Journal of Cellular Biochemistry 45:167–176 (1991)

Extracellular Matrix-Resident Basic Fibroblast Growth Factor: Implication for the Control of Angiogenesis

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Abstract Despite the ubiquitous presence of basic fibroblast growth factor (bFGF) in normal tissues, endothelial cell proliferation in these tissues is usually very low, suggesting that bFGF is somehow sequestered from its site of action. Immunohistochemical staining revealed the localization of bFGF in basement membranes of diverse tissues, suggesting that the extracellular matrix (ECM) may serve as a reservoir for bFGF. Moreover, functional studies indicated that bFGF is an ECM component required for supporting endothelial cell proliferation and neuronal differentiation. We have found that bFGF is bound to heparan sulfate (HS) in the ECM and is released in an active form when the ECM-HS is degraded by heparanase expressed by normal and malignant cells (i.e. platelets, neutrophils, lymphoma cells). It is proposed that restriction of bFGF bioavailability by binding to ECM and local regulation of its release provide a novel mechanism for neovascularization in normal and pathological situations. The subendothelial ECM contains also tissue type- and urokinase type-plasminogen activators which participate in cell invasion and tissue remodeling. These results and studies on the properties of other ECM-immobilized enzymes (i.e. thrombin, plasmin, lipoprotein lipase) and growth factors (GM-CSF, IL-3, osteogenin), suggest that the ECM provides a storage depot for biologically active molecules which are thereby stabilized and protected. This may allow a more localized and persistent mode of action, as compared to the same molecules in a fluid phase.

Key words: endothelial cells, neovascularization, heparan sulfate, heparanase

A role for cell substrate interactions in the control of cell proliferation and differentiation has long been demonstrated [1,2]. In the case of epithelial tissues with a high rate of cell turnover, active cell proliferation is restricted to tall and columnar cells that are in close contact with a basement membrane. In contrast, cells in the upper layers lose their ability to proliferate and gradually adopt a flattened configuration [1,2]. Likewise, cultured cells, in order to proliferate and express their normal phenotype, require, in addition to nutrients and growth factors, an appropriate substratum upon which they can attach and spread [3,4], indicating that the substrate upon which cells rest is a decisive element in their proliferative and differentiation responses. Based on these and other observations, the extracellular matrix (ECM) is regarded as an insoluble complex of factors that regulate cellular growth, morphogenesis, and differentiation.

Our studies on the control of cell proliferation and tumor progression by its local environment focused on the interaction of cells with the ECM produced by cultured corneal and vascular endothelial cells (EC) [5,6]. This ECM closely resembles the subendothelium in vivo in its morphological appearance and molecular composition. It contains collagens (mostly types III and IV, with smaller amounts of types I and V), proteoglycans (mostly heparan sulfate- and dermatan sulfate-proteoglycans with smaller amounts of chondroitin sulfate proteoglycans), laminin, fibronectin, and elastin [4–6]. We have demonstrated that cells plated in contact with the subendothelial ECM, attached, proliferated, and expressed differentiated functions to a more faithful extent as compared to cells plated on

Abbreviations used: bFGF, basic fibroblast growth factor; ECM, extracellular matrix; EC, endothelial cells; HS, heparan sulfate; PA, plasminogen activator.

Received August 13, 1990; accepted October 3, 1990.

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regular tissue culture plastic. Of particular significance is the finding that in some cases cells plated on ECM proliferated in response to plasma and/or plasma proteins and hormones as actively as in the presence of serum [7]. Specific cell surface receptors that recognize various macromolecules in the ECM have been identified and their involvement in transduction of extracellular signals into the cell is being extensively studied [8]. The present article focuses on the identification of ECM-resident basic fibroblast growth factor (bFGF) and its involvement in ECM-induction of cell proliferation and differentiation. The presence and possible function of other ECM-bound growth factors and enzymes is discussed.

