10-1-2012

Umbilical cord blood-derived aldehyde dehydrogenase-expressing progenitor cells promote recovery from acute ischemic injury

David M. Putman
Robarts Research Institute

Kevin Y. Liu
Robarts Research Institute

Heather C. Broughton
Robarts Research Institute

Gillian I. Bell
Robarts Research Institute

David A. Hess
Robarts Research Institute, dhess3@uwo.ca

STEM CELLS®

REGENERATIVE MEDICINE

Umbilical Cord Blood-Derived Aldehyde Dehydrogenase-Expressing Progenitor Cells Promote Recovery from Acute Ischemic Injury

DAVID M. PUTMAN, KEVIN Y. LIU, HEATHER C. BROUGHTON, GILLIAN I. BELL, DAVID A. HESS

Krembil Centre for Stem Cell Biology, Robarts Research Institute, Department of Physiology and Pharmacology, The University of Western Ontario, London, Ontario, Canada

Key Words. Hematopoietic progenitors • Angiogenesis • Xenotransplantation • Umbilical cord blood • Ischemic disease

ABSTRACT

Umbilical cord blood (UCB) represents a readily available source of hematopoietic and endothelial precursors at early ontogeny. Understanding the proangiogenic functions of these somatic progenitor subtypes after transplantation is integral to the development of improved cell-based therapies to treat ischemic diseases. We used fluorescence-activated cell sorting to purify a rare (<0.5%) population of UCB cells with high aldehyde dehydrogenase ($ALDH^{hi}$) activity, a conserved stem/progenitor cell function. $ALDH^{hi}$ cells were depleted of mature monocytes and T- and B-lymphocytes and were enriched for early myeloid (CD33) and stem cell-associated (CD34, CD133, and CD117) phenotypes. Although these cells were primarily hematopoietic in origin, UCB $ALDH^{hi}$ cells demonstrated a proangiogenic transcription profile and were highly enriched for both multipotent myeloid and endothelial colony-forming cells in vitro. Coculture of $ALDH^{hi}$ cells in hanging transwells promoted the survival of human umbilical vein endothelial cells (HUVEC) under growth factor-free and serum-free conditions. On growth factor depleted matrigel, $ALDH^{hi}$ cells significantly increased tube-like cord formation by HUVEC. After induction of acute unilateral hind limb ischemia by femoral artery ligation, transplantation of $ALDH^{hi}$ cells significantly enhanced the recovery of perfusion in ischemic limbs. Despite transient engraftment in the ischemic hind limb, early recruitment of $ALDH^{hi}$ cells into ischemic muscle tissue correlated with increased murine von Willebrand factor blood vessel and $CD31^{+}$ capillary densities. Thus, UCB $ALDH^{hi}$ cells represent a readily available population of proangiogenic progenitors that promote vascular regeneration. This work provides preclinical justification for the development of therapeutic strategies to treat ischemic diseases using UCB-derived $ALDH^{hi}$ mixed progenitor cells. STEM CELLS *2012;30:2248–2260*

Disclosure of potential conflicts of interest is found at the end of this article.

INTRODUCTION

Ischemic disease is characterized by the reduction of blood flow to the heart or peripheral tissues and encompasses life-threatening disorders such as ischemic heart disease and critical limb ischemia. Despite advances in pharmacological and surgical management, ischemic disease remains one of the leading causes of morbidity and mortality worldwide [1, 2]. Thus, novel therapies to promote the regeneration of damaged vasculature are under intense preclinical investigation [3–5].

Angiogenesis, the sprouting of new blood vessels from pre-existing vessels, is a central process for tissue repair, allowing the delivery of circulating proangiogenic cells that form a vascular regenerative microenvironment [5]. Asahara et al. first described human bone marrow (BM)-derived cells that promoted the recovery of blood flow and colocalized with new vessels after transplantation in vivo [6]. Later studies have shown that these rare cells, termed endothelial precursor cells (EPC), express the cell markers CD34, CD133, and VEGFR2 [7]. Because these markers were also expressed on clonally distinct cell types of both hematopoietic and nonhematopoietic lineages, there has been considerable controversy over which cell subtypes best promote vascular regeneration.

Endothelial colony-forming cells (ECFC) are rare blood-derived cells that form late-outgrowth adherent colonies of proliferative cells when propagated under strict endothelial cell (EC) growth conditions and can be isolated at increasing frequency from human peripheral blood, BM, and umbilical cord blood (UCB). ECFCs are distinguished from proangiogenic hematopoietic cell types in human blood as they are exclusively $CD45^{-}$ and can integrate directly into perfused vessels in vivo. Recently, it has been demonstrated that many of the cell types previously referred to as EPC are not EC precursors per se but represent myeloid/macrophage lineage cells that promote vascular repair through proposed paracrine signaling to vessel-derived EC [8, 9, 10–12]. In mouse models, circulating $CXCR4^{+}/VEGFR1^{+}$ hematopoietic cells are recruited to sprouting vessels by secretion of stromal-derived factor 1 or vascular endothelial growth factor-A (VEGF-A)

Author contributions: D.P.: conception and design, collection and assembly of data, data analysis and interpretation, manuscript writing, and final approval of manuscript; K.L. and G.B.: collection and assembly of data and data analysis and interpretation; H.B.: collection and assembly of data; D.H.: conception and design, financial support, collection and assembly of data, data analysis and interpretation, manuscript writing, and final approval of manuscript.

Correspondence: David A. Hess, Ph.D., Krembil Centre for Stem Cell Biology, Robarts Research Institute, Department of Physiology and Pharmacology, The University of Western Ontario, 100 Perth Drive, London, Ontario N6A 5K8, Canada. Telephone: 519-931-5777, ext. 24152; Fax: 519-931-5789; e-mail: dhess@robarts.ca Received May 24, 2012; accepted for publication July 11, 2012; first published online in STEM CELLS *EXPRESS* August 16, 2012. 1066-5099/2012/$30.00/0 doi: 10.1002/stem.1206

produced by damaged vasculature [4, 13]. Studies examining tumor metastasis have demonstrated that even small numbers of these hematopoietic cells play a major role in establishing a permissive niche for tumor vascularization [14–16].

Numerous studies have established that a decreased frequency or impaired function of circulating $CD133^+$ or $CD34^+$ cells is associated with increased cardiovascular risk in patients with ischemic heart disease and diabetes [2, 9, 17–20]. This, in addition to preclinical data showing the proangiogenic potential of BM-derived cells, has led to a number of clinical trials investigating treatment of cardiac [21–23] and limb ischemia [24, 25] by transplantation of heterogeneous human BM mononuclear cells (MNCs). Although these studies have demonstrated safety in the autologous setting, they have shown only modest improvements in clinical endpoints [26]. Thus, purification of specific proangiogenic cellular subtypes from nonautologous sources and further characterization of specific subtype contribution during the coordination of vascular repair are warranted to improve current cell-based therapies for ischemic diseases.

We have recently shown that UCB represents an excellent source of regenerative progenitor cells [27]. Early in ontogeny, UCB has been shown to contain a higher frequency of ECFC [28], and UCB-derived ECFCs have more robust vessel-forming capacity than ECFC from adult blood [9, 29]. Due to recent widespread initiatives to HLA-phenotype and cryopreserve this material, UCB now represents a readily available source of progenitor cells for cell therapy applications. In addition, allogeneic UCB progenitor cell transplantation may have distinct advantages over autologous BM in patients with obesity, chronic diabetes, or vascular disease-related pathologies due to compromised progenitor cell content and function [30–33].

Using high aldehyde dehydrogenase ($ALDH^{hi}$) activity, a cytosolic enzyme involved in retinoic acid metabolism and cellular self-protection from oxidative damage [34–39], we have developed fluorescence-activated cell sorting (FACS) strategies to simultaneously enrich for progenitor cells from hematopoietic, endothelial, and mesenchymal lineages [40]. We have demonstrated that transplanted $ALDH^{hi}$ progenitors reconstitute hematopoiesis [34, 35], exhibit widespread tissue distribution [41], and recruit to areas of ischemia after transplantation [40]. Furthermore, BM-derived $ALDH^{hi}$ cells can augment revascularization and blood flow of ischemic limbs in transplanted mice with acute ischemic injury [40]. Recently, we proposed a model of coordinated neovascularization after human progenitor cell transplantation wherein hematopoietic and nonhematopoietic progenitors act in concert to formulate a proangiogenic niche for vascular regeneration [27].

Here, we show that selection of human UCB cells with high ALDH activity simultaneously purifies a mixed population of primitive hematopoietic colony-forming cells (HCFCs) and ECFCs with potent proangiogenic functions. UCB $ALDH^{hi}$ progenitors demonstrated a proangiogenic transcription profile and supported EC survival in liquid culture and tube-like cord formation in growth factor-reduced matrigel. Transplanted $ALDH^{hi}$ cells improved recovery of blood flow after femoral artery ligation in non-obese diabetic/severe combined immunodeficient (NOD/SCID) mice. Thus, UCB progenitors purified by high ALDH-activity represent an allogeneic population of proangiogenic cells for the revascularization of ischemic tissues.

Materials and Methods

Progenitor Cell Isolation from Human UCB

UCB was collected after informed consent by venipuncture at the London Health Sciences Birthing Centre. The Human Research Ethics Committee at the University of Western Ontario approved all studies. After hypaque-ficoll centrifugation, MNCs were depleted of erythrocytes by ammonium chloride lysis and assayed for ALDH activity using Aldefluor reagent (Stem Cell Technologies, Vancouver, Canada, stemcell.com), as previously described [40]. FACS was performed to isolate cells with low side scatter and high ($ALDH^{hi}$) versus low ($ALDH^{lo}$) ALDH activity to >99% purity. Sorted cells were washed in phosphate-buffered saline (PBS) to allow efflux of Aldefluor substrate via reactivation of inhibited adenosine-5′-triphosphate-binding cassette-transporters.

Cell Surface Phenotype Analysis

UCB MNC, $ALDH^{hi}$ or $ALDH^{lo}$ cells were labeled with anti-human antibodies against pan-leukocyte marker CD45 and colabeled with CD4 and CD8 (T-cells), or CD19 and CD20 (B cells), or CD33 (myeloid) and CD14 (monocytes), or CD31 (PECAM-1) and CD144 (VE-cadherin, GeneTex, Irvine, CA, genetex.com), or CD34 and CD38, or CD117 (c-kit) and CD133 (Miltenyi Biotec, Cologne, Germany, miltenyibiotec.com), or CXCR4 and VEGFR2. All antibodies were from BD Biosciences unless otherwise indicated. Cell surface marker expression was measured using a FACSCalibur cytometer (BD Biosciences, San Jose, CA, www.bdbiosciences.com) and analyzed using FlowJo software (Tree Star, Ashland, OR, flowjo.com).

