Human Peripheral Blood-Derived CD31⁺ Cells Have Robust Angiogenic and Vasculogenic Properties and Are Effective for Treating Ischemic Vascular Disease

Sung-Whan Kim, PhD,* Hyongbum Kim, MD, PhD,* Hyun-Jai Cho, MD, PhD;† Jung-Uek Lee, MS;‡ Rebecca Levit, MD,* Young-sup Yoon, MD, PhD*

Atlanta, Georgia; Seoul, South Korea; and Los Angeles, California

Objectives
This study aimed to determine if CD31 is a novel marker of a circulating angio-vasculogenic cell population and to establish the cells’ therapeutic effects on experimental ischemia.

Background
Emerging evidence suggested that therapeutic mechanisms underlying various bone marrow-derived cells are due to paracrine effects. Furthermore, the vasculogenic potential of these cells is under debate. CD31 is a well-known marker for endothelial cells but is also expressed in a fraction of peripheral blood (PB) mononuclear cells.

Methods
CD31⁺ cells were isolated from human PB by magnetic-activated cell sorting. The gene expression profile was examined by deoxyribonucleic acid microarray and real-time reverse transcriptase polymerase chain reaction. Various in vitro endothelial differentiation or vasculogenic assays were conducted. Finally, cells were directly implanted into a mouse hind limb ischemia model to test angiogenic-vasculogenic and therapeutic effects.

Results
Fluorescent-activated cell sorter analysis revealed that PB-CD31⁻ cells exhibited endothelial and hematopoietic stem/progenitor markers. CD31⁺ cells had higher levels of expression of proangiogenic genes on microarray and real-time reverse transcriptase polymerase chain reaction and generated higher numbers of endothelial progenitor cells than CD31⁻ cells did. CD31⁺ cells spontaneously formed vascular tubelike structures and exhibited an endothelial cell phenotype in vitro. In a hind limb ischemia model, CD31⁺ cell transplantation augmented blood perfusion and prevented limb loss. Both angiogenic cytokines and capillary density were increased, suggesting CD31⁺ cells augmented neovascularization.

Conclusions
CD31 is a novel marker that designates circulating angiogenic and vasculogenic cells. These cells are easily isolated from human PB and thus are a novel candidate for treatment of ischemic cardiovascular disease. (J Am Coll Cardiol 2010;56:593–607) © 2010 by the American College of Cardiology Foundation

Ischemic cardiovascular disease is the most prevalent disease in Westernized society. Despite progress in therapies, nearly 80 million are affected yearly from heart attack, stroke, and ischemic cardiomyopathy (1). As our knowledge of stem cell biology grows, cell-based therapies have become a promising strategy for tissue regeneration. Transplantation of various bone marrow (BM)-derived cells, including mononuclear cells (MNCs), endothelial progenitor cells (EPCs), mesenchymal stem cells, and hematopoietic stem cells (HSCs), were reported to induce therapeutic neovascularization in adult ischemic tissues (2–5). Even though cell-based therapy has great therapeutic potential, several issues remain. The use of unselected BM cells or BM-MNCs was associated with adverse events including calcification, vascular plaque growth, and worsening of tissue ischemia (6–8). Both EPCs and mesenchymal stem cells require ex vivo culture to be used clinically. For all BM-derived cells, their ability to (trans)differentiate into endothelial cells

See page 608
found that CD31+/H11001 progenic and/or vasculogenic capabilities. Kim et al.