Fibroblast growth factors are a family of structurally related polypeptides characterized by high affinity to heparin [9]. They are highly mitogenic for vascular endothelial cells (EC) and are among the most potent inducers of neovascularization [10] and mesenchyme formation [11]. This gene family includes the prototypes aFGF (acidic fibroblast growth factor), bFGF (basic FGF), and three additional FGF-like proteins, hst/KS, int-2, and FGF 5 [9]. Both bFGF and aFGF are potent inducers of EC migration and proliferation and are known to modulate the expression of EC collagenase and plasminogen activator [12]. Recent studies demonstrate that both factors are tightly adsorbed to the ECM, presumably by their avid affinity for heparin-like glycosaminoglycans [13,14]. The association between the FGF prototypes and heparan sulfate was found to protect these polypeptides from proteolytic modification and inactivation [15]. Despite the ubiquitous presence of these potent EC growth factors in normal tissues, EC proliferation in these tissues is usually very low, with turnover time measured in years [16]. This raises the question of how these growth factors are prevented from acting on the vascular endothelium. One possibility is that they are somehow sequestered from their site of action and saved for emergencies such as wound repair and neovascularization [14]. In fact, a striking feature of FGF, either basic or acidic, is that it is mostly an intracellular protein, consistent with the lack of a consensus signal peptide in its gene [9,12]. This implies that FGF is released from cells via a nontraditional mechanism and perhaps in response to sublethal cell damage and leakage associated with tumor necrosis, tissue injury, and inflammation. Our studies indicate that ECM could be a possible storage site for FGF. We have shown that cultured corneal and vascular EC synthesize bFGF but do not secrete it into the medium [13]. They do, however, deposit bFGF into the subendothelial ECM [13]. Likewise, cardiac myocytes were shown to deposit aFGF into their ECM, but not conditioned medium [17]. In the present review we summarize results obtained by us and other investigators on: i) interaction of bFGF with heparan sulfate in ECM; ii) distribution of bFGF in normal tissues; iii) release of ECM-bound FGF by cells, enzymes, and heparin-like molecules; iv) involvement of ECM-resident bFGF in the control of cell proliferation and differentiation; and v) properties and physiological significance of other ECM-bound growth factors and enzymes.

BASIC FGF IS STORED WITHIN BASEMENT MEMBRANES IN VITRO AND IN VIVO

Cultured bovine endothelial cells (EC) lay down an ECM which replaces the requirement that sparsely seeded EC have for FGF in order to proliferate and express their differentiated functions [4]. The possible involvement of ECM-bound growth factors in the induction of cell proliferation has been minimized because ECM treated to inactivate growth factors still supports EC growth. However, the fact that EC growth factors are stabilized and protected by heparan sulfate [15] raised the possibility that ECM contains heparin-binding EC growth factors that are tightly bound and stabilized by the ECM heparan sulfate. We first tested whether bovine aortic and corneal EC synthesize bFGF. For this purpose extracts of cultured EC were analyzed by heparin-Sepharose affinity chromatography for growth factor activity. A single EC growth factor peak was eluted from the column at about 1.5 M NaCl, in a manner similar to a standard bFGF. Anti-bFGF antibodies cross-reacted with a Mr 18,400 polypeptide purified from aortic EC by heparin-Sepharose affinity chromatography [13]. In subsequent experiments, vascular and corneal EC were grown to confluence, the cells solubilized with Triton X-100, or removed intact using 2M urea, and the underlying ECM extracted with collagenase. When ECM extracts were analyzed by heparin-Sepharose chromatography, one single peak of growth-factor activity was eluted at about 1.5 M NaCl, regardless of whether the ECM was denuded with Triton or urea (Fig. 1) [13]. Although the mode of FGF deposition into ECM

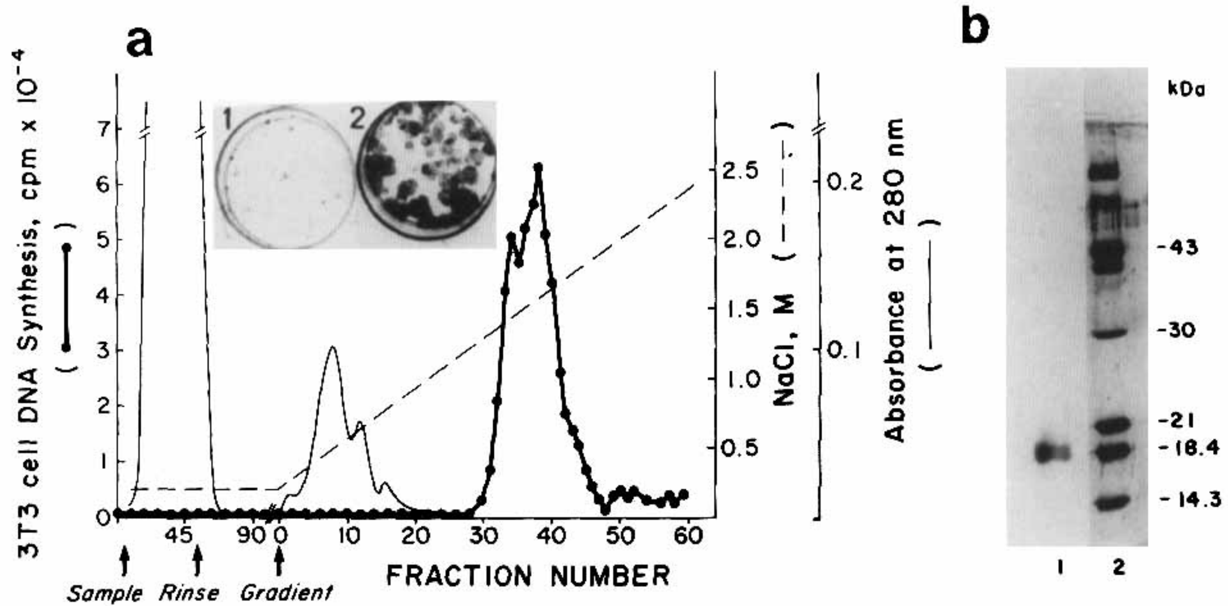

Fig. 1. Heparin Sepharose chromatography, mitogenic activity and electrophoretic transfer blot of ECM-derived bFGF. **a:** ECM extracts applied to heparin-Sepharose and growth factor activity eluted with a salt gradient. **Insert:** bovine aortic endothelial cells seeded at a clonal density and cultured in the absence (1) or presence (2) of ECM derived growth factor. **b:** Electrophoretic transfer blot: Active fractions eluted from heparin-Sepharose were subjected to SDS-PAGE, "Western" blotting and staining with antiserum directed against an internal sequence (positions 33–43) of bFGF (lane 1).