Colony-Forming Cell Assays

UCB MNC, $ALDH^{hi}$ or $ALDH^{lo}$ cells were cultured in methylcellulose (Methocult H4434, Stem Cell Technologies) for 14 days and hematopoietic colony formation was enumerated based on cell morphology identifying burst-forming units of erythrocytes, colony-forming units of granulocytes, macrophages, granulocytes and macrophages, or multilineage mixed colonies containing cells of all three types. ECFCs were enumerated after adherent culture for 14 days in complete endothelial growth media (EGM-2 + 2% fetal bovine serum + epidermal growth factor [EGF], VEGF-A, fibroblast growth factor [FGF], insulin-like growth factor-1 [IGF-1], Lonza, Basel, Switzerland, www.lonza.com), as previously described [9].

mRNA Isolation and Microarray Analyses

mRNA was from three sample-matched UCB $ALDH^{hi}$ and $ALDH^{lo}$ cell cohorts using mRNeasy Mini kits (Qiagen, Dusseldorf, Germany, qiagen.com). Microarrays were performed in triplicate using 18 human gene array chips (Affymetrix, Santa Clara, CA, affymetrix.com) at the London Regional Genomics Facility. Transcripts differentially expressed by UCB $ALDH^{hi}$ versus $ALDH^{lo}$ cells were analyzed using Partek Genomics Suite (Partek Inc., St Louis, MO, partek.com).

Coculture Assays with Human Umbilical Vein ECs

To assess paracrine functions of UCB cells on EC (human umbilical vein EC, HUVEC) survival and proliferation during coculture in vitro, 40×10^3 HUVEC were cultured in endothelial basal media (EBM2, Lonza) with or without angiogenic growth factors (EGF, VEGF-A, FGF, and IGF-1) and serum (2%) supplementation. Under each condition, 10^5 $ALDH^{hi}$ or 10^5 $ALDH^{lo}$ cells were seeded into porous 5 μm hanging transwells suspended above the HUVEC. The number of trypan blue excluding HUVEC present after 72 hours coculture was enumerated by blinded hemocytometer counts. For tube-like cord formation assays, 50×10^3 HUVEC, or HUVEC supplemented with 20×10^3 $ALDH^{hi}$ or $ALDH^{lo}$ cells were seeded on growth factor-reduced Matrigel (BD Biosciences) and cultured for 72 hours. The number of complete branch points in the cord structures formed by HUVEC was enumerated on an inverted microscope by blinded manual counts of four fields of view at 24, 48, and 72 hours. All coculture experiments were performed in triplicate.

Murine Femoral Artery Ligation Surgery and Transplantation of Human UCB Cells

Right femoral and saphenous artery and vein ligation with complete excision of the femoral artery and vein was performed on

anesthetized NOD/SCID (Jackson Laboratory, Bar Harbor, ME, jax.org) or NOD/SCID/mucopolysaccharidosis type VII (MPSVII) mice as previously described [40]. Within 24 hours of surgery, nonirradiated or sublethally irradiated (300 cGy) mice were transplanted by tail vein injection with PBS, 2×10^5 ALDH^{hi} cells, 10×10^6 ALDH^{lo} cells, or 20×10^6 MNC. Cell doses were chosen based on the relative frequency of each cell type within UCB MNC. NOD/SCID mice were used for functional studies to monitor the recovery of perfusion and to quantify blood vessel density as described below. NOD/SCID/MPSVII mice were used to detect human cell engraftment kinetics and localization in muscle sections from ischemic limbs using β-glucuronidase (GUSB) staining as described below.

Quantification of Hind Limb Perfusion Using Laser Doppler Perfusion Imaging

For laser Doppler perfusion imaging (LDPI; MoorLDI-2; Moor Instruments, Devon, U.K., moor.co.uk), anesthetized NOD/SCID mice were heated at 37°C for 5 minutes and blood flow was quantified within the hind quarters of live mice after surgery, and at 3, 7, 14, 21, and 28 days post-transplantation. Perfusion ratios (PRs) comparing blood flow in the ischemic versus nonischemic limb were quantified by averaged units of flux using Moorflow software (Moor Instruments).

Quantification of Blood Vessel Density

Adductor muscles from transplanted mice were embedded and frozen in optimum cutting temperature medium (Tissue Tek, Sakura Finetek, Tokyo, Japan, sakura-finetek.com), cryosectioned, fixed in 10% formalin (Sigma), and blocked with mouse-on-mouse reagent (Vector Labs, Burlingame, CA, vectorlabs.com). Murine blood vessel density in ischemic and nonischemic limbs was quantified at day 28 by counting mouse CD31$^+$ capillaries and mouse von Willebrand factor (vWF$^+$) blood vessels using rat anti-mouse CD31 (1:100; BD Biosciences) or rabbit anti-mouse vWF antibody (1:200; Millipore, Billerica, MA, millipore.com) and peroxidase-labeled anti-rat or anti-rabbit IgG secondary antibodies (Vector Labs) visualized using 3,3′-diaminobenzidine substrate (Vector), and counterstained with hematoxylin. Blood vessel density was counted in a blinded fashion from nine photomicrographic fields using light microscopy.

Quantification of Human Cell Engraftment by Flow Cytometry

BM and adductor muscles from the ischemic and nonischemic limbs were analyzed for human cell engraftment at 3, 7, and 28 days after transplantation by flow cytometric detection of human CD45 and HLA-A,B,C in combination with 7-AAD viability dye (BD Biosciences), as previously described [40]. Muscle samples were digested with type II collagenase (Worthington Biochemical, Lakewood, NJ, worthington-biochem.com) prior to antibody labeling.

Detection of Human Cells by GUSB Activity

Transplanted NOD/SCID/MPSVII mice with unilateral hind limb ischemia were sacrificed at 3, 7, and 28 days after transplantation to allow histochemical detection of single human cell engraftment in ischemic muscle sections (GUSB-deficient) based on the detection of ubiquitous GUSB activity in human cells as previously described [42]. Muscle sections were costained with vWF to assess the distribution of engrafted human cells with respect to murine vasculature and with human CD45 to assess the lineage of engrafted cells.

Statistics

Analysis of significance was performed by one-way analysis of variance (ANOVA) for cell surface marker and progenitor frequency assays and by two-way ANOVA with Bonferroni post hoc tests for all other assays using Graphpad Prism software.

Results

UCB ALDH^{hi} Cells Were Enriched for Myeloid Progenitor Cell Surface Phenotypes

Human UCB MNCs were collected without prior lineage depletion [34] and purified based on cells with low side scatter (nongranular) and with low (ALDH^{lo}, 41.36% ± 3.6%) versus high (ALDH^{hi}, 0.4% ± 0.1%) ALDH activity compared to ALDH-inhibited (diethylaminobenzaldehyde, DEAB) controls ($n = 10$, Fig. 1A, 1B). Compared to unpurified UCB MNC (97.7% ± 0.8% CD45$^+$) or ALDH^{lo} cells (99.1% ± 0.5% CD45$^+$), ALDH^{hi} cells showed lower expression of the pan-leukocyte marker CD45 (93.3% ± 1.9% CD45$^+$; *, $p < .05$; $n = 4$). Thus, sorted cell populations were gated into hematopoietic (CD45$^+$) and nonhematopoietic (CD45$^-$) subsets and further analyzed for primitive versus mature hematopoietic or EC surface marker expression.

CD45$^+$ ALDH^{hi} cells showed reduced expression lineage-specific markers for T- (CD4 and CD8, Fig. 1C) and B-lymphocytes (CD19 and CD20, Fig. 1D) compared to unsorted MNC or ALDH^{lo} cells (**, $p < .01$; Table 1A). More than 90% of CD45$^+$ ALDH^{hi} cells expressed CD33, an early myeloid lineage marker, but coexpression of the mature monocyte marker CD14 was low (3.3% ± 1.0%, Fig. 1E; Table 1A). Similarly, ALDH^{hi} cells showed near homogeneous expression of CD31 or PECAM-1 (96.7% ± 2.9%) but low coexpression of CD144/VE-cadherin (3.2% ± 1.0%, Fig. 1F; Table 1A). Notably, the CD45$^+$ ALDH^{hi} cells were highly enriched for the coexpression of the progenitor markers CD34 (89.6% ± 5.9%, Fig. 1G), CD117 (80.2% ± 4.9%), and CD133 (61.5% ± 2.3%, Fig. 1H) compared to unsorted UCB MNC or ALDH^{lo} cells (*, $p < .05$, Table 1A). CD45$^+$ ALDH^{hi} cells were enriched for SCID repopulating progenitor phenotype (CD34$^+$CD38$^-$, Fig. 1G) [43], and ALDH^{hi}CD34$^+$ cells showed coexpression of the homing marker CXCR4 (14.2% ± 2.9%, Fig. 1I) but low expression of VEGFR2/kinase insert domain receptor (KDR) (0.5% ± 0.1%, Fig. 1J).

CD45$^-$ cells, representing approximately 7% of the ALDH^{hi} population, also showed enriched expression of CD34 (65.0% ± 14.8%), CD117 (64.9% ± 12.4%), and CD133 (22.5% ± 3.9%) progenitor markers (*, $p < .05$) but did not show increased expression of the mature EC-associated adhesion molecules (CD31 and CD144) compared to UCB MNC or ALDH^{lo} cells (Table 1B). Collectively, these data indicate that the hematopoietic fraction of ALDH^{hi} cells was depleted of mature lymphocytes and monocytes yet enriched for primitive myeloid progenitor phenotype, and the CD45$^-$ portion of the ALDH^{hi} cells similarly retained a primitive nonhematopoietic cell phenotype.

