Integrin Profile and In Vivo Homing of Human Smooth Muscle Progenitor Cells

Arjun Deb, MD*; Kimberly A. Skelding, MD*; Shaohua Wang, MD; Margo Reeder, BS; David Simper, MD; Noel M. Caplice, MD, PhD

Background—Recently, we identified circulating smooth muscle progenitor cells (SPCs) in human peripheral blood. The integrin profile of such progenitors is currently unknown and may affect their in vivo homing characteristics. In this study, we determined the integrin profile of vascular progenitors and SPC adhesion to extracellular matrix (ECM) proteins in vitro and in vivo.

Methods and Results—SPCs and endothelial progenitor cells (EPCs) were isolated from peripheral blood of healthy human subjects, and expression of surface integrins and adhesion to several vascular ECM proteins were determined. Homing of SPCs in vivo to specific ECM protein was determined by intracoronary infusion of fluorescent SPCs into porcine coronary arteries containing a fibronectin-coated mesh stent. SPCs had high expression of β1 integrin, moderate expression of α1, low levels of α2β1, and did not express α3β1, α5β1, α6β1, or α6β4 integrins. In contrast, EPCs had high expression of α3β1, α2β1, α5β1, and minimal expression of α6β4. Moreover, SPCs showed increased adherence to fibronectin and collagen type I compared with vitronectin, consistent with their integrin profile, and demonstrated a similar degree of in vivo attachment to fibronectin-coated mesh.

Conclusions—These data for the first time show a spectrum of integrin expression on vascular progenitors and suggest the potential importance of integrins in mediating adherence of SPCs to specific ECM both in vitro and in vivo. (Circulation. 2004;110:2673-2677.)

Key Words: integrins ■ muscle, smooth ■ stem cells ■ extracellular matrix

Emerging experimental evidence suggests that circulating smooth muscle progenitor cells (SPCs) may participate in atherosclerosis biology.1 Currently, there are at least 2 broad classes of molecules that may contribute to maintenance of vascular progenitor cells in the circulation.2 The first group consists of factors with the capacity to stimulate growth, survival, and differentiation of progenitor cells.3 The second group comprises heterodimeric surface adhesion molecules (integrins) known to support physical association between circulating progenitor cells and vascular extracellular matrix (ECM).4 Recently, we described the existence of SPCs in human peripheral blood5 and have also shown that bone marrow–derived smooth muscle cells are highly enriched in the intima of human atherosclerotic vessels.6 Understanding the homing, adhesion, and recruitment characteristics of SPCs may have significant implications for understanding basic mechanisms of atherogenesis and for development of novel therapeutics to treat vascular disease. The full integrin profile of human SPCs and the ECM proteins that allow the best adhesion platform for these cells are currently unknown. Moreover, it remains unclear whether ECM proteins can provide a receptive niche to capture SPCs in vivo.

Methods

Isolation and Identification of SPCs and Endothelial Progenitor Cells

SPCs and endothelial progenitor cells (EPCs) were isolated from peripheral blood of healthy human subjects, expanded in culture, and immunophenotyped as described previously.5 The mononuclear cell population from which EPCs and SPCs are usually derived was also assayed by flow cytometry.7

Analysis of Cell-Surface Integrins of SPCs and EPCs

Fluorescence-activated cell sorter (FACS) analysis was performed to identify the following cell-surface integrins on SPCs and EPCs (passages 2 to 6): αvβ3, αvβ5, α5β1, β3, α1, α6β1 (Chemicon). Human vascular smooth muscle cells, human umbilical vein endothelial cells (HUVECs), and freshly isolated mononuclear cells, respectively, were used as cell controls.

In Vitro Adhesion of SPCs and EPCs to ECM Proteins

The adhesion of SPCs to ECM such as fibronectin, collagen type I (COLL), vitronectin (VN), and fibrin was analyzed on coated 24-well plates in vitro (Calbiochem). SPCs were stained with cell tracker green (CTG; 1 μL/4 mL media; Molecular Probes) and plated at a density of 2×10⁴ cells/well. After 1 hour of incubation at 37°C
in the presence or absence of serum-free EBM-2 media containing magnesium and calcium, unattached cells were washed with PBS, and the number of attached cells was estimated by measuring the fluorescence of each well. In separate experiments, the number of attached SPCs was determined by hemocytometer counting as described previously. Adhesion of cells in the presence or absence of BSA (1%) precoating of wells was also determined.