has not been identified, our studies indicate that bFGF is secreted by intact EC [18].

Bovine Corneas

To determine whether bFGF is normally stored within basement membranes in vivo, we have analyzed the cornea since it contains Descemet's membrane, which is the in vivo counterpart of the ECM produced by cultured corneal EC. Bovine corneas were dissected into three distinct layers. The inner layer consisted almost entirely of Descemet's membrane, the middle layer contained mainly corneal stroma, and the outer layer was composed mostly of Bowman's membrane [19]. When extracts of inner corneal layers were applied to a heparin-Sepharose column, a growth promoting factor which reacted with anti-bFGF antibodies was eluted from the column at about 1.6 M NaCl. Antisera directed against the internal and amino terminal portions of bFGF were used to localize bFGF within frozen sections of whole bovine cornea. Basic FGF appeared to be concentrated in a fine line delineating the outer aspect of Bowman's membrane (Fig. 2b). Intense bFGF staining was observed throughout the entire thickness of Descemet's membrane (Fig. 2d). Stromal staining, when seen, was of low intensity and appeared mainly within the inner half of the corneal stroma [19].

Expression of bFGF in Normal Human Tissues

We characterized the distribution of bFGF in normal human tissues by immunohistochemical staining of unprocessed fresh frozen sections of various organs [20]. Expression of bFGF in normal human tissues was ubiquitously detected in the basement membranes of all size blood vessels, but was not found in epidermal or epithelial basement membranes of a variety of tissues tested. Intensity and patterns of localization in blood vessels was consistent in various tissues, but varied among different regions of the vascular bed. Whereas homogenous and intense immunoreactivity were observed in large and intermediate size blood vessels, heterogeneity of expression was found in capillaries, with the most intense immunoreactivity observed in the anastamosing sites of branching regions of capillary beds [20]. Strong staining for bFGF was also found in cardiac muscle fibers, smooth muscle cells of mid-size blood vessels, the gut, and the myometrium. Basic FGF was also found in subset of central nervous system neurons and cerebellar Purkinje cells (but not on glial cells), and on epithelial cells of the bronchi, colon,

