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# Integrin $\alpha_v\beta_3$ Is Involved in Stimulated Migration of Vascular Adventitial Fibroblasts by Basic Fibroblast Growth Factor but not Platelet-Derived Growth Factor

**Guizhen Liu,[1] Suzanne G. Eskin,[2] and Antonios G. Mikos[1]***

[1]Department of Bioengineering, Rice University, Houston, Texas 77251-1892
[2]Texas Biotechnology Corporation, Houston, Texas 77030

**Abstract** We examined the effects of basic fibroblast growth factor (bFGF) and platelet-derived growth factor (PDGF) on the migration of vascular adventitial fibroblasts (VAFs) isolated from rat aortic adventitiae. Both bFGF and PDGF significantly stimulated VAF migration in vitro. An antibody to rat $\beta_3$ integrin reduced bFGF-stimulated migration in a dose dependent manner. Moreover, VAF migration was inhibited in the presence of cyclic RGD (cRGD) peptide. However, PDGF-directed migration was blocked only by equivalent cRGD peptide but not by antibody to $\beta_3$ integrin. These data suggest that $\alpha_v\beta_3$ integrin mediates VAF migration stimulated by bFGF and that chemoattractant directed migration may be through distinct integrins. J. Cell. Biochem. 83: 129–135, 2001. 

**Key words:** adventitial fibroblasts; migration; bFGF; PDGF; integrins

Restenosis following coronary angioplasty is one of the major problems in cardiology today. The proliferation and migration of medial smooth muscle cells (SMCs) are thought to be responsible in early stages of intimal thickening [Clowes et al., 1983; Ross et al., 1984; Austin et al., 1985; Reidy et al., 1992]. Recent in vivo studies suggested that the vascular adventitial fibroblasts may proliferate and migrate in response to coronary artery injury in a porcine model [Scott et al., 1996; Shi et al., 1996; Wilcox et al., 1996, 1997; De Leon et al., 1997]. These findings demonstrate a new source of cells for vascular repair and indicate that the adventitia may play an important role in restenosis after balloon angioplasty. The mechanisms by which the fibroblasts translocate from the adventitia and contribute to the cellular mass in the neointima, however, are unknown.

Many growth factors have been considered to be involved in cell migration and vascular remodeling. bFGF and PDGF are potent mitogens and chemoattractants for vascular cells. The systemic infusion of recombinant bFGF in a rat model after balloon catheter denudation markedly increased SMC proliferation and the size of the intimal lesion [Lindner et al., 1991; Reidy et al., 1992], whereas the administration of neutralizing antibodies to bFGF caused an 80% reduction in proliferation response after injury to the carotid artery [Lindner and Reidy, 1991]. bFGF induces DNA synthesis and proliferation of fibroblasts and SMCs, and is also a potent mitogen and chemoattractant for endothelial cells in vitro [Folkman and Klagsbrun, 1987]. Vascular adventitial fibroblasts (VAFs) are much less studied than SMCs and endothelial cells. PDGF is a potent mitogen [Ross, 1993] and chemotactic agent [Grotendorst et al., 1982] for both SMCs and fibroblasts in vitro and is identified also as an important chemotactic agent for SMCs in vivo [Reidy et al., 1992]. Moreover, PDGF is a strong mitogen for fibroblasts at the wound site [Pierce et al., 1991]. The adventitia is also the site of PDGF receptor expression as determined by in situ hybridization one week after angioplasty [Scott et al., 1996].

Integrin cell surface proteins can transmit signals both in and out of cells regulating cell adhesion, migration, growth, and differentiation [Hynes, 1992]. Among integrins, $\beta_3$-con-

*Correspondence to: Antonios G. Mikos, Department of Bioengineering, Rice University, MS-142, PO Box 1892, Houston, TX 77251-1892. E-mail: mikos@rice.edu

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taining integrins are critical molecules in cardiovascular diseases including atherosclerosis and restenosis [Brooks et al., 1994; O'Brien et al., 1994; Hoshiga et al., 1995]. Antagonists to $\alpha_v\beta_3$ integrin effectively inhibited the fibroproliferative response in animal models of restenosis [Choi et al., 1994; Matsuno et al., 1994]. Moreover, a humanized $\beta_3$ integrin antibody exhibited a beneficial effect in reducing restenosis in a clinical trial [Topol et al., 1994]. One mechanism by which chemoattractants affect VAF migration may be through the regulation of integrin receptor activity. In other words, the expression and function of integrins may be influenced directly or indirectly by growth factors.

