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Heparanase and Platelet Factor–4 Induce Smooth Muscle Cell Proliferation and Migration *via* bFGF Release from the ECM[1]

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Basic fibroblast growth factor (bFGF) has been shown to play an instrumental role in the cascade of events leading to restenosis; however, the mechanisms of bFGF activation following vascular injury have remained elusive. We have demonstrated that heparanase and platelet factor–4 (PF4), released from activated platelets at the site of injury, liberate bFGF from the extracellular matrix (ECM) of vascular smooth muscle cells (SMC), resulting in the induction of SMC proliferation and migration. Increases in proliferation and migration were inhibited by treatment with a bFGF-neutralizing antibody, suggesting that proliferation and migration in response to heparanase or PF4 are mediated by bFGF activation. When platelets were seeded on top of SMCs, degranulation products were found to release bFGF from the ECM, increasing cell proliferation and cell migration. Again, these increases in SMC proliferation and migration were inhibited by treatment with an anti-bFGF antibody. Furthermore, these increases in proliferation were completely inhibited by treatment with an anti-heparanase antibody. Platelet degranulation products, such as heparanase and PF4, may liberate bFGF from extracellular sequestration, activating the growth factor and inducing the SMC proliferation and migration that contribute to the wound healing response following vascular injury.

Key words: bFGF, ECM, heparanase, PF4, restenosis.

Basic FGF is a prototype of the heparin-binding growth factor family (*1*) that has been determined to be an important vascular SMC mitogen (*2*). Despite the lack of a traditional signal peptide for secretion, bFGF has been localized to the ECM and has been ubiquitously detected in the basement membranes of blood vessels (*3, 4*). Heparan sulfate proteoglycans (HSPG) in the ECM and basement membrane bind bFGF in an inactive form (*5*) and protect it from proteolytic degradation (*6*). ECM sequestration may provide a localized source of growth factors that can be released and activated during developmental and wound healing processes, such as angiogenesis and restenosis. The release of bFGF from the ECM is thought to contribute to the prolonged proliferative response of SMCs following vascular injury (*7*). However, the mechanism of bFGF release from immobilized HSPGs in the ECM in response to vascular injury is currently not known.

Approximately 30–50% of all percutaneous transluminal coronary angioplasty (PTCA) procedures fail due to restenosis, re-narrowing of the vessel lumen (*8*). Restenosis is largely due to SMC proliferation, migration and matrix deposition, resulting in the formation of an occlusive neointimal layer (*9*). bFGF is thought to be an important SMC mitogen in the restenosis process (*7, 10*). For example, the addition of exogenous bFGF has been shown to significantly increase SMC proliferation following balloon injury (*11–13*), and intravenous treatment with bFGF-neutralizing antibodies has been shown to significantly decrease SMC proliferation following vascular injury (*14*). Furthermore, bFGF is stored at high levels in the medial layer of the arterial wall, and bFGF content in the arterial wall has been shown to dramatically decrease following injury (*15, 16*).

The release of bFGF from extracellular sequestration may be initiated by platelet degranulation products at the site of injury. Deendothelialization of the vessel wall following procedures such as balloon angioplasty induces platelet adhesion and the localized release of degranulation products including the heparan sulfate-degrading endoglycosidase, heparanase, and PF4, a heparin-binding protein (*17, 18*). Heparinase, a bacterial analog to mammalian heparanase, has been shown to release a high molecular weight heparan sulfate-bFGF complex that has mitogenic activity (*19*). Release of bFGF as a complex with heparan sulfate

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Abbreviations: ACD, acid citrate dextrose; AEC, aminoethylcarbozole; bFGF, basic fibroblast growth factor; BSA, bovine serum albumin; ECM, extracellular matrix; ELISA, enzyme-linked immunosorbent assay; FBS, fetal bovine serum; FGF, fibroblast growth factor; FGFR, fibroblast growth factor receptor; HASMC, human aortic smooth muscle cell; HBS, hepes buffer solution; HRP, horseradish peroxidase; HS, heparan sulfate; HSPG, heparan sulfate proteoglycan; MEME, Minimum Essential Medium Eagle; PA, plasminogen activator; PBS, phosphate buffered saline; PDGF, platelet derived growth factor; PCNA, proliferating cell nuclear antigen; PF4, platelet factor four; PTCA, percutaneous transluminal coronary angioplasty; SDS-PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; SMC, smooth muscle cell.

(HS) fragments has been suggested to increase the bioactivity of the growth factor by protecting the growth factor from proteolytic degradation (*6, 20*), decreasing re-binding to the ECM (*21*) and enhancing binding to the cell surface fibroblast growth factor receptor (FGFR) (*22*). Recent studies reporting the crystal structure of the FGF-FGFR-heparin ternary complex, where simultaneous binding of FGF and heparin were found to induce receptor dimerization, have provided a structural basis for the essential role of HS in FGF signal transduction (*23*). PF4, a heparin-binding protein found at the site of vascular injury, has been determined to exhibit a greater affinity for HSPGs than bFGF and is proposed to compete with bFGF for HS binding sites in the ECM (*24*), thereby releasing soluble bFGF. Due to the necessity of HS in bFGF signal transduction, soluble bFGF has been proposed to be dependent upon HSPGs in close proximity to cell surface FGFRs for mitogenic signaling (*23*).

While many therapeutic strategies are being developed for the prevention of restenosis, a better understanding of the cascade of events leading to restenosis will permit more rational approaches. In this study, we have shown that heparanase and PF4, released from platelets at the site of vascular injury (*17, 18*), release bFGF from sequestration in the ECM. Furthermore, the specific activation of bFGF has been found to stimulate cell proliferation and is proposed to induce cell migration by increasing plasminogen activator activity. These studies indicate a role for heparanase and PF4 in the induction of intimal thickening following vascular injury *via* the activation of bFGF and subsequent induction of medial SMC proliferation and migration. These studies may also elucidate the mechanisms by which heparin-binding growth factors can be stored in close proximity to cells without emitting a mitogenic signal and can be activated as needed via liberation from the ECM.

EXPERIMENTAL PROCEDURES

Heparanase Purification—Heparanase was purified from human platelets (Human Blood Center, Houston, TX) as described by Freeman and Parish (1998). Platelet lysis was achieved via three freeze/thaw cycles, the platelet homogenate was centrifuged at 35,000 ×*g*, supernatant was collected and the pelleted material was resuspended in 15 mM sodium dimethyl glutarate buffer (Sigma Chemicals), pH 6.0, containing 0.5 M NaCl (Sigma Chemicals). Resuspended homogenate was freeze/thawed, centrifuged, and supernatant was collected as before. This process was repeated a total of three times. Purification was carried out at 4°C by running platelet supernatant through a series of affinity chromatography and size exclusion columns including: concanavalin A-sepharose (Pharmacia Fine Chemicals), Zn^{2+}-chelating sepharose (Pharmacia Fine Chemicals), Blue A-agarose (Pharmacia Fine Chemicals), octyl-agarose (Pharmacia Fine Chemicals), and Superose 12 10/30 chromatography (Pharmacia Fine Chemicals) as described by Freeman and Parish (1998). Enzyme was concentrated by Centricon 30 centrifugation (Amicon). Total protein concentration was determined by bicinchoninic acid analysis (Pierce). Protein purity was examined following reduction with β-mercaptoethanol (Sigma Chemicals), electrophoresis of 1 μg of total protein in a 10% polyacrylamide gel (Bio-Rad Laboratories) and staining with Coomassie brilliant blue R250 (Sigma Chemicals). Platelet-derived heparanase activity was assayed by incubating 2 μg of purified protein with 2 μg of heparan sulfate polysaccharide chains isolated from bovine kidneys (Sigma Chemicals) for 3 h at 37°C. Degradation was examined by reduction with β-mercaptoethanol, electrophoresis of 2 μg of total protein in a 10% polyacrylamide gel, and staining with methylene blue (Sigma Chemicals). Protein activity was assessed by decreased staining and increased mobility of degradation products.

