Stem Cells and Cell Therapy Approaches For Lung Diseases

ISCT, Rotterdam May 2011

Daniel J. Weiss MD PhD
Associate Professor of Medicine

University of Vermont
Vermont Lung Center
Repairing/Regenerating the Lung
Stem Cells and Cell Therapy Approaches

Spectrum of lung diseases
- Different cell therapy approaches
- Endogenous vs exogenous cells
- Embryonic vs adult cells

Engraftment into Lung
- Cystic Fibrosis, Pulmonary Hypertension

Bioengineering Approaches
- Lung Transplantation

Immunomodulation
- Asthma, COPD, ARDS, Lung Cancer
Bioengineering by Manipulation of Endogenous Lung Progenitor Cells

• Function in development, repair, aging (?)

• Lung: complex organ >40 cell types

• Lung progenitors: not well defined

• Most data in mice: little information in humans

• Homeostasis not well understood

• Potential role as lung cancer stem cells

• Many years from clinical manipulation

Rawlins and Hogan, Development. 2006; 133(13):2455-65 (7)
Embryonic Stem Cells

Mouse isolated in 1960’s-70’s;
Human in 1998

Derived from the inner cell mass of a developing embryo

Self renewal

Pluripotent: Differentiate into all tissues

Lanza and Rosenthal
Scientific American 2004
Can Isolated Embryonic Stem Cells Become Lung Epithelium?

- Expose to air-liquid interface
  - Form pseudostratified ciliated epithelium

Resembles tracheal epithelium
Ciliated and basal cells

Coraux et al
Am J Resp Cell and Mol Biol 2005

Generally small numbers unless cells are transduced for selection advantage

Adapted from Roszell et al, Efficient Derivation of Alveolar Type II Cells from Embryonic Stem Cells for In Vivo Application. Tissue Eng 2009
Can Isolated Embryonic Stem Cells Become Lung Epithelium?

- Role of physical environment important
  - Matrix surface and configuration
  - Oxygen tension
  - Stretch

mESCs seeded into 3-dimensional collagen hydrogels and differentiated by sequential exposure to Activin A/Wnt3b and FGF2

Data courtesy of Christine Finck MD, University of Connecticut
Can Isolated iPS Cells Become Lung Epithelium?

Even more difficult than ESCs!

However, can generate disease-specific iPS cells

Adapted from Somers et al Generation of transgene-free lung disease-specific human iPS cells using a single excisable lentiviral stem cell cassette. Stem Cells 2010.
Adult Stem and Progenitor Cells

Bone Marrow Contains Several Stem Cell Populations

- Hematopoietic stem cell (HSC)
- Endothelial progenitor cells (EPC)
- Mesenchymal derived stromal cells (MSC)
- Circulating fibrocytes

Lanza and Rosenthal Scientific American June 2004
Does Administration of Adult Stem Cells Fix Defective Epithelium?

Replace damaged airway epithelium?  Cystic Fibrosis

1 week

Green: Anti-CFTR Ab  Red: Y probe  Blue: Anti-pan-cytokeratin Ab  Light blue: Hoechst nuclear staining


GFP
Bone Marrow Cells
± Myeloablation
± Lung Injury
± Chemotactic Factors

Donor-recipient Model

Donor Mice
Recipient Mice
Bone Marrow Cells
Female CFTR-KO Mice

Morphology
Repopulation of Lung Epithelium by Donor Marrow Cells
Functional (Chloride Current)

IV CB-MSC
Low-dose Irradiation (1 Gy)
NOD/SCID mouse

Donor-derived cells
• β2 microglobulin

Sueblinvong et al AJRCCM 2008

Red = β2 microglobulin, Green = human CFTR, Blue = DAPI
Summary of *In Vivo* Studies
Lung Epithelial Engraftment with Bone Marrow, Cord Blood, Amnion, Placental, Adipose-Derived Adult Cells

- **Can engraft lung epithelium**
  - IV or IT delivery
  - Express lung epithelial-specific proteins (airway and alveolar)
  - Fusion may contribute

- **Small number of cells**
  - <1% airway epithelium
    - Early studies had artifacts
  - Functional significance unclear
  - Need to understand how the cells are recruited to lung and converted into lung cells
Engraftment or Growth Stimulation of the Pulmonary Vasculature

Disease target: Pulmonary Hypertension

EPCs stimulate angiogenesis in models of pulmonary hypertension

Naive  Monocrotaline  Monocrotaline + EPC


Basis of PHaCET Clinical Trial of Autologous EPCs for Pulmonary Hypertension (PI Duncan Stewart MD)