In this study, we examined the effects of bFGF and PDGF on the migration of cultured VAFs, and determined if these actions are regulated by $\alpha_v\beta_3$ integrins.

## MATERIALS AND METHODS

### Materials and Reagents

Recombinant human bFGF and PDGF-BB were obtained from Upstate Biotechnology (Lake Placid, NY). Monoclonal antibody F11, directed against rat $\beta_3$-containing integrins, was purchased from Pharmingen (San Diego, CA). Mouse IgG1 isotype control was from Sigma (St. Louis, MO). Cyclic RGD peptide (GPenGRGDSPCA) was purchased from GIBCO BRL (Frederick, MD). Vitrogen (vitrogen 100 collagen), a 99.9% pure bovine dermal collagen consisting of 95–98% collagen type I with the remainder being type III, was purchased from Collagen Corporation (Palo Alto, CA). All reagents mentioned above were freshly prepared for each experiment. All other reagents and materials for cell culture were from GIBCO BRL unless otherwise indicated.

### Cell Isolation and Culture

Thoracic aortae ($n = 3–4$) of male Sprague–Dawley rats (3–12 months) were harvested and cleaned by dissecting away the fat and connective tissue. Under a dissecting microscope, the adventitia was gently stripped along a cleavage plane beginning at the aortic arch, and placed in Hanks' balanced salt solution at 4°C [Das et al., 1995]. The adventitial strips were either placed as 1–2 mm explants into culture dishes, or enzymatically dissociated in 200 U/ml collagenase (Worthington CLS type I in Dulbecco's modification of Eagle's Medium (DMEM)) in a beaker and agitated in a 37°C water bath for 1.5 h [Battle et al., 1994]. The explants or cell suspensions were maintained in DMEM supplemented with 10% fetal bovine serum (FBS), 1% glutamine and antibiotics (100 U/ml penicillin and 100 μg/ml streptomycin) at 37°C, in a humidified atmosphere of 5% $CO_2$ in air. SMCs were isolated from the media which remained after adventitial stripping, after removing the endothelium with a cotton swab. SMCs were explanted or enzymatically dissociated under the same conditions as fibroblasts. Both cell types were used in migration assays at passages 2–5. Hematoxylin and eosin stained light microscopic sections of adventitial strips were compared to cross sections of intact aorta to confirm separation of adventitia from media.

### Immunofluorescence Staining

VAFs isolated by enzymatic digestion were seeded in 4-well chambers, and on Day 3 they were fixed with 10% formalin for 10 min and blocked with normal nonimmune horse serum (Vector Laboratories, CA) for 30 min. After washing with phosphate-buffered saline (PBS), cells were incubated with primary antibody, a monoclonal mouse 1A4 antibody recognizing α-SM actin (1:100, Sigma), for 1 h at room temperature. Negative controls were incubated with nonimmune serum instead of primary antibody. Subsequently, the slides were washed and incubated with fluorescein tagged goat anti-mouse IgG (H + L) for 1 h, then counter stained with diluted hematoxylin and examined under an Olympus IMT-2 microscope.

### Migration Assay

Migration assays were performed in modified Boyden chambers (Neuroprobe, blind well chambers) [Bilato et al., 1995]. Polycarbonate filters (PVP free, 8 μm pores; OSMONICS, Livermore, CA) were coated overnight with 20 μg/ml of vitrogen and dried in a laminar flow hood. DMEM containing 0.1% bovine serum albumin (BSA) with various concentrations of bFGF or PDGF were placed in the bottom wells of the chambers, and the filters were placed over the bottom wells. Cells were trypsinized, rinsed with 0.1% BSA/DMEM, and washed twice in PBS. A total of 25,000 cells in 100 μl 0.1% BSA/DMEM were then seeded into the upper chambers. (The cell seeding density was kept constant to minimize any effect on the results.)

After 4 h incubation at 37°C, the filters were stained in Diff-Quik (Data Behring, Miami, FL) and mounted on slides, and non-migrated cells were gently scraped from the top sides of the filters with a cotton swab. Migrated cells (four high power fields per filter) were counted at 400× magnification.