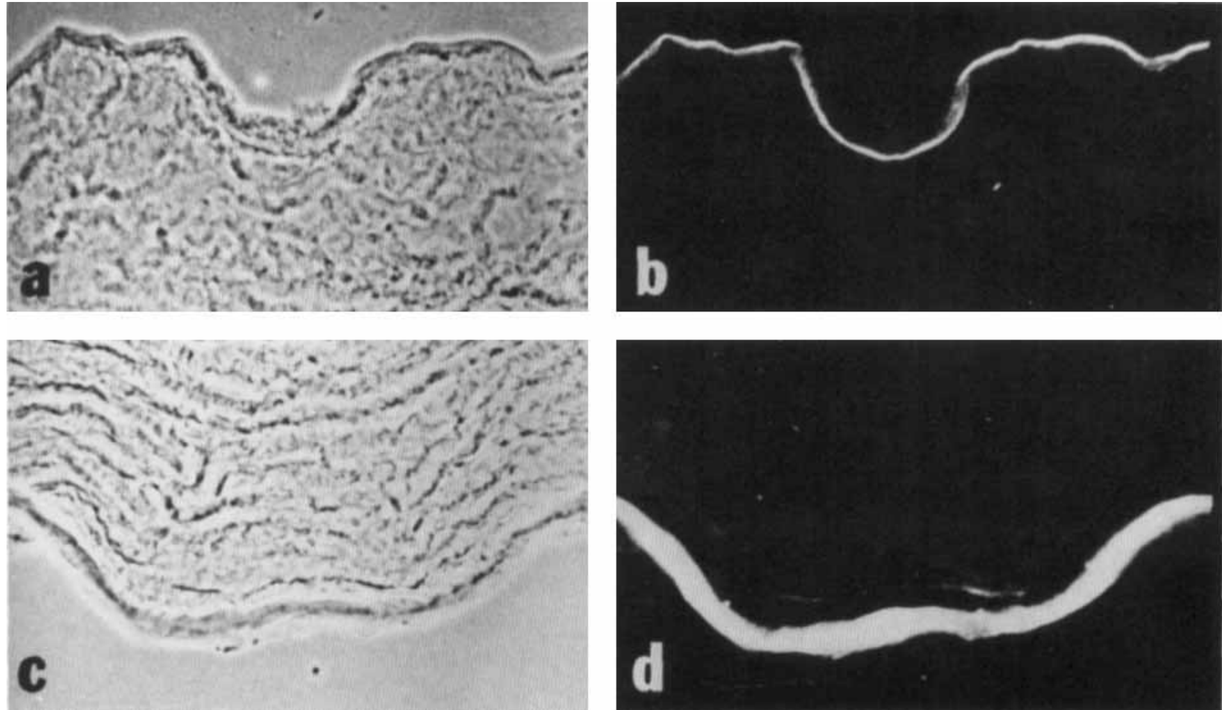

Fig. 2. Storage in basement membranes of the cornea of bFGF-like growth factors. Immunofluorescence (antibodies against internal sequence of bFGF) (b,d) and corresponding phase contrast (a,c) micrographs of bFGF within frozen sections of normal bovine corneas. b: Outer aspect of Bowman's membrane. d: Entire thickness of Descemet's membrane.

endometrium, and sweat gland ducts of the skin [20]. The localization of bFGF in cardiac muscle and nerve cells suggests a role in stimulating myocardial collateral vascularization and neuronal development and regeneration. Immunohistochemical staining performed by other investigators revealed the localization of bFGF in basement membranes of diverse tissues of the rat fetus [21] and in nuclei, intercalated discs, and endomysium of muscle fibers of the bovine heart [22].

EXTRACELLULAR MATRIX BINDING AND RELEASE OF BFGF

Scatchard analysis of ^{125}I-bFGF binding to ECM revealed that bFGF binds to ECM with an affinity (k_D = 610 nM) lower than that reported for binding of FGF to low affinity, presumably heparin-like sites on cell surfaces [23]. It appears that bFGF binds specifically to heparan sulfate (HS), since up to 90% of the bound growth factor was displaced by heparin, HS, or HS-degrading enzymes, but not by unrelated GAGs (i.e. chondroitin sulfate, keratan sulfate, hyaluronic acid), or enzymes (chondroitinases AC and ABC) [23]. Low affinity but yet specific binding are expected features of a physiologically active reservoir of bFGF around cells. These properties enable ECM-bound FGF to be readily available for cells in response to an appropriate signal. By using both in vitro and in vivo experimental systems, we have demonstrated that requirements for release of ECM-bound FGF were the same regardless of whether the FGF was exogenously added and bound to ECM, or was an endogenous constituent of intact basement membranes [19,23].

Heparanase Activity Expressed by Platelets, Neutrophils and Metastatic Tumor Cells Releases Active bFGF from ECM

An endoglycosidase (heparanase) which specifically degrades heparan sulfate was found to be a most efficient specific releaser of active bFGF from ECM [19,23]. Moreover, ECM that was pretreated with heparanase could no longer bind bFGF [23]. Heparanase has been shown to play a role in extravasation of blood-borne tumor cells and activated cells of the immune system [24–26]. Our results suggest that this enzyme

may also participate in tumor angiogenesis, through mobilization of ECM-bound EC growth factors. To investigate whether heparanase, expressed by various normal and malignant cells, is involved in release of bFGF from ECM, we first identified molecules (i.e. carrageenan lambda, N-acetylated heparin) which inhibit the enzyme but do not release the ECM-bound bFGF. Using these inhibitors, we have demonstrated that heparanase activity expressed by platelets (Fig. 3A), neutrophils, and lymphoma cells is involved in release of active bFGF from ECM and Descemet's membrane of bovine corneas [27]. 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, measured by release from ECM of sulfate labeled degradation products (Fig. 3B) [27]. Our results indicate that both endogenous and exogenously added bFGF are accessible to release by heparanase and that the released factor is active in promoting endothelial cell proliferation. We suggest that heparanase activity expressed by tumor cells may not only function in cell migration and invasion [24,25], but at the same time may also elicit an indirect neovascular response by means of releasing the ECM-resident FGF. Alterations in basement membrane structure and turnover that are associated with tumor progression may thus be responsible for the onset of angiogenic activity upon the transition of an in situ carcinoma from the prevascular to the vascularized state. Likewise, platelets, mast cells [28], and activated cells of the immune system (i.e. macrophages, neutrophils, T lymphocytes) that are often attracted by tumor cells may indirectly stimulate tumor angiogenesis by means of their heparanase activity [27]. These cells may also elicit an angiogenic response in the process of inflammation and wound healing.