Promising area for further clinical trials
Cell Therapy Approaches
Immunomodulation of Innate and Adaptive Immune System By MSCs

Immunosuppressive
- Inhibit proliferation and effector functions of immune cells in vitro
  - T cell, B cell, NK cells, dendritic cells, etc

Poor Immunogenicity
- Low levels of MHC I
- No MHC II
- No co-stimulatory molecules
- Successful use of allogeneic MSCs

Systemic administration
- homes to site of injury

Clinical Application
graft vs host, Crohn’s, MS, diabetes, arthritis
cardiac diseases

Use of MSCs in Lung Injury Models
Rapidly Growing Literature

- >40 publications as of 5/11
- Variety of lung injury models including fibrosis, ALI, BPD, COPD, Pulm HTN, Sepsis
- What cells are being utilized: adherence to ISCT criteria?
- Are the effects MSC-specific? Use of cell controls (fibroblasts or other)

<table>
<thead>
<tr>
<th>Acute Lung Injury</th>
<th>ISCT Criteria Met?</th>
<th>Differentiation</th>
<th>Flow Pos</th>
<th>Flow Neg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gupta 2007 IN LPS</td>
<td>No</td>
<td>A/O/myofibroblast</td>
<td>Not specified</td>
<td>CD11b,31,34,45</td>
</tr>
<tr>
<td>Xu 2007 IP LPS</td>
<td>No</td>
<td>C</td>
<td>Not specified</td>
<td>CD11b, 45,TLR4</td>
</tr>
<tr>
<td>McCarter 2007 IT LPS</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Mei 2007 IT LPS</td>
<td>No</td>
<td>A/C/O</td>
<td>CD34, Sca-1</td>
<td>CD11b,45,ICAM-1</td>
</tr>
<tr>
<td>Xu 2008 Nebulized LPS for 7 successive days</td>
<td>No</td>
<td>A</td>
<td>CD31,44,73,105,Sca-1</td>
<td>CD11b,14,45, P-selectin,Ekit,Flk1</td>
</tr>
<tr>
<td>Lee 2009 Intrabronchial LPS</td>
<td>No</td>
<td>Not specified</td>
<td>Not specified</td>
<td>Not specified</td>
</tr>
<tr>
<td>Moodley 2009 Bleomycin</td>
<td>No</td>
<td>O</td>
<td>CD73, 90, 105, 166 HLA A,B,C</td>
<td>CD 14,28,31,34,45, 66, 80, 86 HLA DR</td>
</tr>
<tr>
<td>Iyer 2010 IP LPS</td>
<td>No</td>
<td>C</td>
<td>Not specified</td>
<td>CD11b,45,TLR4</td>
</tr>
</tbody>
</table>
### Reality: Lots of different cells out there

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Derived from</th>
<th>Potency</th>
<th>Surface phenotype</th>
<th>Reference</th>
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</thead>
<tbody>
<tr>
<td>Human mesenchymal stem cell (MSC)(a)</td>
<td>Bone marrow, cord blood, tissue resident</td>
<td>Mesenchymal lineage</td>
<td>CD105(+), CD90(+), CD73(+), CD79a(−), CD45(−), CD34(−), CD19(−), CD14(−), CD11b(−), HLA-DR-</td>
<td>[10–12]</td>
</tr>
<tr>
<td>Human marrow isolated adult multilineage inducible cells (MIAMI)</td>
<td>Bone marrow</td>
<td>Mesoderm, endoderm and ectoderm</td>
<td>CD164(+), CD122(+), CD81(+), SSEA4(+), CD45(−), CD34(−), c-Kit-</td>
<td>[41]</td>
</tr>
<tr>
<td>Human Unrestricted somatic stem cells (USCC)</td>
<td>Cord blood</td>
<td>Mesoderm, endoderm and ectoderm</td>
<td>CD10 low, Flk1 low, CD45(−), CD34(−), c-kit-</td>
<td>[46]</td>
</tr>
<tr>
<td>Human very small embryonic-like (VSEL)</td>
<td>Umbilical cord blood, bone marrow mobilized</td>
<td>Neuroectoderm, pancreatic cells, cardiomyocytes</td>
<td>CXCR4(+), AC133(+), Sca-1(+), CD45(+), SSEA1(+)(mouse), SSEA4(+)(Human), Lin(−), MHC-I-</td>
<td>[42,43]</td>
</tr>
<tr>
<td>Human Multipotent adult stem cells (MASC)</td>
<td>Bone marrow, heart, liver</td>
<td>Pancreatic cells, neuronal, mesenchymal</td>
<td>CD49b(+)/CD90(+), CD13(+), CD105 low, CD73 low, CD44 low, HLA-ABC low, CD133(−), CD45(−), CD34(−), CD14(−), HLA-DR-</td>
<td>[40]</td>
</tr>
<tr>
<td>Human amniotic fluid stem cell (AFS)</td>
<td>Amniotic fluid</td>
<td>Mesenchymal, neuronal, endothelial, hepatic</td>
<td>CD105(+), CD90(+), CD73(+), CD44(+), CD29(+), MHC-I(+), c-Kit(+), MHC-II low, CD133(−), CD45(−), CD34(−)</td>
<td>[45]</td>
</tr>
<tr>
<td>Rodent multipotent adult progenitor cells (MAPC)</td>
<td>Bone marrow, muscle, brain</td>
<td>Mesoderm, endoderm and ectoderm</td>
<td>CD45(+), CD34(−), c-Kit(+), CD9(+), CD13(+), CD31(+), CD44(+), MHC-I(+), CD45(+), Thy1-</td>
<td>[47,49,61,79]</td>
</tr>
</tbody>
</table>