For the inhibition assay, serum-free DMEM with growth factor was placed in the bottom wells of the Boyden chambers. Antibody (F11) directed against rat $\beta_3$-containing integrins or cRGD peptide, which specifically bind to integrin $\alpha_v\beta_1$, $\alpha_v\beta_3$, and $\alpha_v\beta_5$ were added in the upper chambers at the time of cell seeding at the concentrations indicated.

All experiments were run in triplicate and were repeated at least three times with different VAF preparations.

### Statistical Analysis

All results were expressed as means±standard deviation (SD). The comparison of the mean values among the different groups was assessed by a paired Student's test or by an analysis of variance (ANOVA) test when appropriate. A value of $P < 0.05$ was considered significant.

## RESULTS

### Immunostaining

The identity of the VAFs was based on absence of positive α-SM actin immunostaining in primary culture. Primary cultures of SMC were uniformly positive for α-SM actin, whereas in the primary VAFs such staining was rare.

### Migratory Response of VAF vs. SMC to bFGF

VAFs migrated dose dependently to bFGF at the concentrations of 0.1 to 3 ng/ml as compared to control BSA ($13.3 \pm 1.2$ cells/field) (Fig. 1a). Maximum stimulation was observed at 1 ng/ml of bFGF ($30.3 \pm 3.5$ cells/field). SMCs isolated from the same aortae showed no significant response to bFGF stimulation (Fig. 1b), further confirming the phenotypic expression of the isolated cell populations [Grotendorst et al., 1982; Bilato et al., 1995].

### PDGF Stimulates VAF Migration

Using PDGF as the chemoattractant, VAFs markedly migrated to the underside of the filter compared to BSA alone. The VAF migration stimulated by PDGF occurred in a concentration-dependent manner in the range from 1 to 20 ng/ml (Fig. 2). The maximal increase was four-fold (53.6±5.2) compared to control (13.3±2.6).

### Effects of Integrin $\beta_3$ Antibody and cRGD Peptide on bFGF and PDGF-Stimulated VAF Migration

To determine if $\beta_3$ integrins were involved in VAF migration induced by chemoattractants, a monoclonal antibody, F11, directed against rat $\beta_3$-containing integrins, and cRGD peptide targeting $\alpha_v$-containing integrin complexes, were used in migration assays. The results of these experiments are shown in Figure 3. When the rat $\beta_3$ integrin antibody was added to the upper chamber, there was a dose-dependent reduction in bFGF-stimulated migration, with 50% inhibition at 0.5 μg/ml and 68% inhibition at 50 μg/ml. In contrast, equivalent amount of isotype-matched nonimmune mouse IgG1 had no significant effect on bFGF-directed migration (Fig. 3a). Likewise, bFGF-stimulated cell migration was inhibited in the presence of cRGD peptide in a dose-dependent manner (Fig. 3b). Maximum reduction (53%) was seen at a high dose of 2 mg/ml relative to cells exposed to bFGF alone.

Unexpectedly, the antibody to $\beta_3$ integrin had no significant inhibition on PDGF-directed

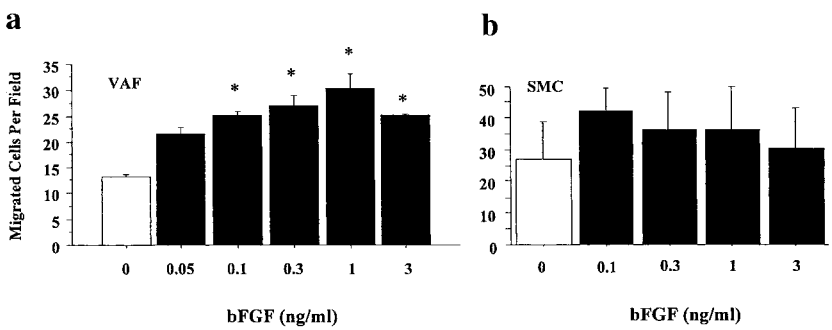


**Fig. 1.** Comparison between adventitial fibroblast (**a**) and smooth muscle cell (**b**) migration in response to bFGF. Plotted values are means±SD for four separate experiments run in triplicate. A value of $^*P < 0.05$ was considered significantly different from control BSA.