Appendix. The following topics are covered there: An expanded Methods section is available in the Online
mentation of neovascularization. Improving ischemia in mouse hind limb ischemia by aug-
cells of the hemangioblast lineage, we hypothesized that
unknown. Based on the conserved expression of CD31 on
the cells expressing this marker in tissue regeneration is
antiapoptotic signaling (20). However, the therapeutic role
transendothelial leukocyte migration, cell-cell adhesion, and
noglobulin superfamily (19). CD31 plays important role for
Endothelial and hematopoietic stem cell characteristics
of PB–CD31+ cells. The FACS analysis on PB–CD31+
cells showed that >95% of magnetic-activated cell sorting–
isolated CD31+ cells express CD31 (Fig. 1A). Approximately
40% of CD31+ cells expressed CD14, a monocyte/macrophase marker and more than 99% of CD31+ cells
expressed CD45, a pan-hematopoietic marker; this suggests that CD31+ cells are not circulating ECs (Figs. 1A and
1C). CD31+ cells preferentially expressed endothelial markers (CD105, CD141, CD144, and von Willebrand
Factor; p < 0.05) and stem cell or progenitor markers (CD34, CD133, CD117, and kinase insert domain receptor [vascular
endothelial growth factor receptor 2]; p < 0.05) (Fig. 1B, Online Fig. 1A). To investigate the hematopoietic progenitor
cell (HPC) properties, a clonogenic assay was performed. CD31+ cells, when compared with CD31– cells, generated a
significantly higher number of hematopoietic colonies such as
colonies forming unit erythroid, burst forming unit erythroid,
colony forming unit granulocyte/macrophase, and colony
forming unit granulocyte/erythroid/macrophase/megakary-
ocyte (p < 0.05) (Fig. 1C, Online Fig. 1B). These data show that CD31+ cells have characteristics of HSC/HPCs as well as ECs.

Enriched angiogenic, cell adhesion, and chemoattraction
genes in CD31+ cells. To compare global gene expression patterns between CD31+ and CD31– cells, we carried out microarray analysis. Hierarchical cluster analysis showed that gene expression in CD31+ cells are distinct from
CD31– cells in that 749 genes were up-regulated and 26
genes were down-regulated by more than 2-fold in the
CD31+ cells than in CD31– cells (Fig. 2A). Further
characterization with the Gene Ontology Database (21)
demonstrated that genes involved in angiogenesis, cell
adhesion, transmembrane structure, chemokine production
and reception, and extracellular matrix were highly and
preferentially expressed in CD31+ cells (Fig. 2B, Table 1,
Online Tables S1 to S3) (22).

Results

Endothelial and hematopoietic stem cell characteristics
of PB–CD31+ cells. We have found that CD31, a ubiquitous EC marker, is
also expressed on the surface of various mouse hematopoietic cell lineages (16), including HSCs (17), and undifferen-
tiated mouse embryonic stem cells (18). CD31, also
known as platelet endothelial cell adhesion molecule 1, is a
130-kDa cell surface molecule and a member of the immu-
noglobulin superfamily (19). CD31 plays important role for
transendothelial leukocyte migration, cell-cell adhesion, and
antipoptotic signaling (20). However, the therapeutic role
of the cells expressing this marker in tissue regeneration is
unknown. Based on the conserved expression of CD31 on
cells of the hemangioblast lineage, we hypothesized that
CD31+ cells in peripheral blood (PB) have higher angiogenic and/or vasculogenic capabilities.

Through a series of in vitro and in vivo experiments, we
found that CD31+ cells isolated from PB showed higher angiogenic activity and vasculogenic potential, effectively
improving ischemia in mouse hind limb ischemia by aug-
mentation of neovascularization.

Methods

An expanded Methods section is available in the Online
Appendix. The following topics are covered there:

- Isolation and culture of CD31+ cells
- Flow cytometry
- Microarray analysis
- Quantitative real-time reverse transcriptase polymerase
  chain reaction (qRT-PCR)
- EPC culture assay and immunocytochemistry
- Cell adhesion assay
- Hematopoietic colony forming unit assays
- Transplantation of the CD31+ and CD31– cells into
  ischemic hind limb
- Histological analysis
- Fluorescent-activated cell sorter (FACS) analysis of
  ECs derived from transplanted CD31+ cells
- Statistical analysis
To confirm the results of microarray data, we performed qRT-PCR analysis. Major angiogenic factors such as vascular endothelial growth factor-A, fibroblast growth factor-2, hepatocyte growth factor, and angiopoietin-1, and an adhesion molecule, VE-cadherin, were more highly expressed in CD31<sup>+</sup> cells than in CD31<sup>-</sup> cells or MNCs (Fig. 2C). Chemokines such as monocyte chemoattractant protein-1 and interleukin-8, which play an important role...
for neovascularization, were also significantly up-regulated (23,24). Collectively, these findings show that CD31+ cells define a population enriched with angiogenic, chemoattractant, and cell adhesion genes.