**In Vitro ECM Adhesion and In Vivo Homing of SPCs to Fibronecin-Coated Mesh Stent**

We have previously described a stainless steel mesh stent that acts as an ideal cell attachment platform when coated with ECM protein. This mesh was coated with several ECM proteins including fibronectin, COLL, and VN. SPCs stained with CTG were seeded at a density of 2.5 x 10^5/cm^2 on the mesh in vitro. After 1 hour, the mesh was washed with PBS, cells were gently trypsinized off the mesh, and the fluorescence of the lifted cells was determined.

For in vivo fluorescence, a fibronectin-coated mesh stent was inserted into the left circumflex coronary artery of normal pigs, followed by intracoronary injection of 2 x 10^6 CTG-labeled SPCs. Stents were explanted 24 hours later, and fluorescence of attached cells on the mesh stent was determined as described for in vitro studies.

**Data and Statistical Analysis**

All experiments were performed in triplicate, and ANOVA was used to determine statistically significant differences in adhesion between the different ECMs. A P<0.05 was determined as statistically significant.

**Results**

**Integrin Profile of EPCs and SPCs**

SPCs showed high expression of β1 integrin, moderate expression of α5, low levels of α6β1, and absence of α6β3, β2, α6β1, and α6β2 integrins (Figure 1). Moreover, apart from β1 integrin expression on SPCs, all other integrins (α5, α6β1, α6β2, and α6β3) were more highly expressed on human vascular smooth muscle cells grown under identical culture conditions. In contrast, EPCs had high expression of α6β1, α6β3, α6β2, α6β1, and α6 minimal expression of α6β1 (Figure 1), with similar levels of these integrins seen on HUVECs with the exception of α6β1, which was more highly expressed on HUVECs (Figure 1). Finally, mononuclear cells were positive for β1 integrin expression in contrast to all other cells analyzed (Figure 1).

**Fibrin Adhesion Properties of SPCs and EPCs**

We determined whether the differential expression of integrins on EPCs and SPCs was reflected in the respective binding properties of these cells to fibrin, which contains a mixture of vascular ECM components. In all experiments, binding of progenitor cells to ECM proteins was unaffected by precoating of wells with BSA. We initially chose fibrin because it plays a central role in atherothrombosis, and freshly formed clot may serve as a niche for incorporation of circulating vascular progenitors. After 1-hour adhesion assay, EPCs showed 2-fold greater adherence to fibrin than SPCs (Figures 2A and 2B). Antibody to α6β1 integrin decreased EPC adhesion to fibrin by 20% compared with isotype IgG control (P<0.05; Figure 2C). Antibodies to other integrins (including β1) did not affect binding of EPCs to fibrin (Figure 2C). In contrast, antibodies to β1 integrin significantly decreased SPC adhesion to fibrin by 35% (P<0.05; Figure 2C). Adhesion to fibrin was not affected by other antibodies to integrins known to be expressed on SPCs, such as α6β1 and α6 (data not shown).

**In Vitro ECM Adhesion and In Vivo Homing of SPCs to Fibronecin-Coated Mesh**

We next determined in vitro adhesion of SPCs to a mesh coated with specific vascular ECM proteins, such as COLL, fibronectin, and VN. SPCs showed increased adherence to fibronectin and COLL compared with VN, consistent with the integrin profile described for these cells (Figure 2D). In a separate series of experiments, adhesion of SPCs to fibronectin was determined in the presence of antibodies to α5 and β1 integrins or control IgG. All integrin antibodies decreased SPC adhesion to fibronectin compared with IgG control antibody, with the combination of both α5 and β1 antibodies being most effective (decreased adhesion by 85%; Figure 2E; P<0.01). Moreover, analysis of fibronectin-coated mesh stents placed in the porcine coronary artery 24 hours after intracoronary infusion of 2 x 10^6 SPCs demonstrated significant in vivo SPC attachment to this mesh stent, as was also observed in vitro (Figure 2, F through J). Approximately 40% of incubated SPCs attached to the fibronectin-coated mesh stent in vitro, whereas 10% of injected SPCs attached to the mesh stent in vivo.

**Discussion**

In this study, we show a distinct surface integrin expression profile in human SPCs compared with EPCs and report for the first time in vivo SPC adhesion to specific ECM protein within the vasculature. These data have implications for understanding in vivo SPC biology and for the development of novel cellular targets in the vessel wall. Moreover, this study provides a window for potential future strategies aimed at device cell interfacing to achieve therapeutic effect.

