CD34⁺ Progenitor to Endothelial Cell Transition in Post-Pneumonectomy Angiogenesis

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In many species, pneumonectomy triggers compensatory lung growth that results in an increase not only in lung volume, but also in alveolar number. Whether the associated alveolar angiogenesis involves the contribution of blood-borne progenitor cells is unknown. To identify and characterize blood-borne progenitor cells contributing to lung growth after pneumonectomy in mice, we studied wild-type and wild-type/green fluorescence protein (GFP) parabiotic mice after left pneumonectomy. Within 21 days of pneumonectomy, a 3.2-fold increase occurred in the number of lung endothelial cells. This increase in total endothelial cells was temporally associated with a 7.3-fold increase in the number of CD34⁺ endothelial cells. Seventeen percent of the CD34⁺ endothelial cells were actively proliferating, compared with only 4.2% of CD34[−] endothelial cells. Using wild-type/GFP parabiotic mice, we demonstrated that 73.4% of CD34⁺ cells were derived from the peripheral blood. Furthermore, lectin perfusion studies demonstrated that CD34⁺ cells derived from peripheral blood were almost uniformly incorporated into the lung vasculature. Finally, CD34⁺ endothelial cells demonstrated a similar profile, but had enhanced transcriptional activity relative to CD34[−] endothelial cells. We conclude that blood-borne CD34⁺ endothelial progenitor cells, characterized by active cell division and an amplified transcriptional signature, transition into resident endothelial cells during compensatory lung growth.

Keywords: endothelial progenitor cells; CD31⁺ CD34⁺ cell; lung angiogenesis; lung regeneration

Within weeks of pneumonectomy, compensatory growth of the remaining lung has been documented in many species, including rats (1), mice (2), dogs (3), cats (4), rabbits (5), and ferrets (6). The mechanism of post-pneumonectomy compensatory growth in the adult mouse lung relies in part on an increase in alveolar size, but also on a significant increase in the number of alveoli derived by neoalveolarization (7). The rapid addition of gas exchange units suggests an extraordinarily efficient process of alveolar capillary angiogenesis. Design-based estimates of capillary length (8) suggest sufficient angiogenesis for more than 3 km of new capillaries. Despite the magnitude of blood vessel growth, the mechanism of alveolar angiogenesis remains unknown.

Post-pneumonectomy lung growth is a special example of angiogenesis, because there is no histologically apparent tissue injury

CLINICAL RELEVANCE

Blood-borne CD34⁺ endothelial progenitor cells contribute to pulmonary angiogenesis. The therapeutic manipulation of these cells may be beneficial in a variety of lung diseases.

or endothelial denudation. Whether regenerative angiogenesis involves the activation of resident endothelial cells, the mobilization of specialized stem cells, or the contribution of blood-borne progenitor cells is unclear. The contribution of mature endothelial cells to regenerative angiogenesis, in the absence of injury, is unlikely because of the low turnover of resident endothelial cells. In normal circumstances, the daily mitotic rates of endothelial cells involve less than 1% of the cell compartment, and the half-life of resident endothelial cells is estimated at 1 to 3 years (9). Nonetheless, studies of the aorta and umbilical vein demonstrated isolated areas of increased mitotic activity (9) and subpopulations of clonogenic cells (10). At the other extreme, hemangioblasts, postulated to be the earliest identifiable common hemato-endothelial stem cell (11), are thought to participate in both developmental (12) and neoplastic (13) vasculogenesis. Neither activated resident endothelial cells nor hemangioblasts have been implicated in lung regeneration after pneumonectomy.

Blood-borne endothelial progenitor cells (EPCs) have been identified in a variety of tissues (14). Despite the varied phenotypes of these cells (15), EPCs are typically associated with remodeling after vascular injury or ischemia. In the lung, the localization of EPCs was demonstrated in acute injury related to transplantation (16, 17) and bacterial pneumonia (18), as well as monocrotaline-induced (19, 20) and hypoxia-induced (21) pulmonary hypertension. In contrast, a post-pneumonectomy model after bone marrow transplantation failed to demonstrate the integration of blood-borne EPCs in the remaining lung 28 days after pneumonectomy (22).