UCB ALDH^{hi} Cells Demonstrated Hematopoietic and Endothelial Colony Formation

UCB MNC, ALDH^{lo} or ALDH^{hi} cells were plated under lineage-specific growth conditions for 14 days to assess HCFC and ECFC frequency. After culture in methylcellulose, ALDH^{hi} cells were highly enriched for myeloid lineage colony-forming cells (one HCFC in 3.6 cells) compared to ALDH^{lo} cells (one HCFC in 6.6×10^3 cells; **, $p < .01$; $n = 4$). ALDH^{hi} HCFC demonstrated multipotent lineage differentiation with increased production of erythroid, macrophage, granulocyte, and mixed morphologies (***, $p < .001$; Fig. 1K). To assess ECFC function in vitro, ALDH-purified UCB cells were cultured under stringent conditions established by Yoder et al. [9]. Colony enumeration revealed increased ECFC content in ALDH^{hi} cells (one ECFC in 4.6×10^4 cells) compared to ALDH^{lo} cells (one ECFC in 2.2×10^5 cells; *, $p < .05$; Fig. 1L). ECFC progenies were highly proliferative, did not express hematopoietic

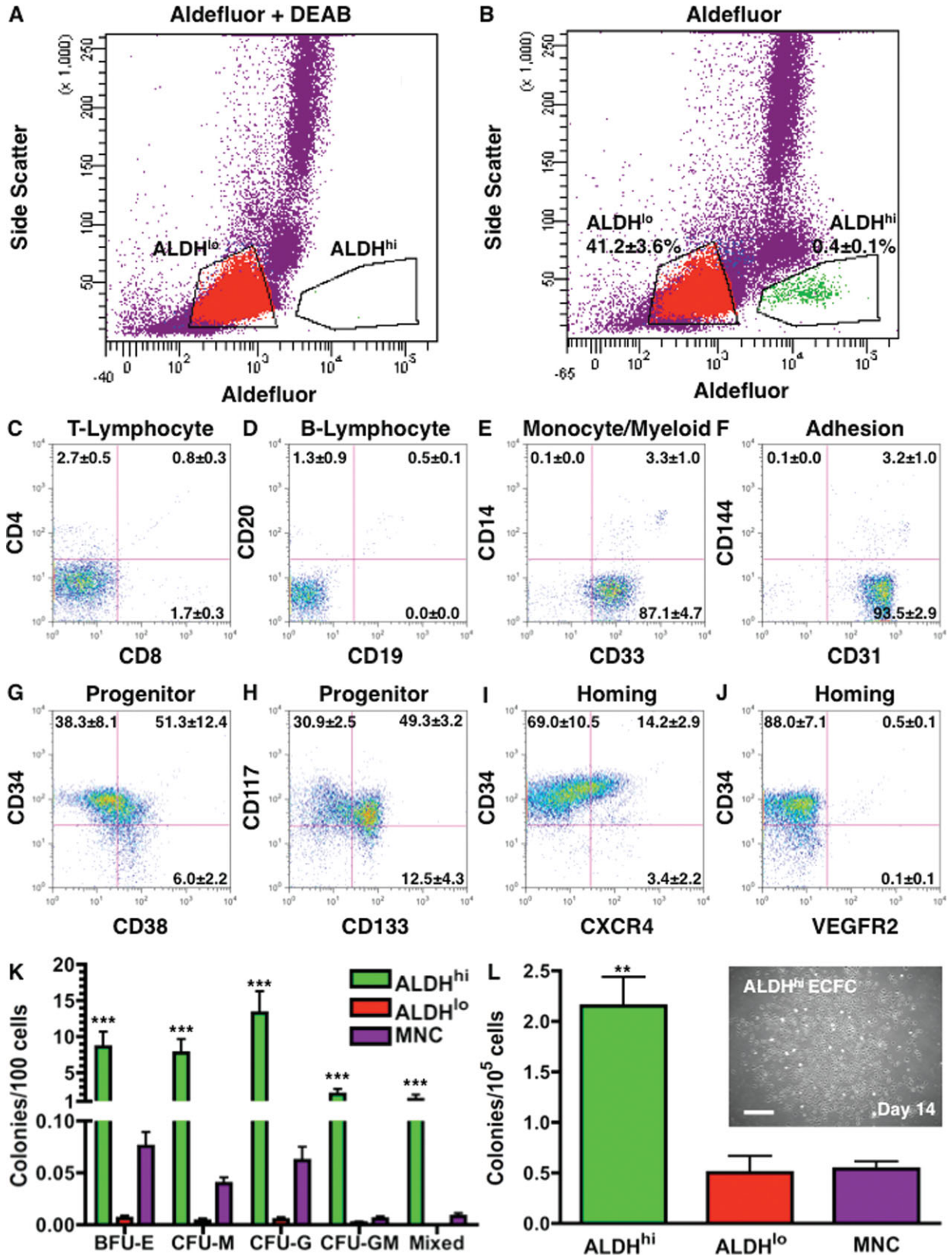

Figure 1. Purification of umbilical cord blood (UCB) ALDH^{hi} cells enriches for hematopoietic colony-forming cells (HCFCs) and ECFCs. **(A,B):** Human UCB MNCs were sorted for low side scatter and low (ALDH^{lo} = 41.2% ± 3.6%) versus high (ALDH^{hi} = 0.4% ± 0.1%) ALDH activity with or without DEAB inhibitor (n = 10). Representative flow cytometry plots show that UCB ALDH^{hi} cells were depleted of cells expressing mature **(C)** T-lymphocyte (CD4 and CD8), **(D)** B-lymphocyte (CD19 and CD20), **(E)** monocyte (CD14), and **(F)** endothelial (VE-cadherin and CD144) cell surface markers and were enriched for cells expressing (E) early myeloid (CD33), (F) adhesion (PECAM-1, CD31), and **(G, H)** primitive progenitor (CD34, CD1117, and CD133) markers. **(I, J):** CD34^{+}ALDH^{hi} cells showed low coexpression of chemokine receptors (CXCR4 and VEGFR2). **(K):** After culture for 14 days in methylcellulose media (n = 7), UCB ALDH^{hi} cells were highly enriched for multipotent HCFC. **(L):** After culture for 14 days in complete endothelial growth media (EGM-2 + IGF, EGF, bFGF, VEGF-A + 2% serum, n = 5), UCB ALDH^{hi} were also enriched for ECFC. Scale bar = 200 μm. Data are expressed as mean ± SEM; **, $p < .01$; ***, $p < .001$. Abbreviations: ALDH, aldehyde dehydrogenase; BFU-E, burst-forming units of erythrocytes; CFU, colony-forming unit; CFU-G, colony-forming units of granulocytes; CFU-M, colony-forming units of macrophages; CFU-GM, colony-forming units of granulocytes and macrophages; DEAB, diethylaminobenzaldehyde (ALDH inhibitor); ECFC, endothelial colony-forming cell; MNC, mononuclear cell.

Table 1. Cell surface marker expression for (A) hematopoietic (gated CD45+) component and (B) nonhematopoietic (gated CD45−) component of human UCB MNC, ALDH^{hi} cells, and ALDH^{lo} cells

Lineage	Markers (%)	MNC	ALDH^{hi}	ALDH^{lo}
(A)				
T-lymphocyte	CD4	47.6 ± 3.2[a]	3.5 ± 0.6**[b]	47.8 ± 2.1[a]
	CD8	24.9 ± 0.9[a]	2.5 ± 0.3**[b]	26.1 ± 3.5[a]
B-lymphocyte	CD19	20.3 ± 1.8[a]	0.6 ± 0.1**[b]	21.1 ± 1.4[a]
	CD20	23.6 ± 1.8[a]	1.8 ± 0.9**[b]	21.1 ± 1.7[a]
Myeloid/monocyte	CD33	20.9 ± 0.9[a]	90.4 ± 4.8**[b]	1.4 ± 0.3**[c]
	CD14	17.7 ± 1.2[a]	3.3 ± 1.1**[b]	0.6 ± 0.2**[b]
Adhesion	CD31	86.5 ± 1.2[a]	96.7 ± 2.9*[b]	81.0 ± 3.5[a]
	CD144	21.5 ± 2.0[a]	3.2 ± 1.0**[b]	4.9 ± 2.9**[b]
Progenitor	CD34	5.1 ± 1.9[a]	89.6 ± 5.9**[b]	0.3 ± 0.1[a]
	CD117/c-kit	15.9 ± 2.8[a]	80.2 ± 4.9**[b]	12.5 ± 5.3[a]
	CD133	0.7 ± 0.3[a]	61.5 ± 2.3**[b]	0.2 ± 0.1[a]
Homing	CXCR4	47.3 ± 6.1[a]	17.7 ± 2.5*[b]	37.8 ± 5.8[a]
	KDR/VEGFR2	0.6 ± 0.3[a]	0.9 ± 0.3[a]	0.3 ± 0.1[a]
(B)				
Adhesion	CD31	50.8 ± 24.9[a]	38.7 ± 16.6[a]	20.3 ± 13.2[a]
	CD144	20.0 ± 12.5[a]	17.8 ± 11.3[a]	6.5 ± 3.8[a]
Progenitor	CD34	17.9 ± 9.2[a]	65.0 ± 14.8*[b]	1.3 ± 0.6**[c]
	CD117/c-kit	22.1 ± 9.8[a]	64.9 ± 12.4*[b]	12.8 ± 3.5[a]
	CD133	6.9 ± 1.5[a]	22.5 ± 3.9*[b]	0.4 ± 0.3[a]
Homing	CXCR4	33.9 ± 10.1[a]	8.8 ± 3.4*[b]	7.4 ± 1.1*[b]
	KDR/VEGFR2	1.8 ± 0.8[a]	1.9 ± 1.0[a]	1.4 ± 1.1[a]

Human UCB MNC, or ALDH^{hi} or ALDH^{lo} cells were stained with anti-human antibodies to assess the cell surface marker phenotype on (A) hematopoietic and (B) nonhematopoietic cells. UCB ALDH^{hi} hematopoietic cells were depleted of mature T- and B-lymphocytes and monocytes and were highly enriched for the early myeloid marker CD33 and platelet-derived endothelial cell adhesion molecule CD31 (PECAM-1). UCB ALDH^{hi} hematopoietic and nonhematopoietic cells were enriched for progenitor cell markers CD34, CD117, and CD133. Data represents mean ± SEM ($n = 4$), different letters denote different expression patterns at *, $p < .05$; **, $p < .01$ compared to unpurified MNC.

Abbreviations: ALDH, aldehyde dehydrogenase; MNC, mononuclear cell; UCB, umbilical cord blood.

markers (CD45 and CD14), and expressed EC markers (CD31, CD105, and CD144, data not shown). Unlike human BM [40], MNC or ALDH-sorted cohorts from UCB did not establish plastic adherent multipotent stromal cell colonies in vitro ($n = 10$). Thus, UCB ALDH^{hi} cells represented a mixture of cells with enriched HCFC and ECFC function.