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 [15]. It is therefore conceivable that bFGF is stored in ECM in a highly stable but relatively inactive

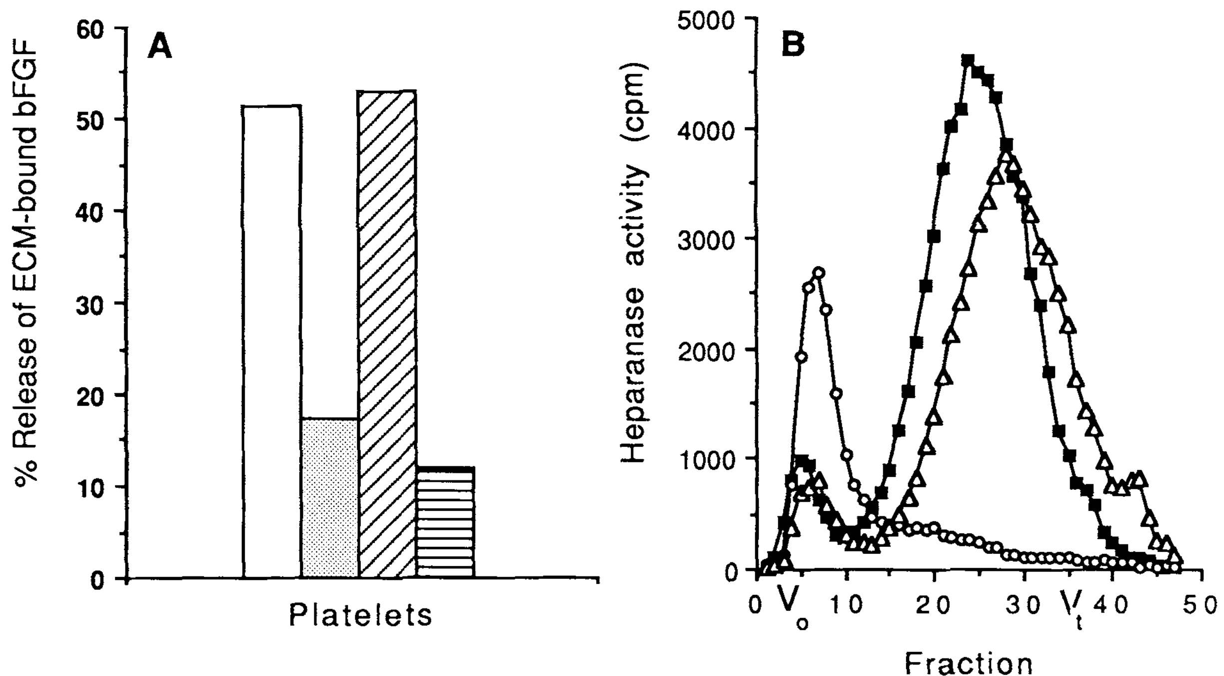

Fig. 3. Heparanase mediated release of ECM-bound bFGF. A: ECM coated wells (4 well plates) were incubated (3 h, 24°C) with ^{125}I-bFGF (1.5×10^4 cpm/well). Unbound bFGF was removed and the ECM incubated (3 h, 37°C, pH 6.8) with 2.5×10^8 platelets in the absence (□) or presence of either 10 ug/ml carrageenan lambda (▒), protease inhibitors (▨), or both (▤). Released ^{125}I-bFGF is expressed as percent of total ECM-bound ^{125}I-bFGF. 100% = 44 pg. B: Heparanase activity. Sulfate labeled ECM was incubated with platelets as described above, in the absence (■) or presence of either 10 ug/ml carrageenan lambda (○), or protease inhibitors (△). Labeled degradation products released into the incubation medium were analyzed by gel filtration on Sepharose 6B.

form, as also indicated by the highly stable ECM-resident growth promoting activity, as compared to that of bFGF in a fluid phase. 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 [12]. Moreover, bFGF complexed to HS fragment should diffuse through the stroma to the target cells more readily than free bFGF since bFGF-HS complexes do not bind to the ECM [12]. Since FGF is a pluripotent factor, restriction of its release at the vicinity of the target cell would ensure that its effect is not systemic [14]. This model (Fig. 4) also provides for a constant extracellular source of the angiogenic stimulus under conditions where the initial release of the growth factor may be as a bolus after cell death [14]. Apart from HS degrading enzymes, ECM-bound bFGF is released by palsmin as a noncovalent complex with HS proteoglycan or GAG [29]. This model (Fig. 4) also provides for a constant extracellular source lates production of plasminogen activator (PA) by EC [29] which may further facilitate release of bFGF from ECM. PA may also stimulate release of ECM-bound FGF through stimulation of heparanase mediated degradation of HS in ECM [30] (Fig. 4).