Cell Culture—Human aortic smooth muscle cells (HASMCs; Clonetics) were maintained in a 5% CO_2 environment at 37°C in Minimum Essential Medium Eagle (MEME; Sigma Chemicals) supplemented with 10% fetal bovine serum (Sigma Chemicals) and 1% L-glutamine-penicillin-streptomycin (Sigma Chemicals). Cells were used at passages 8–12.

Platelet Preparation—Blood was drawn directly into a syringe containing 1:7 volume acid citrate dextrose (ACD; 65 mM citric acid, 85 mM sodium citrate, 111 mM dextrose, pH 4.5; Sigma Chemicals). The pH was then adjusted to 6.5 by addition of ACD. Platelet-rich plasma was prepared by centrifugation at 150 ×*g* for 15 min. Platelets were then pelleted by centrifugation at 1,900 ×*g* for 15 min, washed and resuspended in HEPES buffer solution (HBS; 10 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid, 145 mM NaCl, 5 mM KCl, 0.5 mM Na_2HPO_4, 1 mM $MgSO_4$, 5.5 mM glucose, 3.5 g/liter bovine serum albumin, 0.1 mM $CaCl_2$, 50 U/ml sodium heparin, and 2.5 U/ml apyrase, pH 7.0; Sigma Chemicals). Resuspended platelets were incubated for 45 min at room temperature, repelleted by centrifugation at 1,900 ×*g* for 10 min, and resuspended in HBS which also contained 1 mM $CaCl_2$ at pH 7.4, to initiate platelet degranulation. Fresh platelet preparations were used for all experiments.

bFGF Release—Experiments were either conducted before significant ECM deposition by cells or following the development of a robust ECM in order to distinguish the interaction of heparanase, PF4 or platelet degranulation products with cell surface proteoglycans and the ECM. For experiments with minimal ECM, HASMCs were seeded in tissue culture–treated 24-well plates (Fisher) at 4,000 cells/cm^2, maintained for 3 d and then incubated with heparanase isolated and purified as described above (10 pg/ml, 0.1 ng/ml, 1 ng/ml, 5 ng/ml, 25 ng/ml, and 100 ng/ml), 25 ng/ml heparinase III (Sigma Chemicals) or PF4 (10 pg/ml, 0.1 ng/ml, 1 ng/ml, 5 ng/ml, 25 ng/ml, and 100 ng/ml; Cal Biochem) for 15 min at 37°C. Control wells were left untreated. We chose the 15-min incubation period based on previous studies employing incubation periods ranging from 15 min to 48 h. For experiments in the presence of an ECM, HASMCs were seeded as previously described, allowed to reach confluence (3 d), and maintained for an additional 7 d to allow for the production of a robust ECM. Cells were removed by incubation in 2.5 mM ammonium hydroxide (Sigma Chemicals) for 1 h, and then the remaining ECM structure was washed with 70% ethanol (Fisher Chemicals) prior to incubation with heparanase, heparinase III or PF4 for 15 min as described above. Alternatively, HASMCs were cultured for 10 days to deposit an ECM, cells were removed as described above, and the remaining intact ECM was treated with platelets (500/cm^2), prepared as described above, 25 ng/ml heparanase, or 25 ng/ml PF4 for 4 h. To

exclude the possibility of positive bFGF readings due to serum content, the culture medium was replaced by serum-free medium prior to each treatment. Following incubation, media samples were examined for bFGF levels via ELISA. Media samples and bFGF standards (10 pg/ml to100 ng/ml) coated onto non-tissue culture treated 96-well plates (Nalge Nunc International) were incubated at 4°C overnight. Wells were blocked with incubation buffer (phosphate buffer saline, PBS; pH 7.4, 5 mg/ml bovine serum albumin, 0.05% tween 20, 0.02% NaN_3; Sigma Chemicals) for 1 h at 37°C, incubated with rabbit anti–human bFGF IgG (Biodesign) diluted 1:250 in incubation buffer for 1 h at 37°C, washed 4 times with PBS, incubated with horseradish peroxidase (HRP)–conjugated goat anti–rabbit IgG (Biodesign) diluted 1:250 in incubation buffer for 1 h at 37°C, washed 4 times with PBS, and reacted with 3,3′,5,5′-tetramethylbenzidine (Sigma Chemicals) as the HRP substrate. Absorbance readings were taken on a microplate reader (450 nm; Bio-tek Instruments).

Cell Proliferation—To assess the effects of heparanase and PF4 on cell proliferation in the presence of a minimal ECM, HASMCs were seeded as previously described and immediately incubated with heparanase (10 pg/ml, 0.1 ng/ml, 1 ng/ml, 5 ng/ml, 25 ng/ml, and 100 ng/ml), 25 ng/ml heparinase III or PF4 (10 pg/ml, 0.1 ng/ml, 1 ng/ml, 5 ng/ml, 25 ng/ml, and 100 ng/ml) for 24 h at 37°C. Control wells were either left untreated or received 25 ng/ml bFGF (Sigma Chemicals). Additional studies were carried out where HASMCs were seeded as previously described, allowed to adhere overnight, then treated with platelets (500/cm^2), 25 ng/ml heparanase, 25 ng/ml PF4 or 25 ng/ml bFGF for 24 h at 37°C. For experiments in the presence of an ECM, HASMCs were seeded as previously described, allowed to reach confluence, and maintained for an additional 7 d to allow for the production of a robust ECM. Following ECM deposition, cells were incubated with the concentrations listed above of bFGF, heparanase, heparinase III, PF4 or platelets for 24 h at 37°C. Immunohistochemical staining for proliferating cell nuclear antigen (PCNA) was carried out to examine proliferative activity. Cells were washed with PBS, fixed in 10% buffered formalin (Stephens Scientific) for 10 min, permeabilized with methanol (Sigma Chemicals) for 2 min, and incubated in 3% H_2O_2 (Fisher Chemicals) to quench endogenous peroxidase activity. Cells were incubated with mouse anti–human PCNA IgG (Dako) diluted 1:100 in PBS containing 3% fetal bovine serum (FBS) for 1 h, washed 3 times with PBS, incubated with rabbit anti–mouse IgG HRP (Dako) diluted 1:100 in PBS containing 3% FBS for 1 h, washed 3 times with PBS and incubated with aminoethylcarbazole chromagen (AEC; Dako) for 15 min to form a red precipitate. Cells were counterstained with Mayer's hematoxylin (Dako).

Cell Proliferation Following Anti-bFGF IgG or Anti-Heparanase IgG Treatment—HASMCs were seeded and maintained for 7 d beyond confluence as described above to allow for the production of a robust ECM and then incubated with 0.2 μg/ml anti-bFGF IgG (Biodesign), 25 ng/ml heparanase ± 0.2 μg/ml anti-bFGF IgG, 25 ng/ml heparinase III ± 0.2 μg/ml anti-bFGF IgG, 25 ng/ml PF4 ± 0.2 μg/ml anti-bFGF IgG for 72 h at 37°C, or platelets (500/cm^2) ± 0.2 μg/ml anti-bFGF IgG, 25 ng/ml heparanase ± 0.25 μg/ml anti-heparanase IgG (Sigma Genosys) or platelets (500/cm^2) ± 0.25 μg/ml anti-heparanase IgG for 24 h at 37°C. The anti-bFGF IgG used in this study has been previously shown to neutralize bFGF activity (*14*). The anti-heparanase IgG used in this study was prepared in rabbit against a 20-amino-acid peptide fragment corresponding to the proposed heparanase active site; G^{215} through D^{234} (*26*; Sigma Genosys). Neutralizing activity of this antibody was confirmed using the heparan sulfate degradation assay described above. Negative controls were left untreated, while positive controls were treated with 25 ng/ml bFGF. Proliferation was assessed *via* immunohistochemical staining using the PCNA-HRP conjugate followed by reaction with AEC chromagen as described above.

