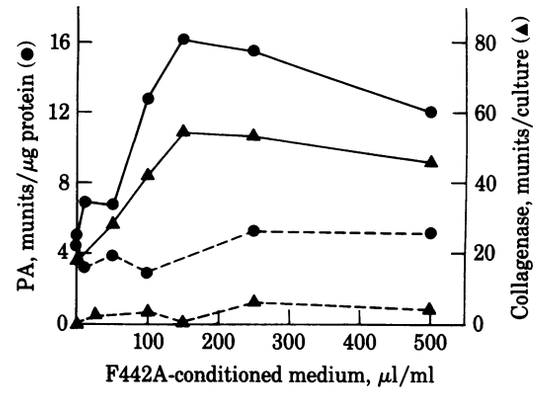

FIG. 2. Effect of 3T3-F442A-conditioned medium on BCE (——) and BAE (---) cell PA (●) and collagenase (▲) activities. Confluent BCE and BAE cell cultures were maintained for 24 hr in Dulbecco modified Eagle medium with 0.5% fetal calf serum and various concentrations of 3T3-F442A adipocyte-conditioned medium (in the same medium). The media from duplicate 35-mm cultures were combined and assayed for collagenase activity. The cell layers from the same cultures were extracted with Triton X-100 and the extracts were assayed for PA. The data represent the mean of duplicate measurements.

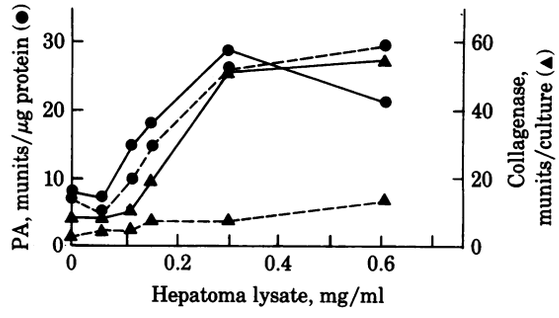

FIG. 3. Effect of human hepatoma cell lysate on BCE (——) and BAE (---) cell PA (●) and collagenase (▲) activities. Confluent BCE and BAE cell cultures were maintained in Dulbecco modified Eagle medium in 5% calf serum (depleted of plasminogen) with various concentrations of human hepatoma lysate (8.6 mg/ml in P_i/NaCl). After 24 hr the media (1.5 ml per culture) from duplicate 35-mm cultures were combined and assayed for collagenase activity. The cell layers from the same cultures were extracted with Triton X-100 and the extracts were assayed for PA. The data represent the mean of duplicate measurements. The collagenase activity endogenous to the hepatoma lysate (5% of total, see text) has been subtracted from the above data.

was a small increase in the amount of collagenase detected in BAE cultures treated with adipocyte-conditioned medium or human hepatoma cell sonicate (Figs. 2 and 3), but the treated BAE cells produced much lower levels of collagenase than did the treated BCE cells. These results suggest that the induction of high levels of both PA and collagenase activities by angiogenic factors is limited to the cells of the microvasculature, which are the cells actually involved in neovascularization.

As a positive control, in all experiments duplicate cultures were treated with 0.2 μM TPA, a known inducer of PA and collagenase in BCE cells (2, 3). In experiments not shown, we determined that the maximal increase in BCE cell protease activities in response to optimal doses of each extract was observed after an incubation period of approximately 24 hr, which is similar to the time course previously demonstrated for TPA stimulation (3). In BCE cell cultures, bovine retinal extract and adipocyte-conditioned medium stimulated PA activity to about the same extent as TPA did (Table 1). However, both bovine retinal extract and adipocyte-conditioned medium were less effective than TPA in stimulating collagenase levels. Human hepatoma cell lysate was consistently more active than TPA in enhancing both PA and collagenase activities of BCE cells. Table 1 also demonstrates that, although PA and collagenase levels of BAE cells were not affected either by bovine retinal extract or by adipocyte-conditioned medium, PA activity could be stimulated by TPA. As reported (2, 3), TPA did not alter the level of detectable collagenolytic activity in BAE cultures.

