CELL REGULATION, Vol. 1, 833–842, October 1990

Heparanase activity expressed by platelets, neutrophils, and lymphoma cells releases active fibroblast growth factor from extracellular matrix

Rivka Ishai-Michaeli*, Amiram Eldor†, and Israel Vlodavsky*
Departments of *Oncology and †Hematology
Hadassah-Hebrew University Hospital
Jerusalem, Israel 91120

Incubation of platelets, neutrophils, and lymphoma cells with Descemet's membranes of bovine corneas and with the extracellular matrix (ECM) produced by cultured corneal endothelial cells resulted in release of basic fibroblast growth factor (bFGF), which stimulated the proliferation of 3T3 fibroblasts and vascular endothelial cells. Similar requirements were observed for release of endogenous bFGF stored in Descemet's membrane and of exogenous bFGF sequestered by the subendothelial ECM. Release of ECM-resident bFGF by platelets, neutrophils, and lymphoma cells was inhibited by carrageenan lambda, but not by protease inhibitors, in correlation with the inhibition of heparanase activity expressed by these cells. Degradation of the ECM-heparan sulfate side chains by this endo-β-D-glucuronidase is thought to play an important role in cell invasion, particularly in the extravasation of blood-borne tumor cells and activated cells of the immune system. We propose that both heparanase and ECM-resident bFGF may modulate the cell response to contact with its local environment. Heparanase-mediated release of active bFGF from storage in basement membranes provides a novel mechanism for a localized induction of neovascularization in various normal and pathological processes, such as wound healing, inflammation, and tumor development.

Fibroblast growth factors (FGF) are a family of structurally related polypeptides characterized by high affinity to heparin (Gospodarowicz *et al.,* 1987; Burgess and Maciag, 1989; Rifkin and Moscatelli, 1989). They are highly mitogenic for vascular endothelial cells (EC) and are among the most potent inducers of neovascularization (Folkman and Klagsbrun, 1987) and mesenchyme formation (Kimelman and Kirschner, 1987). Basic fibroblast growth factor (bFGF) has been extracted from the subendothelial extracellular matrix (ECM) produced in vitro (Baird and Ling, 1987; Vlodavsky *et al.,* 1987) and from basement membranes of the cornea (Folkman *et al.,* 1988), suggesting that ECM may serve as a reservoir for bFGF. In fact, immunohistochemical staining revealed the localization of bFGF in basement membranes of diverse tissues of the rat fetus (Gonzalez *et al.,* 1990) and of blood vessels of human tissues (Cardon-Cardo *et al.,* 1990), as well as in nuclei, intercalated discs, and endomysium of bovine heart muscle fibers (Kardami and Fandrich, 1989). Acidic FGF has been shown to be deposited into the ECM produced by cardiac myocytes (Weiner and Swain, 1989). Despite the ubiquitous presence of bFGF in normal tissues, EC proliferation in these tissues is usually very low (Denekamp, 1984), suggesting that bFGF is somehow sequestered from its site of action. Studies on the interaction of bFGF with ECM revealed that bFGF binds to heparan sulfate proteoglycans (HSPG) in the ECM and can be released by heparin-like molecules, heparan sulfate (HS)-degrading enzymes (Bashkin *et al.,* 1989), or plasmin (Saksela and Rifkin, 1990). These results suggest that the ECM HSPG provide a natural storage depot for bFGF and possibly other growth-promoting factors. Displacement of bFGF from its storage within basement membranes and ECM may provide a novel mechanism for induction of neovascularization in normal and pathological situations (Baird and Walicke, 1989; Vlodavsky *et al.,* 1990). Such a role can be ascribed to an endo-β-D-glucuronidase (heparanase) that degrades HS side chains in the subendothelial ECM (Vlodavsky *et al.,* 1983; Nakajima *et al.,* 1988). Expression of heparanase has been correlated with the ability of blood-borne tumor cells (Vlodavsky *et al.,* 1983; Nakajima *et al.,* 1988), neutrophils (Matzner *et al.,* 1985), and activated cells of the immune system (Naparstek *et al.,* 1984) to extravasate in processes such as tumor metastasis, inflammation, and autoimmunity. In the present study, we demonstrate that heparanase can be utilized by various cell types to release active bFGF from the subendothelial ECM, both in vitro and in vivo.

Table 1. Effect of heparin species and carrageenan on heparanase activity and bFGF release

Compound	Inhibition of heparanase (μg/ml)[a]	Release of ECM-bound bFGF (%)[b]
Heparin	2.5	50–60
N-acetylated heparin	5	10–15
Totally desulfated heparin	>50	<5
Carrageenan lambda	2.5	<5

[a] Concentration required for 100% inhibition of heparanase (ESb lymphoma) activity.

[b] Percent release of ECM-bound ^{125}I-bFGF by the indicated concentrations of each compound. Spontaneous release of bFGF in the presence of incubation medium alone was subtracted and did not exceed 12% of the total ECM-bound bFGF. Heparanase activity and % release of ECM-bound ^{125}I-bFGF were determined as described in Materials and Methods.

Results

We have previously demonstrated that exposure of ECM to either bacterial heparitinase, native heparin, or heparin-like molecules resulted in release of ECM-bound bFGF (Bashkin *et al.*, 1989). Heparin and various nonanticoagulant species of heparin are also potent inhibitors of heparanase activity (Bar-Ner *et al.*, 1987). To investigate whether heparanase expressed by various cell types is involved in release of bFGF from ECM, we first screened for molecules that inhibit the enzyme but, unlike heparin, do not release the ECM-bound bFGF. For this purpose ECM was incubated with ^{125}I-bFGF, washed free of unbound FGF, and incubated with various low-M_r and modified species of heparin to evaluate the extent of bFGF release from ECM. Oligosaccharides derived from depolymerized heparin were inadequate, because molecules as small as the hexasaccharide efficiently released ^{125}I-FGF from ECM (Bashkin *et al.*, 1989). Oligosaccharides of a smaller molecular size did not inhibit the enzyme. Among the various chemically modified heparins, only *N*-desulfated, *N*-acetylated heparin was found to inhibit the enzyme effectively at concentrations that released only a small percentage of the ECM-bound bFGF, compared with native heparin (Table 1). Totally desulfated heparin did not inhibit the enzyme and also failed to release the ECM-bound bFGF (Bar-Ner *et al.*, 1987; Bashkin *et al.*, 1989). We then tested the effect of other polyanionic molecules (i.e., hyaluronic acid, chondroitin sulfate, dermatan sulfate, pentosan sulfate, dextran sulfate, fucoidan, carrageenan lambda, carrageenan alpha, suramin) on both heparanase activity and release of ECM-bound bFGF. Among these compounds, carrageenan lambda was found to inhibit the enzyme efficiently at concentrations that induced little or no release of bFGF from ECM (Table 1). Because this compound yielded the best differential effect, it was used in subsequent experiments to investigate the involvement of cellular heparanase in release of bFGF from the subendothelial ECM produced in vitro and from Descement's membrane of bovine corneas. Whereas the ECM system was used as a model for release of exogenously added bFGF, bovine corneas were utilized to study release of endogenous bFGF from basement membranes.

