Human Lung Stem Cells:Current Status

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- Stem cells,defined as “Cells that have clonogenic and self renewing capabilities and that differentiates into multiple cell lineages”. These can be intrinsic or extrinsic in nature.
- Characteristic feature includes:
  1. Undifferentiated cells
  2. Ability for unlimited self-renewal
  3. Infrequent proliferation.
  4. Replenish progenitor cells

Proc Am Thorac Soc 2008;5:637–67

- Self-renewal can be achieved in two ways:
  - *Asymmetric cell division* produces one daughter cell identical to the parental cell and one daughter cell that is different from the parental cell and is a progenitor or differentiated cell
  - *Symmetric cell division* produces two identical daughter cells. For stem cells to proliferate in vitro, they must divide symmetrically

Few relevant terms

- *Totipotent cells* can form an entire organism autonomously. Only a fertilized egg (zygote) possesses this feature(fig.1)
- *Pluripotent cells* (e.g., ES cells) can form almost all of the body's cell lineages (endoderm, mesoderm, and ectoderm), including germ cells.
- *Multipotent cells* (e.g., HS cells) can form multiple cell lineages but cannot form all of the body's cell lineages
- *Oligopotent cells* (e.g., NS cells) can form more than one cell lineage; called *progenitor cells* or *precursor cells*; lack self-renewing capacity (e.g., myeloid progenitor cells)
- *Unipotent cells* or *monopotent cells* [e.g., spermatogonial stem (SS) cells] can form a single differentiated cell lineage