**CD31+ cells show higher vasculogenic potential and cell adhesion capacity in vitro.** We next investigated the in vitro endothelial differentiation potential of CD31+ cells. First, we performed EPC culture assay. The number of EPCs and EPC colonies were markedly higher in the CD31+ cell group than in the CD31– cell group (all p < 0.01) (Figs. 3A and 3B). Second, we carried out a cell adhesion assay. The number of adhered cells to various extracellular matrix proteins, fibronectin, vitronectin, collagen I, and laminin was significantly higher in the CD31+ cells than in the CD31– cells (all p < 0.001) (Fig. 3C). In ECs, adhesion capacity is an indicator of cell engraftment and cell survival.

To induce EC differentiation, CD31+ cells were cultured in endothelial growth media 2, which is endothelia cell basal media 2 with 15% FBS and cytokine cocktail (SingleQuots, Lonza Cologne AG, Cologne, Germany), for 28 days. CD31+ cells exhibited EC markers such as von Willebrand factor, kinase insert domain receptor, vascular endothelial cadherin, and CD31 by immunocytochemistry (Fig. 3D). CD31– cells were not culturable under these conditions. During this culture, we made an intriguing observation that some CD31+ cells spontaneously formed tubular structures. In the first week of culture, CD31+ cells aggregated; around day 10, the cells started to form tubular structures within the cell clusters; and between days 12 and 16, the cell clusters underwent morphologic changes to form linear tubular structures, mimicking in vivo vasculogenesis (Figs. 3E to 3I). These tubular structures stained positive for lectin and took up 1,1’dioctadecyl-3,3,3’,3’-tetramethylindocarbocyanine-labeled acetylated low-density lipoprotein (DiI-acLDL), characteristics of ECs. We further measured the expression of endothelial and angiogenic genes during the culture of CD31+ cells over 28 days by qRT-PCR. Over 28 days, the expression of kinase insert domain receptor, vascular endothelial cadherin, hepatocyte growth factor, and insulin-like growth factor 1 gradually increased sug-
suggesting differentiation into a more vasculogenic and angiogenic phenotype (Fig. 3J). Together, these data demonstrate the vasculogenic and angiogenic potential of CD31<sup>+</sup>/H11001 cells in vitro.

CD31<sup>+</sup> cells exert favorable therapeutic effects on hind limb ischemia. After identifying the angiogenic and vasculogenic characteristics of CD31<sup>+</sup> cells in vitro, we investigated the therapeutic potential of CD31<sup>+</sup> cells to repair experimentally induced ischemia in vivo. Laser Doppler perfusion image analysis demonstrated that blood perfusion was significantly higher in the CD31<sup>+</sup> cell-injected limbs versus CD31<sup>-</sup> cell- or phosphate-buffered saline (PBS)-injected limbs at days 7, 14, and 21 (Figs. 4A and 4B). The CD31<sup>+</sup> cell-treated group showed a significantly lower limb loss score than the PBS control group did (p < 0.05) (Figs. 4C and 4D). The capillary density and vascular...
maturation in the ischemic hind limb adductor muscle after surgery was also significantly higher in the CD31+ group than in the CD31- group or the PBS group (p < 0.01) (Figs. 5A and 5B, Online Fig. 2). We also found that functional capillaries examined after intracardiac injection of isolecitin B4 (ILB4) were significantly higher in the CD31+ group than in the other groups (Online Fig. 3).