Plasminogen Activator Activity—HASMCs, seeded as described above, were either allowed to adhere overnight or maintained for 10 d to allow for the deposition of a robust ECM. Cell culture medium was replaced with indicator-free MEME (Sigma Chemicals) prior to incubation with heparanase (10 pg/ml to 100 ng/ml), 25 ng/ml heparinase III, PF4 (10 pg/ml to 100 ng/ml) or platelets (500/cm^2) for 18 h at 37°C. In both cases, negative controls were left untreated, while positive controls were treated with 25 ng/ml bFGF. Plasminogen activator activity was assessed via a chromogenic substrate assay (American Diagnostica). Tris-buffered solution (6 mM 6-aminohexanoic acid, Sigma Chemicals; 0.1 mg/ml bovine plasminogen, American Diagnostica; 50 mM Tris buffer, Sigma Chemicals; and 0.1% PEG 6000, pH 7.4, Fluka), filtered media sample and the spectrozyme chromagen (American Diagnostica) were mixed in equal volumes (50 μl each) and incubated for 3 min at 37°C. Absorbance readings were taken on a microplate reader (405 nm; Bio-tek Instruments).

Cell Migration—To assess the effects of heparanase, PF4 or activated platelets on cell migration in the presence of a minimal ECM, HASMCs were seeded on glass slides (Fisher Scientific) fitted with 8-well flexi-perms (Sigma) at 4,000 cells/cm^2 and allowed to adhere overnight. Flexi-perms were then removed, and cells were incubated with 25 ng/ml bFGF, 0.2 μg/ml anti-bFGF IgG, 25 ng/ml heparanase ± 0.2 μg/ml anti-bFGF IgG, 25 ng/ml heparinase III ± 0.2 μg/ml anti-bFGF IgG, 25 ng/ml PF4 ± 0.2 μg/ml anti-bFGF IgG, or platelets (500/cm^2) ± 0.2 μg/ml anti-bFGF IgG for 24 h at 37°C. For experiments in the presence of an ECM, HASMCs were seeded as previously described and maintained for 10 d to allow for the deposition of a robust ECM. Flexi-perms were then removed, and cells were incubated with the previously described concentrations of bFGF, heparanase, heparinase III, PF4, platelets or a combination of each and 0.2 μg/ml anti-bFGF IgG for 24 h at 37°C. Following incubation, cells were washed with PBS, fixed with 10% buffered formalin and stained with Mayer's hematoxylin. Cell migration was quantified by counting the number of cells beyond the initial cell culture perimeter.

Statistical Analysis—All experiments were performed minimally in triplicate. Error bars reflect the standard deviation, and *p*-values were assessed using two-tailed, unpaired *t*-tests. *P*-values less than 0.05 were determined to be significant.

RESULTS

Human Platelet Heparanase Purification and Activity—Recent advances in the purification of the heparanase protein (*25–28*) have enabled us to examine the effects of the

mammalian form of the enzyme. Although both bacterial heparinase and mammalian heparanase have been shown to degrade HSPGs in the ECM and basement membrane, the mammalian enzyme cleaves glycosidic bonds with a hydrolase mechanism distinct from bacterial heparinase, which functions as an eliminase (*29, 30*). This functional difference leads to the generation of different HS-bFGF complexes and may affect FGFR binding and mitogenic potential. Thus, we purified heparanase from human platelet-rich plasma (as described by Freeman and Parish 1998) in addition to conducting studies with flavobacterium-derived heparinase. Protein purity was examined via SDS-PAGE and a single band of the appropriate molecular mass (50 kDa) was obtained when loading 1 μg total protein (Fig. 1). Protein activity was initially investigated by examining the ability of purified heparanase to catalyze the degradation of HS polysaccharide chains isolated from bovine kidneys. HS (M_r ~30,000–100,000) was incubated at 37°C with purified heparanase for 3 h; incubation of HS with heparanase significantly degraded the HS substrate, as indicated by the absence of HS staining in the gel (Fig. 1).

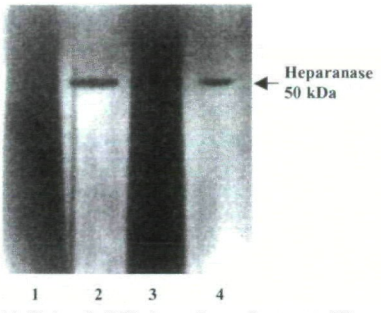

Fig. 1. **SDS-PAGE of HS incubated at 37°C with purified heparanase.** Lane 1, HS; lane 2, heparanase; lane 3, HS incubated with heparanase, t = 0; lane 4, HS incubated with heparanase, t = 3 h. Samples were reduced with beta-mercaptoethanol, electrophoresed in a 10% polyacrylamide gel and stained with methylene blue.

bFGF Release Induced by Heparanase and PF4—To determine whether platelet-derived heparanase or PF4 releases bFGF from the ECM, soluble bFGF levels following exposure to heparanase or PF4 were assessed. To evaluate bFGF release in an experimental setting with minimal ECM deposition, HASMCs were maintained for 3 d and incubated with varying concentrations of heparanase or PF4, and bFGF levels in the media were examined by ELISA. To compare the activity of platelet-derived heparanase and heparinase derived from flavobacterium, cells were also treated with 25 ng/ml heparinase. Treatment with heparanase, heparinase (Fig. 2A) or PF4 (Fig. 2C) was not found to elicit bFGF release in the absence of an ECM.

Following the deposition of an ECM by cultured cells, growth factors such as bFGF are sequestered and available for release. Therefore, further studies were carried out examining soluble bFGF levels after treatment with heparanase or PF4 in an experimental setting where HASMCs were cultured beyond confluence to deposit an ECM. In these studies cells were removed non-enzymatically following ECM deposition, and the remaining intact ECM was incubated with varying concentrations of heparanase, or PF4 or 25 ng/ml heparinase. bFGF levels in the media were examined *via* ELISA. Media samples taken from ECM treated with at least 10 pg/ml heparanase ($p < 0.001$; Fig. 2B), or 1 ng/ml PF4 ($p < 0.05$; Fig. 2D) were found to contain significantly higher levels of bFGF than control sam-