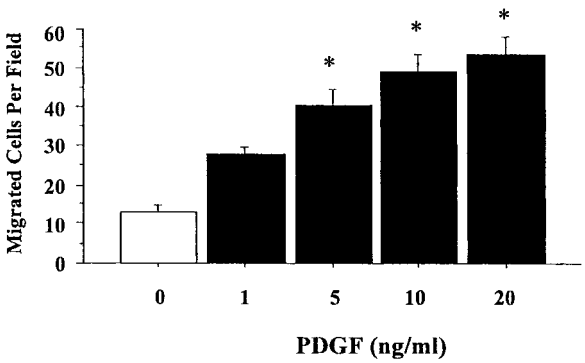


**Fig. 2.** Dose effect of PDGF on migration of cultured rat aortic adventitial fibroblasts. Plotted values are means±SD for four separate experiments run in triplicate. A value of $^*P < 0.05$ was considered significantly different from control BSA.

migration as compared to PDGF alone (Fig. 3c). However, when cRGD was added to the upper chambers, PDGF-directed chemotactic response was significantly blocked, with 38% reduction at a concentration as low as 0.5 mg/ml and 45% reduction at a higher dosage of 2 mg/ml of cRGD (Fig. 3d).

## DISCUSSION

In this study, we have focused on VAF migration and the possible mechanisms involved. Our results demonstrate that bFGF and PDGF are chemoattractants for VAF migration in vitro. An antibody to rat $\beta_3$ integrin inhibited VAF migration induced by bFGF, but not by PDGF. Because the monoclonal antibody specific to rat $\alpha_v\beta_3$ integrin complex is not available, the effect of cRGD peptide, which specifically recognizes integrins $\alpha_v\beta_1$, $\alpha_v\beta_3$, and $\alpha_v\beta_5$ [Pierschbacher and Ruoslahti, 1987; Clyman et al., 1992; Dahm and Bowers, 1998], was examined in these experiments. The cRGD peptide decreased VAF migration stimulated either by bFGF or by PDGF. The fact that both $\beta_3$ integrin antibody and cRGD peptide significantly inhibit bFGF-stimulated VAF migration suggests that the responsible receptors are $\alpha_v\beta_3$ complexes. PDGF-induced VAF migration was reduced only by cRGD peptide but not by an antibody to $\beta_3$ integrin. We speculate that the responsible receptor might be $\alpha_v\beta_1$ or $\alpha_v\beta_5$. However, because the antibodies to rat $\alpha_v\beta_1$ or $\alpha_v\beta_5$ integrin are not available, further studies are needed to clarify the role of these integrins.

The absence of specific markers to distinguish VAFs from SMCs in culture has made in vitro comparisons of these cell types difficult. The presence of desmin, a cytoskeletal protein characteristic of differentiated SMCs, diminishes rapidly in primary cultures of SMCs. The standard marker accepted for SMC identity in culture is presence of α-SM actin