UCB ALDH^{hi} Cells Demonstrated a Proangiogenic Transcription Profile

Affymetrix microarray identified 1,253 transcripts with significantly increased (*, $p < .05$) expression in three UCB ALDH^{hi} cell samples compared to sample-matched ALDH^{lo} cells. These transcripts were first filtered to include unique genes translating to plasma membrane bound (196 hits) or extracellular (108 hits) locations, and subsequently filtered for documented angiogenic biological functions (Supporting Information Tables S1, S2). As a control for the selection of cells with high ALDH-activity, *ALDH1A1* mRNA expression was increased 42-fold in ALDH^{hi} versus ALDH^{lo} cells ($p = 2.08 \times 10^{-5}$). Furthermore, significantly increased *CD34, PROM1*, and *KIT* mRNA expression in ALDH^{hi} cells (Supporting Information Table S1) correlated with increased CD34, CD133, and c-kit/CD117 cell surface protein expression previously observed by FACS (Fig. 1, Table 1). Transcripts associated with early myeloid progenitor differentiation (*FLT3, CSF3R,* and *CD33*), integrin-mediated cell adhesion (*ITGA9, ITGAV,* and *ITGA2B*), and vascular cell signaling (*TIE1, TIE2, FGFR1,* and *endoglin*) were also highly expressed by UCB ALDH^{hi} cells (Supporting Information Table S1). Interestingly, transcripts for secreted cytokines associated with vascular functions (*ANGPT1*, *FGF16*, and *VEGFA*), immune cell migration (*IL18*, *IL1β*, *IL8*, *CSF1*, and *CXCL2*), and modulators of EGF and IGF signaling (*EREG*, *AREG*, *HBEGF*, and *IGFBP7*) were also increased in UCB ALDH^{hi} cells (Supporting Information Table S2). Collectively, these data suggested that UCB ALDH^{hi} cells possessed a proangiogenic transcription profile compared to ALDH^{lo} cells.

UCB ALDH^{hi} Cells Augment the Survival of HUVEC In Vitro

To assess the paracrine functions of ALDH-purified UCB cells on EC survival and proliferation in vitro, 40×10^3 HUVECs were seeded into transwell cocultures for 72 hours using a porous 5 μm hanging insert containing 10^5 UCB ALDH^{hi} or ALDH^{lo} cells (or basal media control) suspended above HUVEC. Under optimal conditions containing growth factors (GF$^+$) and serum, noncontact coculture with ALDH^{lo} or ALDH^{hi} cells did not impact the fivefold expansion of HUVEC observed over 72 hours (Fig. 2A, 2E). Conversely, in GF$^-$/serum-free conditions with or without ALDH^{lo} cell supplementation, HUVEC invariably died within 72 hours (Fig. 2B, 2E). In contrast, coculture with ALDH^{hi} cells under GF$^-$/serum-free conditions preserved HUVEC viability for 72 hours (*, $p < .05$; Fig. 2B, 2E). Under GF$^+$/serum-free conditions, ALDH^{hi} cell coculture promoted a modest expansion (1.7-fold) of HUVEC ($67.4 \pm 7.1 \times 10^3$ cells), compared to more than threefold HUVEC loss with basal media ($12.4 \pm 2.6 \times 10^3$ cells) or ALDH^{lo} cell supplementation ($14.4 \pm 2.8 \times 10^3$ cells; *, $p < .05$; Fig. 2C, 2E). Under GF$^-$/serum$^+$ conditions, ALDH^{lo} or ALDH^{hi} coculture did not significantly alter HUVEC numbers (Fig. 2D, 2E). Thus, noncontact coculture with human ALDH^{hi} cells promoted HUVEC survival under GF$^-$/serum-free conditions. These effects were consistent with the prosurvival stimulus in HUVEC provided by VEGF-A treatment [44], a central proangiogenic cytokine with increased mRNA-expression in UCB ALDH^{hi} cells (Supporting Information Table S2).

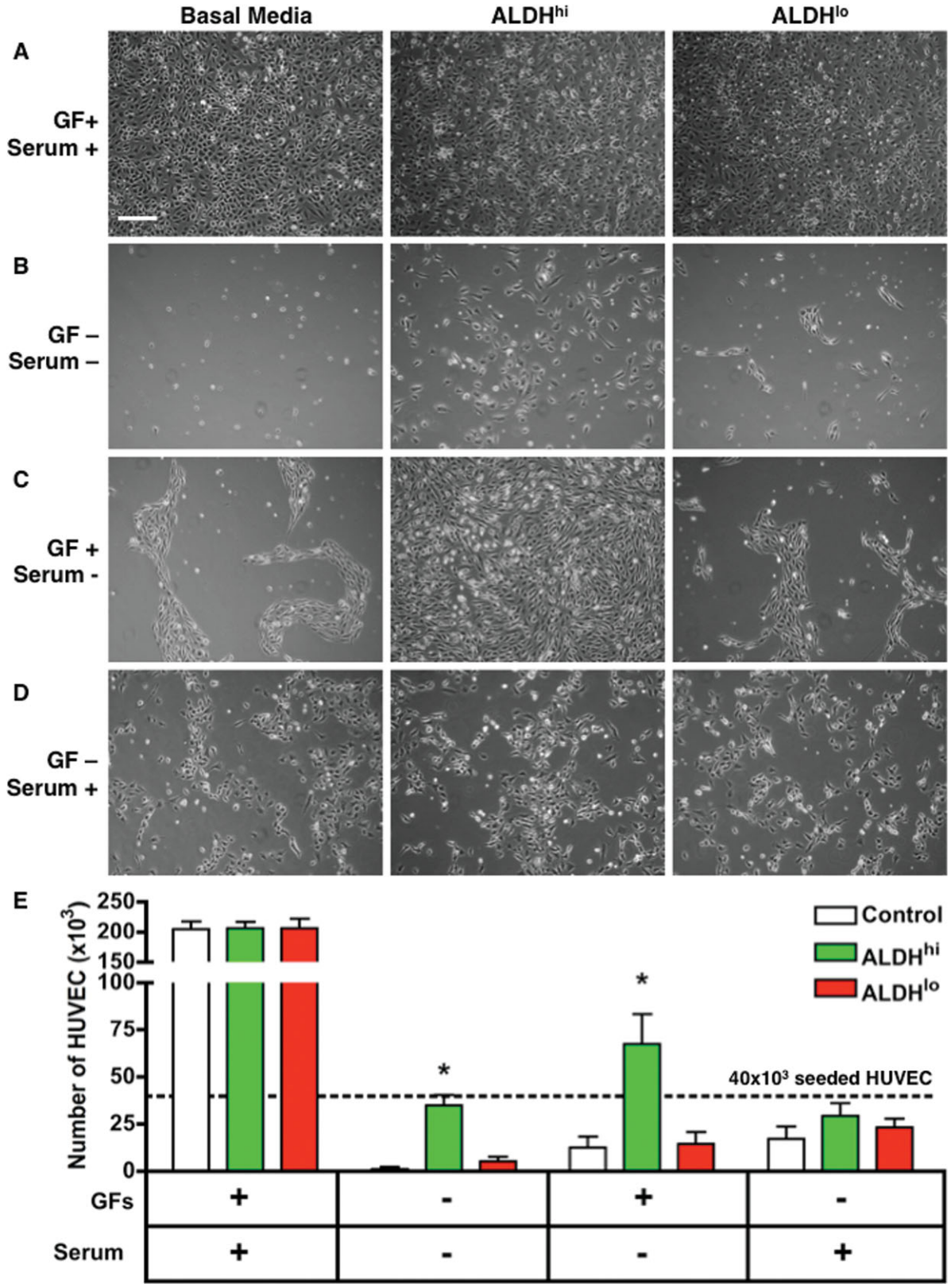

Figure 2. Umbilical cord blood (UCB) ALDH^{hi} cells promote endothelial cell survival under growth factor and serum-free conditions. **(A--D):** Representative photomicrographs of HUVEC density after 72 hours coculture with UCB ALDH^{lo} or ALDH^{hi} cells in hanging transwells with or without growth factor or serum supplementation. Scale bar = 200 μm. **(E):** Noncontact coculture with human UCB ALDH^{hi} cells promoted the survival of HUVEC under growth factor-free, serum-free conditions ($n = 5$). Data are expressed as mean ± SEM; *, $p < .05$. Abbreviations: ALDH, aldehyde dehydrogenase; GF, growth factor; HUVEC, human umbilical vein endothelial cell.