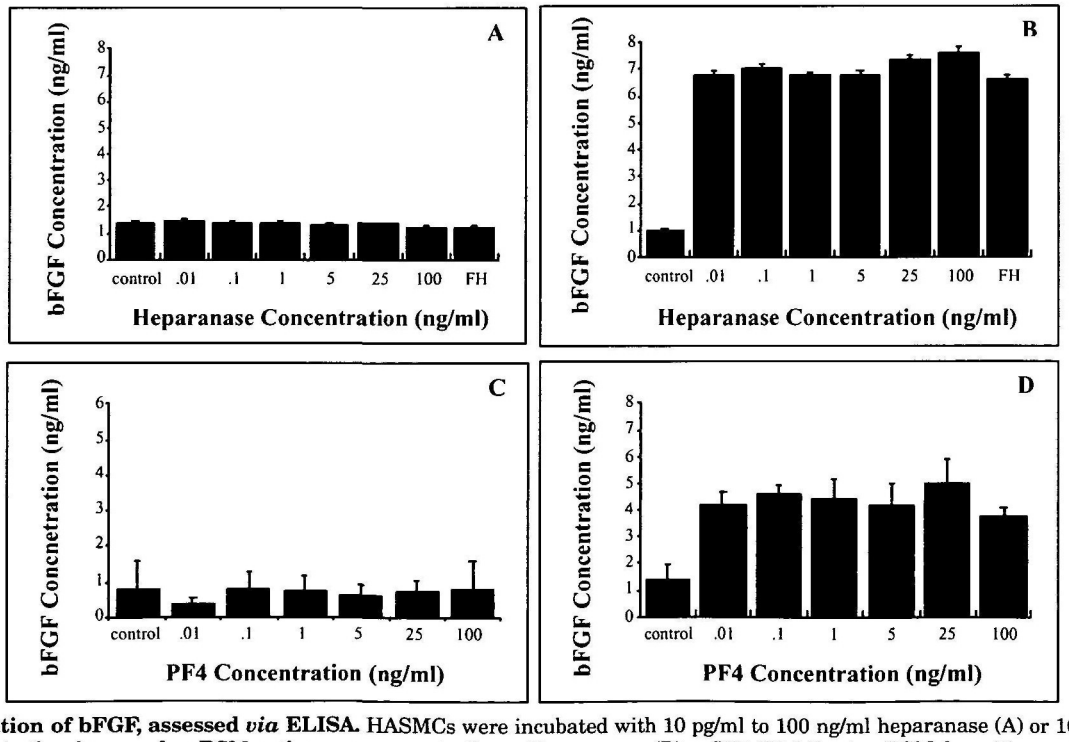

Fig. 2. **Liberation of bFGF, assessed *via* ELISA.** HASMCs were incubated with 10 pg/ml to 100 ng/ml heparanase (A) or 10 pg/ml to 100 ng/ml PF4 (C) in the absence of an ECM or the same concentrations of heparanase (B) or PF4 (D) following ECM deposition and cell removal. Controls were left untreated. FH indicates 25 ng/ml flavobacterium heparinase.

ples. Media samples taken from ECM treated with 25 ng/ml heparinase were found to contain similar levels of bFGF to that observed following treatment with heparanase (Fig. 2B).

Proliferation Following Exposure to Heparanase—To evaluate the effects of heparanase and growth factor release on cell proliferation, cultured HASMCs were treated with varying concentrations of heparanase in both the presence and absence of an ECM. In the absence of an ECM, no significant increases in HASMC proliferation were observed with heparanase or heparinase treatment. However, in the experimental setting where cells were incubated with the same concentrations of heparanase following ECM deposition, HASMCs were found to undergo significant increases in proliferation ($p < 0.04$; Fig. 3E). In addition, mammalian heparanase was found to induce significantly greater proliferation than treatment with 25 ng/ml bFGF ($p < 0.03$). Heparanase treatment may induce greater proliferation than soluble bFGF due to the presence of HS fragments, which have been proposed to increase the bioactivity of the growth factor (*6, 20–22*). Furthermore, while heparanase and heparinase were previously found to release similar levels of bFGF from the ECM, heparanase exposure was found to induce significantly greater increases in proliferation than flavobacterium-derived heparinase ($p < 0.001$). Heparanase treatment may produce HS-bFGF complexes distinct from and more active in the induction of cell proliferation than those obtained following heparinase treatment.

Proliferation Following Exposure to PF4—PF4, released by platelets following vascular injury (*18*), is thought to bind HSPGs in the ECM, thereby displacing bFGF (*24*). To evaluate the effects of PF4 and growth factor release on cell proliferation, HASMCs were treated with varying concentrations of PF4 in both the presence and absence of an ECM. In the experimental setting where cells were exposed to PF4 after minimal ECM production, no significant increases in cell proliferation were observed. However, in the experimental setting where cells were exposed to PF4 following ECM deposition, HASMCs treated with at least 1 ng/ml PF4 were found to undergo significant increases in proliferation ($p < 0.02$; Fig. 4). Furthermore, increases in proliferation following PF4 treatment were found to be similar to proliferation increases induced by the administration of soluble bFGF, supporting the hypothesis that PF4 releases soluble bFGF from the ECM, activating the growth factor and inducing cell proliferation.

Proliferation Following ECM Deposition and Treatment with Anti-bFGF IgG—To determine if the increased proliferation after treatment with heparanase, heparinase or PF4 was due to bFGF, we simultaneously treated HASMCs

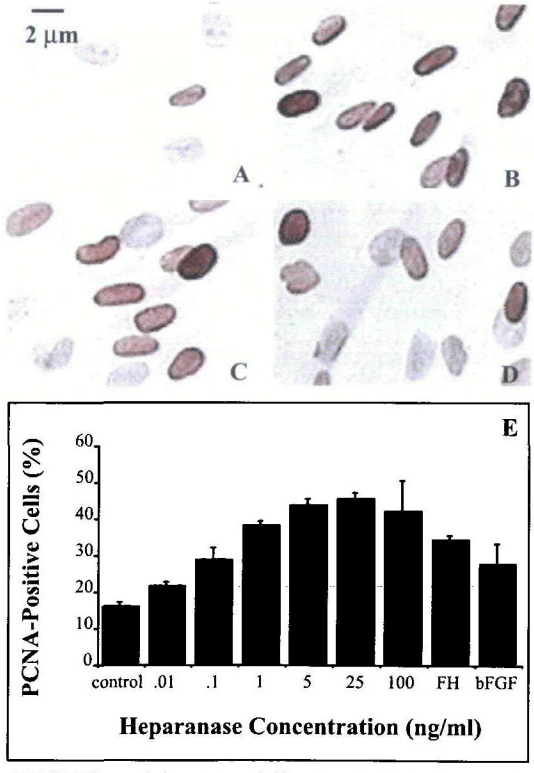

Fig. 3. **HASMC proliferation following ECM deposition and treatment with platelet-derived heparanase, heparinase, or bFGF.** Immunohistochemical staining for PCNA; A, control (untreated); B, 25 ng/ml platelet-derived heparanase; C, 25 ng/ml heparinase; or D, 25 ng/ml bFGF. The number of cells in S-phase was determined by PCNA-HRP staining following treatment with 10 pg/ml to 100 ng/ml heparanase, 25 ng/ml flavobacterium heparinase (FH), or 25 ng/ml bFGF (E).

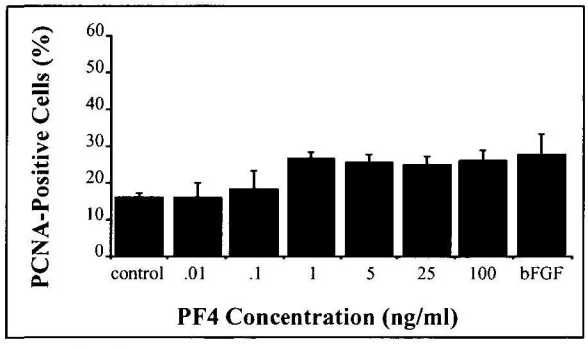

Fig. 4. **HASMC proliferation following ECM deposition and treatment with PF4 or bFGF.** The number of cells in S-phase was determined by PCNA-HRP staining following treatment with 10 pg/ml to 100 ng/ml PF4 or 25 ng/ml bFGF. Controls were left untreated.