In addition, terminal deoxyribonucleotidyl transferase (TdT)-mediated dUTP-digoxigenin nick end labeling (TUNEL) assay, together with ILB4 staining, demonstrated that transplantation of CD31+ cells significantly reduced endothelial cell apoptosis when compared to control groups, suggesting protective effects on existing vessels (Online Fig. 4). Collectively, these findings suggest that CD31+ cells can augment functional neovascularization and preserve existing vessels in vivo and are effective for treating ischemic vascular disease.

Multiple angiogenic factors are up-regulated after CD31+ cells transplantation. To determine the effects of CD31+ cell transplantation on cytokine expression in ischemic hind limbs, mice were sacrificed and hind limb tissues collected. The expression levels of vascular endothelial growth factor-A, angiopoietin-1, fibroblast growth factor-2, stromal cell-derived factor-1, and CD31 were significantly increased in the CD31+ cell-injected limbs compared with the CD31- cell- or PBS-injected limbs (Fig. 5C). These data suggest that CD31+ cell transplantation up-regulates the expression of multiple biological factors associated with neovascularization and bone marrow cell mobilization.

### Table 1: Genes Associated With Angiogenesis Significantly Up- or Down-Regulated in CD31+ Cells Compared to CD31- Cells

<table>
<thead>
<tr>
<th>Probe Set ID</th>
<th>Gene Symbol</th>
<th>Description</th>
<th>Fold Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>7932985</td>
<td>NRP1</td>
<td>Neurepin-1</td>
<td>11.8</td>
</tr>
<tr>
<td>7977046</td>
<td>TNFAIP2</td>
<td>Tumor necrosis factor, alpha-induced protein-2</td>
<td>7.4</td>
</tr>
<tr>
<td>7991335</td>
<td>ANPEP</td>
<td>Alanine (membrane) aminopeptidase</td>
<td>6.6</td>
</tr>
<tr>
<td>8095680</td>
<td>IL8</td>
<td>Interleukin-8</td>
<td>6.5</td>
</tr>
<tr>
<td>8108217</td>
<td>TGFB1</td>
<td>Transforming growth factor beta-induced</td>
<td>6.5</td>
</tr>
<tr>
<td>8140556</td>
<td>HGF</td>
<td>Hepatocyte growth factor</td>
<td>5.8</td>
</tr>
<tr>
<td>8103399</td>
<td>PDGF-C</td>
<td>Platelet-derived growth factor C</td>
<td>5.2</td>
</tr>
<tr>
<td>8119898</td>
<td>VEGFA</td>
<td>Vascular endothelial growth factor A</td>
<td>4.7</td>
</tr>
<tr>
<td>8156278</td>
<td>EDG3</td>
<td>Endothelial differentiation, sphingolipid G-protein coupled receptor-3</td>
<td>4.2</td>
</tr>
<tr>
<td>8017599</td>
<td>PECAM1</td>
<td>Platelet/endothelial cell adhesion molecule-1</td>
<td>3.9</td>
</tr>
<tr>
<td>7970737</td>
<td>FLT3</td>
<td>Fms-related tyrosine kinase-3</td>
<td>3.5</td>
</tr>
<tr>
<td>8040473</td>
<td>RHOB</td>
<td>Ras homolog gene family, member B</td>
<td>3.5</td>
</tr>
<tr>
<td>7973084</td>
<td>ANG</td>
<td>Angiogenin</td>
<td>3.2</td>
</tr>
<tr>
<td>7969414</td>
<td>KLF5</td>
<td>Kruppel-like factor-5</td>
<td>3</td>
</tr>
<tr>
<td>7951886</td>
<td>IL1B</td>
<td>Interleukin-1B</td>
<td>2.5</td>
</tr>
<tr>
<td>8096160</td>
<td>ARHGAP24</td>
<td>Rho GTPase activating protein-24</td>
<td>2.4</td>
</tr>
<tr>
<td>8092552</td>
<td>IGF2BP2</td>
<td>Insulinlike growth factor-2 mRNA binding protein-2</td>
<td>2.2</td>
</tr>
<tr>
<td>8152297</td>
<td>ANGPT1</td>
<td>Angiopoietin-1</td>
<td>2.2</td>
</tr>
<tr>
<td>8064978</td>
<td>JAG1</td>
<td>Jagged-1</td>
<td>2.1</td>
</tr>
<tr>
<td>8137670</td>
<td>PDGF-A</td>
<td>Platelet-derived growth factor alpha polypeptide</td>
<td>2</td>
</tr>
<tr>
<td>7951351</td>
<td>PDGF-D</td>
<td>Platelet-derived growth factor D</td>
<td>-2</td>
</tr>
<tr>
<td>8099471</td>
<td>FGFBP2</td>
<td>Fibroblast growth factor binding protein-2</td>
<td>-2.2</td>
</tr>
</tbody>
</table>