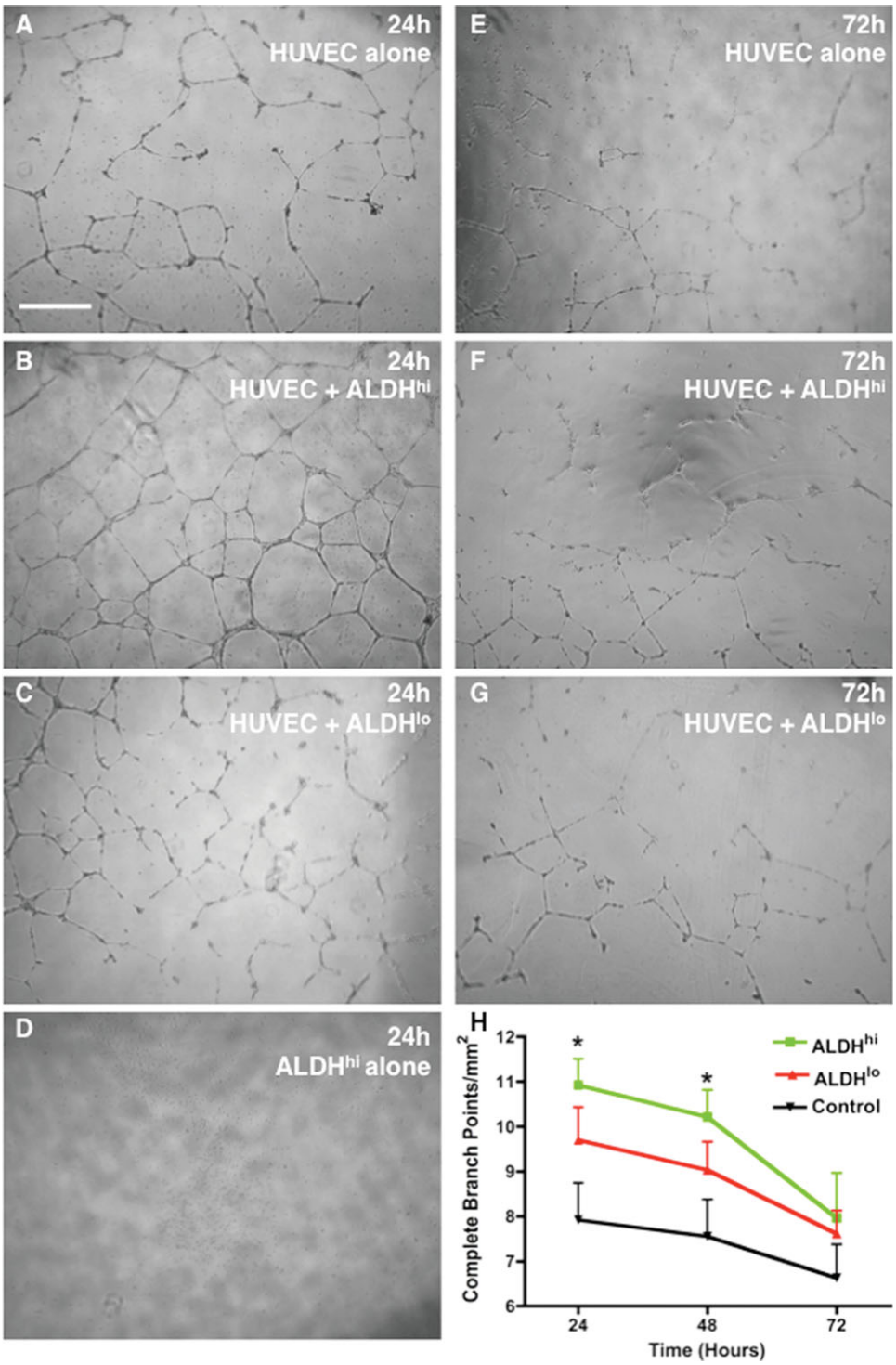

Figure 3. Umbilical cord blood ALDH^{hi} cells augmented tube-like cord formation by HUVEC. Representative photomicrographs of tubule formation by HUVEC after 24 **(A--C)** and 72 **(E--G)** hours coculture with ALDH^{lo} or ALDH^{hi} cells or media control. Scale bar = 500 μm. **(D):** ALDH^{hi} cells plated without HUVEC did not form cord structures. **(H):** Coculture with ALDH^{hi} cells augmented the formation of complete tubule networks formed by HUVEC at 24 and 48 hours, but did not augment tubule stability after 72 hours coculture. Data are expressed as mean ± SEM; *, $p < .05$. Abbreviations: ALDH, aldehyde dehydrogenase; HUVEC, human umbilical vein endothelial cell.

UCB ALDH^{hi} Cells Augment Tube-Like Cord Formation by HUVEC In Vitro

HUVECs (50×10^3) were cultured alone or cocultured with ALDH^{hi} or ALDH^{lo} cells (20×10^3) on growth factor-reduced Matrigel. HUVEC established partially completed cord networks within 24 hours (Fig. 3A). Although coculture of HUVEC with UCB ALDH^{lo} cells did not significantly alter cord formation, supplementation with ALDH^{hi} cells augmented HUVEC tube-like cord formation at 24 hours (Fig. 3B, 3C). Although UCB ALDH^{hi} cells established

ECFC in liquid culture (Fig. 1L, inset), UCB ALDH^{lo} or ALDH^{hi} cells seeded alone on growth factor-reduced Matrigel did not spontaneously align to form cord networks (Fig. 3D), and tube-like cord stability of HUVEC was not enhanced by coculture with UCB ALDH^{lo} or ALDH^{hi} at 72 hours (Fig. 3E--3H). Enumeration of completed branch points at 24 and 48 hours revealed that coculture with UCB ALDH^{hi} cells augmented cord formation by HUVEC compared to media controls ($n = 6$; *, $p < .05$, Fig. 3H). Therefore, coculture with UCB ALDH^{hi} cells promoted the cord-forming function of HUVEC in vitro.

Transplanted UCB ALDH^{hi} Cells Enhanced the Recovery of Hind Limb Perfusion

To determine whether ALDH-purified human UCB cells could support blood vessel regeneration after intravenous transplantation in vivo, we induced acute unilateral hind limb ischemia in NOD/SCID mice by right femoral artery ligation with complete excision of a 0.5-cm section of the femoral artery and vein. Blood flow was assessed by LDPI and the PR in the ischemic versus control limb was decreased >10-fold (PR = 0.08 ± 0.02) postsurgery confirming consistent induction of unilateral hind limb ischemia (Fig. 4). Within 24 hours of surgery, mice were tail vein injected with PBS vehicle control, unsorted UCB MNC, ALDH^{hi} cells, or ALDH^{lo} cells. The representative LDPI shown in Figure 4 documents the recovery of limb perfusion after transplantation. PBS-injected control mice showed recovery of PR from 0.07 ± 0.01 after surgery to 0.36 ± 0.05 by day 7 postsurgery without subsequent improvement at later time points (Fig. 4A, 4E). This baseline recovery of perfusion was sufficient to prevent excess morbidity or limb loss in PBS transplanted mice. Mice transplanted with 20×10^6 MNC or 10×10^6 ALDH^{lo} cells showed equivalent recovery of limb perfusion similarly compared to PBS controls (Fig. 4B, 4D, 4E). In contrast, mice transplanted with 50–100-fold fewer ALDH^{hi} cells (2×10^5) showed increased perfusion by day 14 post-transplantation (PR = 0.58 ± 0.07) compared to all other treatments and augmented perfusion was maintained for 28 days post-transplantation (PR = 0.64 ± 0.06; **, $p < .01$; Fig. 4C, 4E). Thus, intravenous transplantation of human UCB ALDH^{hi} cells improved the limb perfusion in mice with acute unilateral hind limb ischemia.

Transplanted UCB ALDH^{hi} Cells Increased Capillary Density in the Ischemic Limb

To address the recovery of blood vessels in the ischemic hind limb, sections from the adductor muscle at the site of artery ligation were stained for both murine CD31$^+$ capillary (Fig. 5A--5C) and vWF$^+$ blood vessel (Fig. 5D--5F) density. Compared to the nonischemic limb at day 28, PBS-injected mice showed significant reduction (*, $p < .05$) of both CD31$^+$ capillaries (Fig. 5G) and vWF$^+$ blood vessels (Fig. 5F) within the ischemic hind limb. However, mice transplanted with UCB ALDH^{hi} cells had significantly more CD31$^+$ capillaries compared to mice transplanted with ALDH^{lo} cells (*, $p < .05$) or PBS (**, $p < .01$; Fig. 5G). Similarly, mice transplanted with ALDH^{hi} cells showed higher vWF$^+$ blood vessel density than mice injected with ALDH^{lo} cells or MNC (*, $p < .05$), or PBS-injected controls (***, $p < .001$, Fig. 5H). Despite only partial recovery of perfusion, CD31$^+$ and vWF$^+$ vessel density in the ischemic limbs of ALDH^{hi} cell-treated mice were equivalent to the vessel density of the nonischemic control limb at day 28 (Fig. 5G, 5H). Thus, UCB ALDH^{hi} cell transplantation increased both CD31$^+$ capillary and vWF$^+$ blood vessel density in the ischemic hind limb after femoral artery ligation.

Transplanted UCB ALDH^{hi} Cells Were Recruited to the Ischemic Muscle at Low Frequency

In order to correlate the recovery of perfusion with human cell engraftment, murine BM and adductor muscle were collected at 3, 7, and 28 days after transplantation and analyzed for the presence of human CD45$^+$ or HLA-A,B,C$^+$ cells by flow cytometry. Despite consistent human cell hematopoietic chimerism in the BM of mice transplanted with ALDH^{hi} cells (Fig. 6A, 6C) or MNC (Fig. 6C), human cells were not significantly detected in the adductor muscle of ischemic limbs by flow cytometry (Fig. 6B, 6D) at day 28. In addition, mice processed for engraftment at earlier time points showed no evidence of human cells in the ischemic limb at three ($n = 3$) or 7 days ($n = 3$) post-transplantation by FACS. Therefore, we repeated engraftment experiments using identical cell doses in the GUSB-deficient NOD/SCID/MPSVII recipient to permit the detection of transplanted human cells at single-cell resolution. At 3 and 7 days post-transplantation, the detection of GUSB-expressing cells confirmed the recruitment of human cells to the ischemic hind limbs of mice that received MNC ($n = 4$, Fig. 6E, 6F) or ALDH^{hi} cells ($n = 4$, Fig. 6H, 6I). However, human cells were not detected in the ischemic limbs of mice that received ALDH^{lo} cells ($n = 4$). Although transplanted cells were never detected in the nonischemic limb (data not shown), engrafted MNC and ALDH^{hi} cells were observed in ischemic muscle sections surviving out to 28 days (Fig. 6G, 6J). Staining for GUSB-activity combined with murine vWF showed engraftment of single human cells between muscle fibers (Fig. 6K) and in association with the connective tissue surrounding blood vessels (Fig. 6L). Also, the majority of GUSB$^+$ human cells surviving at day 28 costained for CD45 (Fig. 6M). Despite low frequency engraftment in the ischemic region, early recruitment of ALDH^{hi} progenitor cells to the site of ischemic injury was sufficient to augment blood vessel density and improve limb perfusion.

Sublethal Irradiation Did Not Improve Perfusion in the Ischemic Hind Limb

The requirement for preparative irradiation is an important consideration in the design of clinically applicable cell therapies for ischemic diseases. In an attempt to improve human cell engraftment in the ischemic limb, transplantation experiments were repeated after sublethal irradiation (300 cGy) administered as a means to reduce xenorejection by residual innate immunity or natural killer-cell activity in the NOD/SCID model. Surprisingly, in mice receiving UCB ALDH^{hi} cells, perfusion at early time points (days 3, 7, and 14) was similar for all transplanted groups (Supporting Information Fig. S1A--S1E). Although perfusion improved in the ALDH^{hi} cell transplanted cohort at day 21, perfusion was reduced to baseline by day 28 (Supporting Information Fig. S1E), indicating that only a transient improvement in limb perfusion was observed. Compared to mice transplanted without preparative irradiation (Fig. 4), irradiated mice receiving an equal dose of UCB ALDH^{hi} cells actually showed delayed recovery of perfusion at day 14 ($PR_{300\ cGy} = 0.40 \pm 0.05$ vs. $PR_{0\ cGy} = 0.58 \pm 0.07$). Nonetheless, at day 28, irradiated mice injected with ALDH^{hi} cells still showed improved vWF$^+$ blood vessel density (Supporting Information Fig. S2) similar to nonirradiated mice.