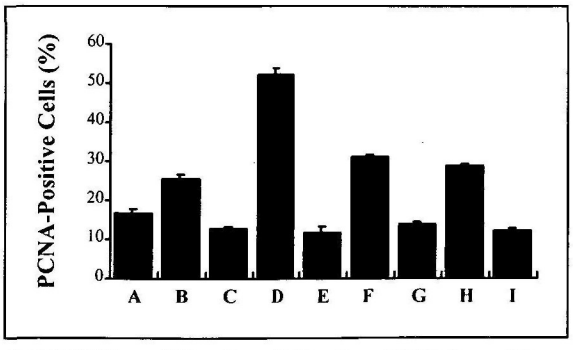

Fig. 5. **HASMC proliferation following ECM deposition and treatment with a bFGF-neutralizing antibody.** The number of cells in S-phase was determined by PCNA-HRP staining following treatment with: A, control (untreated); B, 25 ng/ml bFGF; C, 0.2 μg/ml anti-bFGF IgG; D, 25 ng/ml heparanase; E, 25 ng/ml heparanase + 0.2 μg/ml anti-bFGF IgG; F, 25 ng/ml heparinase; G, 25 ng/ml heparinase + 0.2 μg/ml anti-bFGF IgG; H, 25 ng/ml PF4; I, 25 ng/ml PF4 + 0.2 μg/ml anti-bFGF IgG.

with heparanase, heparinase or PF4 and a bFGF-neutralizing antibody. While HASMCs treated with 25 ng/ml heparanase, 25 ng/ml heparinase or 25 ng/ml PF4 alone were found to experience significant increases in proliferation ($p < 0.007$), addition of 0.2 μg/ml bFGF-neutralizing antibody was found to completely inhibit these increases (Fig. 5).

Plasminogen Activator Activity after Exposure to Heparanase or PF4—During restenosis, medial SMCs migrate from the medial layer of the vessel wall to the intima. Increased plasminogen activator (PA) activity has been shown to correlate with bFGF activity and is indicative of a migratory phenotype (*31*). Thus, cultured HASMCs were evaluated for PA activity following exposure to varying concentrations of heparanase or PF4 or 25 ng/ml heparinase. PA activity was found not to increase following treatment with heparanase, heparinase, or PF4 in the absence of an ECM, but to increase significantly following heparanase ($p < 0.002$; Fig. 6A), heparinase ($p < 0.01$; Fig. 6A), or PF4 ($p < 0.0005$; Fig. 6B) treatment in the presence of an ECM.

SMC Migration after Exposure to Heparanase or PF4—Given the indication that heparanase and PF4 may induce cell migration by activating bFGF and increasing PA activity, we directly examined cell migration following heparanase or PF4 treatment in both the presence and absence of

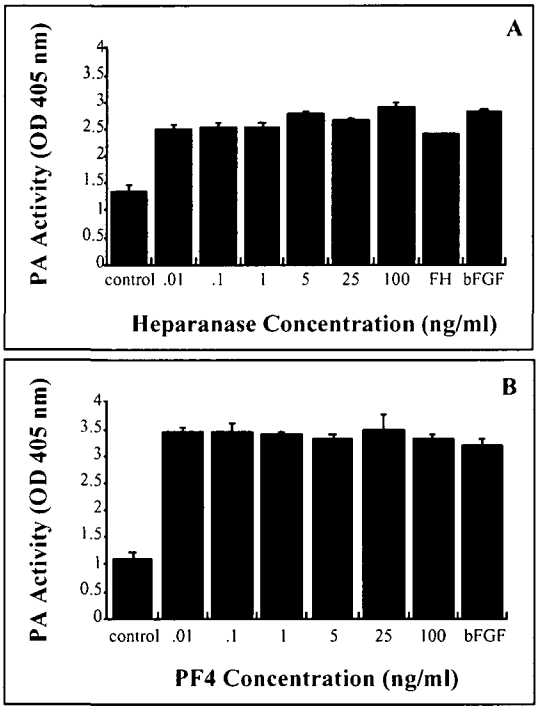

Fig. 6. **PA activity following ECM deposition.** HASMCs were incubated with 10 pg/ml to 100 ng/ml heparanase (A), or 10 pg/ml to 100 ng/ml PF4 (B). Negative controls were left untreated, while positive controls were treated with 25 ng/ml bFGF. FH indicates 25 ng/ml flavobacterium heparinase.

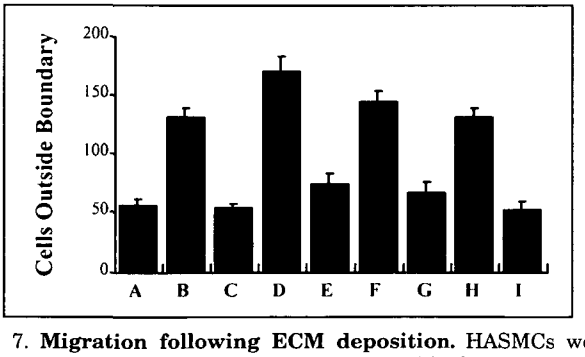

Fig. 7. **Migration following ECM deposition.** HASMCs were treated with: A, control (untreated); B, 25 ng/ml bFGF; C, 0.2 μg/ml anti-bFGF IgG; D, 25 ng/ml heparanase; E, 25 ng/ml heparanase + 0.2 μg/ml anti-bFGF IgG; F, 25 ng/ml heparinase; G, 25 ng/ml heparinase + 0.2 μg/ml anti-bFGF IgG; H, 25 ng/ml PF4; I, 25 ng/ml PF4 + 0.2 μg/ml anti-bFGF IgG. Migration was assessed by quantifying the number of cells beyond the initial cell culture perimeter.

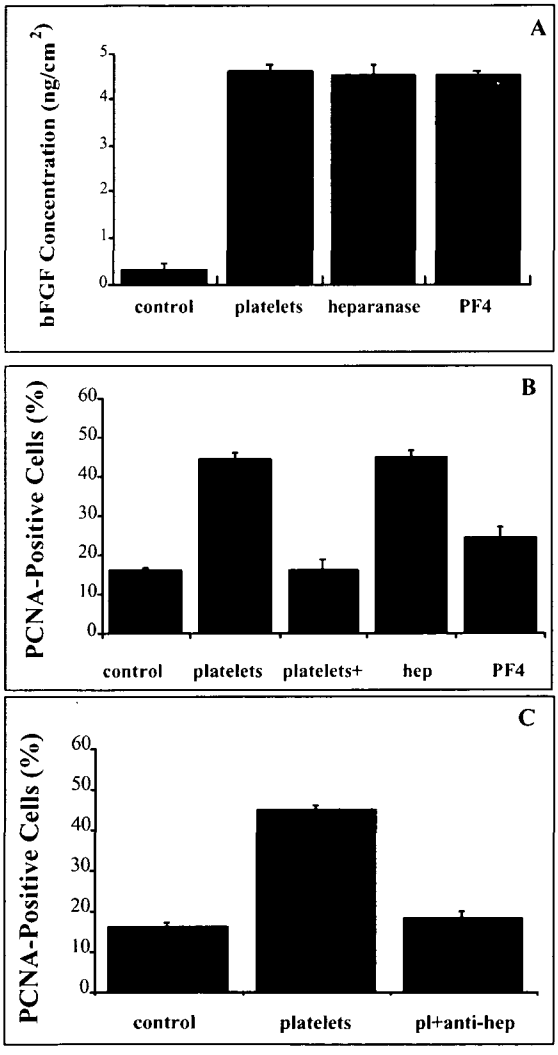

Fig. 8. **Platelets liberate bFGF and stimulate HASMC proliferation following ECM deposition.** A: Liberation of bFGF, assessed via ELISA, from HASMCs incubated with: control (untreated), platelets (500/cm²), 25 ng/ml heparanase, or 25 ng/ml PF4 following ECM deposition and cell removal. B: HASMC proliferation following ECM deposition and treatment with: control (untreated), platelets (500/cm²), platelets (500/cm²) + 0.2 μg/ml anti-bFGF IgG (+), 25 ng/ml heparanase (hep) or 25 ng/ml PF4. C: HASMC proliferation following ECM deposition and treatment with: control (untreated), platelets (500/cm²), platelets (500/cm²) + 0.25 μg/ml anti-heparanase IgG (pl + anti-hep). The number of cells in S-phase was determined by PCNA-HRP staining.

an ECM. In the absence of an ECM, bFGF increased cell migration but treatment with heparanase, heparinase or PF4 did not. However, in the presence of an ECM, heparanase, heparinase, PF4 and bFGF were all found to significantly increase cell migration (Fig. 7; $p < 0.0000008$). Furthermore, treatment with the bFGF-neutralizing antibody was shown to significantly decrease migration in all cases (Fig. 7), suggesting that migration is facilitated by bFGF activation.