Sohni et al 2011 Best Practice & Research Clinical Haematology 24 (2011) 3–11
Considerations for Clinical Investigations/Trials with MSCs in Lung Diseases

- **Source**
  - Which cells?
  - Autologous vs Allogeneic
  - Which tissue? marrow vs adipose vs cord blood vs other
    - Similar but different
  - Commercial vs Academic

- **Manufacturing**
  - GMP
  - Scale-up

- **Route of Administration**
  - Intravenous?
  - Intratracheal?

- **Dosing**

- **Monitoring/Safety**
  - Infusional toxicity
  - Short-term
  - Long-term: potential tumorigenesis

- **Mechanisms**

Ripe area for clinical trials!
Large number of trials
Acute MI as an inflammatory event

Single IV infusion 1-10 days after acute MI

Safety: No infusional toxicity, attributable SAEs, ectopic tissue formation

Efficacy: Improved LVEF, fewer PVCs, improved global assessment

Improved FEV1
Mesenchymal stromal cells for the treatment of moderate to severe COPD

- Phase II multicenter, double-blind, placebo-controlled trial
- 65 patients enrolled at 6 sites
- Patients received a total of 4 infusions over the course of 4 months
- Secondary outcomes included pulmonary function tests, exercise capability, and health-related quality of life
- Patients were followed for 2 years after the first infusion

Used with permission of Osiris Therapeutics Inc
Osiris COPD Trial: 6 month interim analysis

- The trial met its primary goal of demonstrating the safety of Prochymal in patients with compromised pulmonary function at the six-month evaluation point.

- Prochymal significantly decreased systemic inflammation in patients when compared to those receiving placebo, as determined by C-reactive protein (CRP).

- Trend towards improvement in global outcomes such as 6 minute walk

- Despite the reduction in inflammation, pulmonary function in patients receiving Prochymal was not significantly improved compared to those receiving placebo.

Trial is underpowered to detect changes in PFTs

2 year trial period was completed in Fall 2010

Press release
June 23, 2009
Is COPD an Optimal Target for Immunomodulation with MSCs?

• Although chronic lung and systemic inflammation, primarily a destructive disease

• Will need large number of patients to show efficacy, particularly with lung function evaluations

• No clear idea of optimal dosing strategies

• Nonetheless: 3rd most prevalent cause of death by 2020

Other Diseases More Amenable to MSC Immunomodulation

Adult respiratory distress syndrome (ARDS): 30% mortality

Many animal studies

Promising area for clinical trials
Asthma – Target for MSC Immunomodulation

Syngeneic/Allogeneic
Sensitization vs Challenge

MSCs are not inhibiting antigen presentation or CD4 T cell clonal expansion in vivo.

Likely no effects on DC antigen presentation or T reg functions

MSCs do not suppress generation of Th2 CD4 T cells in vivo

MSCs promote a Th1-mediated immune response and IFNγ is necessary/sufficient to mediate some of MSC effects
Bioengineering the Lungs Ex Vivo: Trachea and Diaphragm

MSCs from various sources

Clinical application in 2009: Bronchial stenosis

Macchiarini et al., *Clinical transplantation of a tissue-engineered airway*. Lancet, 2009
Bioengineering the Lungs Ex Vivo

All those different cell types!