Release of ECM-bound FGF by platelets

Incubation (3 h, 37°C) of increasing amounts of lysed platelets with ECM that was first incubated with ^{125}I-bFGF resulted in release of up to 60% of the ECM-bound FGF in a dose-dependent manner (Figure 1). This release was inhibited by 85–95% in the presence of 10 μg/ml carrageenan lambda (Figures 1 and 2), which also completely inhibited release of HS degradation products by means of the platelet heparitinase (Figure 3A). In contrast, both degradation of HS and release of ECM-bound bFGF were not inhibited in the presence of carrageenan kappa. We have previously demonstrated that HS in subendothelial ECM is available for degradation by the platelet heparitinase

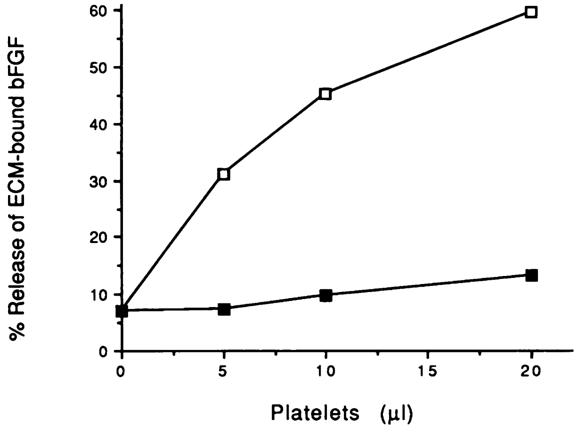

Figure 1. **Release of ECM-bound ^{125}I-bFGF by lysed platelets.** ECM-coated wells (4-well plates) were incubated (3 h, 24°C) with ^{125}I-bFGF (2.5 × 10^4 cpm/well). Unbound FGF was washed (4 times), and the ECM was incubated (2 h, 37°C) with increasing amounts of lysed platelets (1 μl = 1.5 × 10^6 lysed platelets) in the absence (□) or presence (■) of 10 μg/ml carrageenan lambda. Released ^{125}I-bFGF was counted in a γ-counter. Radioactivity released into the incubation medium is expressed as percent of total ECM-bound ^{125}I-FGF (100% = 75 pg).

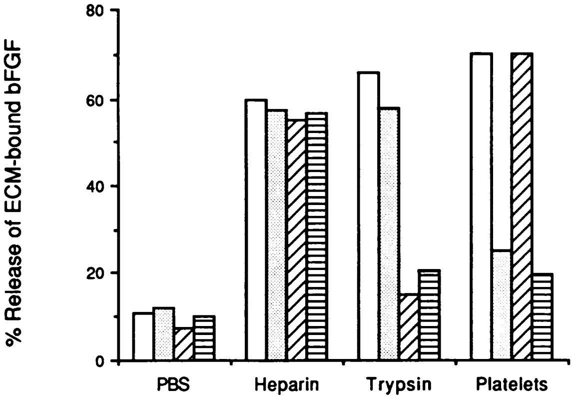

***Figure 2.* Effect of carrageenan and protease inhibitors on release of ECM-bound bFGF by heparin, trypsin, and platelets.** ECM that was preincubated with ^{125}I-bFGF and washed free of unbound FGF (legend to Figure 1) was incubated (2 h, 37°C) with PBS alone or with PBS containing 10 µg/ml heparin, 10 µg/ml trypsin, or 3.5 × 10^7 lysed platelets. Incubations were performed in the absence (☐) or presence of either 10 µg/ml carrageenan lambda (░), protease inhibitors (2 mM PMSF and 100 KIU/ml aprotinin) (▨), or both (▤). Released ^{125}I-bFGF was counted in a γ-counter. Radioactivity released into the incubation medium is expressed as percent of total ECM-bound ^{125}I-FGF.

(Yahalom *et al.,* 1984). As demonstrated in Figure 3A, incubation (3 h, 37°C) of sulfate-labeled ECM with platelets resulted in release of low-M_r sulfate-labeled degradation products (peak II, Kav ~ 0.75 on Sepharose 6B). This material has previously been characterized as HS degradation fragments, five to eight times smaller than intact ECM-derived HS side chains (Yahalom *et al.,* 1984). Carrageenan lambda, but not protease inhibitors (2 mM PMSF and 100 KIU/ml aprotinin), inhibited both degradation of HS (Figure 3A) and release of ECM-bound bFGF by lysed (Figure 2) or intact (Figure 4) platelets. Incubation of ECM with phosphate-buffered saline (PBS) or carrageenan (10–25 µg/ml) alone resulted in release of 10–15% of the ECM-bound ^{125}I-bFGF (Figures 1 and 2). This basal release of FGF may be due to an endogenous proteolytic activity (Bar-Ner *et al.,* 1986) because it was reduced to <5% when the ECM was heat treated (70°C, 10 min) before incubation with the labeled bFGF. Exposure of ECM to heparin resulted in release of 60–70% of the ECM-bound bFGF. This release was, however, not affected by carrageenan (Figure 2), indicating that carrageenan does not hinder release of bFGF because of a possible masking of the ECM-bound FGF. Trypsin-mediated release of bFGF from ECM was inhibited by serine protease inhibitors, but not by carrageenan lambda (Figure 2). Accordingly, digestion of ^{3}H-globin (Bar-Ner *et al.,* 1986) by trypsin was completely blocked by protease inhibitors, but there was no inhibition, and even a slight stimulation, in the presence of carrageenan lambda. Lysed platelets incubated with ^{3}H-globin exhibited <3% of the proteolytic activity expressed by 10 µg/ml trypsin under the same conditions. This proteolytic activity was inhibited by protease inhibitors, but there was no effect to carrageenan.