BASIC FGF IS AN ECM COMPONENT REQUIRED FOR SUPPORTING ENDOTHELIAL CELL PROLIFERATION AND NEURONAL DIFFERENTIATION

To investigate the involvement of the ECM-associated bFGF in its induction of cell proliferation and differentiation, we compared the ECM produced by PF-HR-9 mouse endodermal carcinoma cells, which do not synthesize bFGF, to ECM produced by PF-HR-9 cells transfected with the gene for bFGF [18]. PF-HR-9 cells secrete an underlying basement membrane-like ECM which is not mitogenic for vascular EC. This ECM also failed to induce extension of

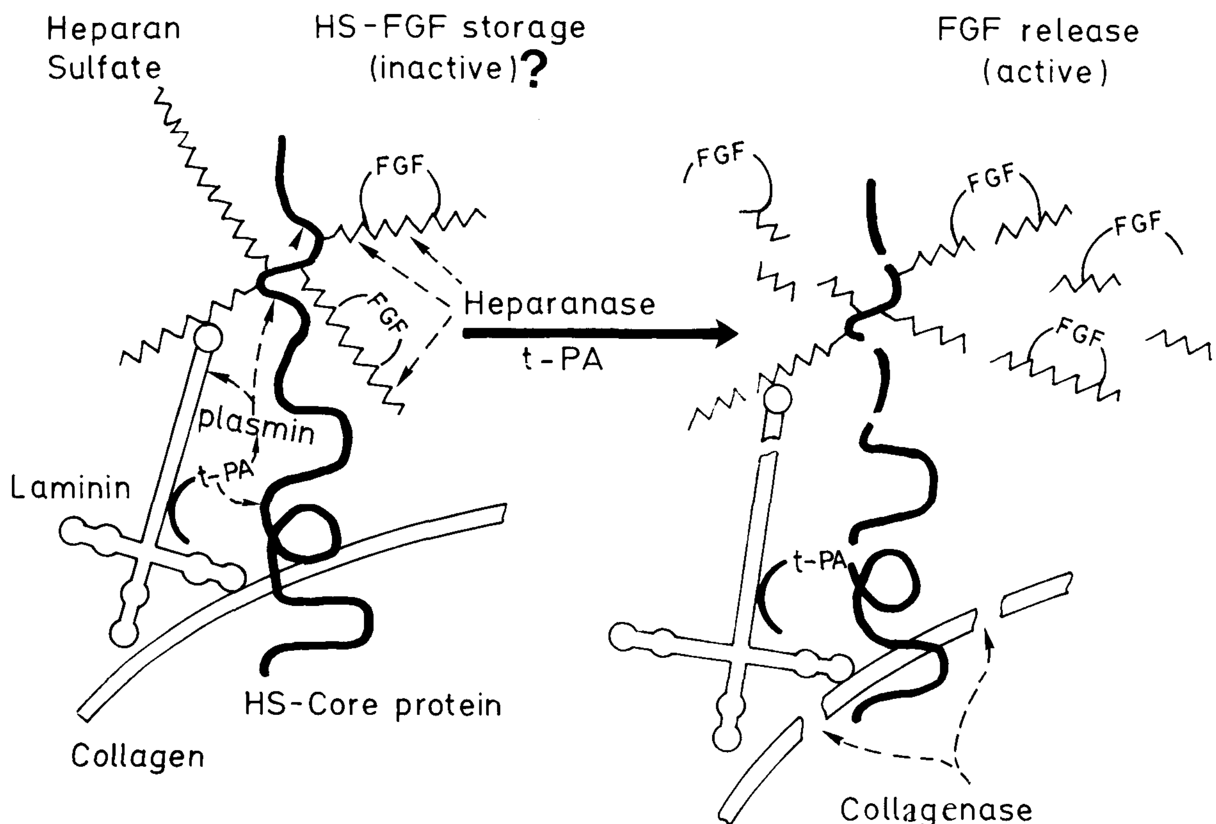

Fig. 4. Scheme describing the presence of heparan sulfate-bound FGF in ECM and release of FGF by heparanase. The ECM also contains plasminogen activator which participates in sequential degradation of heparan sulfate in ECM.

lysates of HR9 cells were subjected to heparin-Sepharose chromatography, no growth factor activity for vascular EC was eluted with a salt gradient ranging from 0.2 to 3.0 M NaCl. In contrast, a clonal cell population (HR9/bFGF) derived by transfecting HR9 cells with bFGF expression vector produced bFGF. ECM deposited by HR9 cells had little or no effect on both clonal growth of vascular EC and neurite outgrowth by PC12 cells. In contrast, ECM produced by HR9/bFGF cells induced clonal growth of vascular EC and extensive neuronal differentiation of PC12 cells [18]. These effects were inhibited in the presence of anti-bFGF antibodies, indicating that bFGF is an ECM component required for supporting proliferation of EC and differentiation of PC12 cells.

The mechanism by which components of the ECM may modulate the activity and bioavailability of bFGF is the subject of current investigations. In a recent study it was established that bFGF is synthesized as a phosphoprotein by bovine capillary EC [31]. Basic FGF was found to be a substrate for both PK-A and PK-C. PK-A phosphorylates bFGF in the receptor binding domain, resulting in a greater affinity for its cell surface receptor. When bFGF is associated with ECM proteins (i.e. fibronectin, laminin, collagen) the site of phosphorylation is masked and the mitogen is no longer a substrate for PK-A. In the presence of heparin, bFGF is phosphorylated by PK-A at a cryptic site that is not a PK-A consensus sequence. These results suggest that the bioavailability and function of bFGF are regulated by a complex array of biochemical interactions with the proteins, proteoglycans, and glycosaminoglycans present in the extracellular milieu and the cytoplasm [31].