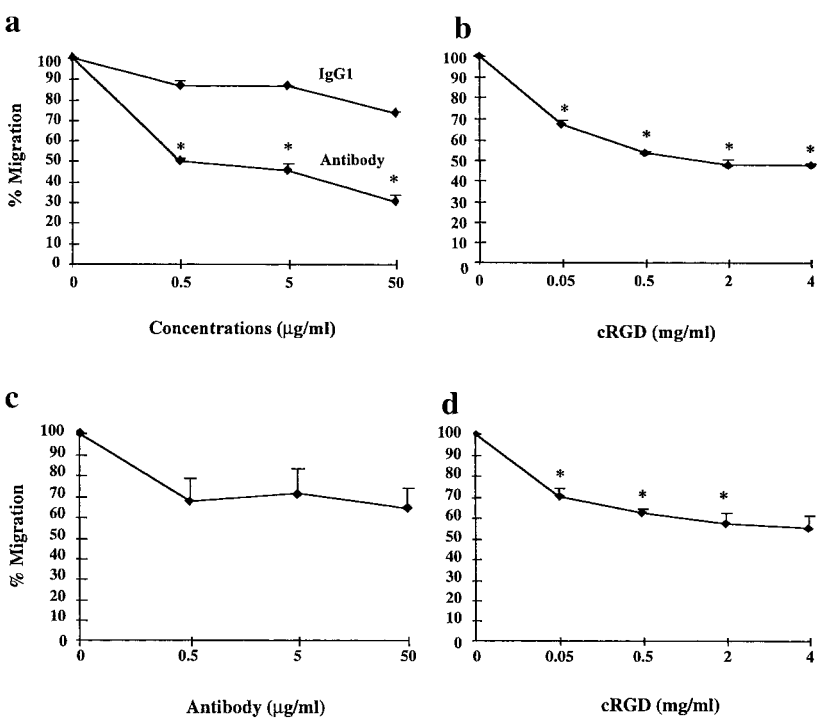


**Fig. 3.** **a:** $\beta_3$ integrin antibody inhibits bFGF-directed migration in rat aortic adventitial fibroblasts; no significant inhibition was observed for IgG1. **b:** Inhibitory effect of cRGD peptide on bFGF-directed adventitial fibroblast migration. **c:** Effects of $\beta_3$ integrin antibody, and (**d**) cRGD peptide on PDGF-stimulated adventitial fibroblast migration. Results represent at least three independent experiments, and are expressed relative to migration in the absence of antibody or peptide after subtraction of background migration in response to 0.1% BSA. Values are means±SD. A value of $^*P < 0.05$ was considered significantly different from bFGF or PDGF alone.

[Skalli et al., 1986]. However, in primary culture VAFs acquire the ability to synthesize α-SM actin, and it may be that a subpopulation of VAFs possess α-SM actin in vivo [Skalli et al., 1986; Desmouliere and Gabbiani, 1992]. The separation between media and adventitia can be distinguished in cross sections of the intact aortic wall, thus examination of adventitial strips from which fibroblasts are harvested seems an appropriate means of distinguishing populations of VAFs from SMCs. Contamination of the VAF cultures with SMCs and vice versa is still possible. However, as shown in Figure 1, the VAFs migrate dose dependently to bFGF, while the SMCs do not.

A number of studies have demonstrated that bFGF alone is not chemotactic for SMCs isolated from the vessels of human [Pickering et al., 1997], cattle [Grotendorst et al., 1982], and rat [Ferns et al., 1991; Bilato et al., 1995], even though other investigators have shown indirect effects of bFGF for primate or mouse [Herbert et al., 1997; Kenagy et al., 1997]. In this present study, we found that bFGF alone did not stimulate migration of rat aortic SMCs. The migration of SMCs in response to PDGF is well established [Grotendorst et al., 1981; Ferns et al., 1991; Kundra et al., 1994]. We have shown that the dose response of VAFs to PDGF as chemoattractant is similar to that of SMCs.

The molecular mechanisms for vascular cell migration are complex and poorly understood. Chemoattractants, integrins, as well as many other factors including extracellular matrix (ECM), calcium, urokinase type plasminogen activator (uPA), and protein kinase may all be involved. The vascular components of extracellular matrix including fibronectin (FN), laminin (LN), vitronectin (VN), and collagen type I (I) and IV(IV) [Ross and Klebanoff, 1971; Heickendorff, 1988; de Reeder et al., 1989] can mediate cell adhesion and migration [Schor et al., 1981; Goodman and Newgreen, 1985; Lanir et al., 1988; Nickoloff et al., 1988; Aznavoorian et al., 1990]. A study by Clyman et al. [1992] demonstrated that SMC adhesion to FN, LN, I, and IV, but not VN, mainly depended on functioning $\beta_1$ integrin; while cell migration over these substrates depended exclusively on the $\alpha_v\beta_3$ receptor. These data suggest that $\alpha_v\beta_3$ can mediate cell migration on various extracellular matrix coated substrates. In our experiments, bFGF-directed VAF migration on coated collagen type I was inhibited by either anti-rat $\beta_3$ and cRGD peptide, which demonstrates that it was regulated by $\alpha_v\beta_3$. Additional studies need to be conducted to shed light on the effect of the substrate on the results.

Previous studies showed that bFGF upregulated the expression and mRNA level of $\alpha_v\beta_3$ in cultured vascular endothelial cells [Sepp et al., 1994], and that the $\alpha_v\beta_3$ promoted human vascular endothelial cell migration is in a calcium-dependent manner [Leavesley et al., 1993]. Moreover, calcium/calmodulin-dependent protein kinase II reversed PDGF-directed SMC migration, which had been inhibited by anti-$\alpha_v\beta_3$ [Bilato et al., 1997]. Furthermore, the expression of uPA·uPAR (uPA bound to its receptor) induced by growth factor is a prerequisite for $\alpha_v\beta_5$-dependent migration but not for $\alpha_v\beta_3$-dependent migration [Yebra et al., 1996]. Our data support previous findings on SMCs and endothelial cells that growth factor stimulated vascular cell migration may be mediated through different integrins and distinct signal pathways. We also believe that the same stimuli may influence distinct integrin expression in different cell types, and different cell types may respond to the same stimuli differently. Integrin $\alpha_v\beta_3$, for example, regulates PDGF-stimulated SMC migration [Bilato et al., 1997], but not VAF migration, as shown in our study. In addition, bFGF can stimulate VAF migration but not SMC migration. The molecular mechanism for VAF migration and the integrin-mediated signal pathways will be investigated further.

