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Myeloid lineage of high proliferative potential human smooth muscle outgrowth cells circulating in blood and vasculogenic smooth muscle-like cells *in vivo*

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Abstract

Emerging experimental data supports a circulating precursor origin for some smooth muscle cells that participate in vasculogenesis but uncertainty exists on the precise phenotype and lineage of these vascular precursors. We determined the lineage of human smooth muscle outgrowth cells (SOC) derived from circulating blood mononuclear cells and smooth muscle-like cells present in regions of vasculogenesis in diseased arteries. Immunophenotypic characterization of SOC was performed using FACS and immunofluorescence (IF). An SOC hierarchy was determined based on *in vitro* clonogenic and proliferative potential. Lineage of smooth muscle-like cells in vasculogenic regions *in vivo* was also determined by dual IF for myeloid and smooth muscle specific markers combined with FISH for the X and Y chromosome in diseased vessel of human subjects who had undergone gender mismatched cardiac transplantation. We show here that primary high proliferative potential smooth muscle outgrowth cells (HPP-SOC) expanded in culture from human peripheral blood mononuclear cells (PBMC) and recipient-derived chimeric smooth muscle cells participating in vasculogenesis *in vivo* share a myeloid phenotype (CD68 and CD14 positivity). Moreover, HPP-SOC *in vitro* are distinct in being negative for several myeloid markers such as CD11b, CD13 and CD33, and CD45 surface antigens and chimeric SMC *in vivo* show no evidence of cell fusion propensity. This study provides evidence of a possible myeloid subpopulation origin for smooth muscle outgrowth cells in blood and vasculogenic smooth muscle-like cells in the intima and adventitial microvasculature of diseased arteries. These data have significant implications for understanding the role myeloid cells play in smooth muscle cell biology and vascular remodelling.

Keywords: Vascular biology; Myeloid lineage; Smooth muscle outgrowth

1. Introduction

Experimental evidence from a number of animal models of atherosclerosis suggests that circulating precursors of smooth muscle cells integrate into remodelling vasculature [1,2]. We have previously shown that isolated smooth muscle outgrowth cells (SOC) can be cultured from human peripheral blood mononuclear cells [3] and that human donor derived smooth muscle cells originating from cells administered at bone marrow transplantation are enriched in intimal and adventitial microvessels of atherosclerotic coronary arteries [4]. Together, these data support a circulating precursor origin of some vessel wall smooth muscle cells. Although the lineage of such cells remains unclear numerous possible candidates exist; including cells of hematopoietic, mesenchymal, or angioblastic origin.

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Early experimental animal data supported a haematopoietic origin for vascular precursors and more recently the additional possibility has arisen that cells of myelomonocytic lineage may differentiate into smooth muscle cells [5–9]. Since myeloid cells are abundant in the peripheral circulation the clinical implications of significant vascular cell differentiation from such cells would be great. However, to date, no evidence exists to support high proliferative potential myeloid lineage-smooth muscle cell differentiation in peripheral blood or within the vasculature of human subjects. To explore this possibility we undertook two sets of experiments aimed at determining whether (a) clonal smooth muscle outgrowth cells of myeloid immunophenotype with high proliferative potential and extensive self renewal capacity exist in human blood and whether (b) chimeric smooth muscle cells seen within the neovasculature of gender mismatched cardiac transplant arteriopathy subjects share a common myeloid lineage with these cells. We show here that single cell-derived SOC grown from human peripheral blood mononuclear cells (PBMC) exhibit a hierarchy of self-renewal capacity *in vitro* including high proliferative potential (HPP) exceeding the Hayflick limit, and that these HPP-SOC and recipient-derived chimeric smooth muscle cells participating in vasculogenesis *in vivo* share a myeloid immunophenotype. Moreover, chimeric myeloid marker positive smooth muscle cells in the vessel wall microvasculature show no evidence of tetraploidy or cell fusion. Together these data support a highly robust smooth muscle cell differentiation pathway with fundamental implications for our understanding of the role of myeloid subpopulations in vascular biology.

2. Methods

2.1. Patient blood and autopsy tissue

Coronary artery specimens from five male subjects who had received a gender mismatched cardiac transplant were studied. Twenty healthy volunteer blood donors were used to study blood outgrowth cells. The institutional review board at Mayo Clinic approved the use of all autopsy specimens and donor blood. All transplant patients gave consent pre-surgery for their tissue to be used in research studies.

2.2. PBMC isolation and SOC culture

We isolated peripheral blood mononuclear cells (PBMC) from healthy blood donor buffy coats in ficoll-paque as previously described [10]. Freshly isolated MNC were seeded in 12-well plates coated with collagen type I and cultured in EGM-2 medium (Cambrex) at five million cells per well. Three weeks later, smooth muscle outgrowth cells (SOC) were expanded from colonies and maintained in EGM-2 supplemented with PDGF-BB (50 ng/ml, R&D systems) as previously described [3]. PDGF-BB was essential for SOC cultivation as attempts to grow SOC in the presence of VEGF, FGF or TGF-β failed in the absence of co-incubation with PDGF-BB (data not shown). On average, 1–2 SOC colonies were obtained per million cells seeded. Primary SOC used in this study were between passages 3 and 6. Human coronary arterial smooth muscle cells (HSMC) were obtained from Clonetics.

2.3. Clonogenic and proliferative potential assays

To examine the clonogenic capacity and proliferative potential of SOC, a single cell deposition assay was used in a modification of previously described methodology [11]. Briefly early passage SOC were plated into 96-well plates at a density of 0.3 cell/well in EGM-2 medium supplemented with PDGF. Individual wells were examined under a contrast microscope to ensure that only one single cell was placed into each well. In parallel early passage SOC were transduced with a GFP–expressing lentiviral vector pHR'SIN-CSGW provided by Dr. A. Thrasher (Institute of Child Heath, London). The packaging (pCMVR8.91) and VSV-G (pMDG) plasmids were provided as a gift and to make infectious vectors, 293 T cells were cotransfected with pHRSIN-CSGW, pCMVR8.91 and pMDG plasmids. Two days after transfection the culture supernatants were harvested and passed through a 0.45 μm pore size filter.

Lentiviral GFP transduced single cells were identified by positive immunofluorescence. Medium was changed every 3 days and after 2 weeks, each well was examined for colony growth. Colonies were graded after 14 days culture according to size into various categories: 2–50, 50–200, 200–2000, 2000–10,000, >10,000 cells. The colonies with over 2000 cells were expanded into 24-well plate and further expanded into T-75 cm^2 flasks for long-term culture.

2.4. Antibodies and lineage specific markers

The following specific antibodies and mRNA expression markers were used for lineage determination by FACS, in situ immunofluorescence staining and PCR: α-smooth muscle actin (SMA), myosin heavy chain (MHC) and calponin for smooth muscle lineage, CD11b (MAC-1), CD13, CD14, CD33, and CD68 for myeloid lineage, and CD45 for hematopoietic lineage. The primers used for determination of mRNA expression profiles in blood outgrowth and mature vascular cells are shown on Table 1.

2.5. Myeloid colony forming ability

SOC were assessed for their ability to form colonies of myeloid lineage. 1000 SOC, PBMC, and $CD34^+$ cells (isolated from PBMC with Miltenyi bead isolation kit) were used in the MethoCult® assay (StemCell Technologies Inc.) as per manufacturer's instructions. Number of colones were counted 11 days after initial seeding of cells.