All preparations were tested for endogenous PA and collagenase activities. Only hepatoma cell lysates contained such activities. Although PA and collagenase were added to BCE cultures along with the hepatoma cell lysate, the PA and collagenase activities observed in human hepatoma cell lysate-stimulated BCE cells appeared to be due mainly to induced BCE cell proteases for the following reasons. First, the amount of collagenase activity in the hepatoma cell lysate added to the culture medium could account for no more than 5–10% of the total activity detected in the medium of BCE cell cultures incubated for 24 hr with the lysate. Thus, the majority (90–95%) of the collagenase activity in culture medium from hepatoma lysate-treated BCE cells was produced by the BCE cells. Moreover, upon removal of the extract after a suitable time of stimulation, collagenase levels in the culture medium continued to increase (data not shown). Second, the PA from the human hepatoma cells could be distinguished from the PA of BCE cells by its different apparent molecular weight as determined by Na-DodSO$_4$/polyacrylamide gel electrophoresis (3). Human hepatoma cell lysate PA migrated with three peaks of activity with apparent molecular weights (approximately 100,000, 64,000, and 51,000) which were greater than the apparent molecular weight of the cell-associated BCE cell PA induced by hepatoma cell lysate (approximately 45,000). This suggests that the enhanced PA activity of hepatoma lysate-treated BCE cells is induced, not simply adsorbed by the cells from the human hepatoma lysate PA in the culture medium. Thus, the incubation of BCE cells with hepatoma lysate resulted in bona fide inductions of BCE cell PA and collagenase activities.

Table 1. Effect of angiogenic preparations on BCE and BAE cell PA and collagenase activities

Addition	BCE cells		BAE cells	
	PA, munits/μg	Collagenase, munits/culture	PA, munits/μg	Collagenase, munits/culture
None	2.8	4.5	2.4	0
Retinal extract (133 μg/ml)	11.0	28.5	2.4	0
TPA (0.2 μM)	6.2	156.0	4.3	0
None	4.9	9.0	4.4	0
F442A adipocyte-conditioned medium (150 μl/ml)	16.1	54.0	3.6	0.6
TPA (0.2 μM)	12.5	114.0	16.3	2.4
None	2.9	3.0	7.0	2.7
Hepatoma lysate (570 μg/ml)	28.6	166.0	29.6	4.8
TPA (0.2 μM)	14.0	52.5	16.3	2.4

Confluent BCE and BAE cells were maintained for 24 hr in Dulbecco modified Eagle medium containing 5% calf serum (depleted of plasminogen) with the additions as shown. The culture fluids and detergent-extracted cell monolayers were assayed for latent collagenase and PA activities, respectively. The data represent the mean of duplicate measurements on each of two cultures.

Extracts of cultured human hepatoma cells were compared to extracts of cultured cells of nontumor origin for their ability to enhance PA and collagenase activities of BCE cells. Extracts of primary cultures of rat hepatocytes stimulated PA activity of BCE cells at the same concentrations that were effective for extracts of cultured human hepatoma cells (Table 2). Collagenase activity was increased with even lower concentrations of hepatocyte extract than hepatoma cell extract. In a second experiment, extracts of human embryo fibroblasts and extracts of human hepatoma cells gave parallel dose-dependent stimulations of BCE PA and collagenase levels. Thus, the ability to augment PA and collagenase activities of BCE cells is not limited to tumor cells. At least two cultured cell types not derived from tumors contain factors that will stimulate PA and collagenase levels in BCE cells.

Because neovascularization involves the proliferation of endothelial cells, it is possible that increases in PA and collagenase activities were due to an increased rate of endothelial cell multiplication induced by prolonged exposure to the angiogenic extracts (4, 7). However, the following experiments suggest that the increased BCE cell PA and collagenase levels were not dependent upon cell proliferation. First, molecules that are known to stimulate cell growth (18–21) but are not angiogenic were tested for their ability to affect BCE cell PA and latent collagenase levels. BCE cells were exposed for 24 hr to various growth-promoting factors, in concentration ranges known to promote cell proliferation, and cell-associated PA and secreted latent collagenase activities were then measured. BCE cell PA and latent collagenase levels were unaffected by all mitogens tested (Table 3). Second, BCE cells were pretreated with two inhibitors of DNA synthesis, mitomycin-C or cytosine arabinoside, and then subsequently challenged with TPA to induce increased BCE cell PA and collagenase levels. Preincubation with mitomycin-C and cytosine arabinoside inhibited BCE cell [^{3}H]thymidine incorporation (data not shown) but had no effect on the induction of PA and collagenase activities by TPA (Table 4). Together, these results demonstrate that the stimulation of BCE cell PA and collagenase activities is unaffected by several purified mitogens and is independent of cell proliferation (DNA synthesis).