This is the first report that bFGF-stimulated VAF migration is $\alpha_v\beta_3$ dependent and enhances our understanding of the pathology of vascular diseases, particularly restenosis following balloon angioplasty, in which VAF migration may be a contributing factor. The elucidation of the cellular mechanisms involved in this response may offer helpful new evidence for therapy of restenosis in the future.

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## REFERENCES

Austin GE, Ratliff NB, Hollman J, Tabei S, Phillips DF. 1985. Intimal proliferation of smooth muscle as an explanation for recurrent coronary artery stenosis after

percutaneous transluminal coronary angioplasty. J Am Coll Cardiol 6:369–375.

Aznavoorian S, Stracke ML, Krutzsch H, Schiffmann E, Liotta LA. 1990. Signal transduction for chemotaxis and haptotaxis by matrix molecules in tumor cells. J Cell Biol 110:1427–1438.

Battle T, Arnal JF, Challah M, Michel JB. 1994. Selective isolation of rat aortic wall layers and their cell types in culture-application to converting enzyme activity measurement. Tissue and Cell 26:943–955.

Bilato C, Pauly RR, Melillo G, Monticone RE, Gorelick-Feldman D, Gluzband YA, Sollott SJ, Ziman B, Lakatta EG, Crow MT. 1995. Intracellular signal pathways required for rat vascular smooth muscle cell migration. J Clin Invest 96:1905–1915.

Bilato C, Curto KA, Monticone RE, Pauly RR, White AJ, Crow MT. 1997. The inhibition of vascular smooth muscle cell migration by peptide and antibody antagonists of the $\alpha_v\beta_3$ integrin complex is reversed by activated calcium/calmodulin-dependent protein kinase II. J Clin Invest 100:693–704.

Brooks PC, Clark RAF, Cheresh DA. 1994. Requirement of vascular integrin $\alpha_v\beta_3$ for angiogenesis. Science 264:569–571.

Choi ET, Engel L, Callow AD, Sun S, Trachtenberg J, Santoro S, Ryan US. 1994. Inhibition of neointimal hyperplasia by blocking $\alpha_v\beta_3$ integrin with a small peptide antagonist GpenGRGDSPACA. J Vasc Surg 19:125–134.

Clowes AW, Reidy MA, Clowes MM. 1983. Kinetics of cellular proliferation after arterial injury. I. Smooth muscle growth in the absence of endothelium. Lab Invest 49:327–333.

Clyman RI, Mauray F, Kramer RH. 1992. $\beta_1$ and $\beta_3$ integrins have different roles in the adhesion and migration of vascular smooth muscle cells on extracellular matrix. Exp Cell Res 200:272–284.

Dahm LM, Bowers CW. 1998. Vitronectin regulates smooth muscle contractility via $\alpha_v$ and $\beta_1$ integrin. J Cell Sci 111:1175–1183.

Das M, Stenmark KR, Dempsey EC. 1995. Enhanced growth of fetal and neonatal pulmonary artery adventitial fibroblasts is dependent on protein kinase C. Am J Physiol 269:L660–L667.

De Leon H, Scott NA, Martin F, Simonet L, Bernstein KE, Wilcox JN. 1997. Expression of nonmuscle myosin heavy chain-B isoform in the vessel wall of porcine coronary arteries after balloon angioplasty. Circ Res 80: 514–519.

de Reeder EG, Poelmann RE, van Munsteren JC, Patterson DF, Gittenberger-de Groot AC. 1989. Ultrastructural and immunohistochemical changes of the extracellular matrix during intimal cushion formation in the ductus arteriosus of the dog. Atherosclerosis 79:29–40.

Desmouliere A, Gabbiani G. 1992. The role of arterial smooth muscle cells in the pathogenesis of atherosclerosis. Cerebrovasc Dis 2:63–71.