GTPase = guanosine triphosphatase; mRNA = messenger ribonucleic acid.

**Contribution of CD31+ cells into vasculogenesis.** We further sought to determine the potential and magnitude of the contribution of CD31+ cells to ECs’ generation or vasculogenesis. To track the transplanted cells, we intramuscularly injected 1 × 10⁶ DiI-labeled CD31+ cells into the ischemic hind limb of nude mice. Histologic analysis demonstrated that a larger part of the injected CD31+ cells were concentrated in the pericytic or perivascular areas (Figs. 6A to 6D, Online Video 1) and a smaller population of CD31+ cells exhibited an EC’s specific marker, ILB4, within the vascular structure at 2 to 8 weeks (Figs. 6E to 6G, Online Video 2). This suggests that CD31+ cells generated ECs in vivo. To confirm this, we performed fluorescent in situ hybridization (FISH) using human Y chromosome probe (25,26). The FISH data showed that Y chromosome signals from transplanted human CD31+ cells were detected within the nuclei of ECs (Fig. 6I). Collectively, these data indicate that CD31+ cells can give rise to ECs, suggesting their vasculogenic potential.

**Quantitative analysis of endothelially transdifferentiated CD31+ cells.** It has been difficult to quantify the rate of differentiation of BM-derived cells in vivo. Here, to more accurately quantify the number of functional ECs derived from transplanted CD31+ cells, we conducted FACS analysis on enzymatically digested muscle following systemic injection of fluorescein isothiocyanate-labeled ILB4. The FACS analysis revealed that 2.2 ± 0.8% of the cells were double positive for DiI and ILB4 at 4 weeks, suggesting that significant portion of functional
Figure 3 In Vitro Vasculogenic Properties of CD31⁺ Cells

(A, B) Uptake of 1,1’dioctadecyl-3,3,3’,3’-tetramethylindocarbocyanine-labeled acetylated low-density lipoprotein (red fluorescence) and binding to Ulex europaeus agglutinin 1 lectin (green fluorescence) identified endothelial progenitor cells. CD31⁺ cells gave rise to higher number of endothelial progenitor cells (A) and endothelial progenitor cell colonies (B). n = 5 per group. **p < 0.01. Bars: 200 μm. (C) Cell adhesion assay showed higher adhesion of CD31⁺ cells than CD31⁻ cells to all tested extracellular matrix proteins. n = 5 per group. Bars: 200 μm. (D) CD31⁺ cells expressed endothelial proteins von Willebrand factor, kinase insert domain receptor, vascular endothelial cadherin, and CD31 in culture with endothelial growth media 2 for 6 days. Bars: 200 μm. Continued on next page.
ECs were derived from transplanted CD31⁺ cells (Fig. 7A). Next, to further confirm whether these double positive cells were truly derived from the injected human CD31⁺ cells, they were subjected to FISH with human Y chromosome. FISH demonstrated that the ILB4- and Dil-positive cells from recipient mice were of human donor origin, confirming differentiation of CD31⁺ cells into ECs (Fig. 7B).
Discussion

In this study, we show that CD31 is a unique and comprehensive marker to represent potent angiogenic and vasculogenic cells in human PB and that these cells are therapeutically effective for recovering tissue from ischemia. First, we demonstrated that human CD31^+ H11001 cells highly express HSC/HPC and EC markers. Second, CD31^+ H11001 cells are enriched with proangiogenic genes. Third, compared with CD31^− cells, CD31^+ cells generated more EPCs and demonstrated EC differentiation and vascularlike tube formation in culture. Fourth, implantation of CD31^+ cells into ischemic hind limb enhanced recovery of tissue from ischemic injury and increased the rate of limb salvage as well as the expression of angiogenic factors. Fifth, implanted CD31^+ cells show clear vasculogenic potential in ischemic limb tissues.