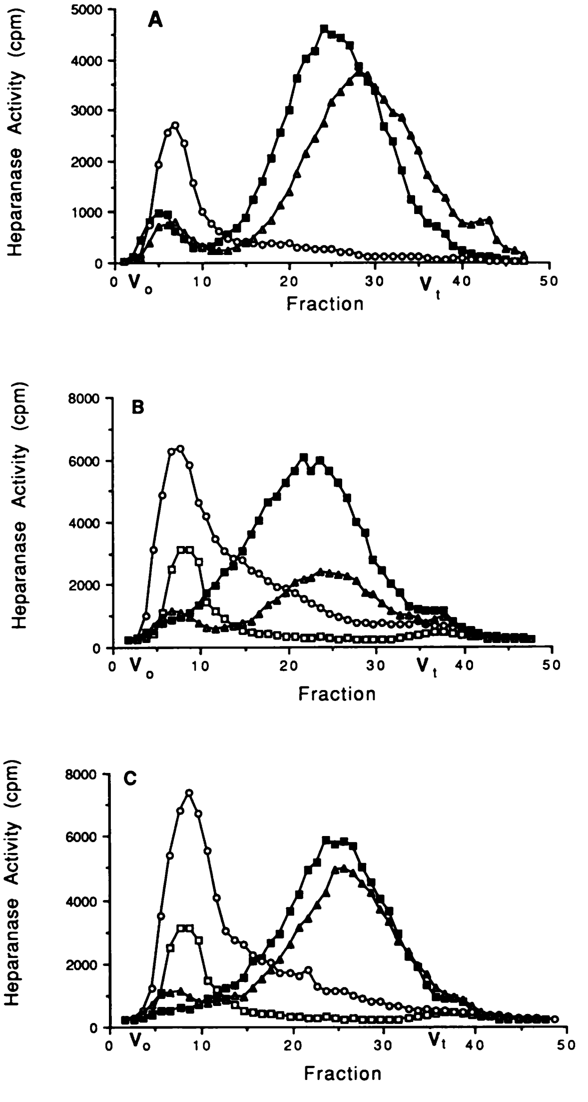

***Figure 3.* Effect of carrageenan and protease inhibitors on heparanase-mediated degradation of HS in ECM.** Sulfate-labeled ECM (35-mm plates) was incubated (3 h, 37°C, pH 6.8) with (A) 2.5 × 10^8 platelets; (B) supernate fraction of 5 × 10^6 neutrophils that were preincubated (4 h) at 4°C; and (C) 5 × 10^6 intact neutrophils. Incubations on the labeled ECM were performed in the absence (■) or presence of either 10 µg/ml carrageenan lambda (○) or a mixture of protease inhibitors (2 mM PMSF, 10 µg/ml leupeptin, 100 KIU/ml aprotinin, 100 µg/ml benzamidine, and 5 mM EGTA) (△). The ECM was also incubated with PBS alone in the absence of cells (☐). Labeled degradation products released into the incubation medium were analyzed by gel filtration on Sepharose 6B, as described in Materials and methods.

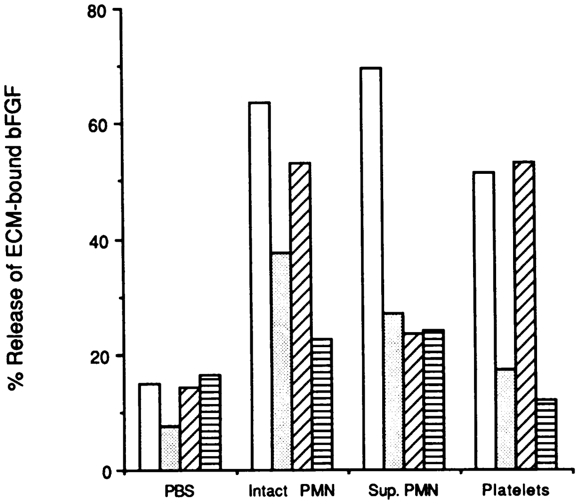

***Figure 4.* Heparanase-mediated release of ECM-bound bFGF. ECM-coated wells (4-well plates) were incubated (3 h, 24°C) with ^{125}I-bFGF (1.5 × 10^4 cpm/well).** Unbound bFGF was removed and the ECM incubated (3 h, 37°C, pH 6.8) with PBS, human polymorphonuclear neutrophils (PMN), supernate fraction of neutrophils (sup. PMN), or platelets, as described in the legend to Figure 3. Incubations were performed in the absence (□) or presence of either 10 μg/ml carrageenan lambda (□), a mixture of protease inhibitors (legend to Figure 3) (▨), or both (▤). Released ^{125}I-bFGF, expressed as percent of total ECM-bound ^{125}I-bFGF, was counted in a γ-counter. 100% = 44 pg.

Efficient release of ECM-bound ^{125}I-bFGF was induced by a purified preparation of bacterial heparitinase (EC 4.2.2.8, Flavobacterium heparinum) and by a nearly pure preparation of heparanase isolated from a human hepatoma cell line (Sk-hep-1). This release was inhibited by carrageenan lambda. In contrast, bacterial heparinase (EC 4.2.2.7) or chondroitinase ABC failed to release ^{125}I-bFGF from ECM above the basal amount of bFGF released during incubation with PBS alone (not shown). Release of ECM-bound ^{125}I-bFGF occurred also on incubation of the ECM with intact platelets. Results were similar to those obtained with cell lysates in that carrageenan lambda, but not protease inhibitors, inhibited release of both bFGF (Figure 4) and HS degradation fragments from the ECM (Figure 3A).

Release of ECM-bound FGF by neutrophils

Human polymorphonuclear neutrophils (PMN) express heparanase activity, which degrades heparan sulfate in the subendothelial ECM (Matzner *et al.,* 1985). The enzyme is found mainly in the PMN-specific granules, and up to 25% of its cellular content is preferentially released on incubation of the cells at 4°C (Matzner *et al.,* 1985). As demonstrated in Figure 3B, degradation of the ECM HS by the released enzyme(s) was, unlike degradation by intact neutrophils (Figure 3C), inhibited by protease inhibitors. These inhibitors had no effect on the neutrophil heparanase itself, but rather inhibited the activity of protease(s) that generated a more accessible substrate for subsequent degradation by the heparanase enzyme (Matzner *et al.,* 1985; Bar-Ner *et al.,* 1986). This type of sequential degradation is reflected by the accumulation of high-M_r labeled material in the presence of carrageenan but not of protease inhibitors, which inhibited release from ECM of both high- and low-M_r material (Figure 3B). We compared the effect of carrageenan and protease inhibitors on degradation of HS (Figure 3) and release of ECM-bound FGF (Figure 4) by intact neutrophils and by a neutrophil supernate fraction. As demonstrated in Figure 4, supernate fraction obtained after incubation (4 h) of human neutrophils at 4°C released 70% of the ECM-bound ^{125}I-bFGF. This release was inhibited by ~80% (not taking into account spontaneous release from ECM alone) in the presence of either carrageenan lambda or protease inhibitors, in correlation with the inhibition of HS degradation under the same conditions (Figure 3B). Incubation of ECM with intact neutrophils resulted in degradation of HS (Figure 3C) and release of ^{125}I-bFGF (Figure 4) to an extent that was similar to that obtained by the neutrophil supernatant. Release of ECM-bound bFGF by intact PMN was inhibited by carrageenan, although to a smaller extent (55%) compared with the neutrophil supernatant. There was only a small inhibitory effect to a mixture of protease inhibitors (Figure 4), as was also observed when intact PMN were incubated with sulfate-labeled ECM under the same conditions (Figure 3C).

Growth-promoting activity of bFGF released from ECM

We have previously reported that the ECM produced by cultured corneal EC contains bFGF that promotes cell proliferation and differentiation (Vlodavsky *et al.,* 1987; Rogelj *et al.,* 1989). Preliminary experiments revealed that material released from this ECM (2-cm^2 ECM-coated wells) during incubation with heparanase exhibited only a low mitogenic activity (~2% the effect of bFGF or 10% serum) when aliquots were tested on vascular EC or 3T3 fibroblasts. This was due to insufficient amounts of released bFGF and/or to release of an inactive form of bFGF. To investigate whether bFGF is released from ECM in an active form, ECM was prein-

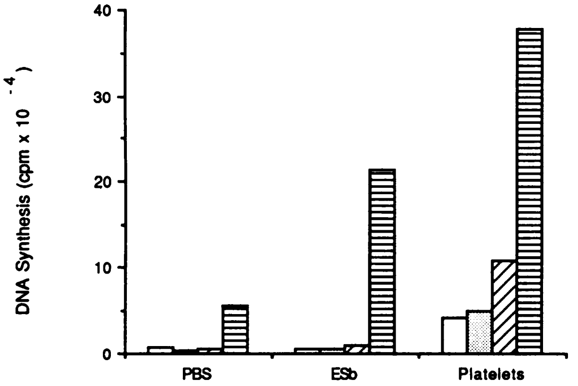

***Figure 5.* Mitogenic activity of ECM-bound bFGF released by platelets and ESb lymphoma cells.** ECM-coated wells (4-well plates) were incubated (20 h, 4°C) with bFGF (20 ng/well). ECM was washed free of unbound bFGF and incubated (4.5 h, 37°C) (▤) with PBS, 1.3 × 10^6 ESb lymphoma cells, or 8 × 10^7 platelets. Cells were also incubated on regular tissue culture plastic wells (☐), tissue culture plastic that was preexposed to bFGF (☐), and ECM that was not exposed to exogenous bFGF (▨). Aliquots (20 μl) of the incubation media were tested for stimulation of ^{3}H-thymidine incorporation in growth-arrested 3T3 fibroblasts.