Ferns GA, Reidy MA, Ross R. 1991. Balloon catheter deendothelialization of the nude rat carotid. Am J Pathol 138:1045–1057.

Ferns GA, Raines EW, Sprugel KH, Motani AS, Reidy MA, Ross R. 1991. Inhibition of neointimal smooth muscle accumulation after angioplasty by an antibody to PDGF. Science 253:1129–1132.

Folkman J, Klagsbrun M. 1987. Angiogenic factors. Science 235:442–447.

Goodman SL, Newgreen D. 1985. Do cells show an inverse locomotory response to fibronectin and laminin substrates? EMBO J 4:2769–2771.

Grotendorst GR, Seppa HEJ, Kleinman HK, Martin GR. 1981. Attachment of smooth muscle cells to collagen and their migration towards PDGF. Proc Natl Acad Sci USA 78:3669–3672.

Grotendorst GR, Chang T, Seppa HE, Kleinman HK, Martin GR. 1982. Platelet-derived growth factor is a chemoattractant for vascular smooth muscle cells. J Cell Physiol 113:261–266.

Heickendorff L. 1988. Laminin, fibronectin and type IV collagen in BM-like material from cultured arterial smooth muscle cells. Int J Biochem 20:381–386.

Herbert JM, Lamarche I, Carmeliet P. 1997. Urokinase and tissue-type plasminogen activator are required for the mitogenic and chemotactic effects of bovine fibroblast growth factor and platelet-derived growth factor-BB for vascular smooth muscle cells. J Biol Chem 272:23585–23591.

Hoshiga M, Alpers CE, Smith L, Giachelli CM, Schwartz SM. 1995. $\alpha_v\beta_3$ integrin expression in normal and atherosclerotic artery. Circ Res 77:1129–1135.

Hynes RO. 1992. Integrins: versatility, modulation, and signaling in cell adhesion. Cell 69:11–25.

Kenagy RD, Hart CE, Stetler-Stevenson WG, Clowes AW. 1997. Primate smooth muscle cell migration from aortic explants is mediated by endogenous platelet-derived growth factor and basic fibroblast growth factor acting through matrix metalloproteinases 2 and 9. Circulation 96:3555–3560.

Kundra V, Escobedo JA, Kazlauskas A, Kim HF, Rhee SAG, Williams LT, Zetter BR. 1994. Regulation of chemotaxis by the platelet-derived growth factor receptor-beta. Nature 367:474–476.

Lanir N, Ciano PS, Van de Water L, McDonagh J, Dvorak AM, Dvorak HF. 1988. Macrophage migration in fibrin gel matrices. II. Effects of clotting factor XIII, fibronectin, and glycosaminoglycan content on cell migration. J Immunol 140:2340–2349.

Leavesley DI, Schwartz MA, Rosenfeld M, Cheresh DA. 1993. Integrin $\beta_1$ and $\beta_3$-mediated cell migration is triggered through distinct signal mechanisms. J Cell Biol 121:163–170.

Lindner V, Reidy MA. 1991. Proliferation of smooth muscle cell after vascular injury is inhibited by an antibody against basic fibroblast growth factor. Proc Natl Acad Sci USA 88:3739–3743.

Lindner V, Lappi DA, Baird A, Majack RA, Reidy MA. 1991. Role of basic growth factor in vascular lesion formation. Circ Res 68:106–113.

Matsuno H, Stassen JM, Vermylen J, Deckmyn H. 1994. Inhibition of integrin function by a cyclic RGD-containing peptide prevents neointimal formation. Circulation 90:2203–2206.

Nickoloff BJ, Mitra RS, Riser BL, Dixit VM, Varani J. 1988. Modulation of keratinocyte motility. Correlation with production of extracellular matrix molecules in response to growth promoting and antiproliferative factors. Am J Pathol 132:543–551.

O'Brien ER, Garvin MR, Stewart DK, Hinohara Y, Simpson JB, Schwartz SM, Giachelli CM. 1994. Osteopontin is

synthesized by macrophage, smooth muscle, and endothelial cells in primary and restenotic human coronary atherosclerotic plaques. Arteri Thromb Vasc Biol 14:1648–1656.

