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SHORT REPORT

Feeder-independent derivation of induced-pluripotent stem cells from peripheral blood endothelial progenitor cells

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Abstract Induced-pluripotent stem cells (iPSCs) are a potential alternative cell source in regenerative medicine, which includes the use of differentiated iPSCs for cell therapies to treat coronary artery and/or peripheral arterial diseases. Late-outgrowth endothelial progenitor cells (late-EPCs) are a unique primary cell present in peripheral blood that exhibit high proliferative capacity, are being used in a wide variety of clinical trials, and have the ability to differentiate into mature endothelial cells. The objective of this study was to reprogram peripheral blood-derived late-EPCs to a pluripotent state under feeder-free and defined culture conditions. Late-EPCs that were retrovirally transduced with OCT4, SOX2, KLF4, c-MYC, and iPSC colonies were derived in feeder-free and defined media conditions. EPC-iPSCs expressed pluripotent markers, were capable of differentiating to cells from all three germ-layers, and retained a normal karyotype. Transcriptome analyses demonstrated that EPC-iPSCs exhibit a global gene expression profile similar to human embryonic stem cells (hESCs). We have generated iPSCs from late-EPCs under feeder-free conditions. Thus, peripheral blood-derived late-outgrowth EPCs represent an alternative cell source for generating iPSCs.

Abbreviations: ALP, alkaline phosphatase; EBs, embryoid bodies; GMP, good manufacturing practice; H&E, hematoxylin and eosin; hESC, human embryonic stem cells; iPSC, induced-pluripotent stem cells; Late-EPC, late-outgrowth endothelial progenitor cells; SMA, smooth muscle actin.

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Introduction

Cell therapies hold great promise for the treatment of numerous human diseases and disorders. For example, atherosclerotic vascular disease is a major cause of heart failure and contributes importantly to the growing burden of chronic disease. The use of stem cells to promote cardiac repair or restore perfusion to tissues represents an important strategy to avoid the deleterious consequences of occlusive vascular disease. In preclinical studies, the administration of adult stem and progenitor cells has been shown to improve cardiac function post acute myocardial infarction and restore perfusion to the ischemic hindlimb; however, their efficacy in clinical trials has been at best modest (Lasala and Minguell, 2011). These include endothelial progenitor cells (EPCs) that can be isolated from bone marrow or circulating mononuclear cells by surface marker selection of culture specification. The latter can be classified into early outgrowth EPCs (or circulating angiogenic cells) that appear within 3 days of culture, and late outgrowth EPCs (or blood outgrowth endothelial cells) that only appear after 2 weeks and then rapidly overgrow the early growth cells. Unlike the early cells, late-outgrowth EPCs are highly differentiated to an endothelial phenotype and exhibit high growth and colony forming potential (Lavoie and Stewart, 2012).

The recent discovery of iPSCs provides a potential alternative cell source for cell therapies and regenerative medicine (Takahashi and Yamanaka, 2006; Takahashi et al., 2007). However, a more immediate impact of iPSCs is its application to modeling human diseases and disorders (Chang et al., 2012; Grskovic et al., 2011); for example, iPSCs derived from patients harboring genetic perturbations that result in premature atherosclerosis can be used to screen for novel anti-atherosclerosis therapies. Since the initial demonstration of somatic reprogramming to a pluripotent state by the exogenous expression of Oct4, Sox2, Klf4, and c-Myc in dermal fibroblasts (Takahashi et al., 2007), numerous additional somatic cell types have been reprogrammed into iPSCs including neural progenitors (Eminli et al., 2008), B-cells (Choi et al., 2011), T-cells (Brown et al., 2010; Loh et al., 2010; Seki et al., 2010), adipocytes (Sun et al., 2009), and endothelial cells (Ho et al., 2010). Although some of these cell types can be reprogrammed with high efficiency, many are not readily accessible. Blood cells are perhaps the most easily accessible but lymphocytes, the targets of most established blood-derived iPSCs, have limited proliferative capacity preventing banking of sufficient primary cells for future reprogramming with improved technologies and use in genomic and proteomic assays. Alternatively, late-outgrowth EPCs (late-EPCs) can also be isolated from peripheral blood, in fact, from as little as 3–5 ml of blood (Martin-Ramirez et al., 2012). These cells exhibit high proliferative capacity; for example, 10^{19} cells can be generated from a starting 100 ml culture within 2 months (Lin et al., 2000). Interestingly, peripheral blood late-EPC progenitor frequencies are often altered in disease states. However, in general, pathogenic states increase the frequency of circulating late-EPC progenitors (Jodon de Villeroche et al., 2010; Thill et al., 2008) making them generally accessible from controls and patient's blood samples of 20–30 ml. In this report, we isolate late-EPCs from peripheral blood and reprogram these cells into iPSCs under feeder-free and defined media conditions.