cubated (18 h, 4°C) with unlabeled FGF and washed free of unbound FGF, and material released by cells and cell extracts was tested for its growth-promoting activity. Incubation (3 h, 37°C) of ESb lymphoma cells (Vlodavsky et al., 1983) or washed platelets with ECM that was first exposed to excess of bFGF and washed free of unbound bFGF resulted in release of 45–80% (4.2–7.2 ng bFGF/well) of the ECM-bound mitogenic activity, as indicated by a marked stimulation of DNA synthesis in both growth-arrested 3T3 fibroblasts (Figure 5) and sparsely seeded bovine aortic EC (not shown). Incubation of ESb lymphoma cells on ECM alone, or on regular tissue culture plastic that was first exposed to excess of bFGF, failed to elicit any mitogenic response in both 3T3 fibroblasts and bovine aortic EC. A relatively low degree of mitogenic activity for 3T3 fibroblasts was released during incubation of platelets with ECM that was not exposed to exogenous bFGF, compared with ECM that was first incubated with bFGF (Figure 5). Because release of endogenous bFGF from ECM could hardly be detected under our experimental conditions, this activity may be due primarily to platelet-derived growth factor (PDGF) released during incubation and activation of platelets on the ECM.

In a second set of experiments, intact cells or cell lysates were incubated in contact with the Descemet's membrane side of bovine corneas (Folkman *et al.,* 1988; Bashkin *et al.,* 1989). Descemet's membrane has been shown to contain ~10-fold higher amounts of bFGF per unit area, compared with its in vitro counterpart, the ECM produced by corneal EC (Folkman *et al.,* 1988). Based on the mitogenic activity of corneal extracts compared with recombinant bFGF, it was estimated that the average amount of bFGF per cornea ranged from 30 to 40 ng. Incubation of washed platelets on top of the Descemet's membrane surface of bovine corneas resulted in a time-dependent release of growth-promoting activity for 3T3 fibroblasts (Figure 6) and vascular EC. Endogenous mitogenic activity released from bovine corneas during incubation with platelets was inhibited by 70–80% in the presence of neutralizing rabbit anti-human bFGF antibodies. Although Descemet's membrane may contain growth-promoting factors other than bFGF, the induction of EC proliferation and its inhibition by anti-bFGF antibodies indicate that the observed stimulation of cell proliferation was elicited primarily by FGF. Likewise, ESb lymphoma cells or neutrophils released mitogenic factor(s) from Descemet's membrane that stimulated the proliferation of vascular EC (Figure 7) and 3T3 fibroblasts (not shown). Based on the mitogenic effect of aliquots of the incubation media relative to that of recombinant bFGF, it was estimated that up to ~20% (i.e., 5–8 ng) of the total amount of bFGF per cornea was released under the experimental conditions described above. These

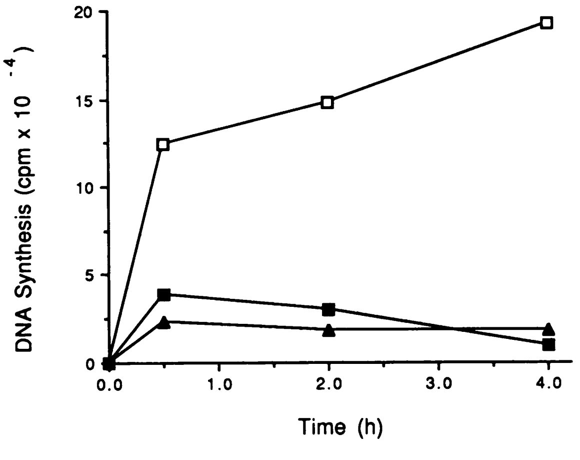

***Figure 6.* Release of growth-promoting activity from bovine corneas by intact platelets.** Platelets (1.5 × 10^8 in 0.5 ml medium) were incubated (37°C) on top of the inner side of bovine corneas (☐) or in regular tissue culture plastic wells (▲). Bovine corneas were also incubated with 0.5 ml PBS alone (■). Aliquots (20 μl) of the incubation media were taken at various time periods, centrifuged, and tested for stimulation of ^{3}H-thymidine incorporation in growth-arrested 3T3 fibroblasts, as described in Materials and methods. Each point is the average of six wells (3 wells for each cornea). The variation between different determinations did not exceed 15% of the mean.

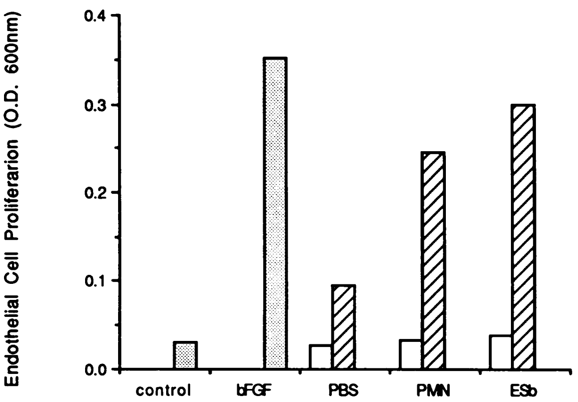

***Figure 7.* Release of endothelial cell growth-promoting activity from bovine cornea by neutrophils and ESb lymphoma cells.** Neutrophils (5 × 10^6) (PMN), ESb lymphoma cells (5 × 10^6), or PBS alone were incubated (3 h, 37°C) on top of the inner side of bovine corneas (▨) or in regular tissue culture plastic wells (☐). Aliquots (20 μl) of the 0.5-ml incubation medium were added to sparsely seeded endothelial cells on days 2 and 4 after seeding and tested for stimulation of endothelial cell proliferation as described in Materials and methods. Endothelial-cell proliferation was also measured in the absence (control) and presence (bFGF) of 5 ng/ml bFGF added on days 2 and 4 after seeding. Each point is the average of triplicate wells, and the variation in cell number (i.e., uptake of metylene blue) was <10%.

results indicate that normal and malignant cells are capable of releasing endogenous active bFGF from its storage in the subendothelial ECM.