In this report, we identified a significant population of CD34⁺ cells migrating into the lung after pneumonectomy. These cells—characterized by increased proliferative activity, a distinctive surface phenotype, and amplified transcriptional signature—integrated into the vascular lining of the regenerating lung. The frequency and proliferative capacity of these migrating cells suggest that the blood-borne CD34⁺ cell population is an important contributor to lung angiogenesis.

MATERIALS AND METHODS

Mice

C57BL/6 wild-type and GFP⁺C57BL/6-Tg (ubiquitin C promoter [UBC]-green fluorescent protein [GFP]) 30Scha/J (Jackson Laboratories, Bar

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Harbor, ME) mice with similar weights were used for the experiments. The care of the animals was consistent with guidelines of the American Association for Accreditation of Laboratory Animal Care (Bethesda, MD).

Parabiotic Surgery and Pneumonectomy

Animals were paired and pneumonectomized, based on modifications of a previously described technique (23–25). Details are described in the online supplement.

Lung Digestion

The lung was processed in a modification of a procedure previously described (26) (details are described in the online supplement). Cell counts and calculations were performed as described in the online supplement.

Monoclonal Antibodies

Information on monoclonal antibodies (mAbs) is detailed in the online supplement.

Lectins

To identify vessel integration, three endothelial-binding lectins were injected into the circulation: biotinylated *Lycopersicon esculentum* lectin (LEL), biotinylated *Bandeiraea simplicifolia*-1 lectin (BS-1), and biotinylated *Ricinus communis* agglutinin (RCA), purchased from Vector Laboratories (Burlingame, CA). Each lectin was injected into the tail vein 7 days after pneumonectomy. Control mice received a vehicle injection alone. After a circulation period of 10 minutes, the lung was digested and preserved in staining buffer (BD Biosciences, Franklin Lakes NJ) for subsequent analyses. Flow cytometry gating controls demonstrated that the potential contamination of blood cells involved less than 0.05% of the endothelial cell events.

Flow Cytometry

For standard phenotyping, cells were incubated with a 5-fold excess of anti-mouse antibodies. The cells, labeled with biotinylated antibodies, were washed with staining buffer (BD Biosciences, San Jose, CA) and subsequently stained with streptavidin-PE-Cy7 (Biolegend, San Diego, CA) at a concentration of 0.1 $\mu\text{g}/\text{ml}$. For detecting lectin-positive cells *in vivo*, lectin-perfused lungs were digested and stained with anti-CD45 mAb, anti-CD31 mAb, anti-CD34 mAb, and streptavidin-PE-Cy7. The cells were analyzed by FACSCanto II (BD Biosciences) with tri-excitation laser (407 nm, 488 nm, and 633 nm excitation). The data were analyzed by FCS Express 4 software (De Novo Software, Los Angeles, CA). In all analyses, debris were eliminated by gating the alive cell population of side and forward light scatter, and were further eliminated by gating the population negative for 7-aminocinactinomycin D (7AAD) (BD Biosciences). The methods of cell isolation and DNA cell-cycle analysis are described in the online supplement.

Immunohistochemical Staining

Methods of immunohistochemical staining are described in the online supplement.

PCR Array

Commercially available PCR arrays were obtained from SA Biosciences (Frederick, MD) and included the Angiogenesis Array (catalogue number PAMM-024), and the Stem Cell Signaling Array (catalogue number PAMM-047). PCR was performed on ABI 7300 Real-Time PCR System (Applied Biosystems, Carlsbad, CA). The two sets of triplicate control wells (reverse transcription controls and positive PCR controls) were also examined for inter-well and intra-plate consistency. Standard deviations of the triplicate wells were uniformly less than 1 cycle threshold (C_t). Five genes were common to both arrays (*Eng*, *Fgfr3*, *Fzd5*, *Smad5*, and *Tgfb β 1*). The variance of C_t values was uniformly less than

0.5 C_t . To reduce variance and improve inferences per array, a design strategy was used that combined pooled samples (typically three mice) as well as individual mice.

Statistical Analysis

The methods used in our statistical analysis are described in the online supplement.

RESULTS

Endothelial Response to Pneumonectomy

Consistent with previous work demonstrating an increase in lung volumes and lung weights (27, 28), left pneumonectomy in mice led to an increase in the total number of lung cells (Figure 1A). Paralleling the increase in total lung cells was an increase in lung endothelial cells identified by the surface molecule CD31 ($r^2 = 0.92$) (Figure 1B). The measured 3.2-fold increase in the number of endothelial cells (Figure 1B) suggested the possible contribution of endothelial progenitor cells. Flow cytometry analysis of the lung 7 days after pneumonectomy demonstrated that 12% of CD31⁺ endothelial cells expressed the CD34 cell surface molecule (Figures 1C–1E).