DISCUSSION

The response of endothelial cells in capillaries to angiogenic factors *in vivo* can be divided into three components: penetration of the surrounding tissue, migration, and proliferation. These responses have possible correlates in cultured endothelial cells

Table 2. Comparison of stimulation of PA and collagenase activities in BCE cells by extract of hepatoma cells and normal cells

Addition	PA, munits/μg	Collagenase, munits/culture
None	14.8	10.0
Human hepatoma cell lysate:		
0.02 mg/ml	16.4	9.0
0.04	44.8	5.6
0.08	52.4	11.2
0.25	96.3	24.0
0.5	94.5	37.0
Rat hepatocyte lysate:		
0.02 mg/ml	28.9	16.0
0.04	41.8	25.0
0.08	70.2	25.0
0.25	97.8	25.0
TPA:		
0.2 μM	112.6	22.0
None	1.4	22.5
Human hepatoma cell lysate:		
0.04 mg/ml	1.6	15.0
0.10	4.4	45.0
0.19	7.3	75.0
0.38	13.5	105.0
Human embryo fibroblast lysate:		
0.04 mg/ml	1.7	15.0
0.10	3.1	45.0
0.19	3.4	75.0
0.38	7.1	112.5
TPA:		
0.2 μM	9.9	135.0

BCE cells were grown and prepared for experimental use as described (3). Confluent cultures were maintained for 24 hr in Dulbecco modified Eagle medium containing 5% calf serum (without plasminogen) with the above additions (1.5 ml per culture). The culture fluids and detergent-extracted cells were assayed for latent collagenase and PA activities, respectively. The data represent the mean of duplicate measurements on each of two cultures.

exposed to angiogenic stimuli: production of proteases, increased motility, and increased rate of multiplication. Others have shown that preparations that stimulate angiogenesis *in vivo* will stimulate motility and multiplication in cultured endothelial cells (4, 6, 7, 11, 12). Here we demonstrate that preparations that stimulate angiogenesis *in vivo* also stimulate increased levels of two proteases, PA and latent collagenase, in cultured capillary endothelial cells.

An increase in PA and latent collagenase levels may provide the proteolytic activities necessary for the penetration of endothelial cells into surrounding tissues. PA converts plasminogen, which is abundant in the serum and tissue spaces, to plasmin. Plasmin, a general protease, is able to digest most tissue proteins. In addition, plasmin converts latent collagenase to active collagenase (22) which can degrade the major plasmin-resistant tissue protein, collagen. We have previously shown that the BCE cell collagenase activity measured in the experiments presented here is a typical vertebrate collagenase capable of degrading interstitial collagens (types I, II, and III) (3). It has recently been reported that endothelial cells stimulated by bovine retinal extract produce a collagenase activity capable of degrading basement membrane collagens (types IV and V) (23). It is not yet known whether adipocyte-conditioned medium or human hepatoma cell sonicate also stimulates endothelial cell production of collagenases active against basement membrane collagens.

Table 3. Effect of mitogens on BCE cell PA and collagenase activities

Addition	PA, munits/μg	Collagenase, munits/culture
Insulin:		
0 μg/ml	5.8	12.3
0.01	3.4	3.7
0.10	6.0	9.6
1.0	7.8	10.2
10.0	7.4	11.9
TPA:		
0.2 μM	45.9	38.6
EGF:		
0 ng/ml	2.1	9.8
1.0	1.4	9.1
2.5	2.0	15.7
25.0	2.6	8.7
100.0	2.2	7.2
TPA:		
0.2 μM	5.5	191.0
FGF:		
0 ng/ml	2.0	0
0.01	1.1	0.7
0.10	1.4	0
1.0	1.6	0.2
10.0	1.1	0
100.0	1.8	2.5
TPA:		
0.2 μM	7.3	62.7
ECGS:		
0 μg/ml	1.5	0
0.10	1.6	0
1.0	1.7	0
10.0	1.9	0
100.0	0.2	0
TPA:		
0.2 μM	4.6	25.5

BCE cells were grown and prepared for experimental use as described (3). Confluent cultures were maintained for 24 hr in Dulbecco modified Eagle medium containing 5% calf serum (without plasminogen) with the additions shown (1.5 ml per culture). The culture fluids and detergent-extracted cells were assayed for latent collagenase and PA activities, respectively. The data represent the mean of duplicate measurements on each of two cultures. EGF, epidermal growth factor; FGF, fibroblast growth factor; ECGS, endothelial cell growth supplement.