Irradiated mice also showed enhanced recruitment of ALDH^{hi} cells to the BM at day 7 (1.3% ± 0.2%, Supporting Information Fig. S3A, S3C), resulting in extensive hematopoietic repopulation by day 28 (44.7% ± 2.0%, Supporting Information Fig. S3A, S3C). Unexpectedly, human cells in the ischemic limb were not detected by FACS or GUSB staining at day 7 (Supporting Information Fig. S3B, S3D, S3E) and were only rarely

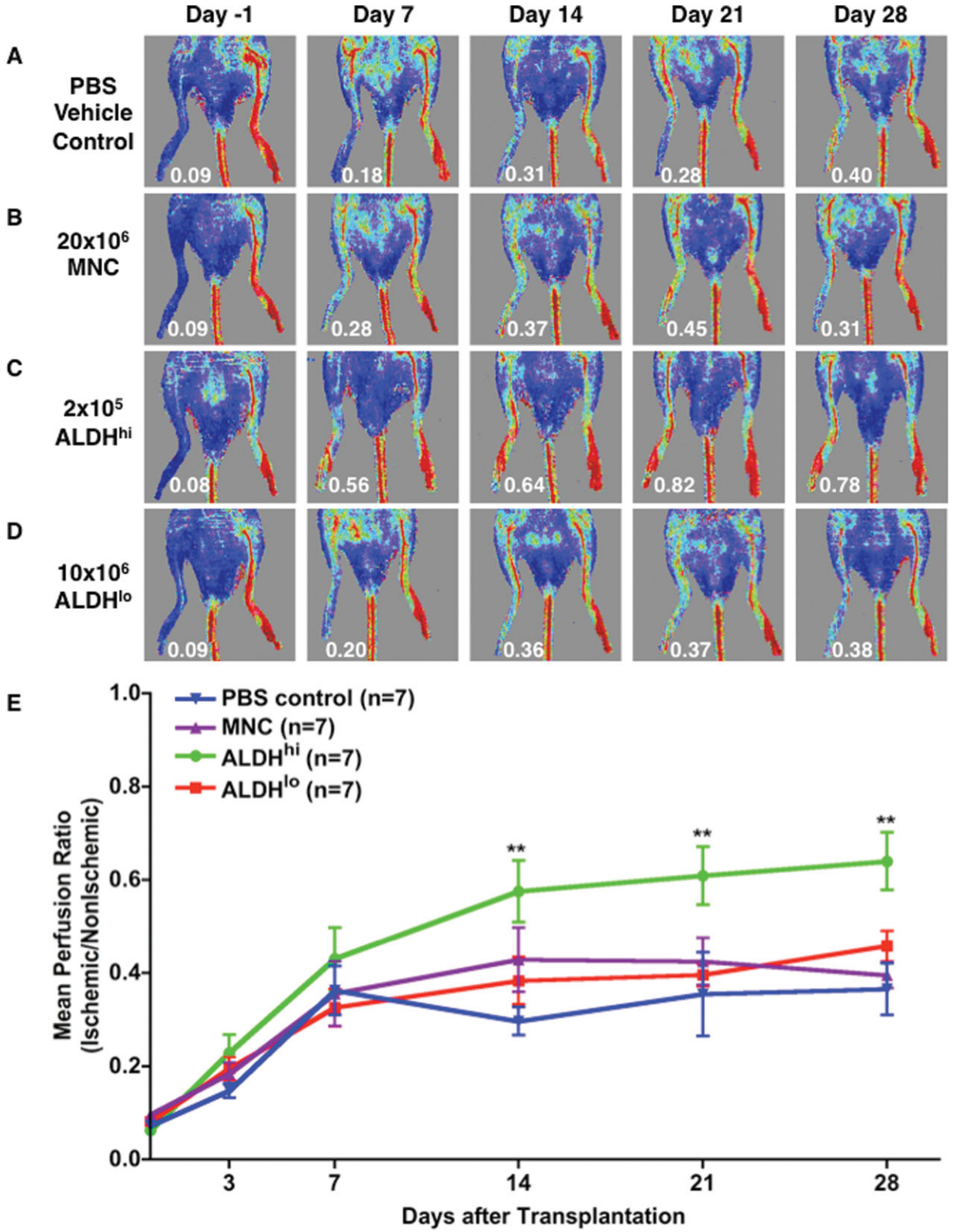

Figure 4. Transplanted umbilical cord blood (UCB) ALDH^{hi} cells augmented perfusion in ischemic hind limbs. Representative LDPI following right femoral artery ligation and tail vein injection of **(A)** saline vehicle control ($n = 7$), **(B)** 20×10^6 unsorted UCB MNC ($n = 7$), **(C)** 2×10^5ALDH^{hi} cells ($n = 7$), or **(D)** 10×10^6 ALDH^{lo} cells ($n = 7$). Numbers in lower left of each image indicate perfusion ratio of the ischemic versus the nonischemic hind limb. **(E):** Compared to all other treatments, transplantation of UCB ALDH^{hi} cells promoted improved recovery of perfusion in ischemic mouse hind limbs by day 14 and augmented perfusion was maintained to day 28. Data are expressed as mean ± SEM; **, $p < .01$. Abbreviations: ALDH, aldehyde dehydrogenase; MNC, mononuclear cell; PBS, phosphate-buffered saline.

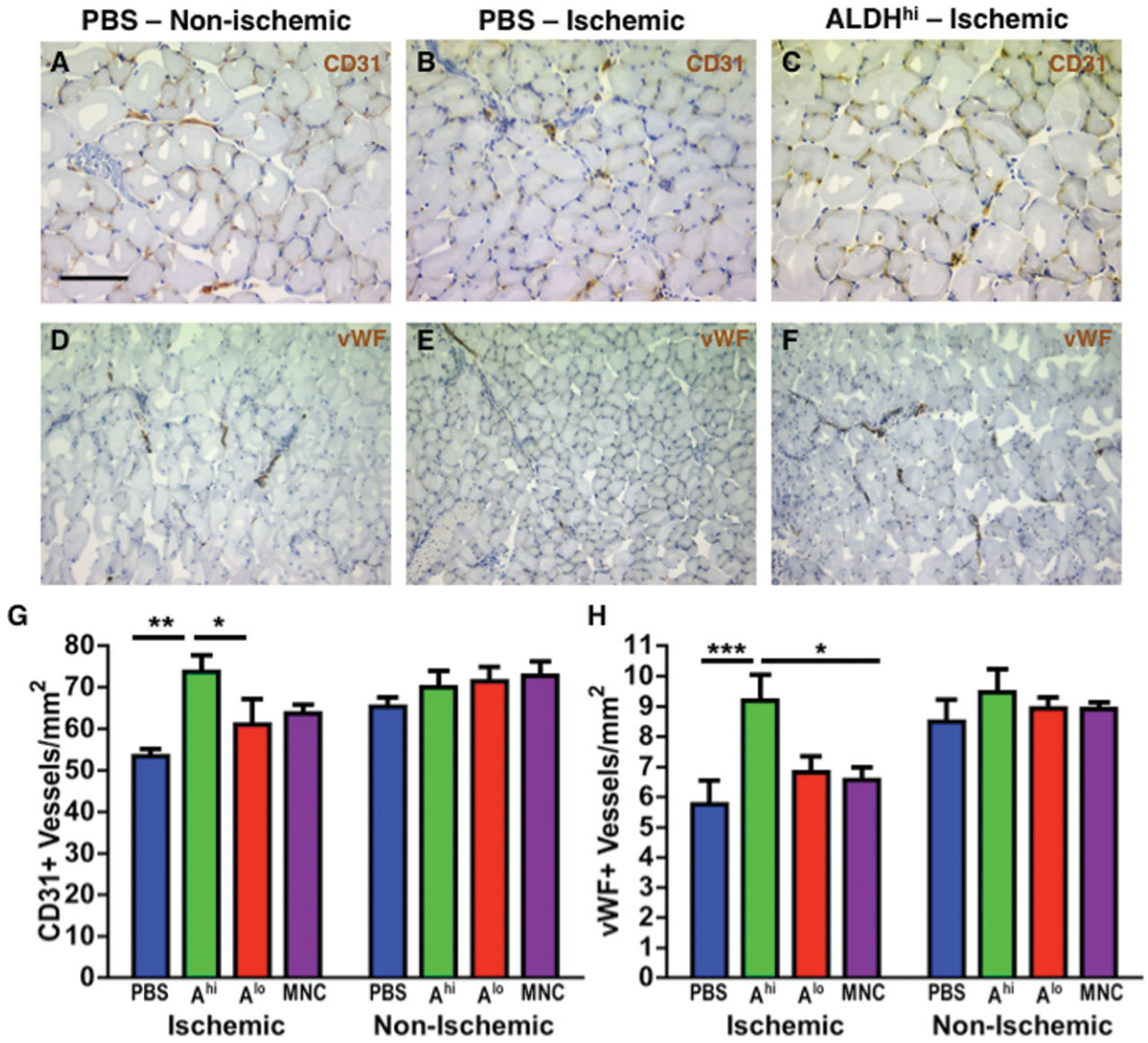

Figure 5. Transplanted umbilical cord blood (UCB) ALDH^{hi} cells augmented blood vessel density within ischemic hind limbs. Representative photomicrographs of adductor muscle sections from the ischemic and nonischemic limbs of mice injected with PBS or UCB ALDH^{hi} cells stained for **(A--C)** CD31^{+} capillaries or **(D--F)** vWF^{+} blood vessels at day 28 post-transplantation. Scale bar = 100 μm. Compared to all other treatments, **(G)** CD31^{+} capillary density and **(H)** vWF^{+} blood vessel density were increased in the ischemic limb of mice transplanted with human UCB ALDH^{hi} cells. Data are expressed as mean ± SEM; *, $p < .05$; **, $p < .01$; ***, $p < .001$. Abbreviations: ALDH, aldehyde dehydrogenase; MNC, mononuclear cell; PBS, phosphate-buffered saline; vWF+, von Willebrand factor.

detected at day 28 (Supporting Information Fig. S3F). Thus, irradiation prior to ALDH^{hi} cells transplantation did not augment engraftment or the recovery of perfusion in the ischemic limb.

Discussion

In this study, we demonstrate that ALDH-expressing cells from human UCB, representing <0.5% of total UCB cells and primarily composed of primitive myeloid progenitor cells, demonstrated increased expression of mRNA transcripts for several angiogenic cytokines and stimulated the survival and tubule-forming functions of EC during coculture in vitro. Intravenous transplantation of UCB ALDH^{hi} cells also augmented recovery of limb perfusion induced by femoral artery ligation and transection. Although the engraftment of human cells within ischemic muscle was infrequent, UCB ALDH^{hi} cells were specifically recruited to the ischemic limb within 3 days of transplantation and stimulated the recovery of blood vessel and capillary density. Furthermore, ALDH^{hi} cells function within the pancreas to promote islet revascularization after transplantation into streptozotocin-treated mice [45, 46]. Collectively, these data suggest that UCB ALDH^{hi} progenitor cells represent a readily available population of proangiogenic cells for the development of cellular therapies to promote endogenous revascularization.