Materials and methods

Derivation and cell culture of late-outgrowth EPCs

Three healthy donors were used to derive late-EPCs with informed consent. Leukapheresis product (lines 106 and 124) was isolated from patients using a Cobe Spectra 7.0 Apheresis System. Approximately 200 ml of mononuclear cell volume was collected from each patient. To derive late-EPCs from leukapheresis product, 40 ml from each sample was diluted with 3 volumes of Dulbecco's phosphate-buffered saline (DPBS) and centrifuged in a Ficoll-Paque PLUS (GE Healthcare) density gradient according to the manufacturer's instructions, whereas the PB1 late-EPC line was derived from 30 ml of whole peripheral blood. Mononuclear cells (MNCs) were fractionated from other components of peripheral blood by centrifugation on Ficoll-Paque PLUS. MNCs can be used immediately for late-EPC isolation or frozen in 10% DMSO without affecting isolation of late-EPCs or affecting the capacity of the late-EPCs to be reprogrammed. MNCs were then cultured on fibronectin-coated plates (Roche Applied Science) in Endothelial Growth Medium-2MV (CC-3202, Lonza) (basal endothelial media-2 (EBM), 20% human serum, VEGF, bFGF, IGF, EGF, gentamicin, ascorbic acid, and hydrocortisone). Late-outgrowth EPCs (late-EPCs) formed approximately 18 days after culturing in EGM-2MV. Late-EPCs exhibited a high proliferative capacity and had a cobblestone appearance. All experiments were performed with passage 7 or earlier late-EPCs. To passage late-EPC, cells were washed with PBS and trypsinized with 0.05% Trypsin-EDTA (25300120, Life Technologies).

In vitro endothelial cell tube formation assay

Endothelial tube formation assays were performed in 96-well plates coated with Matrigel (354230, Becton Dickinson), a reconstituted basement membrane matrix. Approximately, 2×10^4 late-EPCs were seeded into each well with 200 μL EGM-2MV + 20% human serum media. Capillary-like networks were then monitored and imaged 16 h later.

Reagents and cell culture of EPC-iPSCs

EPC-iPSCs were maintained on matrigel (BD Biosciences) in E8 media (DMEM/F12, L-ascorbic acid-2-phosphate magnesium) (Sigma), sodium selenium (14 μg/l, Sigma), FGF2 (100 μg/l, Life Technologies), insulin (19.4 mg/l, Roche), $NaHCO_3$ (543 mg/l, Sigma), transferrin (10.7 mg/l, Sigma), and TGFβ1 (2 μg/l, Life Technologies) (Chen et al., 2011) under hypoxic conditions (10% CO_2, 5% O_2). EPC-iPS colonies were initially passaged mechanically and then enzymatically with collagenase (#07923 or #07909, Stem Cell Technologies). Enzymatic passaging was performed by first washing the cells with 2 ml of PBS and then 1 ml of collagenase was added and the iPSCs were incubated at 37 °C for 10 min. The collagenase solution was removed and cells were rinsed once with DMEM/F12. Colonies were scraped with a cell scraper, dissociated to smaller clumps by resuspending E8 medium.