Discussion

We have previously reported that the subendothelial ECM (Vlodavsky *et al.*, 1987) and Descemet's membranes of bovine corneas (Folkman *et al.*, 1988) contain bFGF-like factors that participate in induction of cell proliferation and differentiation by the ECM (Rogelj *et al.*, 1989). We have also demonstrated that bFGF is bound to HS in the ECM and is readily released when the ECM HS is degraded by heparanase (Bashkin *et al.*, 1989). Release of FGF was also brought about by native heparin and by various heparin-like molecules that bind FGF with high affinity compared with HS in the ECM (Bashkin *et al.*, 1989). In the present study, we investigated the ability of various normal and malignant cells to release bFGF from the subendothelial ECM and, in particular, the involvement of a cellular endoglycosidase (heparanase) in this release. Heparanase activity correlates with the ability of blood-borne tumor cells and activated cells of the immune system to leave the circulation and reach their target sites (Parish *et al.*, 1987; Nakajima *et al.*, 1988; Lider *et al.*, 1990). Native heparin and various heparin-like molecules are both potent inhibitors of heparanase activity (Bar-Ner *et al.*, 1987) and efficient releasers of ECM-bound bFGF (Bashkin *et al.*, 1989). In contrast, *N*-acetylated heparin and, to a higher extent, carrageenan lambda were found to effectively inhibit heparanase-mediated degradation of the ECM HS at concentrations that released little or no bFGF from ECM. Using carrageenan lambda as a potent inhibitor of heparanase, we have demonstrated that heparanase activity expressed by normal and malignant cells is involved in release of bFGF from ECM and Descemet's membrane of bovine corneas. Although carrangeenan specifically inhibited heparanase-mediated release of ECM-bound FGF, it had no effect on FGF release caused by high-affinity binding to heparin, or by proteolytic digestion of the ECM.

A striking feature of bFGF is that it is mostly an intracellular protein, consistent with the lack of a consensus signal peptide in its gene (Abraham *et al.*, 1986). Although the mode of FGF deposition into ECM has not yet been identified, our studies on the FGF content and growth-promoting activity of ECM that was denuded from cells several hours compared with several days after the ECM-producing cells had reached confluency, strongly suggest that bFGF is deposited by intact EC (Rogelj *et al.*, 1989). Apart from deposition by cells that synthesize the ECM, bFGF liberated in response to cell damage or death is likely to be sequestered by the ECM HS and is thereby stabilized and protected (Gospodarowicz and Cheng, 1986; Saksela *et al.*, 1988). As demonstrated in the present study, both endogenous and exogenously added bFGF were accessible to release by heparanase—although a more efficient release of bFGF (up to 70% of the ECM-bound factor) was achieved in the case of added bFGF compared with endogenous bFGF, where up to about 20% of the total amount of factor was released. These results may reflect the different requirements for release of surface-bound factors, as opposed to bFGF that is deeply embedded in the tissue. It should be emphasized, however, that the estimated amounts of endogenous bFGF released from corneas during incubation with cells are physiologically relevant, because bFGF has been shown to induce EC proliferation and angiogenesis at picomolar (1–10 ng/ml) amounts (Folkman and Klagsbrun, 1987). Regardless of the source of heparanase and of whether release of bFGF was brought about by a pure enzyme, intact cells, or cell lysates, inhibition of FGF release correlated with inhibition of heparanase activity as measured by release from ECM of sulfate-labeled degradation products. It should be noted, however, that whereas heparanase activity was almost fully inhibited by carrageenan lambda, release of ECM-bound bFGF was inhibited to a lesser degree. In fact, a strict correlation between the total amounts of released sulfate-labeled material and iodinated bFGF is not expected, mainly because HSPG within the ECM may not bind the exogeneously added bFGF, but yet are susceptible to degradation by proteases and heparanase. Our results suggest, for example, that only some of the high-M_r material that accumulates in the presence of carrageenan is associated with iodinated bFGF. Release of this high-M_r material occurs even in the absence of cells (Figure 3, B and C) because of a proteolytic activity residing in the ECM itself (Bar-Ner *et al.,* 1986) and is further stimulated by cellular proteases. Furthermore, a proteolytic activity may release bFGF that is bound to ECM components other than HS.

We suggest that heparanase activity expressed by tumor cells may function not only in cell migration and invasion (Vlodavsky *et al.,* 1983; Nakajima *et al.,* 1988), but also, may at the same time, elicit an indirect neovascular response by means of releasing the ECM-resident FGF. Likewise, platelets and activated cells of the immune system (i.e., macrophages, neutrophils, and T lymphocytes) (Naparstek *et al.,* 1984; Yahalom *et al.,* 1984; Matzner *et al.,* 1985) that are often attracted by tumor cells may indirectly stimulate tumor angiogenesis by means of their heparanase activity. These cells may also elicit an angiogenic response in the process of inflammation and wound healing. We have recently reported that mast cells express heparanase activity (Bashkin *et al.,* 1990). It is conceivable that mast cell heparin and/or heparanase may be involved in angiogenesis by liberating the ECM-resident bFGF. Mast cells are widely distributed in perivascular connective tissues, especially in areas of active tumor growth and vascular reactivity. Heparanase, released on degranulation of mast cells (Bashkin *et al.,* 1990), may thus play a role in the long-proposed mast cell–mediated stimulation of neovascularization (Kessler *et al.,* 1976).

Several studies and our own results indicate that heparin and HS inhibit the mitogenic activity of bFGF but at the same time stabilize and protect the molecule from inactivation (Gospodarowicz and Cheng, 1986; Saksela *et al.,* 1988). It is therefore conceivable that bFGF is stored in ECM in a highly stable but relatively inactive form, as also indicated by the highly stable ECM-resident growth-promoting activity compared with that of bFGF in a fluid phase (Fridman *et al.,* 1985). Release from ECM of bFGF as a complex with HS fragment may yield a form of bFGF that is more stable than free bFGF and yet capable of binding the high-affinity plasma-membrane receptors (Saksela *et al.,* 1988). Moreover, bFGF complexed to HS fragment should diffuse through the stroma to the target cells more readily than free bFGF because bFGF-HS complexes do not bind to the ECM (Rifkin and Moscatelli, 1989). Although the present study emphasizes the role of heparanase in bFGF release, it does not exclude the involvement of other enzymes. Thus, ECM-bound bFGF is released by plasmin as a noncovalent complex with HSPG or glycosaminoglycans (GAG) (Saksela and Rifkin, 1990). Basic FGF complexed to HSPG stimulates production of plasminogen activator (PA) by EC (Saksela *et al.,* 1988), possibly yielding an autocatalytic process (Saksela and Rifkin, 1990). Moreover, heparanase-mediated degradation of ECM-bound HS has been shown to be facilitated markedly by both cellular and ECM-associated PA (Bar-Ner *et al.,* 1986). Whereas proteolytic digestion of the ECM releases bFGF (Bashkin *et al.,* 1989; Saksela and Rifkin, 1990)—but at the same time degrades major constituents of the ECM, resulting in significant structural and functional alterations—heparanase activity is restricted to cleavage of HS side chains so that the treated ECM retains, to a large extent, its native morphological appearance and biological activity.

In addition to bFGF, both granulocyte-macrophage colony-stimulating factor (GM-CSF) and interleukin-3 also bind to HS on stromal cells (Roberts *et al.,* 1988). These factors may be released by heparanase and presented to hematopoietic cells. It has also been reported that both TGF-β and its proteoglycan receptor (Betaglycan) are present in the ECM of various cells and tissues (Andres *et al.,* 1989). Likewise, various heparin-binding growth factors are tightly associated with bone matrix. Among these is osteogenin, an ECM-associated bone-inductive protein that was isolated from demineralized bone by heparin affinity chromatography (Sampath *et al.,* 1987). Heparanase may thus participate in cellular responses to interaction with the local environment by means of releasing active growth and differentiation factors that are firmly sequestered by HS in the ECM.