bFGF Release and SMC Proliferation after Exposure to Platelets—Following vascular injury, platelets adhere to the subendothelial vascular wall, aggregate and release degranulation products, many of which are believed to increase SMC proliferation. To mimic this process, we seeded platelets on top of cultured HASMCs following ECM deposition, and assessed bFGF release and cell proliferation. Exposure of the ECM produced by cultured HASMCs to platelets resulted in significant bFGF release (Fig. 8A; $p < 0.000002$). In addition, platelet degranulation products were determined to release similar levels of bFGF as 25 ng/ml heparanase, and 25 ng/ml PF4. Platelet degranulation products alone did not contain measurable amounts of bFGF. Treatment of HASMCs with platelets in the presence of an ECM was also found to induce significant increases in proliferation (Fig. 8B; $p < 0.000005$), and these increases in proliferation were similar to those obtained by the addition of 25 ng/ml heparanase. Furthermore, proliferation increases were found to be completely inhibited by the addition of 0.2 μg/ml bFGF-neutralizing antibody (Fig. 8B), suggesting that the increases in cell proliferation were mediated in large part by bFGF activation. Further studies were carried out where HASMCs were treated with platelets and an antibody against heparanase following ECM deposition. While platelets alone were found to significantly increase SMC proliferation (Fig. 8C), joint treatment with 0.25 μg/ml anti-heparanase IgG was found to completely inhibit these increases in proliferation (Fig. 8C). HASMCs treated with platelets in the absence of an ECM did not increase proliferation, further indicating that increased proliferation in the presence of an ECM was specifically due to the release of endogenous bFGF and not due to additional platelet-derived mitogenic factors at this low dosage of platelets.

PA Activity and SMC Migration after Exposure to Platelets—PA activity in cultured HASMCs was significantly increased after exposure to platelets when an ECM was present (Fig. 9A; $p < 0.000006$). Additionally, exposure to platelets increased the number of migrating SMCs when an ECM was present (Fig. 9B; $p < 0.003$) and these increases in migration were similar to those obtained by the addition of 25 ng/ml heparanase. Furthermore, platelet-induced migration was inhibited by the addition of 0.2 μg/ml bFGF-neutralizing antibody, suggesting that the increases in cell migration were largely due to bFGF activity.

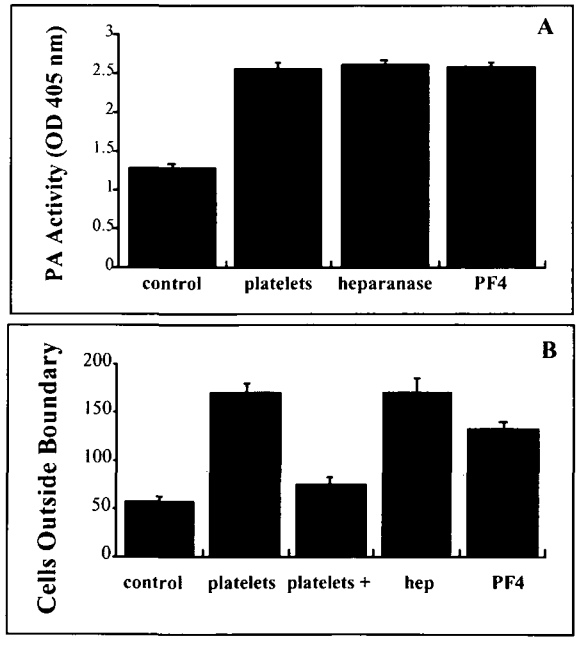

Fig. 9. **Platelets increase PA activity and migration following ECM deposition.** A: PA activity of HASMCs incubated with: control (untreated), platelets (500/cm^2), 25 ng/ml heparanase, or 25 ng/ml PF4. B: HASMC migration following treatment with: control (untreated), platelets (500/cm^2), platelets (500/cm^2) + 0.2 μg/ml anti-bFGF IgG (+), 25 ng/ml heparanase (hep) or 25 ng/ml PF4. Migration was evaluated by quantifying the number of cells beyond the initial cell culture perimeter.

DISCUSSION

The ECM is a biologically active constituent of the arterial wall. Dynamic interactions between cells and the surrounding ECM are critical for the maintenance of cell functions, developmental processes, and the response of tissues to injury (*5, 32*). Heparan sulfate proteoglycans (HSPG) located on the cell surface and in the ECM play crucial roles in cell–cell and cell–matrix interactions. HS binds and localizes structural proteins, such as fibronectin and collagen, in the ECM and tethers chemokines and growth factors, such as bFGF, vascular endothelial growth factor, and hepatocyte growth factor, to the ECM, which then can function as a storage depot for bioactive signaling molecules (*5, 33*). Tissue injury triggers the rapid localized release of these growth factors to facilitate cellular responses such as proliferation and migration (*32–35*), though the mechanisms of this are as yet poorly understood.

bFGF is an important SMC mitogen that has been determined to be present in the arterial media both intracellularly and extracellularly (*36, 37*). HSPGs in the ECM, basement membrane and on the cell surface bind bFGF in inactive form, sequestering the growth factor (*5*) and protecting it from denaturation (*34*) and proteolytic degradation (*6, 20*). Sequestration of bFGF in the ECM has been suggested to inhibit ongoing proliferation under normal conditions (*5, 37*). The liberation of bFGF from the ECM may provide a mechanism for the induction of cell proliferation and migration during events such as angiogenesis and wound healing (*5*). One source of bFGF following injury is thought to be due to cellular damage in which punctured cells release intracellular stores of the growth factor (*34*). Another source of bFGF is proposed to be due to the disruption of the ECM and basement membrane where sequestered bFGF is liberated, thus becoming biologically available to nearby cells (*37*). Liberation from the ECM may occur by displacement or by enzymatic activity. Studies carried out by West and Hubbell (1996), employing a nonthrombogenic hydrogel barrier formed on the luminal

surface of the rat carotid artery following balloon injury, found that SMC proliferation and migration were not induced in the absence of blood contact, and further, that bFGF was not liberated. This suggests that a luminal factor may be required for bFGF mobilization and subsequent activation.

Platelets are a rich repository of growth factors, such as PDGF, and have been determined to play an important role in driving the early events following vascular injury (*16, 18, 38, 39*). PDGF has been determined to function as a mitogen and chemoattractant for vascular SMCs *in vitro* but has been found to induce minimal increases in medial SMC proliferation following vascular injury in the rat carotid artery (*38*). Additionally, PDGF has been found to increase intimal thickening through the induction of SMC migration from the media to the intima rather than through increased proliferation (*38*). Furthermore, while PDGF-BB has been found to enhance intimal thickening following vascular injury, it was found not to induce intimal thickening in the absence of additional luminal factors (*16*).

Evidence suggests bFGF to play a crucial role in early SMC proliferation and possibly SMC migration and matrix elaboration (*13–16*). The dominant effects of bFGF on SMC proliferation following arterial injury have been demonstrated in studies where treatment with bFGF-neutralizing antibodies after injury were shown to decrease SMC proliferation by approximately 80% (*14*). Undetectable amounts of bFGF are released from platelets at the site of vascular injury, but the ECM in the medial layer of the vessel wall is a rich source of this factor (*2*). However, the mechanisms by which this stored bFGF is released and activated following vascular injury remain unclear. The objective of our study was to investigate the role of platelet-derived factors on bFGF liberation from the ECM and the subsequent induction of cell proliferation and migration. Specifically, we have evaluated the effects of platelet-derived heparanase and PF4.