The preparations tested in these experiments have been shown by others (4, 7, 11, 12) to stimulate endothelial cell migration or proliferation. The concentrations at which these extracts stimulate migration or multiplication correlate reasonably well with the concentrations needed to stimulate BCE cell PA and collagenase activities. For instance, bovine retinal extract at 125 μg/ml maximally enhanced BAE cell thymidine incorporation (5), whereas BCE cell protease levels were maximally enhanced with retinal extract at 100–133 μg/ml. Similarly, half-maximal stimulation of BCE and BAE cell growth was observed with adipocyte-conditioned medium at 10 μl/ml (7), whereas half-maximal induction of BCE cell protease activities occurred at approximately 75 μl/ml. Finally, crude human hepatoma cell lysate at 300 μg/ml stimulated BCE cell PA and collagenase activities to the greatest extent. Olander *et al.* (12) demonstrated that a preparation of human hepatoma cell lysate enhanced BAE and BCE cell multiplication rate at 50 μg/ml and Zetter (11) observed that human hepatoma cell lysate increased BCE cell motility maximally at 100 μg/ml. The minor differ-

Table 4. Effect of DNA synthesis inhibitors on BCE cell PA and collagenase activities

Addition	TPA	PA, munits/μg	Collagenase, munits/culture
None	−	18.9	4.5
	+	57.2	51.8
Mitomycin-C	−	15.8	6.0
(1.0 μg/ml)	+	37.5	75.0
Cytosine arabinoside	−	15.7	4.5
(0.067 μg/ml)	+	43.8	59.3
Cytosine arabinoside	−	12.8	13.5
(0.67 μg/ml)	+	32.6	95.3

BCE cells were prepared for experimental use as described (3). Duplicate confluent 35-mm cultures were pretreated with mitomycin-C (6 hr) or cytosine arabinoside (19 hr) in Dulbecco modified Eagle medium with 5% calf serum (without plasminogen). Cultures were washed three times with P_i/NaCl and incubated for an additional 24 hr in Dulbecco modified Eagle medium with 5% calf serum (without plasminogen) with or without 0.2 μM TPA. The culture fluids (1.5 ml per culture) and detergent-extracted cells were assayed for latent collagenase and PA activities, respectively. The data are the mean of duplicate determinations on each of two cultures.

ences in the effective concentrations of these extracts are likely due to variations in cellular response (i.e., clonal variation) and in the history and preparation of the extracts. The fact that endothelial cell motility, proliferation, and protease levels all are stimulated by approximately equal concentrations of these preparations suggests that these responses all may be stimulated by a single factor in each of the preparations.

The following observations suggest that BCE cells differ dramatically from BAE cells in their response to angiogenic preparations. First, two of the angiogenic preparations, bovine retinal extract and adipocyte-conditioned medium, had no effect on BAE cell PA activity. Second, the three angiogenic preparations were able to induce much higher levels of collagenase in BCE cells than in BAE cells. Third, some angiogenic preparations are able to stimulate increased motility in BCE cells but not BAE cells (11). It may be that capillary endothelial cells are simply much more responsive to these factors than BAE cells and that, with very high concentrations of the active agent, even BAE cells would respond. However, these differences in cell sensitivity are consistent with the observation that new blood vessels arise primarily from the microvasculature and suggest that only endothelial cells from the microvasculature react to angiogenic factors with a full complement of responses.

Although endothelial cell motility, proliferation, and protease activities may be coordinately stimulated by angiogenic factors, these responses are not always linked. Fibroblast growth factor and endothelial cell growth supplement stimulate multiplication of endothelial cells (20, 21) but do not affect PA and collagenase levels in BCE cells. TPA is a potent stimulator of BCE cell PA and collagenase activities but does not alter BCE motility (D. Mullins, personal communication). Angiogenic preparations seem to be uniquely able to stimulate all of the components of the angiogenic response.

The finding that extracts of two normal cell types enhance PA and collagenase levels in BCE cells suggests that these tissues may also contain angiogenic factors, although neither of these extracts has been tested directly for stimulation of angiogenesis. Extracts of normal tissues have been shown to contain chemoattractants for BAE cells which are assumed to be angiogenic factors (6). Although angiogenesis is a common pathological process in the retinae of diabetics, an angiogenic factor can also be extracted from retinae of normal individuals (4). Perhaps all tissues contain angiogenic factors which are only released upon damage or pathological alteration of the tissue. This is consistent with the finding that the factor that stimulates PA and collagenase activity in BCE cells can be readily detected in cell extracts of hepatoma cells but is not easily detected in medium conditioned by these cells (unpublished data).

These observations suggest that the stimulation of PA and collagenase activities in BCE cells can be used for a rapid screening of substances for angiogenic activity. Substances that stimulate PA and collagenase activities can then be tested directly in the more laborious angiogenesis assays. Likewise, the stimulation of BCE cell PA and collagenase may afford a rapid and quantitative assay for the isolation and purification of angiogenic molecules.

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