Materials and methods

Materials

Partially purified bFGF was isolated from bovine brain, as described (Gospodarowicz *et al.,* 1978). Recombinant human bFGF was kindly provided by Takeda Chemical Industries (Tokyo, Japan). Bacterial heparinase (EC 4.2.2.7) and heparitinase (EC 4.2.2.8) (Flavobacterium heparinum) were obtained from Seikagaku Kogyo (Tokyo, Japan). Dulbecco's modified Eagle's medium (DMEM, 1 g glucose/l), calf serum, fetal calf serum (FCS), penicillin, and streptomycin were obtained from GIBCO (Grand Island, NY). Saline containing 0.05% trypsin, 0.01 M sodium phosphate, and 0.02% EDTA (STV) was obtained from Biological Industries (Beit-Haemek, Israel). Tissue culture dishes were obtained from Falcon Labware Division, Becton Dickinson (Oxnard, CA). Four-well tissue culture plates were from Nunc (Roskilde, Denmark). ^3H-methyl thymidine (500 mCi/mmol) and $Na_2{}^{35}SO_4$ (540–590 mCi/mmol) were obtained from New England Nuclear (Boston, MA). *N*-acetylated heparin and totally desulfated heparin were kindly prepared and provided to us by Dr. Lina Wasserman (Beilinson Medical Center, Petah-Tikva, Israel), as described (Bar-Ner *et al.*, 1987; Bashkin *et al.*, 1989). Sodium heparin was from Diosynth (Oss, Holland) and aprotinin [Trasylol, 7142 Kallikrein inhibitor units (KIU)/mg] from Bayer (Leverkusen, FRG). Carrageenan lambda, triton X-100, dextran T-40, phenylmethylsulfonyl fluoride (PMSF), leupeptin and all other chemicals were of reagent grade, purchased from Sigma (St. Louis, MO).

Cells

Cultures of bovine corneal endothelial cells were established from steer eyes as previously described (Gospodarowicz *et al.*, 1977). Stock cultures were maintained in DMEM supplemented with 10% bovine calf serum, 5% FCS, penicillin (50 U/ml), and streptomycin (50 μg/ml) at 37°C in 10% CO_2 humidified incubators. Brain-derived bFGF (100 ng/ml) was added every other day during the phase of active cell growth. Bovine aortic endothelial cells (BAEC) were cultured as described (Vlodavsky *et al.*, 1987). Mouse methylcholanthrene-induced ESb T lymphoma cells were cultured as described (Vlodavsky et al., 1983).

Preparation of platelet-rich plasma (PRP)

Blood from healthy individuals who had a history of no drug ingestion for at least 10 d before testing was obtained by venipuncture with the two-syringe technique and mixed with 0.1 vol of 3.2% trisodium citrate (Yahalom *et al.*, 1984). PRP was prepared by centrifugation at 150 g for 10 min at room temperature. This preparation yielded a concentration of 2–3 $\times 10^5$ platelets/μl, as determined by counting with a Coulter Counter (Coulter Electronics, Hialeah, FL). Crude preparation of the platelet heparitinase was obtained by subjecting PRP to three cycles of freezing (liquid nitrogen) and thawing (37°C). Ten–20 μl of the lysed platelet preparation were taken for each determination of bFGF release and heparanase activity. For preparation of washed platelets, PRP was centrifuged (1100 g, 15 min, 20°C), the platelet-poor plasma was removed, and the platelet pellet was washed twice and resuspended in acid citrate dextrose (ACD)-buffered saline (pH 6.5), to yield the original concentration of 2–3 $\times 10^5$ platelets/μl (Yahalom *et al.*, 1984).

Neutrophils

Neutrophils were prepared from fresh blood samples obtained from healthy human donors and were purified by dextran sedimentation followed by hypotonic lysis of contaminating erythrocytes and centrifugation over Ficoll-Hypaque, as described (Matzner *et al.*, 1985). The granulocyte pellet was washed in Ca^{2+}- and Mg^{2+}-free PBS and suspended at 5×10^6 cells/ml in PBS containing Ca^{2+} and Mg^{2+}. Preparations obtained in this manner contained >95% neutrophils. Preferential release of the neutrophil heparanase was obtained by incubation of the cell suspension at 4°C for 4 h, followed by low-speed centrifugation (300 g, 10 min). The supernate fraction contained 20–30% of the total cellular heparanase activity (Matzner *et al.*, 1985). Samples (0.2–0.4 ml) of this supernatant were taken for determinations of bFGF release and heparanase activity.

Preparation of dishes coated with ECM

Bovine corneal endothelial cells were dissociated from stock cultures (2nd–5th passage) with STV and plated into 35-mm or 4-well plates at an initial density of 5×10^4 cells/ml. Cells were maintained as described above, except that 5% dextran T-40 was included in the growth medium. Six–8 d after the cells reached confluency, we exposed the subendothelial ECM by dissolving (3 min, 22°C) the cell layer with a solution containing 0.5% Triton X-100 and 20 mM NH_4OH in PBS followed by four washes in PBS (Vlodavsky *et al.*, 1987). For preparation of sulfate-labeled ECM, we added $Na_2[^{35}S]O_4$ (40 uC_i/ml) 3 and 7 d after seeding the cells, and the cultures were incubated with the label with no medium change. Ten–12 d after seeding, we dissolved the cell layer and exposed the ECM as described above. Of the total ECM-associated radioactivity, 70–75% was incorporated into HS side chains (Kramer *et al.*, 1982; Vlodavsky *et al.*, 1983). The ECM remained intact, free of cellular debris, and firmly attached to the entire area of the tissue culture dish. The presence of nuclei or cytoskeletal elements could not be detected in the denuded ECM when plates were examined by phase-contrast microscopy, scanning electron microscopy, or indirect immunofluorescence using anti-actin and anti-vimentin antibodies or the benzimidazole derivative Hoechst 33258 for nuclear staining. No serum proteins could be identified in the ECM (Gospodarowicz *et al.*, 1983). Main constituents of the corneal endothelial ECM were fibronectin; laminin; collagen types I, III, and IV; elastin; and sulfated proteoglycans (i.e., heparan sulfate, dermatan sulfate, and chondroitin sulfate proteoglycans).

Degradation of sulfated proteoglycans

$[^{35}S]O_4^=$-labeled ECM was incubated (3 h, 37°C, 10% CO_2 incubator) with intact cells, cell lysates, or heparanase preparations in PBS containing 0.02% gelatin adjusted to pH 6.8 with 20 mM phosphate buffer. To evaluate the occurrence of proteoglycan degradation, the incubation medium was collected and applied for gel filtration on Sepharose 6B columns (0.9 × 30 cm). Fractions (0.2 ml) were eluted with PBS at a flow rate of 5 ml/h and counted for radioactivity using Bio-fluor scintillation fluid. The excluded volume (V_o) was marked by blue dextran and the total included volume (V_t) by phenol red. The latter was shown to comigrate with free $[^{35}S]O_4^=$ (Kramer *et al.*, 1982; Vlodavsky *et al.*, 1983; Matzner *et al.*, 1985). Degradation fragments of HS side chains eluted from Sepharose 6B at $0.5 < Kav < 0.8$ (peak II) (Kramer *et al.*, 1982; Vlodavsky *et al.*, 1983). A nearly intact HSPG released from ECM by trypsin—and, to a lower extent, during incubation with PBS alone—was eluted next to V_o (peak I) (Vlodavsky *et al.*, 1983). Recoveries of labeled material applied on the columns ranged from 85 to 95% in different experiments. Each experiment was performed at least three times and the variation of elution positions (Kav values) did not exceed ±15%.