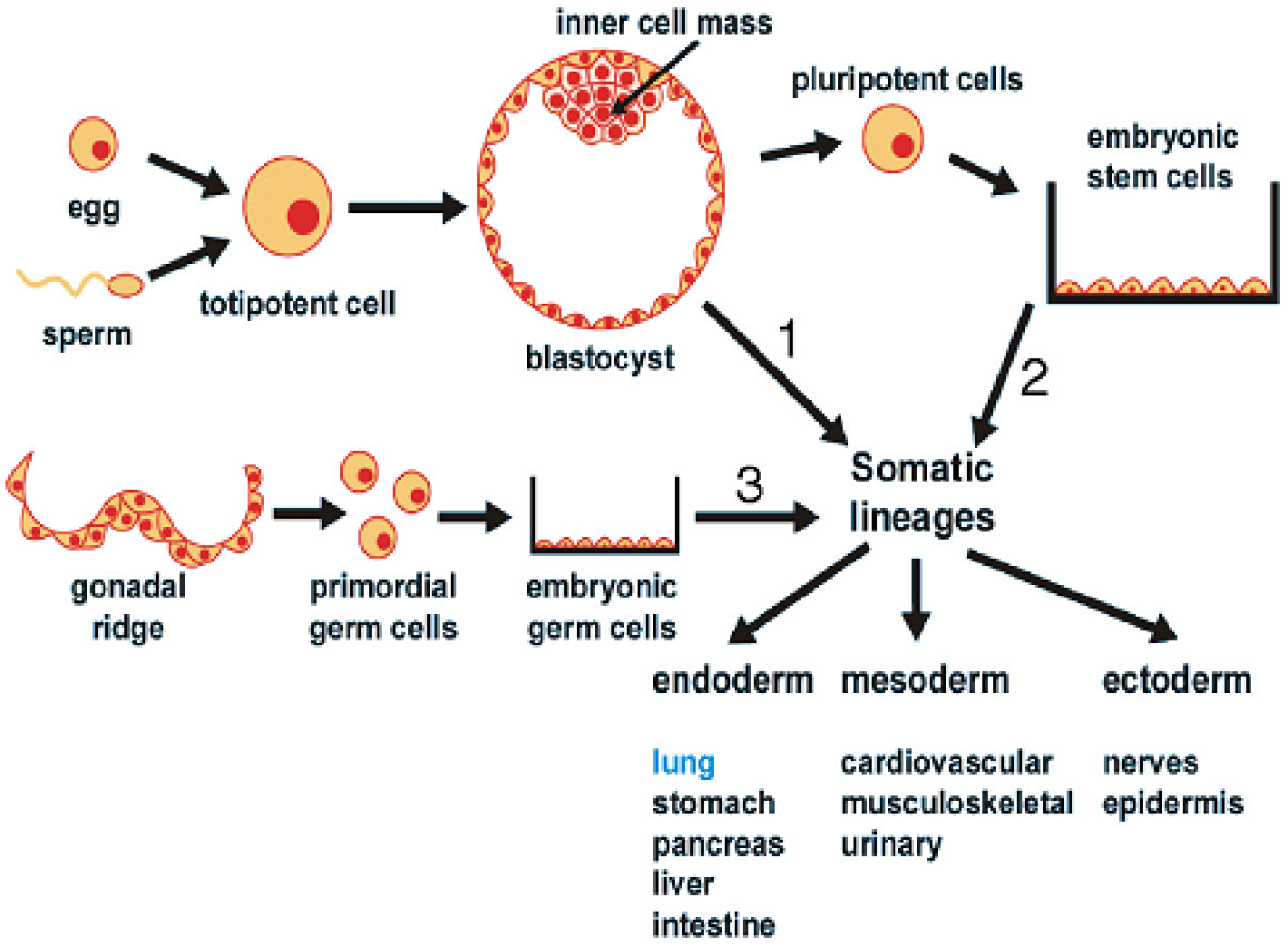


Figure 1

Cell lineage determination during embryogenesis and generation of pluripotent embryonic cells. The three primary germ layers form during normal development (path 1). Embryonic stem cells from the inner cell mass (path 2) or embryonic germ cells from the gonadal ridge (path 3) can be cultured and manipulated to generate cells of all three lineages.

Types of stem cell

- Embryonic stem cell: inner mass of developing blastocyst, capacity for self-renewal , pluripotent, able to differentiate to cells of all embryologic lineage and adult cell types
- Adult stem cell: Cells from adult tissues like bone marrow, adipose tissue,nervous tissue,skin,umbilical cord blood, and placenta,multipotent,some like mesenchymal stem cells show a range of lineage differentiation
- Adult tissue-specific stem cell:has defined tissue specificity, within a stem cell niche e.g.hematopoietic stem cell
- Progenitor cell:any cell with capacity to divide into different cell lineages within a tissue, have limited or no self-renewal capacity, senesce after multiple division

Stem cell identification

- Identification of stem cells requires their separation and purification,based on specific cell-surface markers
- These *isolated stem cells* [e.g., hematopoietic stem (HS) cells] can be studied in detail and used in clinical applications, such as bone marrow transplantation
- Lack of specific cell-surface markers for other types of stem cells has made it difficult to isolate them in large quantities.
- Challenge partially addressed in animal models by genetically marking cell types with green fluorescence protein driven by cell-specific promoters
- Putative stem cells have been isolated from a variety of tissues as side population (SP) cells using fluorescence-activated cell sorting after staining with Hoechst 33342 dye

- Tissue stem cells,considered lineage-committed multipotent cells, possess the capacity to differentiate into cell types outside their lineage restrictions (*transdifferentiation*)
- HS cells may be converted into neurons as well as germ cells.
- It provide tissue stem cells derived from a patient for therapeutic purposes,eliminating need for embryonic stem cells or nuclear reprogramming of a patient's somatic cell
- May reflect cell fusion,contamination with progenitors of other cell lineage,persistence of pluripotent EC in adult organs
- Whether *transdifferentiation* exists and can be used for therapeutic purposes remains to be determined conclusively

- Cultured stem cells derived from resident stem cells are often called *adult stem cells* to distinguish them from *embryonic stem* (ES) and *embryonic germ* (EG) *cells*
- But considering embryo-derived, tissue-specific stem cells [e.g., trophoblast stem (TS) cells] and similar cells from an embryo/fetus [e.g., neural stem (NS) cells], more appropriate to use the term, *tissue stem cells*.