The UCB ALDH^{hi} population was significantly enriched for human hematopoietic progenitor function. Approximately one in four ALDH^{hi} cells demonstrated multipotent CFU capacity in methylcellulose cultures. UCB ALDH^{hi} cells also showed increased expression of primitive cell surface markers (CD34, c-kit/CD117, and CD133), and >90% of UCB ALDH^{hi} cells expressed the early myeloid marker CD33, indicating a substantial enrichment of early myeloid progenitors. At the same time, UCB ALDH^{hi} cells were depleted of mature T- and B-lymphocytes as well as CD14-expressing monocytes. This is notable as

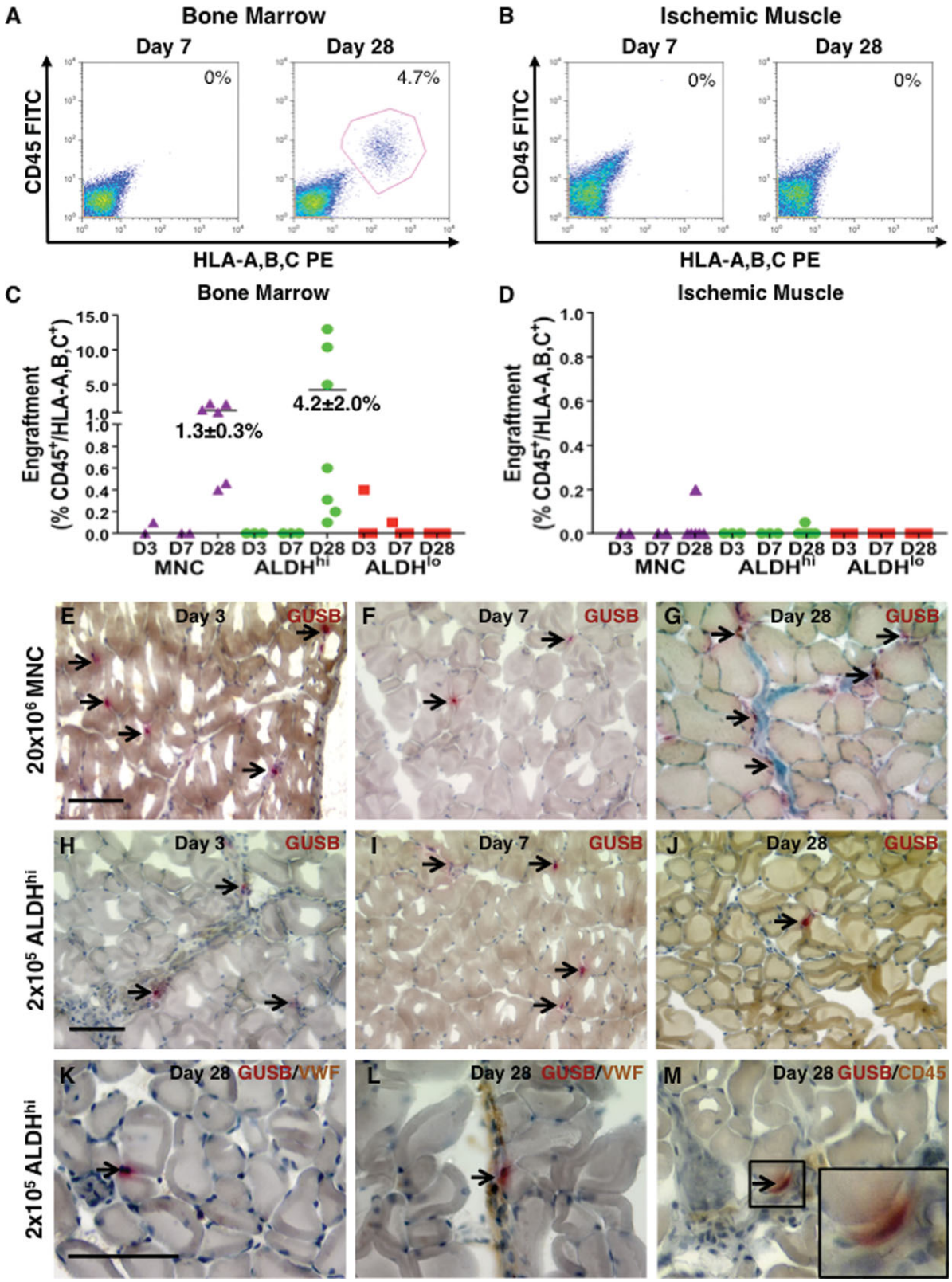

Figure 6. Transplanted human umbilical cord blood (UCB) ALDH^{hi} cells recruited to the ischemic hind limb. Representative flow cytometry plots illustrating the detection of human cells (CD45^{+}/HLA-A,B,C^{+}) cells within **(A)** the bone marrow (BM) and **(B)** ischemic hind limbs of mice transplanted with UCB ALDH^{hi} cells. **(C, D):** Despite engraftment of human cells in the mouse BM, human cells were rarely detected in the ischemic hind limb by flow cytometry. Representative photomicrographs showing the detection of transplanted human cells (red) in the adductor muscle of the ischemic hind limb of mice transplanted with **(E--G)** UCB MNC or **(H--J)** UCB ALDH^{hi} cells at 3, 7, and 28 days post-transplantation. GUSB^{+} human cells (arrows) were detected in the ischemic limb of mice injected with UCB MNC or ALDH^{hi} cells, but not in mice injected with UCB ALDH^{lo} cells. Costaining for GUSB activity with murine vWF at day 28 showed **(K)** engraftment of single GUSB^{+} cells between muscle fibers and **(L)** association with vWF^{+} blood vessels. **(M):** GUSB^{+} human cells surviving at day 28 costained for CD45. Scale bars = 100 μm. Abbreviations: ALDH, aldehyde dehydrogenase; GUSB, β-glucuronidase; MNC, mononuclear cell; vWF, von Willebrand factor.

$CD14^+$ monocytes have been specifically implicated in the paracrine support of angiogenesis [47]. However, transplantation of UCB $ALDH^{hi}$ cells, containing only 3% $CD14^+$ monocytic cells, significantly improved perfusion and blood vessel density in the ischemic limb, whereas transplantation of mice with 100-fold higher doses of unsorted MNC, containing more than 17% $CD14^+$ monocytes, failed to promote recovery from hind limb ischemia. Recent studies have demonstrated that murine common myeloid progenitors preferentially differentiate into proangiogenic monocytes that support neovessel formation in vivo [48]. Although UCB $ALDH^{hi}$ cells may be capable of differentiating into monocytes in vivo, our data indicate that primitive UCB $ALDH^{hi}$ myeloid progenitors play a significant role in support of angiogenesis in vivo.

UCB $ALDH^{hi}$ cells also possessed enriched ECFC capacity in vitro. In contrast to the dominant myeloid progenitor composition, ECFCs were infrequent at approximately one ECFC in 50,000 $ALDH^{hi}$ cells. Although this represents a significant ECFC enrichment from unpurified UCB MNC [9], it is unlikely that the few ECFCs within 40×10^3 $ALDH^{hi}$ cells make a major contribution toward the maintenance of HUVEC survival and the induction of tubule-forming function that we observed under growth factor and serum-starved conditions in vitro. However, paracrine contributions of the nonhematopoietic component of the $ALDH^{hi}$ population should not be overlooked in vivo. Approximately 7% of the UCB $ALDH^{hi}$ population was $CD45^-$, and these cells demonstrated high coexpression of primitive endothelial precursor markers (CD34 and CD133). Therefore, we propose that UCB $ALDH^{hi}$ cells represent a heterogeneous mixture of primitive hematopoietic and nonhematopoietic progenitors that may act in synergy to generate a supportive microenvironment to promote EC survival and revascularization function.

After intravenous transplantation, UCB $ALDH^{hi}$ cells induced stable recovery from acute ischemic injury within 2 weeks of transplantation into mice with unilateral hind limb ischemia. However, permanent engraftment of $ALDH^{hi}$ cells within the ischemic muscle was below the threshold of detection by flow cytometry. Notably, transplantation of 50–100-fold greater cell doses of unpurified UCB MNC or $ALDH^{lo}$ cells also showed little engraftment by FACS. Using NOD/SCID/MPSVII mice, a unique cell tracking model capable of detecting GUSB-expressing human cell at the single-cell level, we demonstrate that intravenous-injected UCB $ALDH^{hi}$ cells specifically recruited into the ischemic limb at 3 and 7 days after transplantation, and these cells survived in ischemic muscle tissue, albeit at low frequencies, for up to 28 days. Nonetheless, this low frequency engraftment of $ALDH^{hi}$ cells in the ischemic region at early time points was sufficient to augment the revascularization of ischemic muscle indicating a vascular stabilization was mediated by the $ALDH^{hi}$ cells.

Recent models have proposed a transient role for proangiogenic hematopoietic cells in the stabilization of injured vasculature and in the activation and recruitment of vessel resident endothelial precursors to the sprouting vessel branch [8]. In support of this concept, we show that sublethal irradiation prior to transplantation of $ALDH^{hi}$ cells reduced recruitment of human cells to the ischemic region, increased engraftment of $ALDH^{hi}$ cells in the murine BM, and delayed the functional recovery of perfusion. Therefore, we propose that early recruitment of $ALDH^{hi}$ cells to the ischemic region is critical for vascular recovery, allowing $ALDH^{hi}$ cells to release proangiogenic stimuli that activate an endogenous program for collateral vessel formation resulting in improved limb perfusion. Thus, strategies to improve the efficiency $ALDH^{hi}$ progenitor cell delivery, or to prolong survival at or near the site of ischemic injury, are expected to further augment vascular regeneration via paracrine or contact dependent mechanisms.

Conclusions

Strategies that purify proangiogenic cell subtypes from alternate human sources are required to improve the efficacy of human cell therapy trials [26]. Our studies outline several important caveats relevant to the development of cellular therapies to treat ischemic diseases. First, we have identified a mixed population of hematopoietic/myeloid and nonhematopoietic/endothelial $ALDH^{hi}$ progenitor cells from human UCB as a readily available and clinically applicable cell population with potent proangiogenic function. Second, the potential use of allogeneic UCB cells will open new avenues toward clinical cell-based therapies for ischemic disease, as mounting evidence indicates the potential for progenitor deletion and dysfunction of autologous BM cells in patients with severe diabetes and cardiovascular disease [2, 18–20]. Third, we have shown that neither high-level nor permanent engraftment of human cells were necessary to mediate augmentation of perfusion and improved vessel density in vivo. In an allogeneic context, such as intramuscular delivery of UCB $ALDH^{hi}$ cells in patients with ischemic heart disease or critical limb ischemia [49, 50], future clinical transplantation strategies using UCB may require only short-term immune suppression to initiate potent revascularization. Future studies will increase our understanding of the cellular constituents and paracrine pathways that modulate regenerative angiogenesis and will lead to novel therapies to improve tissue vascularization during ischemic diseases.