Organized into this complicated three-dimensional structure!
Clinical Applications: Many

- Many lung diseases are progressive and incurable
  - Chronic obstructive pulmonary disease (COPD): emphysema
  - Idiopathic pulmonary fibrosis
  - Cystic fibrosis

- Lung transplantation only option
  - Critical shortage of donor lungs
    - Only a few thousand transplants performed each year
  - Lifelong immunosuppression
  - 5 year survival approximately 50%
    - Acute and chronic rejection (bronchiolitis obliterans)

- Need way to increase supply of non-immunogenic donor lungs
3-Dimensional Scaffolds and Lung Parenchymal Regeneration

- Variety of synthetic matrices and polymers
  - Glycosaminoglycans
  - Polyglycolic acid
  - Sponges

- Various cell types
  - Mature lung cells
  - Fetal lung homogenates
  - (Putative) endogenous lung progenitor cell populations

- Express markers of airway and pulmonary vascular cells in vitro

- Form airway and vascular-like structures when implanted in vivo

- Challenges:
  - Functional lung cells
  - Functional intact airway and vascular systems
  - Functional pulmonary biology
Gelfoam and Lung Tissue Engineering with MSCs

MSC in Gelfoam

Sueblinvong et al 2008
3-Dimensional Scaffolds and Lung Parenchymal Regeneration

The ultimate scaffold: Whole de-cellularized lungs

Perfuse

H and E
Elastin
Collagen

Ventilate

Lung Mechanics
De-cellularized Lungs can be Surgically Implanted

De-cellularized rat lung (arrow) surgically implanted (airway anastomosis) following unilateral pneumonectomy.

Anastomosed de-cellularized lungs ventilate

Rat

Sheep
Re-Cellularization and Implantation of De-Cellularized Lungs

Ott et al
Nature Med
2010

HUVEC, A549, fetal rat lung cells

Petersen et al
Science 2010

Neonatal lung homogenates, A549, cord blood endothelial cells
Re-Cellularization of De-Cellularized Lungs with Autologous Stem or Progenitor Cells: MSCs

1 week

Normal lung  De-Cell  1 week Re-Cell  1 week Re-cell

Pro-SPC
DAPI
Re-Cellularization of De-Cellularized Lungs: mESCs

Day 0  + Activin A /Wnt3b
Embryonic Stem Cell

Day 5  + FGF-2
Single Cell Seeding
Definitive endoderm
Fox A2, ECadherin

Day 11
Type II Alveolar Cells
Pro SpC

Jensen et al, manuscript submitted
De-Cellularized Human Lungs

H and E
Intact Lung
Lung Slice

Elastin

Trichrome Collagen
Human Lung De-Cellularization and Re-Cellularization

- Will all lungs be equivalent? Not homogenous laboratory mice
  - Age, disease, smoking, etc

<table>
<thead>
<tr>
<th>Protein (Gene Symbol)</th>
<th># Peptides Identified</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt; 2 fold less in Smoking Lung</td>
<td></td>
</tr>
<tr>
<td>&gt; 2 fold less in Non-Smoking Lung</td>
<td></td>
</tr>
</tbody>
</table>

ELN = Elastin
COL4A1/2 = Collagen IV alpha-1 or 2
SLPI = Antileukoproteinase
FBN1 = Fibrillin-1
HSPG2 = Basement membrane-specific heparan sulfate proteoglycan
Actin = Actin
BGN = Biglycan
MYH9 = Myosin Heavy Chain 9
AnnexA2 = Annexin A2
TIMP3 = Metalloproteinase inhibitor 3
NPNT = Nephronectin
COL6A1 = Collagen VI alpha-1
LAMB2 = Laminin subunit beta-2
LAMC1 = Laminin subunit gamma-1
VTN = Vitronectin
LAMA5 = Laminin subunit alpha-5
TGM2 = Protein-glutamine gamma-glutamyltransferase 2
FGA = Fibrinogen alpha chain
LMNA = Laminin A/C
POSTN = Periostin
POTE = Ankyrin domain family member E
De-Cellularization: Different for human lungs

- Presumable goal: maintain native ECM

Mouse

Native

De-cellularized

Human

Fibronectin
Summary: Stem Cells and Cell Therapy Approaches for Lung Diseases

• Desperate need for new therapeutics for lung diseases

• MSC immunomodulation and *ex vivo* lung bioengineering most promising current approaches

• Promising area for more clinical investigations/trials
  – Lung diseases lag behind

• Many questions
  – Specific use of cells or cell products
  – Cell type, dose, administration
  – Long term safety
  – Mechanisms
Stem Cells and Cell Therapies in Lung Biology and Disease Conferences


NHBLI
NIBIB
NASA
FDA
Resp Disease Foundations

Adult Stem Cells, Lung Biology, and Lung Disease Conference

July 25-27, 2005
Burlington, Vermont
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