Origin of CD34⁺ Cells

Defining endothelial cells as CD31⁺ and CD45[−], the CD34⁺ endothelial cells were prominent during the rapid phase of lung growth. Both the percentage (Figure 2A) and absolute number (Figure 2B) of CD34⁺ endothelial cells peaked on Day 7 after pneumonectomy. Flow cytometric cell cycle analysis demonstrated that CD34⁺ endothelial cells were actively proliferating (Figures 2C and 2D). Cell cycle activity in the CD34⁺ cells was 4-fold greater than in CD34[−] endothelial cells (Figures 2C and 2E). Because of the CD34⁺ cell kinetics, subsequent experiments focused on Day 7 after pneumonectomy.

The lung migration of blood-borne CD34⁺ progenitor cells was investigated using a parabiotic (wild-type/GFP) pneumonectomy model (Figure 3A). On Day 7 after pneumonectomy, lung cells expressing GFP in the wild-type parabiont were poorly visualized by fluorescence microscopy, but were detectable in the alveolar septae by immunohistochemistry (Figure 3B). Flow cytometry demonstrated that 10.9% $\pm$ 6.0% (range, 4–20%; $n = 5$) of CD31⁺ endothelial cells in the lung were GFP⁺ and were derived from the peripheral blood (Figures 3C and 3D). More than 36.7% $\pm$ 14.1% of CD34⁺ endothelial cells in the regenerating lung were GFP⁺ (Figures 3E and 3F). Because both wild-type (GFP[−]) and GFP⁺ parabionts contributed equally to the peripheral blood (24), an average of 21.8% (range, 8–39%) of all endothelial cells and 73.4% (range, 44–96%) of all CD34⁺ cells were derived from the peripheral blood.

CD34⁺ Vascular Lining Cells

In addition to expressing the CD31 cell surface molecule, the CD34⁺ cells expressed higher concentrations of the vascular endothelial growth factor receptor-1 (VEGFR1) and LYVE-1 molecules than did resident CD34[−] endothelial cells (Figure 4). To determine if the migrating cells were actually incorporated into the vessel and functioning as vascular lining cells, biotinylated lectins were injected into the blood circulation. After a brief circulation time (10 minutes), the lung endothelial cells were isolated by flow cytometry gating and examined for lectin binding (Figure 5). Nearly uniform lectin binding to both CD34⁺ and CD34[−] endothelial cells was observed, a finding consistent with integration of the CD34⁺ endothelial cells into

Figure 1. Cell composition of the post-pneumonectomy lung. After enzymatic digestion, the total numbers of lung-digested cells (A, open circles) and CD31⁺ (CD45⁻) endothelial cells (B, solid circles) were determined 0, 7, 14, and 21 days after pneumonectomy ($n = 4$). The total number of lung-digested cells from sham-operated mice was determined 7 days after pneumonectomy (gray square and triangle, $n = 4$). Error bars reflect the mean $\pm$ 1 SD. (C) The flow cytometry gate defining both CD34⁻ (blue) and CD34⁺ (purple) endothelial cells was both CD45⁻ and CD31⁺. (D and E) The CD34⁺ endothelial cells were identified by a statistical confidence level of 0.005, based on isotype controls.

the vascular lining. All three lectins (LEL, BS-1, and RCA) demonstrated higher levels of lectin binding to CD34⁺ cells than to CD34⁻ endothelial cells (Figures 5G–5I). Lectin binding by peripheral blood cells within the CD34⁺ or CD31⁺ flow cytometry gates was negligible ($<0.005\%$; data not shown).