Acknowledgments

This work was supported by the Canadian Institutes of Health Research (MOP#86759) and by a grant-in-aid (NA 7295) and the MacDonald scholarship/new investigator salary award from the Heart and Stroke Foundation of Canada to D.A.H. D.M.P. received support from a Canadian Institutes of Health Research studentship and an Ontario Graduate Scholarship. We wish to acknowledge the contributions of Drs. Kristin Chadwick and Karen Morley at the London Regional Flow Cytometry Facility and David Carter at the London Regional Genomics Facility.

Disclosure of Potential Conflicts of Interest

The authors indicate no potential conflicts of interest.

References

1 Dotsenko O. Stem/progenitor cells, atherosclerosis, cardiovascular regeneration. Open Cardiovasc Med J 2010;4:97–104.

2 Bozdag-Turan I, Turan RG, Turan CH et al. Relation between the frequency of CD34 bone marrow derived circulating progenitor cells and the number of diseased coronary arteries in patients with myocardial ischemia and diabetes. Cardiovasc Diabetol 2011;10:107.

3 Dimmeler S, Zeiher AM, Schneider MD. Unchain my heart: The scientific foundations of cardiac repair. J Clin Invest 2005;115:572–583.

4 Jin DK, Shido K, Kopp HG et al. Cytokine-mediated deployment of SDF-1 induces revascularization through recruitment of CXCR4+ hemangiocytes. Nat Med 2006;12:557–567.
5 Rafii S, Lyden D. Therapeutic stem and progenitor cell transplantation for organ vascularization and regeneration. Nat Med 2003;9:702–712.
6 Asahara T, Murohara T, Sullivan A et al. Isolation of putative progenitor endothelial cells for angiogenesis. Science 1997;275:964–967.
7 Peichev M, Naiyer AJ, Pereira D et al. Expression of VEGFR-2 and AC133 by circulating human CD34(+) cells identifies a population of functional endothelial precursors. Blood 2000;95:952–958.
8 Richardson MR, Yoder MC. Endothelial progenitor cells: Quo vadis? J Mol Cell Cardiol 2011;50:266–272.
9 Yoder MC, Mead LE, Prater D et al. Redefining endothelial progenitor cells via clonal analysis and hematopoietic stem/progenitor cell principals. Blood 2007;109:1801–1809.
10 Capoccia BJ, Shepherd RM, Link DC. G-CSF and AMD3100 mobilize monocytes into the blood that stimulate angiogenesis in vivo through a paracrine mechanism. Blood 2006;108:2438–2445.
11 Estes ML, Mund JA, Mead LE et al. Application of polychromatic flow cytometry to identify novel subsets of circulating cells with angiogenic potential. Cytometry A 2010;77:831–839.
12 Urbich C, Heeschen C, Aicher A et al. Relevance of monocytic features for neovascularization capacity of circulating endothelial progenitor cells. Circulation 2003;108:2511–2516.
13 Grunewald M, Avraham I, Dor Y et al. VEGF-induced adult neovascularization: Recruitment, retention, and role of accessory cells. Cell 2006;124:175–189.
14 Kaplan RN, Riba RD, Zacharoulis S et al. VEGFR1-positive haematopoietic bone marrow progenitors initiate the pre-metastatic niche. Nature 2005;438:820–827.
15 Kaplan RN, Rafii S, Lyden D. Preparing the "soil": The premetastatic niche. Cancer Res 2006;66:11089–11093.
16 Rafii S, Lyden D. Cancer a few to flip the angiogenic switch. Science 2008;319:163–164.
17 Urbich C, Dimmeler S. Endothelial progenitor cells: Characterization and role in vascular biology. Circ Res 2004;95:343–353.
18 Vasa M, Fichtlscherer S, Aicher A et al. Number and migratory activity of circulating endothelial progenitor cells inversely correlate with risk factors for coronary artery disease. Circ Res 2001;89:E1–7.
19 Schatteman GC, Hanlon HD, Jiao C et al. Blood-derived angioblasts accelerate blood-flow restoration in diabetic mice. J Clin Invest 2000; 106:571–578.
20 Tepper OM, Carr J, Allen RJ, Jr. et al. Decreased circulating progenitor cell number and failed mechanisms of stromal cell-derived factor-1alpha mediated bone marrow mobilization impair diabetic tissue repair. Diabetes 2010;59:1974–1983.
21 Janssens S, Dubois C, Bogaert J et al. Autologous bone marrow-derived stem-cell transfer in patients with ST-segment elevation myocardial infarction: Double-blind, randomised controlled trial. Lancet 2006;367:113–121.
22 Wollert KC, Meyer GP, Lotz J et al. Intracoronary autologous bone-marrow cell transfer after myocardial infarction: The BOOST randomised controlled clinical trial. Lancet 2004;364:141–148.
23 Schachinger V, Erbs S et al. Intracoronary bone marrow-derived progenitor cells in acute myocardial infarction. N Engl J Med 2006;355: 1210–1221.
24 Tateishi-Yuyama E, Matsubara H, Murohara T et al. Therapeutic angiogenesis for patients with limb ischaemia by autologous transplantation of bone-marrow cells: A pilot study and a randomised controlled trial. Lancet 2002;360:427–435.
25 Higashi Y, Kimura M, Hara K et al. Autologous bone-marrow mononuclear cell implantation improves endothelium-dependent vasodilation in patients with limb ischemia. Circulation 2004;109:1215–1218.
26 Rosenzweig A. Cardiac cell therapy–mixed results from mixed cells. N Engl J Med 2006;355:1274–1277.
27 Putman DM, Bell GI, Hess DA. Blood-derived ALDHhi cells in tissue repair. In: Allan DS, Strunk D, eds. Regenerative Therapy Using Blood-Derived Stem Cells. Humana Press, 2012:21–34.
28 Ingram DA, Mead LE, Tanaka H et al. Identification of a novel hierarchy of endothelial progenitor cells using human peripheral and umbilical cord blood. Blood 2004;104:2752–2760.
29 Alves G, Wu WW, Wang G et al. Enhancing peptide identification confidence by combining search methods. J Proteome Res 2008;7: 3102–3113.
30 Werner N, Wassmann S, Ahlers P et al. Endothelial progenitor cells correlate with endothelial function in patients with coronary artery disease. Basic Res Cardiol 2007;102:565–571.
31 Werner N, Kosiol S, Schiegl T et al. Circulating endothelial progenitor cells and cardiovascular outcomes. N Engl J Med 2005;353: 999–1007.
32 Fadini GP, Sartore S, Albiero M et al. Number and function of endothelial progenitor cells as a marker of severity for diabetic vasculopathy. Arterioscler Thromb Vasc Biol 2006;26:2140–2146.
33 Jialal I, Devaraj S, Singh U et al. Decreased number and impaired functionality of endothelial progenitor cells in subjects with metabolic syndrome: Implications for increased cardiovascular risk. Atherosclerosis 2010;211:297–302.
34 Hess DA, Meyerrose TE, Wirthlin L et al. Functional characterization of highly purified human hematopoietic repopulating cells isolated according to aldehyde dehydrogenase activity. Blood 2004;104: 1648–1655.
35 Hess DA, Wirthlin L, Craft TP et al. Selection based on CD133 and high aldehyde dehydrogenase activity isolates long-term reconstituting human hematopoietic stem cells. Blood 2006;107:2162–2169.
36 Cai J, Cheng A, Luo Y et al. Membrane properties of rat embryonic multipotent neural stem cells. J Neurochem 2004;88:212–226.
37 Pearce DJ, Taussig D, Simpson C et al. Characterization of cells with a high aldehyde dehydrogenase activity from cord blood and acute myeloid leukemia samples. Stem Cells 2005;23:752–760.
38 Storms RW, Green PD, Safford KM et al. Distinct hematopoietic progenitor compartments are delineated by the expression of aldehyde dehydrogenase and CD34. Blood 2005;106:95–102.
39 Corti S, Locatelli F, Papadimitriou D et al. Transplanted ALDHhiSSClo neural stem cells generate motor neurons and delay disease progression of nmd mice, an animal model of SMARD1. Hum Mol Genet 2006;15:167–187.
40 Capoccia BJ, Robson DL, Levac KD et al. Revascularization of ischemic limbs after transplantation of human bone marrow cells with high aldehyde dehydrogenase activity. Blood 2009;113:5340–5351.
41 Hess DA, Craft TP, Wirthlin L et al. Widespread nonhematopoietic tissue distribution by transplanted human progenitor cells with high aldehyde dehydrogenase activity. Stem Cells 2008;26:611–620.
42 Hofling AA, Vogler C, Creer MH et al. Engraftment of human CD34+ cells leads to widespread distribution of donor-derived cells and correction of tissue pathology in a novel murine xenotransplantation model of lysosomal storage disease. Blood 2003;101:2054–2063.
43 Bhatia M, Bonnet D, Murdoch B et al. A newly discovered class of human hematopoietic cells with SCID-repopulating activity. Nat Med 1998;4:1038–1045.
44 Gerber HP, McMurtrey A, Kowalski J et al. Vascular endothelial growth factor regulates endothelial cell survival through the phosphatidylinositol 3′-kinase/Akt signal transduction pathway. Requirement for Flk-1/KDR activation. J Biol Chem 1998;273:30336–30343.
45 Bell GI, Meschino MT, Hughes-Large JM et al. Combinatorial human progenitor cell transplantation optimizes islet regeneration through secretion of paracrine factors. Stem Cells Dev 2012;21:1863–1876.
46 Bell GI, Putman DM, Hughes-Large JM et al. Intrapancreatic delivery of human umbilical cord blood aldehyde dehydrogenase-producing cells promotes islet regeneration. Diabetologia 2012;55:1755–1760.
47 Awad O, Dedkov EI et al. Differential healing activities of $CD34^+$ and $CD14^+$ endothelial cell progenitors. Arterioscler Thromb Vasc Biol 2006;26:758–764.
48 Wara AK, Croce K, Foo S et al. Bone marrow-derived CMPs and GMPs represent highly functional proangiogenic cells: Implications for ischemic cardiovascular disease. Blood 2011;118:6461–6464.
49 Balber AE. Concise review: Aldehyde dehydrogenase bright stem and progenitor cell populations from normal tissues: Characteristics, activities, and emerging uses in regenerative medicine. Stem Cells 2011; 29:570–575.
50 Perin EC, Silva G, Gahremanpour et al. A randomized, controlled study of autologous therapy with bone marrow-derived aldehyde dehydrogenase bright cells in patients with critical limb ischemia. Catheter Cardiovasc Interv 2011;78:1060–1067.


See www.StemCells.com for supporting information available online.