In examining the hypothesis that heparanase and PF4 release bFGF from sequestration in the ECM, thereby activating the growth factor and inducing mitogenesis, proliferation was assessed following exposure to heparanase or PF4. While heparanase and PF4 were found not to induce proliferation in the absence of an ECM, both were found to induce significant increases in proliferation following ECM deposition. These data indicate that heparanase and PF4 do not directly induce mitogenesis, but that proliferation increases are achieved following interaction with the ECM. Because these proliferation increases were completely inhibited by the addition of a bFGF-neutralizing antibody, we suggest that heparanase and PF4 induce cell proliferation solely by activating bFGF. These data correlate with the inhibition of medial SMC proliferation and intimal thickening observed by Lindner and Reidy (1991) when administering a bFGF-neutralizing antibody to the rat carotid artery following injury. In the experimental setting where platelets were seeded on top of HASMCs, an extremely low number of platelets were used to eliminate the effects of platelet-derived mitogens (approximately 1 platelet per 40 SMCs). For example, 500 platelets are estimated to release only 0.00003 ng of PDGF (*40*), whereas heparanase released from 500 platelets was found to release approximately 4.5 ng of bFGF. These data correlate with studies where low platelet concentrations (1 to 11 platelets per SMC) were found not to induce proliferation in SMCs cultured in the absence of a robust ECM (*41*).

Heparanase and PF4 have been determined to release similar concentrations of bFGF, but heparanase has been found to induce significantly greater proliferation than PF4. This difference in mitogenic potential is thought to be due to the increased bioactivity of the growth factor in the presence of HS fragments. While soluble bFGF has been proposed to be dependent on HSPGs in close proximity to cell surface FGFRs to mediate signal transduction (*22, 23*), the release of bFGF as a complex with HS by heparanase negates this limitation. Release of bFGF as a complex with HS fragments has been suggested to increase the bioactivity of the growth factor by protecting it from proteolytic degradation (*6, 20*), decreasing re-binding to the ECM (*21*) and enhancing binding to the cell surface FGFR (*22*). Thus bFGF activity, thought to be mediated by HS fragments, is affected by enzymes such as heparanase that modulate HS fragment conformation and dynamics. Mammalian heparanase cleaves glycosidic bonds with a hydrolase mechanism distinct from bacterial heparinase, which functions as an eliminase (*29*), and this functional difference is thought to lead to the generation of different HS-bFGF complexes. It is this difference in HS conformation that is thought to regulate bFGF binding affinity for cell surface FGFRs and subsequent mitogenic potential. In addition, cell- and tissue-specific variations in HS structure may further modulate bFGF activity (*5, 19–23, 30, 37*). High concentrations of PF4 have shown to inhibit cell proliferation (data not shown), while exposure to lower concentrations has been found to stimulate cell proliferation. This may be due to the saturation of cell-surface HSPGs required to facilitate bFGF signaling. The inhibitory effects of high concentrations of PF4 can be overcome by exogenous heparin (*42*), further suggesting that PF4 inhibition of mitogenesis is caused by binding of PF4 to cell-surface HSPGs. PF4 releases soluble bFGF, and rapid re-binding to the ECM may contribute to the difficulties in bFGF detection during release studies.

Plasminogen activator converts inactive plasminogen to active plasmin, which has been shown to degrade ECM components and to activate matrix metalloproteinases (*43*). Activation of these proteolytic enzymes correlates with the ability of cells to migrate through an intact ECM into the arterial wound (*44*). Furthermore, PA activity that is indicative of a migratory phenotype and has been shown to correlate with bFGF activity and SMC migration into the arterial intima, has been found to be inhibited by a plasmin inhibitor (*31, 44*). We found PA activity to increase following heparanase and PF4 treatment in the presence of an ECM, indicating a role for heparanase and PF4 in the induction of migration through bFGF activation. Further studies were carried out showing the direct induction of cell migration following heparanase or PF4 treatment in the presence of an ECM, where migration was primarily attributed to bFGF activation. These data correlate with the inhibition of SMC migration and intimal thickening observed by Jackson and Reidy (1993) when administering a bFGF-neutralizing antibody to the rat carotid artery following injury. Heparan sulfate is an important constituent of the ECM and basement membrane of blood vessels presenting a physical barrier (*25*). Administration of the bFGF-neutralizing antibody in conjunction with hepara-

nase was found not to completely inhibit migration, suggesting that the ECM-degrading activity of heparanase may contribute to ability of cells to migrate.

We have confirmed a role for heparanase and discovered a novel role for PF4 in the induction of cell proliferation by releasing active bFGF from sequestration in the ECM. Given our preliminary studies, we propose that heparanase and PF4, released from activated platelets following vascular injury, liberate bFGF in active form from the subendothelial basement membrane and ECM of blood vessels, thereby inducing the medial SMC proliferation and migration that contributes to the formation of a neointima during restenosis. Furthermore, sequestration of heparin-binding growth factors in the ECM provides a local source of mitogenic and chemotactic agents that may be mobilized by heparan sulfate-degrading enzymes and heparan sulfate-binding proteins, such as heparanase and PF4 in response to vascular injury and during vascular development. In addition, similar regulatory mechanisms of heparin-binding growth factor sequestration and activation may be utilized in many tissues during proliferation and differentiation processes.