Transcriptional Profile of CD34⁺ Endothelial Cells

To define the endothelial transcriptional signature of CD34⁺ and CD34⁻ endothelial cells, quantitative RT-PCR arrays were compared with the transcription profile of CD11b⁻ and CD31⁻ lung cells (presumed epithelial or smooth muscle cells) also

Figure 2. The frequency (A, open circles) and absolute number (B, open circles) of CD34⁺ endothelial cells were determined by flow cytometry of lung digests at 0, 3, 7, 14, and 21 days after pneumonectomy ($n = 4$). The results of sham pneumonectomy controls (gray squares) are presented. Error bars reflect the mean $\pm$ 1 SD. (C) Cell-cycle profiles of CD31⁺ endothelial cells on Day 7 after pneumonectomy. Cells were obtained by enzymatic digestion, and were analyzed using flow cytometry and cell-cycle software (ModFit; Verity Software House) ($P < 0.006$; $n = 3$). (D and E) After the exclusion of digestion debris by light scatter gating, automated analysis of the ModFit model components, processed by a Marquardt nonlinear least-squares analysis, excluded aggregates (Agg.; green) and identified S-phase (S; gray arrow) and G2-phase (G2; gray arrow) cells on Day 7.

Figure 3. Parabolic demonstration of blood-borne CD34⁺ endothelial cells on Day 7 after pneumonectomy. (A) Schematic of experimental design involving a left pneumonectomy in the wild-type parabiont of wild-type/GFP⁺ parabiotic twins (C57/B6). Parabiosis was established for 28 days prior to left pneumonectomy. The remaining lung was studied 7 days after pneumonectomy. (B) Anti-GFP avidin-biotin complex (ABC) immunohistochemical staining demonstrated GFP⁺ cells within the alveolar septae. Flow cytometry of the lung digests demonstrated GFP⁺ cells that were CD45⁻ (C) and CD31⁺ (D). (E and F) Analysis of CD31⁺ lung endothelial cells demonstrated that most of the GFP expression occurred in the CD34⁺ endothelial cell population ($P < 0.01$; $n = 5$).

derived from post-pneumonectomy lungs (Figure 6). In angiogenesis arrays, the CD34⁺ cells expressed an endothelial cell transcription profile consistent with the observed surface expression of CD31 and VEGFR1 (*Pecam1* and *Flt1*) (Figure 6A). In addition, the CD34⁺ cells were generally more transcriptionally active than CD34⁻ cells; a notable exception was *Tbx1* ($P < 0.006$) (Figure 6A). In stem cell arrays, the expression of CD34⁺ was generally higher than that of resident endothelial cells, with several notable exceptions. *Bmpr1a* ($P < 0.04$), *Fzd2* ($P < 0.0007$), and *Gli1* ($P < 0.007$) were significantly down-regulated relative to CD34⁻ endothelial cells.

DISCUSSION

In this report, we studied the contribution of endothelial progenitor cells in a unique example of adult tissue regeneration—namely, murine post-pneumonectomy lung growth. Lung growth after pneumonectomy is a rapid process of tissue morphogenesis that occurs in the absence of histologically apparent tissue injury or reparative inflammation. In our studies, the selective migration of blood-borne progenitor cells was tracked without the coincident migration of inflammatory cells. Moreover,

Figure 4. Phenotype of CD34⁺ and CD34⁻ lung endothelial cells, 7 days after pneumonectomy. After gating exclusion of the CD45⁺ cells, CD31⁺ endothelial cells were separately analyzed as CD34⁻ (A, C, E, and G) and CD34⁺ (B, D, F, and H) populations. The enzymatically digested lungs were stained with anti-vascular endothelial growth factor receptor-1 (VEGFR1) monoclonal antibody (mAb) (A and B), anti-VEGFR2 mAb (C and D), anti-CXCR4 mAb (E and F), and anti-LYVE-1 mAb (G and H), as described in MATERIALS AND METHODS. The expression of VEGFR1 and LYVE-1 antigens was highly significant ($P < 0.0001$). Representative histograms of a single mouse are shown.

the unique structure of the lung facilitated not only the isolation of these migrating progenitor cells, but also the characterization of their transcriptional profile.

Our study demonstrated that blood-derived cells, expressing the CD34 surface molecule, directly contributed to murine post-pneumonectomy angiogenesis, based on several lines of evidence: (1) The peak of CD34⁺ cell migration was at 7 days—timing that coincided with the peak of lung angiogenesis (29). (2) Approximately 17.0% of migrating CD34⁺ cells were undergoing active cell division. (3) The CD34⁺ cells directly integrated into the vascular lining. (4) The GFP marker, indicating a peripheral blood origin, was present not only in CD34⁺ cells, but also in “mature” (CD34⁻ CD31⁺) endothelial cells. (5) The CD34⁺ cells had a similar, but enhanced, transcriptional signature relative to resident endothelial cells. Collectively, these findings suggest that blood-borne endothelial progenitor cells make a quantitative contribution to the endothelial component of neoalveolarization, and may play a regulatory role in post-pneumonectomy angiogenesis.