Iodination of bFGF

Recombinant bFGF was iodinated with ^{125}I and IodoGen (Pierce Chemical) as described (Bashkin *et al.*, 1989). Briefly, bFGF (3.3 μg in 50 μl of 10 mM tris(hydroxymethyl)-aminomethane–HCl (Tris-HCl), pH 7.1, and 2 M NaCl), together with 60 μl of 0.2 M sodium phosphate, pH 7.2, was added to a glass tube containing 1.6 μg Iodogen. The reaction was started by the addition of a twofold molar excess of Na^{125}I and stopped after 15 min at room temperature by the addition of 60 μl of 0.1% sodium metabisulfite and 30 μl of 0.1 mM KI. The reaction mixture was applied onto a small (0.3 ml) heparin-Sepharose column and the ^{125}I-FGF eluted with 1.5 ml buffer containing 20 mM sodium phosphate, pH 7.2, 2 M NaCl, and 0.2% gelatin. The specific activity was usually 1.2×10^5 cpm/ng FGF and the labeled preparation was kept for up to 3 wk at 4°C. The iodinated material yielded a single band (18.4 kDa) when subjected to NaDodSO$_4$-PAGE and autoradiography.

Release of ECM-bound FGF

ECM-coated wells (4-well plates) were incubated with either unlabeled bFGF (20 ng in 0.5 ml DMEM per well, overnight, 4°C) or with iodinated FGF (1.5–2.5×10^4 cpm/well, 3 h, room temperature) and the unbound factor washed four times with PBS containing 0.02% gelatin. ECM was then incubated (3 h, 37°C) with intact cells, cell lysates, or heparanase preparations; and aliquots (20 μl) of the 0.5 ml incubation medium were tested for mitogenic activity on 3T3 fibroblasts and vascular EC, or amount of released iodinated material. The remaining ECM was washed twice with PBS and solubilized with 1 M NaOH, and the radioactivity was counted in a γ-counter. The percentage of released ^{125}I-FGF was calculated from the total ECM-associated radioactivity. "Spontaneous" release in the presence of incubation medium alone was 7–12% of the total ECM-bound bFGF. Each data point is the average of triplicate wells, and the standard deviation did not exceed 6%. Intact cells and heparanase preparations were incubated under the same conditions with sulfate-labeled ECM, and the released radioactivity was analyzed for presence of HS degradation products, as described above.

Release of FGF from bovine corneas

Bovine corneas were placed in 35-mm culture dishes with their Descemet's membrane side upwards. The corneas were washed three times in PBS and incubated (37°C) with 0.5 ml of PBS (0.02% gelatin) alone, intact cells, or cell lysates. The incubation mixtures were tested for stimulation of ^{3}H-thymidine incorporation by 3T3 cells and proliferation of vascular EC. Mitogenic activity released from corneas in the presence of PBS alone did not exceed 20% of that released by intact or lysed cells.

Growth factor activity

Assay for DNA synthesis in 3T3 cells was performed as described (Vlodavsky *et al.*, 1987). Briefly, Balb/C 3T3 cells were seeded at half confluence into 0.3 cm^2 microtiter wells in DMEM supplemented with 10% calf serum. After reaching confluence (2–3 d), the cells were further incubated for a minimum of 5 d. Samples and ^{3}H-thymidine (1 μCi/well) were then added to the quiescent cells, and, after an incubation period of 32–40 h, DNA synthesis was assayed by measuring the radioactivity incorporated into trichloroacetic acid (TCA)-insoluble material. For measurements of EC proliferation, cells were seeded at a low density (2×10^2 cells per well of a 96-well plate) in 0.2 ml DMEM containing 10% heat-inactivated calf serum. Samples (10–20 μl) were added on day 2 and 4, and the cultures were fixed (2.5% formaldehyde in PBS) on day 6. The plates were immersed in 0.01 M borate buffer (pH 8.5), stained (10 min, 24°C) with 0.1 ml/well methylene blue (1% in 0.1 M borate buffer, pH 8.5), and washed four times in borate buffer. This procedure removed practically all non–cell-bound dye. Specific cell-incorporated methylene blue was dissolved with 0.2 ml of 0.1 N HCl (40 min, 37°C) and determined by reading the absorbance at 600 nm. Uptake of methylene blue was linearly correlated to the number of viable cells (Goldman and Bar-Shavit, 1979).

Acknowledgments

The excellent assistance of Ms. Ester Hy-Am is gratefully acknowledged. This work was supported by Public Health Service grant CA30289 awarded to I.V. by the National Cancer Institute, DHHS; and by grants from the USA-Israel Binational Science Foundation (grant # 8600135) and the German-Israel Foundation for Scientific Research and Development.

Received: July 10, 1990.
Revised and accepted: August 28, 1990.

References

Abraham, J.A., Mergia, A., Whang, J.L., Tumolo, A., Friedman, J., Hjerrild, K.A., Gospodarowicz, D., and Fiddes, C. (1986). Nucleotide sequence of a bovine clone encoding the angiogenic protein basic fibroblast growth factor. Science *233*, 545–548.

Andres, J.L., Stanley, D., Cheifetz, S., and Massague, J. (1989). Membrane-anchored and soluble forms of betaglycan, a polymorphic proteoglycan that binds transforming growth factor-β. J. Cell Biol. *109*, 3137–3145.

Baird, A., and Ling, N. (1987). Fibroblast growth factors are present in the extracellular matrix produced by endothelial cells in vitro: implications for a role of heparinase-like enzymes in the neovascular response. Biochem. Biophys. Res. Commun. *142*, 428–435.

Baird, A., and Walicke, P.A. (1989). Fibroblast growth factors. Br. Med. Bull. *45*, 438–452.

Bar-Ner, M., Mayer, M., Schirrmacher, V., and Vlodavsky, I. (1986). Involvement of both heparanase and plasminogen activator in lymphoma cell mediated degradation of heparan sulfate in the subendothelial extracellular matrix. J. Cell. Physiol. *128*, 299–307.

Bar-Ner, M., Eldor, A., Wasserman, L., Matzner, Y., and Vlodavsky, I. (1987). Inhibition of heparanase mediated degradation of extracellular matrix heparan sulfate by modified and non-anticoagulant heparin species. Blood *70*, 551–557.

Bashkin, P., Klagsbrun, M., Doctrow, S., Svahn, C-M., Folkman, J., and Vlodavsky, I. (1989). Basic fibroblast growth factor binds to subendothelial extracellular matrix and is released by heparanase and heparin-like molecules. Biochemistry *28*, 1737–1743.

Bashkin, P., Razin, E., Eldor, A., and Vlodavsky, I. (1990). Degranulating mast cells secrete an endoglycosidase which degrades heparan sulfate in subendothelial extracellular matrix. Blood *75*, 2204–2212.

Burgess, W.H., and Maciag, T. (1989). The heparin-binding (fibroblast) growth factor family of proteins. Annu. Rev. Biochem. *58*, 575–606.

Cardon-Cardo, C., Vlodavsky, I., Haimovitz-Friedman, A., Hicklin, D., and Fuks, Z. (1990). Expression of basic fibroblast growth factor in normal human tissues. Lab. Invest. (in press).