OTHER ECM-IMMOBILIZED GROWTH FACTORS AND ENZYMES

We have previously demonstrated that heparanase mediated degradation of heparan sulfate in ECM is markedly enhanced in the presence of plasminogen and inhibited by aprotinin, suggesting a role for plasminogen activator (PA) in sequential degradation of the ECM-heparan sulfate [30]. Subsequent studies revealed that PA activity is associated with the ECM itself. Incubation of plasminogen with ECM, but not with regular tissue culture plastic, resulted in plasmin generation, as evidenced by its ability to degrade fibrin. Heating the ECM inactivated its ability to generate plasmin from plasminogen, but this activity was not inhibited when the matrix was preincubated with DFP or aprotinin [30]. To characterize the ECM associated PA, ECM extracts were subjected to SDS-PAGE zymography. Two main bands of proteolytic activity were observed when the gel was co-polymerized in the presence, but not in the absence of plasminogen (Fig. 5). The high MW protein cross-reacted with anti-human t-PA antiserum and co-migrated with a purified preparation of human melanoma t-PA. The lower molecular weight PA activity cross reacted with anti-human u-PA antibodies and migrated on an activity gel with a MW slightly lower than that of human u-PA (Fig. 5). The presence of t-PA and u-PA in the subendothelial ECM was also demonstrated by inhibition of the ECM mediated plasminogen activation in the presence of both anti t-PA and anti u-PA antibodies. In addition to its role in fibrinolysis, the ECM PA

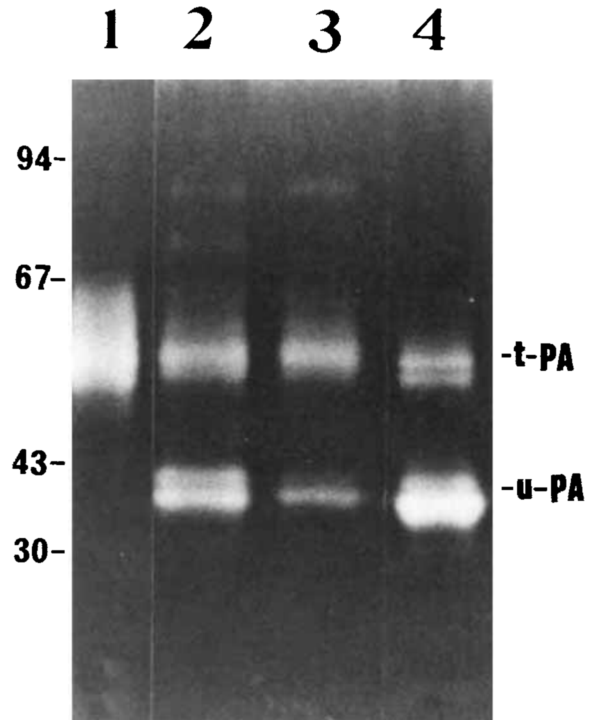

Fig. 5. Identification of ECM-associated PA by SDS-PAGE zymography. SDS-PAGE was performed on 10% polyacrylamide gels copolymerized with plasminogen. **Lane 1:** 15 mIU human melanoma t-PA; **lane 2:** ECM exposed by Triton/NH_4OH and solubilized in sample buffer; **lane 3:** ECM exposed by 2M urea and solubilized in sample buffer; **lane 4:** 15 ul aliquot of corneal endothelial cell conditioned medium. When plasminogen was not copolymerized in the gel, no lysis zones were detected.

may be involved in turnover of ECM components and in local dissolution of ECM and basement membranes associated with tumor metastasis, inflammation, and other processes involving cell migration and tissue remodeling. A functionally active PA inhibitor (PAI-1) has been identified in the ECM of cultured vascular endothelial and smooth muscle cells [32]. It has been demonstrated that PAI-1 binds efficiently to vitronectin [33] and that both vitronectin and PA-1 in the complex retained their cell attachment promoting activity and capacity to inhibit u-PA and t-PA, respectively [33]. A remarkable example for a basement membrane situated enzyme is acetylcholinesterase. This enzyme is secreted by the muscle cell and becomes anchored by a short collagen-like tail to the basal lamina that lies between the nerve terminal and the muscle cell membrane [34]. This localization is maintained even after degeneration of the muscle cell and nerve [34].