The characteristics of the blood-derived CD34⁺ endothelial cells in this study were consistent with the contemporary definition of a progenitor or precursor cell. In contrast to heman-gioblast stem cells with potentially unlimited self-renewal and multilineage differentiation, the blood-derived CD34⁺ endothelial

Figure 5. Intravascular labeling of vascular lining cells of mice after pneumonectomy. Biotinylated lectins were injected into tail vein of the pneumonectomized mice on Day 7, and allowed to circulate for 10 minutes before flushing and harvest. Digested lung cells were stained with anti-CD45 mAb, anti-CD31 mAb, anti-CD34 mAb, and avidin-PE-Cy7. Data were analyzed by flow cytometry (see MATERIALS AND METHODS). (A–C) The CD31⁺ CD45[−] endothelial-cell population was studied with anti-CD34 (purple) and avidin-PE-Cy7 to detect lectin binding. (D–F) Dual-parameter histograms of anti-CD31 and the lectins *Lycopodium esculentum* (LEL) (D), *Bandeiraea simplicifolia*-1 (BS-1) (E), and *Ricinus communis* agglutinin (RCA) (F). (G–I) Single-parameter histograms of LEL, BS-1, and RCA binding are shown to facilitate comparisons of lectin binding with endothelial cells; gray indicates lectin control sample (C). CD34⁺ endothelial cells (purple) and CD34[−] endothelial cells (blue) are compared with lectin controls (gray).

cells in this study demonstrated an increased, but modest, capacity for cell division. Although the enzymatic technique of lung digestion limited the numerical precision, we estimated that approximately half of the new endothelial cells within the first 2 weeks after pneumonectomy were derived from the peripheral blood, and the remainder were derived from cell division. Further, the transcriptional profile of CD34⁺ endothelial cells was notably similar to the profile of resident endothelial cells. The most striking difference between CD34⁺ and CD34[−] cells was the enhanced transcriptional activity of CD34⁺ endothelial cells.

Importantly, the expression of GFP provided a marker of peripheral blood origin, irrespective of the expression of membrane molecules. Although most of the GFP⁺ endothelial cells in our study were also CD34⁺, the expression of the CD34 surface molecule is not restricted to endothelial progenitor cells. The finding that the expression of endothelial CD34 is linked to a variety of proliferative conditions and cell-cell interactions (30) suggests that CD34 is potentially up-regulated in resident endothelial cells, depending on the regenerative microenvironment and vascular milieu. Although endothelial progenitor cells are enriched in the CD34⁺ cell population, we recommend caution in interpreting the functional implications of CD34 expression alone.

Endothelial progenitors have been identified among human mononuclear cells enriched for CD34-expressing (CD34⁺) cells (31, 32) and in the buffy coat fractions of peripheral blood (33);

however, prior evidence for the incorporation of the CD34⁺ cell into the microvasculature has been limited (34–36). Our parabiotic model (24), with cross-circulation of a GFP⁺ and wild-type blood, provided confirmation that CD34⁺ EPC were: (1) derived from the peripheral blood, and (2) incorporated into the lung. Further, GFP provided an opportunity to track the fate of blood-derived endothelial cells and confirm their integration into the vascular lining. An interesting finding was that the intensity of GFP fluorescence in CD34[−] GFP⁺ endothelial cells—the cells integrated into the vascular lining—was 10- to 100-fold lower than in CD34⁺ GFP⁺ endothelial cells (not shown). Presumably reflecting the *in situ* maturation of the blood-borne progenitor cells, this speculation is also consistent with the muted transcriptional signature of CD34[−] endothelial cells in the growing lung.

