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 \( \beta_1 \) integrin, moderate expression of \( \alpha_1 \), low levels of \( \alpha_2 \), and did not express \( \alpha_3 \), \( \beta_3 \), \( \alpha_5 \), \( \alpha_6 \), or \( \alpha_\beta_1 \) integrins. In contrast, EPCs had high expression of \( \alpha_\beta_1 \), \( \alpha_\beta_2 \), \( \alpha_\beta_3 \), \( \alpha_\beta_5 \), \( \beta_1 \), and \( \alpha_1 \) and minimal expression of \( \alpha_\beta_1 \). 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): \( \alpha_\beta_1 \), \( \alpha_\beta_2 \), \( \alpha_\beta_3 \), \( \alpha_\beta_5 \), \( \beta_1 \), \( \alpha_1 \), \( \alpha_\beta_1 \); (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 \( \mu \)M 4 mL media; Molecular Probes) and plated at a density of \( 2 \times 10^5 \) 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 Fibronectin-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, COL, and VN. SPCs stained with CTG were seeded at a density of 2.5×10^5/cm² 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 2×10^6 CTG-labeled SPCs (n=3). 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 α1, low levels of αβ3, and absence of αβ3, β1, αβ1, and αβ1 integrins (Figure 1). Moreover, apart from β1 integrin expression on SPCs, all other integrins (α1, αβ1, αβ3, and αβ3) were more highly expressed on human vascular smooth muscle cells grown under identical culture conditions. In contrast, EPCs had high expression of αβ1, αβ1, αβ1, β1, and α1 and minimal expression of αβ1 (Figure 1), with similar levels of these integrins seen on HUVECs with the exception of αβ1, 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 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 α1 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 α1 and β1 integrins. In contrast, fibrin binding of EPCs that expressed much less β1 integrin was not inhibited by β1 antibody. SPCs lacked significant αβ1 integrin inhibition, 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 α1β1 integrin, which is a significant mediator of SPC adhesion to fibronectin compared with IgG control antibody, with the combination of both α1 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 2×10^6 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.
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,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.
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.

Acknowledgments
This work was supported in part by grants from the National Institutes of Health (HL66958-NMC) and the Mayo Foundation. We thank Toni Higgins for her assistance in preparing this manuscript.

References
Integrin Profile and In Vivo Homing of Human Smooth Muscle Progenitor Cells
Arjun Deb, Kimberly A. Skelding, Shaohua Wang, Margo Reeder, David Simper and Noel M. Caplice

Circulation. 2004;110:2673-2677; originally published online August 16, 2004;
doi: 10.1161/01.CIR.0000139842.15651.B2
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2004 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/110/17/2673

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation is online at:
http://circ.ahajournals.org//subscriptions/