Because CD31 is a ubiquitous endothelial marker, we tested the hypothesis that the cells expressing CD31 in PB have angiogenic and/or vasculogenic capabilities. FACS showed that the CD31^+ cells expressed HSC/HPC markers and well-known EC markers, suggesting dual properties.
of CD31+ cells. Unbiased microarray data and qRT-PCR results indicated that angiogenic and other genes beneficial for vascular regeneration are highly enriched in the CD31+ cell population. Another distinguishing feature of CD31+ cells is that they gave rise to EPCs in the culture whereas CD31– cells did not. CD31+ cells frequently formed vascularlike tubes in culture without the aid of biomaterials such as Matrigel (BD Biosciences, San Jose, California), mimicking the developmental process of vascular tube formation from angioblasts or EPCs (Figs. 3E to 3I). This finding is one of the most unprecedented of this study, supporting the vasculogenic potential of CD31+ cells. These data suggest that CD31 is an antigenic signpost for robust proangiogenic and vasculogenic cells in PB.

In vivo cell transplantation studies further confirmed these results. CD31+ cell transplantation induced higher expressions of multiple paracrine factors in the ischemic tissues during the critical period of ischemia repair. Our data suggest that the major mechanism of CD31+ cells would be a nondifferentiation mechanism because the rate of endothelial differentiation was too small to account for the magnitude of therapeutic effects seen. CD31+ cells highly expressed crucial angiogenic and arteriogenic factors including vascular endothelial growth factor, angiopoietin-1, fibroblast growth factor-2, and monocyte chemoattractant protein-1. Synergistic effects of these angiogenic factors have been reported to induce therapeutic neovascularization (27). In addition, tissues
Figure 6 Engraftment and Differentiation of Transplanted CD31⁺ Cells Into ECs in Ischemic Hind Limbs

(A to D) Localization of CD31⁺ cells in the perivascular regions. Confocal microscopic images showed that 1,1′dioctadecyl-3,3′,3′,3′-tetramethylindocarbocyanine (Dil)-labeled CD31⁺ cells were more preferentially localized to the pericytic or perivascular areas (arrowheads). One colocalization with endothelial cells is shown by an arrow. Bars: A, C, D, 10 μm; B, 20 μm. Continued on next page.
Endothelial cells differentiation of CD31⁺ cells. Three-dimensional z-stacked orthogonal and multipanel images clearly demonstrated that a part of the engrafted CD31⁺ cells were incorporated into vascular structures and exhibit an endothelial cell marker, suggesting endothelial differentiation of CD31⁺ cells. Green, isolectin B4; Red, Dil-labeled CD31⁺ cells; Blue, 4',6-diamidino-2-phenylindole. Bars: E, 500 μm; F, G, 10 μm. (H, I) Fluorescent in situ hybridization on CD31⁺ cell transplanted hind limb tissues. One cell (H, arrowhead), which was localized at the pericytic area, exhibited red fluorescence for human Y chromosome. The other cell (I, arrows) exhibited a fluorescent in situ hybridization signal (red) within the nuclei of an isolectin B4–stained capillary (arrows), suggesting differentiation of injected CD31⁺ cells into endothelial cells in vivo. Isolectin B4 (green), 4',6-diamidino-2-phenylindole (blue). Bars: H, I, 100 μm. ECs = endothelial cells; ILB4 = isolectin B4; other abbreviations as in Figure 3.
Figure 7 Verification and Quantification of ECs Generated From Transplanted CD31⁺ Cells