Our studies suggest several explanations for previously unsuccessful attempts to identify blood-borne endothelial progenitor cells in the post-pneumonectomy lung. First, the decreased expression of the cytoplasmic fluorochrome in GFP⁺CD34[−] endothelial cells provides a potential explanation for the limited detection of fluorescence in endothelial cells 28 days after bone marrow transplantation (22). Second, the lower level of GFP expression, combined with the autofluorescence of lung elastin (37), complicates the spatial detection of the thin profile of resident lung endothelial cells. Third, the CD34 cell surface expression suggests that the endothelial progenitor cells were

Figure 6. Expression of angiogenesis-related genes (A) and stem cell signaling genes (B) in CD34⁺ cells after pneumonectomy (see MATERIALS AND METHODS). CD34⁺ and CD34⁻ endothelial cell gene transcription was compared with an unrelated post-pneumonectomy CD31⁻ CD11b⁻ cell population (presumed epithelial and smooth muscle cells). The comparison cell population was isolated by cell sorting, and the transcriptional profile was characterized by quantitative RT-PCR. The genes from CD34⁻ (light bars) and CD34⁺ (dark bars) endothelial cells demonstrating statistically significant expression (*t* test, *P* < 0.05) are shown. Each data point represents triplicate or quadruplicate arrays of 3–5 mice for each gene.

derived from the bone marrow. It is possible that this progenitor population was selectively compromised during bone marrow engraftment. Finally, an advantage of our study was the use of flow cytometry—a high-throughput technology that examines tissues on a per cell basis. Flow cytometry provided not only a sensitive and quantitative measure of cell fluorescence, but also a cell isolation technique that facilitated transcriptional analysis.

Post-pneumonectomy lung growth is remarkable not only for the amount of angiogenesis, but also the stringent functional constraints of new vessel growth. Within 10 to 14 days of pneumonectomy, there is a significant increase in the total number of endothelial cells (29). This increase in endothelial cells parallels a 25% and 33% increase in the number of alveoli at Days 6 and 21 following pneumonectomy, respectively (7). Matching the angiogenesis of the capillary endothelium and the septation of neoalveolarization suggests an active process of alveolar construction that facilitates new alveolar growth without compromising gas exchange. A potential mechanism for optimizing this process of alveolar construction is the dynamic alteration of the resident endothelial cell population within the lungs. Our flow cytometry data, using the nucleic acid intercalating dye 7AAD, indicated a significant population of 7AAD-dim CD31⁺ cells (not shown). Previously demonstrated to be suggestive of DNA fragmentation and apoptosis (38), the 7AAD-dim CD31⁺ cells may indicate dynamic population changes and network remodeling (39) during the phase of maximal lung growth.

Another potential mechanism for maintaining tissue function during the rapid expansion of the microcirculation is intussusceptive angiogenesis. Originally identified in the developing rodent lung (40, 41), intussusceptive angiogenesis is a dynamic process that can produce two lumens from a single vessel (42, 43). The central structural feature of intussusceptive angiogenesis is the development of a pillar that bridges the opposing sides of the microvessel lumen (44). Physical expansion of the pillar eventually leads to the division of the vessel into two lumens (44). *In vitro* studies of the intravascular pillar have demonstrated that particle flow trajectories lead to prolonged pillar contact and increased residence time at the vessel bifurcation (45). In short, the intravascular pillars provide a mechanism for localizing the distribution of blood-borne progenitors while maintaining organ perfusion. Of interest, the original description of blood-borne

endothelial cells used surgical sutures reminiscent of intravascular pillars (46).

Finally, the transcriptional profile of CD34⁺ and CD34⁻ endothelial cells was similar; the notable difference was a generalized increase in transcriptional activity in the CD34⁺ cells. Of particular interest, four genes demonstrated a different pattern of activity. The genes *Tbx1*, *Bmpr1a*, *Fzd2*, and *Gli1* demonstrated a marked decrease in transcriptional activity relative to resident endothelial cells. An intriguing possibility is that these genes, and their respective signaling pathways, may provide clues to the regulatory “switch” that controls conversion of progenitor to mature endothelial cells. For example, *Tbx1*, *Bmpr1a*, and *Gli1* are genes associated with stem cell behavior and early lung development (47–49). The decreased transcriptional activity in these genes is consistent with a transition of the CD34⁺ blood-borne progenitor cells to mature endothelial cells. A functional explanation of diminished *Fzd2* transcriptional activity is less clear; however, *Fzd2* has been linked to lung regeneration (50). Investigating these genes, and their associated signaling pathways, will likely provide important insights into the regulation of endothelial progenitor cells.

Author disclosures are available with the text of this article at www.atsjournals.org.

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