Circulating and Endogenous Progenitor cells in Lung Injury and Repair

Vivek Balasubramaniam, MD
Associate Professor of Pediatrics
Pediatric Heat Lung Center
University of Colorado
Lung injury and repair

• Only the neonatal lung has the capacity for growth
• Adult rodent lungs grow new alveoli after pneumonectomy
• Recapitulation of neonatal patterns of growth
• Understanding lung growth may provide insight into mechanisms of lung repair

Bronchopulmonary Dysplasia
Characterized by abnormal lung structure
Impaired lung growth in Bronchopulmonary Dysplasia (BPD)

Normal BPD

Antiangiogenesis agents impair Alveolarization

(Jakkula, 2000)
VEGF receptor inhibition during infancy decreases Alveolarization

Vascular Hypothesis in the pathogenesis of BPD

- Disruption of angiogenesis impairs alveolarization
- Preservation of vascular growth and endothelial cell survival will promote normal lung growth

Abman SH; AJRCCM 2001
Mechanisms of Lung Vascular Growth

- Angiogenesis
  - Sprouting of new blood vessels from pre-existing vessels
- Vasculogenesis
  - Formation of blood vessels from circulating or resident angioblastic progenitor cells (endothelial progenitor cells [EPCs], other angiogenic cells).

Endothelial Progenitor Cells (EPC)

- Circulating highly proliferative cells (CD34+) differentiate into endothelial cells (Asahara, 1997)
- Bone Marrow cells (CD133+/VEGFR-2+) differentiate into endothelial cells (Reyes, 2002)
- EPCs isolated from Cord blood (Ingram & Yoder, 2004)
  - Do not express hematopoietic marker CD45
EPCs in Tissue repair

- EPC home to sites of injury (cardiac, ischemic limb). (Kawamoto A; Circulation 2001; Kocher AA; Nat Med 2001)
- EPCs are able to differentiate into endothelial cells.
EPCs in Lung Disease

- Clinical Studies
  - Increased numbers of EPC correlated with survivors of Acute Lung Injury. (Burnham EL; 2005 AJRCCM)
  - Lower circulating EPC numbers correlate with developing fibrosis in adults recovering from pneumonia. (Yamada M; 2005 Thorax)
  - EPC number decreased in patients with COPD and restrictive lung disease. (Fadini GP; 2006 Stem Cells)

Hyperoxia results in simplified lung structure in neonatal mice that persists in juvenile mice

Balasubramaniam, 2007
Hyperoxia reduces vessel density

Room Air

Hyperoxia

Neonatal hyperoxia reduces EPCs in the circulation, bone marrow and lungs of infant mice

Balasubramaniam, 2007
How to define the EPC?

- AC133+
- Tie-2+
- CD45-
- CD90-
- CD117(c-kit)+

**EPC**

- CD105+
- ICAM-1+

CD34+  CD146+  VEGFR-2+  CD31+  VE-Cadherin+

Prater DN, Leukemia 2007
Is an EPC an EPC?!?

- Early outgrowth cells - CFU-Hill
  - low proliferative rate
  - spindle appearance
  - express endothelial (CD31,CD105,CD144,CD146), and macrophage markers (CD45,CD14) and engulf bacteria
  - promote blood vessels growth

- Late outgrowth cells - ECFC
  - highly proliferative
  - Cobblestone appearance
  - endothelial markers (CD31, CD105,CD144,CD146) but not macrophage markers (CD45,CD14)
  - incorporate into endothelial layer of blood vessel

EPCs are increased in preterm infants

Baker CB, AJRCCM 2009
ECFCs are reduced in premature infants with BPD

Borghesi A, AJRCCM 2009

Preterm ECFC proliferate faster than term ECFC

Baker CB, AJRCCM 2009
Preterm ECFC are more sensitive to hyperoxia

Neonatal hyperoxia reduces EPCs in the circulation, bone marrow and lungs of infant mice
Isolation of bone marrow vascular progenitor cells

Transfer to: 6 well tissue culture plate on feeder layer of MEF

BM derived colonies

BM derived Tie2-GFP cells
Bone marrow derived angiogenic cells (BMDAC)
Phenotypic characterization of BMDAC

Myeloid Progenitor Cell

BM derived cells improve lung structure after neonatal hyperoxia

Neonatal hyperoxia w/ RA recovery
Neonatal hyperoxia w/ RA recovery + BMDAC
Increase in vascular density after BMDAC injection

BMDAC cells engraft in perivascular location
BMAC cells engraft in close proximity to alveolar type II epithelial cells

Does the ECFC enhance lung growth?
ECFCs enhance the recovery of lung structure after neonatal hyperoxia

Neonatal hyperoxia w/ RA recovery

Neonatal hyperoxia w/ RA recovery + ECFC

Circulating Pro-Angiogenic Cells

Circulating Endothelial Progenitor Cells