The components of the vasculature targeted by SPC homing are currently unknown. Such homing may involve exit of progenitor cells from the circulation, with attachment to ECM proteins and incorporation into vascular tissue. Integrins are known to play a key role in mediating anchorage between progenitor and vascular cells and ECM, potentiating cell differentiation, cell migration, and proliferation. In vitro matrix models suggest different vascular cells share several integrins with potentially overlapping matrix-binding characteristics, and indeed, the constituents of the matrix environment are a strong determinant of specific integrin interaction.

In the present study, SPCs expressed high levels of β1 and α5 integrins, both of which are known to facilitate fibronectin interfacing, and it is possible these integrins are also significant determinants of SPC–fibronectin interaction. Indeed, our SPC binding data support this concept, with significant inhibition of SPC–fibronectin binding by combined antibodies to α5 and β1 integrins. In contrast, fibrin binding of EPCs that expressed much less β1 integrin was not inhibited by β1 antibody. SPCs lacked significant α5β1 and α5β2 integrins, which have been shown to play a key role in angiogenesis, whereas EPCs known to participate in postnatal neovascularization strongly expressed these integrins. Moreover, the α5β1 integrin, which is a significant mediator of
Figure 1. Flow cytometry analysis of surface integrin expression of SPCs, EPCs, and mononuclear cells (MNC) compared with differentiated vascular smooth muscle cell (VSMC) and HUVEC populations. Experiments were performed for each antibody on at least 3 separate occasions. In each case, filled histogram indicates isotype-matched IgG control antibody.
collagen binding,\textsuperscript{12} was expressed at high levels in EPCs but absent in SPCs. Together, these data suggest differential integrin-matrix binding between SPCs and EPCs.

Although SPC adherence to fibrin was more potent than to fibronectin, the heterogeneous nature of fibrin (which consists of a large number of different proteins) and its prothrombotic potential makes interpretation of this integrin-matrix interaction in vivo much more complex. Given the in vivo SPC adhesion to fibronectin-coated mesh stent in the present study, it is tempting to speculate whether fibronectin within the diseased vessel wall may also provide a receptive niche for SPC attachment. Atherosclerotic plaques are prone to fissure with exposure of underlying fibronectin, and it is conceivable that homing of SPCs to fibronectin-rich atherosclerotic lesions may be amenable to pharmacological manipulation.

In summary, we report distinct integrin profiles for human SPCs and EPCs and demonstrate differences in ECM attachment.

Figure 2. Adhesion properties of SPCs and EPCs to various ECMs and mesh stent in vitro and in vivo. A, Immunofluorescence staining of SPCs and EPCs for smooth muscle (SM) and endothelial lineage markers. HC indicates heavy chain; VECAD, vascular endothelial cadherin. B, EPCs show greater binding to fibrin than SPCs. F.U. indicates fluorescence units. α, αβ (open bars) and β1 (hatched bars) antibodies decrease EPC and SPC binding, respectively, to fibrin compared with isotype-matched IgG control antibodies (solid bars). Ctrl indicates control. D, Adhesion properties of SPCs to various ECM proteins, demonstrating strongest and weakest adhesion to fibronectin (FN) and VN, respectively. E, SPC adhesion to fibronectin (FN) matrix in presence of various integrin antibodies (α, β, and α+β) compared with IgG control (Ctrl) antibody. F through J, Successful adhesion of SPC to fibronectin-coated mesh stent in vivo compared with in vitro adhesion to similar fibronectin-coated mesh (E through G). G, Confocal microscopy of mesh stent after explantation from porcine coronary artery shows CTG-labeled SPC (green) within mesh framework (100×). H, Same cell at high magnification (400×), staining positive for CTG (upper panel) and Hoechst (blue; lower panel). I and J, SPCs staining positive for CTG and α-smooth muscle actin (αSMA; 200×). Experiments in all cases were performed on at least 3 separate occasions. For in vitro assays, each individual experiment was performed in triplicate. Adhesion experiments were performed after preblocking with BSA.
ment of these cells that may have in vivo biological relevance. Further investigation will be necessary to determine whether homing properties of vascular progenitors can be altered pharmacologically or genetically by targeted inhibition or potentiation of integrin–matrix interactions.

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