Apart from the angiogenic factor bFGF, heparan sulfate proteoglycans may function in localizing colony-stimulating factors (GM-SCF, Interleukin-3) to the stromal cell matrix where interaction with stem cells occurs [35]. These growth factors, once bound and localized, can be presented in an active form to haemopoietic cells [35]. While heparan sulfate side chains seems to exhibit relatively broad binding specificity and high capacity, the binding site for transforming growth factor-β (TGF-β) resides not in the GAG side chains but in the 110-130-kDa core protein of a cell surface chondroitin/heparan sulfate proteoglycan that binds various forms of TGF-β with high affinity and specificity [36]. Moreover, both TGF-β and its proteoglycan receptor (betaglycan) are present in the ECM of various cells and tissues [36]. Osteogenin, an ECM associated bone inductive protein, has been isolated from demineralized bone matrix by heparin affinity chromatography [37]. Other heparin binding growth factors tightly associated with bone matrix have been identified, suggesting that the bone matrix is a repository for chemotactic, mitogenic, and differentiation inducing factors involved in endochondral bone formation. A growth promoting activity may also be present within a large structural protein of the ECM. Thus, fragments of laminin which consist of cysteine-rich "EGF-like" repeats were found to stimulate thymidine incorporation in cultured cells possessing EGF receptors [38].

In addition to molecules that are secreted by the ECM producing cells, ECM may serve as a storage depot for active molecules produced by other cell types. We have recently demonstrated that thrombin, a serine protease which also exerts growth promoting and chemotactic activities, binds with high affinity to dermatan sulfate in the subendothelial ECM [39]. While ECM-bound thrombin retained its biological activities (i.e. esterolytic activity, platelet activation, stimulation of vascular smooth muscle cell proliferation), it was protected from inactivation by its physiological circulating inhibitor anti-thrombin III [39]. Other plasma proteins participating in the hemostatic process were also shown to bind specifically to the ECM. These include von Willebrand factor mediating platelet adhesion to the vascular subendothelium, and plasminogen participating in the fibrinolytic system [40]. Immobilized plasminogen was found to be a better substrate for t-PA than soluble plasminogen and was protected from its inhibitor α_2-plasmin inhibitor [40]. Likewise, we have demonstrated that lipoprotein lipase (LPL) binds to the subendothelial ECM and retains its catalytic activity for a longer period than the soluble enzyme [41]. Thus, a concept emerges where the ECM not only binds and localizes various active proteins, but also modulates their mode of action and provides a protective environment from the circulating plasma inhibitors.

CONCLUDING REMARKS AND PROSPECTIVES

The microvascular system remains quiescent for prolonged periods. However, on short notice it appears capable of responding with rapid capillary growth to physiological demands such as ovulation, as well as to pathological conditions such as wounds, chronic inflammation, certain immune reactions, and tumors. While bFGF is a potent angiogenic factor, its lack of a signal sequence raises questions concerning how and when bFGF is released and whether results obtained after the addition of exogenous bFGF represent the normal role of the protein. It may well be that bFGF is stored intracellularly and is utilized primarily as an autocrine repair factor in response to cell injury. In fact, we have demonstrated that irradiated endothelial cells release bFGF [42] and exhibit increased production of bFGF mRNA (Fuks et al., submitted).

The localization of bFGF in basement membranes and its interaction with heparan sulfate proteoglycans are indicative of a novel extracellular control of bFGF bioavailability, which has not been described for other growth factors. While cell damage and death may provide a source for the ECM-resident bFGF, our results suggest that FGF is deposited by intact cells, although the mode of its deposition has not been elucidated. Likewise, while release of active bFGF from ECM can be brought about by cellular heparanase [23,27] and/or plasmin [29], other modes of interactions, not involving ECM degradation, may elicit cellular responses to ECM-bound bFGF. Studies are under way to elucidate the involvement of heparanase in neovascularization, tumor cell invasion, and autoimmune disorders. The enzyme has been partially purified from a human hepatoma cell line and normal human neutrophils towards a better understanding of its molecular nature and regulation of gene expression. Preparation of anti-heparanase antibodies will enable studies on its tissue distribution and content in normal and pathological situations. Because bFGF stimulates neovascularization in all the currently used experimental models, it is conceivable that it might have important therapeutic applications in clinical paradigms where an enhancement of vascularization is beneficial (i.e. tissue repair in the reticulo-endothelium, recovery from ischemic lesions, and in some types of ovarian dysfunction) [14]. Because FGF is a pluripotent factor it may also function in the repair of connective tissues and injury to the nervous system [14]. Local release of ECM-bound bFGF is expected to yield better clinical results as compared to a systemic administration of exogenous bFGF, since the tissue responds to its endogenous growth factor that becomes available in appropriate amounts and over an extended period of time. This may also minimize the incidence of cell transformation which may occur in response to over expression of bFGF. Of particular interest in this regard is to elucidate the mode of autocrine cell transformation by bFGF and the interaction of bFGF with the cell nucleous [43] as a possible pathway for induction of cell proliferation and expression of differentiated functions.

ACKNOWLEDGMENTS

This work was supported by Public Health Service Grants CA-30289 (I.V.); CA-37392 (M.K.) and CA-52462 (Z.F.) awarded by the National Cancer Institute, DHHS; and by grants from the USA-Israel Binational Science Foundation and the German-Israel Foundation for Scientific Research and Development (I.V.).

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