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

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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 $\alpha_5\beta_1$, $\alpha_6\beta_1$, $\alpha_\delta$, or $\alpha_{\gamma}$. In contrast, EPCs had high expression of $\alpha_2\beta_1$, $\alpha_\gamma$, and $\alpha_5\beta_3$ and $\alpha_\delta$ and $\alpha_{\gamma}$. Moreover, SPCs showed increased adherence to fibronectin and collagen type I compared with vitronectin, consistent with their integrin profile, and demonstrated a significant 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.

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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. Currently, there are at least 2 broad classes of molecules that may contribute to maintenance of vascular progenitor cells in the circulation. The first group consists of factors with the capacity to stimulate growth, survival, and differentiation of progenitor cells. The second group comprises heterodimeric surface adhesion molecules (integrins) known to support physical association between circulating progenitor cells and vascular extracellular matrix (ECM).

Recently, we described the existence of SPCs in human peripheral blood and have also shown that bone marrow-derived smooth muscle cells are highly enriched in the intima of human atherosclerotic vessels. 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. The mononuclear cell population from which EPCs and SPCs are usually derived was also assayed by flow cytometry.

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): $\alpha_5\beta_1$, $\alpha_6\beta_1$, $\alpha_\delta$, $\alpha_{\gamma}$, $\alpha_6\beta_3$; 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 $\mu$L/4 mL media; Molecular Probes) and plated at a density of $2 \times 10^3$ 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, COLL, and VN. SPCs stained with CTG were seeded at a density of 2.5x10⁴/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 2x10⁶ 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 α₂β₁, and absence of α₂β₃, β₂, α₁β₂, and α₂β₃ integrins (Figure 1). Moreover, apart from β₁ integrin expression on SPCs, all other integrins (α₁, α₃β₁, α₄β₁, and α₅β₁) were more highly expressed on human vascular smooth muscle cells grown under identical culture conditions. In contrast, EPCs had high expression of α₁β₂, α₁β₃, α₁β₄, β₁, and α₁ and minimal expression of α₂β₁ (Figure 1), with similar levels of these integrins seen on HUVECs with the exception of α₁β₂, 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 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 vascular progenitors.

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 α₁β₂, α₁β₃, and α₂β₃ 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 α₁β₁ 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 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. C, αβ1 (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 (α1, β1, and α1+β1) 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 (α-SMA; 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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References
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