(A) Hind limb tissues were harvested 4 weeks after injection with Dil-labeled CD31⁺ cells and digested with an enzyme cocktail. The fluorescent-activated cell sorter analysis showed that 2.2% of endothelial cells in hind limb muscles were derived from injected CD31⁺ cells. P1 gating represents ILB4⁺ fraction and P2 gating indicates double positive population for Dil (CD31⁺ cells) and isolectin B4 (endothelial cells), n = 5. (B) Fluorescent-activated cell-sorted cells showing double positivity for Dil and isolectin B4 were subjected to fluorescent in situ hybridization. Fluorescent in situ hybridization showed that these double positive cells derived from ischemic hind limb of nude mice were of human origin. Bars: upper B, 200 μm; lower B, 20 μm. FACS = fluorescent-activated cell sorter; FISH = fluorescent in situ hybridization; other abbreviations as in Figure 6.
injected with CD31\(^+\) cells expressed high levels of stromal cell-derived factor-1, which are known to contribute to a pro-neovascularization environment. For optimal neovascularization, such an environment is required to recruit necessary cell types including monocytes and macrophages (28,29). Another characteristic that may make CD31\(^+\) cells particularly effective is their high adhesion capacity. Cell adhesion signals control cell viability and are essential for regulated angiogenesis and vasculogenesis via cell–cell and cell–matrix interactions (9,11,15).

The controversy remains regarding the (trans)differentiation potential of BM-derived cells into ECs. To clarify this, we meticulously evaluated hind limb muscles using multiple techniques. First, we used 3-dimensional reconstruction of confocal microscopic images to examine at least 1,200 tissue sections collected from 10 animals from each group. Although most CD31\(^+\) cells were localized to the interstitium or perivascular region, some cells were incorporated into vasculature and expressed functional EC markers (Fig. 7A). Second, FISH using Y chromosome probes showed positive donor cell-derived signals within the nuclei of ECs, suggesting endothelial differentiation of CD31\(^+\) cells. Third, FACS analysis confirmed and quantified ECs derived from CD31\(^+\) cells injected into hind limb ischemia. The FACS analysis revealed 2.2% of ECs in hind limb muscle were derived injected into hind limb ischemia. The FACS analysis PB-derived CD31\(^+\)/H11001 can be used for cell therapy.

These cells do not require mobilization to obtain enough to recruit necessary cell types including monocytes and macrophages (28,29). Another characteristic that may make CD31\(^+\) cells and macrophages (28,29). Another characteristic that may make CD31\(^+\) cells do not transdifferentiate into cardiac myocytes in myocardial infarcts. Nature 2004;428:664–8.


Cho HJ, Lee N, Lee JY, et al. Akt-modified mesenchymal stem cells can exert most of the beneficial effects of both cell types while still providing additional paracrine or vascularizing effects through other cell types. Clinically, as CD31\(^+\) cells occupy approximately 30% of PB-MNCs, these cells do not require mobilization to obtain enough to be used for cell therapy.

**Conclusions**

PB-derived CD31\(^+\) cells contain robust angiogenic and vasculogenic abilities that ameliorate hind limb ischemia. Although we only showed ischemic limb recovery in this study, this therapy would also be effective for ischemic heart disease given their broad neovascularizing, paracrine, and cell adhesion capacities. Accordingly, these cells can serve as a highly promising and novel therapeutic option for ischemic cardiovascular disease. Further investigation will be needed to determine if CD31\(^+\) cells are capable of treating human ischemic cardiovascular diseases.

**Reprint requests and correspondence:** Dr. Young-sup Yoon, Division of Cardiology, Department of Medicine, Emory University School of Medicine, 1639 Pierce Drive, WMRB 3309, Atlanta, Georgia 30322. E-mail: yyoon5@emory.edu.

**REFERENCES**


Key Words: CD31 • endothelial cells • angiogenesis • vasculogenesis • ischemic hind limb.

APPENDIX

For an expanded Methods section and supplemental figures and tables, please see the online version of this article.