Stem Cell Research Timeline

- **February 1, 1961: Till & McCulloch establish the foundation for stem cell science,Ontario Cancer Institute**
- **1998: James Thomson Isolates Human Embryonic Stem Cell,** University of Wisconsin, initiates the ethical debate on hESC research as his team derives cells destroying human embryos
- **November 2007: Yamanaka and Thomson Independently Derive iPS Cells** from skin cells that had 4 genes inserted via viruses,the cells acquiring properties similar to ESC,coaxing them into becoming beating heart cells and nerve cells
- **October 11, 2010: Geron Initiates Clinical Trial of Human Embryonic Stem Cell-Based Therapy**
- **November 22, 2010: FDA Approval To Test Stem Cell Therapy For Degenerative Eye Disease**

Lung epithelial structure

- Proximal conducting airways--trachea and main bronchi, display a columnar epithelium of ciliated,secretory,basal cells, and submucosal glandular epithelium
- Distal conducting airways devoid of basal cells,populated on epithelial surface with increasing ratio of secretory (Clara) to ciliated epithelial cells
- At the BADJ, the airway epithelium changes to a distal lung epithelium organized into functional alveoli
- Alveolar epithelium consists of flat type I cells which comprise the majority of the gas-exchange surface area of the lung, and cuboidal surfactant-expressing type II cells

Stem cell in lung

- Multipotent adult stem cells found in bone marrow,heart, brain,liver
- But presence of human lung stem cell a matter of controversy
- No classical stem cell hierarchy has yet been described for the maintenance of this essential tissue
- But a number of lung cell types are able to proliferate and reconstitute the lung epithelium
- Differentiated mature epithelial cells and newly recognized local epithelial progenitors residing in specialized niches may participate in lung repair process