Denekamp, J. (1984). Vasculature as a target for tumor therapy. Prog. Appl. Microcirc. *4*, 28–38.

Folkman, J., and Klagsbrun, M. (1987). Angiogenic factors. Science *235,* 442–447.

Folkman, J., Klagsbrun, M., Sasse, J., Wadzinski, M., Ingber, D., and Vlodavsky, I. (1988). A heparin-binding angiogenic protein—basic fibroblast growth factor—is stored within basement membrane. Am. J. Pathol. *130,* 393–400.

Fridman, R., Ovadia, H., Fuks, Z., and Vlodavsky, I. (1985). Differential structural requirements for the induction of cell attachment, proliferation and differentiation by the extracellular matrix. Exp. Cell. Res. *157,* 181–194.

Goldman, R., and Bar-Shavit, Z. (1979). Dual effect of normal and stimulated macrophages and their conditioned media on target cell proliferation. J. Natl. Cancer Inst. *63,* 1004–1016.

Gonzalez, A-M., Buscaglia, M., Ong, M., and Baird, A. (1990). Distribution of basic fibroblast growth factor in the 18-day rat fetus: localization in the basement membranes of diverse tissues. J. Cell Biol. *110,* 753–765.

Gospodarowicz, D., Mescher, A.R., and Birdwell, C.R. (1977). Stimulation of corneal endothelial cell proliferation in vitro by fibroblast and epidermal growth factors. Exp. Eye Res. *25,* 75–82.

Gospodarowicz, D., Bialecki, H., and Greenburg, G. (1978). Purification of the fibroblast growth factor activity from bovine brain. J. Biol. Chem. *253,* 3736–3842.

Gospodarowicz, D., Gonzalez, R., and Fujii, D.K. (1983). Are factors originating from serum, plasma or cultured cells involved in the growth promoting effect of the extracellular matrix produced by cultured bovine corneal endothelial cells. J. Cell. Physiol. *114,* 191–201.

Gospodarowicz, D., and Cheng, J. (1986). Heparin protects basic and acidic FGF from inactivation. J. Cell. Physiol. *128,* 475–484.

Gospodarowicz, D., Ferrara, N., Schweigerer, L., and Neufeld, G. (1987). Structural characterization and biological functions of fibroblast growth factor. Endocr. Rev. *8,* 95–114.

Kardami, E., and Fandrich, R.R. (1989). Basic fibroblast growth factor in atria and ventricles of the vertebrate heart. J. Cell Biol. *109,* 1865–1875.

Kessler, D.A., Langer, R.S., Pless, N.A., and Folkman, J. (1976). Mast cells and tumor angiogenesis. Int. J. Cancer *18,* 703–710.

Kimelman, D., and Kirschner, M. (1987). Synergistic induction of mesoderm by FGF and TGF-beta and the identification of an mRNA coding for FGF in the early *Xenopus* embryo. Cell *51,* 869–877.

Kramer, R.H., Vogel, K.G., and Nicolson, G.L. (1982). Solubilization and degradation of subendothelial matrix glycoproteins and proteoglycans by metastatic tumor cells. J. Biol. Chem. *257,* 2678–2683.

Lider, O., Mekori, Y.A., Vlodavsky, I., Naparstek, Y., Baharav, E., and Cohen, I.R. (1990). Inhibition of T-lymphocyte heparanase by heparin prevents T cell migration and T cell–mediated immunity. Eur. J. Immunol. *20,* 493–499.

Matzner, Y., Bar-Ner, M., Yahalom, J., Ishay-Michaeli, R., Fuks, Z., and Vlodavsky, I. (1985). Degradation of heparan sulfate in the subendothelial basement membrane by a readily released heparanase from human neutrophils. J. Clin. Invest. *76,* 1306–1313.

Nakajima, M., Irimura, T., and Nicolson, G.L. (1988). Heparanases and tumor metastasis. J. Cell. Biochem. *36,* 157–167.

Naparstek, Y., Cohen, I.R., Fuks, Z., and Vlodavsky, I. (1984). Activated T lymphocytes produce a matrix-degrading heparan sulfate endoglycosidase. Nature *310,* 241–243.

Parish, C.R., Coombe, D.R., Jakobsen, K.B., and Underwood, P.A. (1987). Evidence that sulphated polysaccharides inhibit tumor metastasis by blocking tumor cell–derived heparanase. Int. J. Cancer *40,* 511–517.

Rifkin, D.B., and Moscatelli, D. (1989). Recent developments in the cell biology of basic fibroblast growth factor. J. Cell Biol. *109,* 1–6.

Roberts, R., Gallagher, J., Spooncer, S., Allen, T.D., Bloomfield, F., and Dexter, T.M. (1988). Heparan sulphate bound growth factors: a mechanism for stromal cell mediated haemopiesis. Nature *332,* 376–378.

Rogelj, S., Klagsbrun, M., Atzmon, R., Kurokawa, M., Haimovitz, A., Fuks, Z., and Vlodavsky, I. (1989). Basic fibroblast growth factor is an extracellular matrix component required for supporting the proliferation of vascular endothelial cells and the differentiation of PC12 cells. J. Cell Biol. *109,* 823–831.

Saksela, O., Moscatelli, D., Sommer, A., and Rifkin, D.B. (1988). Endothelial cell–derived heparan sulfate binds basic fibroblast growth factor and protects it from proteolytic degradation. J. Cell Biol. *107,* 743–751.

Saksela, O., and Rifkin, D.B. (1990). Release of basic fibroblast growth factor–heparan sulfate complexes from endothelial cells by plasminogen activator–mediated proteolytic activity. J. Cell Biol. *110,* 767–775.

Sampath, T.K., Muthukumaran, M., and Reddi, A.H. (1987). Isolation of osteogenin, an extracellular matrix associated bone inductive protein, by heparin affinity chromatography. Proc. Natl. Acad. Sci. USA *84,* 7109–7113.

Vlodavsky, I., Folkman, J., Sullivan, R., Fridman, R., Ishai-Michaelli, R., Sasse, J., and Klagsbrun, M. (1987). Endothelial cell–derived basic fibroblast growth factor: synthesis and deposition into subendothelial extracellular matrix. Proc. Natl. Acad. Sci. USA *84,* 2292–2296.

Vlodavsky, I., Fuks, Z., Bar-Ner, M., Ariav, Y., and Schirrmacher, V. (1983). Lymphoma cell mediated degradation of sulfated proteoglycans in the subendothelial extracellular matrix: relationship to tumor cell metastasis. Cancer Res. *43,* 2704–2711.

Vlodavsky, I., Korner, G., Ishai-Michaeli, R., Bashkin, P., Bar-Shavit, R., and Fuks, Z. (1990). Extracellular matrix resident growth factors and enzymes: possible involvement in tumor metastasis and angiogenesis. Cancer Met. Rev. (in press).

Weiner, H.L., and Swain, J.L. (1989). Acidic fibroblast growth factor mRNA is expressed by cardiac myocytes in culture and the protein is localized to the extracellular matrix. Proc. Natl. Acad. Sci. USA *86,* 2683–2687.

Yahalom, J., Eldor, A., Fuks, Z., and Vlodavsky, I. (1984). Degradation of sulfated proteoglycans in the subendothelial extracellular matrix by human platelet heparitinase. J. Clin. Invest. *74,* 1842–1849.