Pickering JG, Uniyal S, Ford CM, Chau T, Laurin MA, Chow LH, Ellis CG, Fish J, Chan BM. 1997. Fibroblast growth factor-2 potentiates vascular smooth muscle cell migration to platelet-derived growth factor: upregulation of $\alpha_2\beta_1$ integrin and disassembly of actin filaments. Circ Res 80:627–637.

Pierce GF, Berg JV, Rudolph R, Tarpley J, Mustoe TA. 1991. Platelet derived growth factor-BB and transforming growth factor beta-1 selectively modulate glycosaminoglycans collagen, and myofibroblasts in excisional wounds. Am J Pathol 138:629–646.

Pierschbacher MD, Ruoslahti E. 1987. Influence of stereochemistry of the sequence Arg-Gly-Asp-Xaa on binding specificity in cell adhesion. J Biol Chem 262:17294–17298.

Reidy MA, Fingerle J, Lindner V. 1992. Factors controlling the development of arterial lesions after injury. Circulation 86: Suppl. 6 III43–III46.

Ross R. 1993. The pathogenesis of atherosclerosis: a perspective for the 1990s. Nature 362:801–809.

Ross R, Klebanoff SJ. 1971. The smooth muscle cell. I. In vivo synthesis of connective tissue proteins J Cell Biol 50:159–171.

Ross R, Wight TN, Strandness E, Thiele B. 1984. Human Atherosclerosis, I: cell constitution and characteristics of advanced lesions of the superficial femoral artery. Am J Pathol 114:79–93.

Schor SL, Schor AM, Bazill GW. 1981. The effects of fibronectin on the migration of human foreskin fibroblasts and Syrian hamster melanoma cells into three-dimensional gels of native collagen fibres. J Cell Sci 48:301–314.

Scott NA, Cipolla GD, Ross CE, Dunn B, Martin FH, Simonet L, Wilcox JN. 1996. Identification of a potential role of the adventitia in vascular lesion formation after balloon overstretch injury of porcine coronary arteries. Circulation 93:2178–2187.

Sepp NT, Li LJ, Lee KH, Brown EJ, Caughman SW, Lawley TJ, Swerlick RA. 1994. Basic fibroblast growth factor increases expression of the $\alpha_v\beta_3$ integrin complex on human microvascular endothelial cells. J Invest Dermatol 103:295–299.

Shi Y, O'Brien JE, Fard A, Mannion JD, Wang D, Zalewski A. 1996. Adventitial myofibroblasts contribute to neointimal formation in injured porcine coronary arteries. Circulation 94:1655–1664.

Shi Y, Pieniek M, Fard A, O'Brien J, Mannion JD, Zalewski A. 1996. Adventitial remodeling after coronary arterial injury. Circulation 93:340–348.

Skalli O, Ropraz P, Trzeciak A, Benzonana G, Gillesen D, Gabbiani G. 1986. A monoclonal antibody against alpha-smooth muscle actin: a new probe for smooth muscle differentiation. J Cell Biol 103:2787–2796.

Skalli O, Bloom WS, Ropraz P, Azzarone B, Gabbiani G. 1986. Cytoskeletal remodeling of rat aortic smooth muscle cells in vitro: relationships to culture conditions and analogies to in vivo situations. J Submicrosc Cytol 18:481–493.

Topol EJ, Califf RM, Weisman HF, Ellis SG, Tcheng JE, Worley S, Ivanhoe R, George BS, Fintel D, Weston M. et al. 1994. Randomized trial of coronary intervention with antibody against platelet IIb/IIIa integrin for reduction of clinical restenosis: result at six month. Lancet 343:881–886.

Wilcox JN, Waksman R, King SB, Scott NA. 1996. The role of the adventitia in the arterial response to angioplasty: the effect of intravascular radiation. Int J Rad Oncol Biol Phys 4:789–796.

Wilcox JN, Cipolla GD, Martin FH, Simonet L, Dunn B, Ross CE, Scott NA. 1997. Contribution of adventitial myofibroblasts to vascular remodeling and lesion formation after experimental angioplasty in pig coronary arteries. Ann N Y Acad Sci 811:437–447.

Yebra M, Parry GCN, Stromblad S, Mackman N, Rosenberg S, Mueller BM, Cheresh DA. 1996. Requirement of receptor-bound urokinase-type plasminogen activator for integrin $\alpha_v\beta_5$-directed cell migration. J Biol Chem 271:29393–29399.