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REFERENCES

1. Bikfalvi, A., Klein, S., Pintucci, G., and Rifkin, D.B. (1997) Biological roles of fibroblast growth factor-2. *Endocrine Rev.* **18**, 26–45
2. Baird, A., Esch, F., Mormede, P., Ueno, N., Ling, N., Bohlen, P., Ying, S.Y., Wehrenberg, W.B., and Guillemin, R. (1986) Molecular characterization of fibroblast growth factor: distribution and biological activities in various tissues. *Recent Prog. Horm. Res.* **42**, 143–205
3. Aktas, G. and Kayton, R. (2000) Ultrastructural immunolocalization of basic fibroblast growth factor in fibroblasts and extracellular matrix. *Histochem. Cell Biol.* **113**, 227–233
4. Cordon-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.* **63**, 832–840
5. Vlodavsky, I., Maio, H., Medalion, B., Danagher, P., and Ron, D. (1996) Involvement of heparan sulfate and related molecules in sequestration and growth promoting activity of fibroblast growth factor. *Canc. Met. Rev.* **15**, 177–186
6. Sommer, A. and Rifkin, D.B. (1989) Interaction of heparin with human basic fibroblast growth factor: protection of the angiogenic protein from proteolytic degradation by a glycosaminoglycan. *J. Cell. Physiol.* **138**, 215–220
7. Lindner, V. and Reidy, M.A. (1993) Expression of basic fibroblast growth factor and its receptor by smooth musde cells and endothelium in injured rat arteries an en face study. *Circ. Res.* **73**, 589–595
8. Lee, M., Simon, A.D., Stein, C.A., and Rabbani, L.E. (1999) Antisense strategies to inhibit restenosis. *Antis. Nucl. Acid Drug Dev.* **9**, 487–492
9. Chandrasekar, B. and Tanguay, J.F. (2000) Platelets and restenosis. *J. Am. Coll. Cardiol.* **35**, 555–562
10. Medalion, B., Merin, G., Aingorn, H., Miao, H.Q., Nagler, A., Elami, A., Ishai-Michaeli, R., and Vlodavsky, I. (1997) Endogenous basic fibroblast growth factor displaced by heparin from the luminal surface of human blood vessels is preferentially sequestered by injured regions of the vessel wall. *Circulation* **95**, 1853–1862
11. Parish, M.A., Grossi, E.A., Baumann, F.G., Asai, T., Rifkin, D.B., Colvin, S.B., and Galloway, A.C. (1995) Effects of a single administration of fibroblast growth factor on vascular wall reaction to injury. *Ann. Thorac. Surg.* **59**, 948–954
12. Edelman, E.R., Nugent, M.A., Smith, L.T., and Karnovsky, M.J. (1992) Basic fibroblast growth factor enhances the coupling of intimal hyperplasia and proliferation of vasa vasorum in injured rat arteries. *J. Clin. Invest.* **89**, 465–473
13. Lindner, V., Lappi, D.A., Baird, A., Majack, R.A., and Reidy, M.A. (1991) Role of basic fibroblast growth factor in vascular lesion formation. *Circ. Res.* **68**, 106–113
14. Lindner, V. and Reidy, M.A. (1991) Proliferation of smooth muscle cells after vascular injury is inhibited by an antibody against basic fibroblast growth factor. *Proc. Natl. Acad. Sci USA* **88**, 3739–3743
15. Olson, N.E., Chao, S., Lindner, V., and Reidy, M.A. (1992) Intimal smooth muscle cell proliferation after balloon catheter injury: The role of basic fibroblast growth factor. *Am. J. Pathol.* **140**, 1017–1023
16. West, J.L. and Hubbell, J.A. (1996) Separation of the arterial wall from blood contact using hydrogel barriers reduces intimal thickening after balloon injury in the rat: The roles of medial and luminal factors in arterial healing. *Proc. Natl. Acad. Sci. USA* **93**, 13188–13193
17. Oosta, G.M., Favreau, L.V., Beeler, D.L., and Rosenberg, R.D. (1982) Purification and properties of human platelet heparitinase. *J. Biol. Chem.* **257**, 11249–11255
18. Files, J.C., Malpass, T.W., Yee, E.K., Ritchie, J.L., and Harker, L.A. (1981) Studies of human platelet alpha-granule release in vivo. *Blood* **58**, 607–618
19. Bashkin, P., Doctrow, S., Klagsbrun, M., Svahn, C.M., Folkman, J., and Vlodavsky, I. (1989) Basic fibroblast growth factor binds to subendothelial extracellular matrix and is released by heparitinase and heparin-like molecules. *Biochemistry* **28**, 1737–1743
20. 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
21. Flaumenhaft, R., Moscatelli, D., and Rifkin, D.B. (1990) Heparin and heparan sulfate increase the radius of diffusion and action of basic fibroblast growth factor. *J. Cell Biol.* **111**, 1651–1659
22. Kan, M., Wang, F., Xu, J., Crabb, J.W., Hou, J., and McKeehan, W.L. (1993) An essential heparin-binding domain in the fibroblast growth factor receptor kinase. *Science* **259**, 1918–1921
23. Pellegrini, L., Burke, D.F., von Deift, F., Mulloy, B., and Blundell, T.L. (2000) Crystal structure of fibroblast growth factor receptor ectodomain bound to ligand and heparin. *Nature* **407**, 1029–1034
24. Brown, K.J. and Parish, C.R. (1994) Histidine-rich glycoprotein and platelet factor 4 mask heparan sulfate proteoglycans recognized by acidic and basic fibroblast growth factor. *Biochemistry* **33**, 13918–13927
25. Freeman, C. and Parish, C.R. (1998) Human platelet heparanase: purification, characterization and catalytic activity. *Biochem. J.* **330**, 1341–1350
26. Hulett, M.D., Freeman, C., Hamdorh, B.J., Baker, R.T., Harris, M.J., and Parish, C.R.. (1999) Cloning of mammalian heparanase, an important enzyme in tumor invasion and metastasis. *Nat. Med.* **5**, 803–809
27. Freeman, C., Browne, A.M., and Parish, C.R. (1999) Evidence that platelet and tumor heparanases are similar enzymes. *Biochem. J.* **342**, 361–368
28. Vlodavsky, I., Friedmann, Y., Elkin, M., Aingorn, H., Atzmon, R., Ishai-Michaeli, R., Bitan, M., Pappo, O., Peretz, T., Michal, I., Spector, L., and Pecker, I. (1999) Mammalian heparanase: gene cloning, expression and function in tumor progression and metastasis. *Nat. Med.* **5**, 793–802
29. Ernst, S., Langer, R., Cooney, C.L., and Sasisekharan, R. (1995) Enzymatic degradation of glycosaminoglycans. *Crit. Rev. Biochem. Mol. Biol.* **30**, 387–444
30. Bame, K.J., Venkatesan, I., Stelling, H.D., and Tumova, S. (2000) The spacing of S-domains on HS glycosaminoglycans determines whether the chain is a substrate for intracellular

heparanases. *Glycobiology* **10**, 715–726
31. Reidy, M.A., Irvin, C., and Lindner, V. (1996) Migration of arterial wall cells: expression of plasminogen activators and inhibitors in injured rat arteries. *Circ. Res.* **78**, 405–414
32. Dempsey, L.A., Brunn, G.J., and Platt, J.L. (2000) Heparanase, a potential regulator of cell-matrix interactions. *Trends Biochem. Sci.* **25**, 349–351
33. Gospodarowicz, D., Delgado, D., and Vlodavsky, I. (1980) Permissive effect of the extracellular matrix on cell proliferation. *Proc. Natl. Acad. Sci. USA* **77**, 4094–4098
34. Crowley, S.T., Ray, C.J., Nawaz, D., Majack, R.A., and Horwitz, L.D. (1995) Multiple growth factors are released from mechanically injured vascular smooth muscle cells. *Am. J. Physiol.* **269**, 1641–1647
35. Vlodavsky, P., Bashkin, P., Ishai-Michaeli, R., Chajek-Shaul, T., Bar-Shavit, R., Haimovitz-Friedman, A., Klagsbrun, M., and Fuks, Z. (1991) Sequestration and release of basic fibroblast growth factor. *Ann. N.Y. Acad. Sci.* **638**, 207–220
36. Gospodarowicz, D. and Cheng, J. (1986) Heparin protects basic and acidic FGF from inactivation. *J. Cell Physiol.* **128**, 475–484
37. Ishai-Michaeli, R., Svahn, C.M., Weber, M., Chajek-Shaul, T., Korner, G., Ekre, H., and Vlodavsky, I. (1992) Importance of size and sulfation of heparin in release of basic fibroblast growth factor from the vascular endothelium and extracellular matrix. *Biochemistry* **31**, 2080–2088
38. Jawien, A., Bowen-Pope, D.F., Lindner, V., Schwartz, S.M., and Clowes, A.W. (1992) Platelet-derived growth factor promotes smooth muscle migration and intimal thickening in a rat model of balloon angioplasty. *J. Clin. Invest.* **89**, 507–511
39. Faxon, D.P., Sanborn, T.A., Haudenschild, C.C., and Ryan, T.J. (1984) Effect of antiplatelet therapy on restenosis after experimental angioplasty. *Am. J. Cardiol.* **53**, 72C–76C
40. Ross, R., Raines, E.W., and Bowen-Pope, D.F. (1986) The biology of platelet derived growth factor. *Cell* **46**, 155–169
41. Loppnow, H., Bil, R., Hirt, S., Schonbeck, U., Herzberg, M., Werdan, K., Reitschel, E.T., Brandt, E., and Flad, H.D. (1998) Platelet-derived interleukin-1 induces cytokine production, but not proliferation of human vascular smooth muscle cells. *Blood* **91**, 134–141
42. Watson, J.B., Getzler S.B., and Mosher, D.F. (1994) Platelet factor 4 modulates the mitogenic activity of basic fibroblast growth factor. *J. Clin. Invest.* **94**, 261–268
43. Dano, K., Andreasen, P.A., Grondahl-Hansen, J., Kristensen, P., Nielsen, L.S., and Skriver, L. (1985) Plasminogen activators, tissue degradation and cancer. *Adv. Cancer Res.* **44**, 139–266
44. Jackson, C.L. and Reidy, M.A. (1993) Basic fibroblast growth factor: its role in the control of smooth muscle cell migration. *Am. J. Pathol.* **143**, 1024–1031