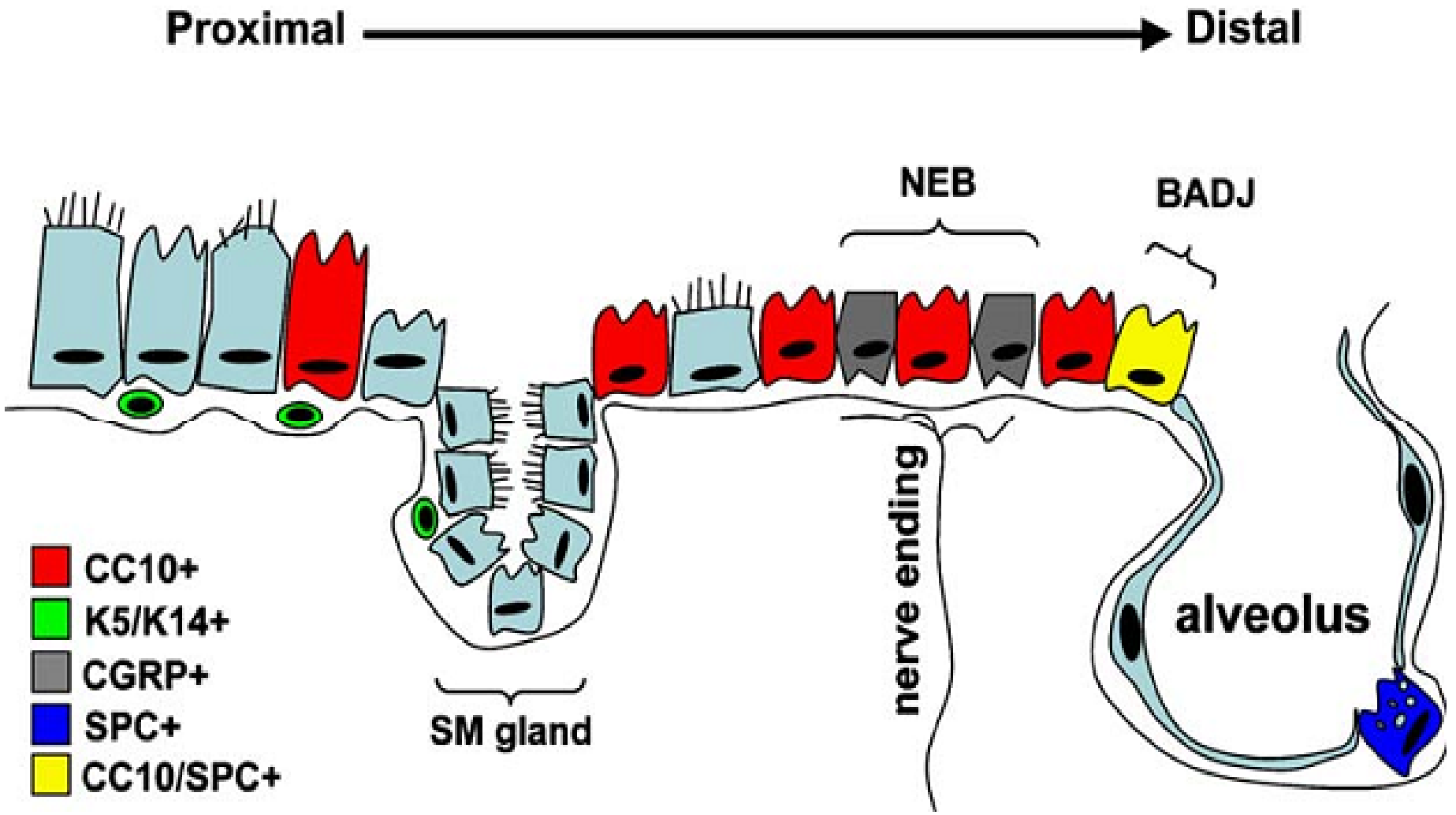
Endogenous stem cells of human lung

- These cells exhibit self renewal capacity, produce more unspecialized cells,can also give rise to daughter cells known as progenitor cells or transit-amplifying cells
- At least three distinct regions have been described that supports populations of lung tissue stem cells:

  -intercartillagenous regions of tracheobronchial airways

  -neuroepithelial bodies (NEB) in bronchiole

  - and the bronchoalveolar duct junction (BADJ).
- Progenitor cells have a finite life span,more robust proliferative potential e.g. toxin-resistant cells, Clara cells, basal cells,etc



Basal (green; keratins K5 and K14),non-basal cells and cells within the submucosal gland (SM gland) duct,Clara cells (red; CC10+),variant-Clara(NEB), type II alveolar epithelial cell (blue;surfactant protein-C, SPC), variant-Clara cell in the BADJ niche co-expresses both CC10 and SPC, functions as a multipotent broncho-alveolar stem cell (yellow)

Cell Tissue Res (2008)

- Adult lung epithelial cells are significantly more quiescent, with turnover times possibly greater than 100 days
- A classical stem cell hierarchy not been easily identified in the lung
- Researchers have focused instead on characterizing the relatively differentiated epithelial cell types that appear to proliferate in response to airway or alveolar injuries

Exp Cell Res1967;46:144–154

Studies on lung epithelial injury and repair

- Data suggests that the type of airway injury is an important determinant of the type of progenitor cell activation
- Evans and colleagues have demonstrated that secretory cells of rat airways function as the principal airway epithelial progenitor following injury with nitrogen dioxide or ozone
- Following naphthalene injury in the tracheal epithelium, basal cells have been proposed as possessing progenitor capacity
- In rat tracheal xenograft, studies suggest that both basal cells or non-basal columnar cells populations can restore the proximal airway epithelium

Am J Physiol Lung Cell Mol Physiol 2003;286:L643–L649

- In diffuse airway injury as in naphthalene exposure, Clara cells within neuropepithelial bodies proliferate to contribute to distal airway epithelial repair
- Naphthalene toxin metabolized by the CYP 4502F2;Clara cells expressing this CYP are killed by former but variant cells with marker CC10(called CCSP, CCA or Scgb1a1)resist injury
- These variants residing within localized anatomical niches appear to function as transit-amplifying progenitors activated after certain types of airway injury
- Information for lineage relationships, self-renewal properties, clonality of these progenitors, and whether these cells play a role in normal tissue maintenance,unclear.

Am J Respir Cell Mol Biol 2003;24:671681

- Controversy persist over whether ciliated epithelial cells are able to contribute to airway epithelial reconstitution after injury
- Generally agreed that ciliated airway epithelial cells flatten and change their gene expression patterns in order to cover the injured airway following Clara cell ablation
- Animal models employed to examine airway epithelial reconstitution indicate that several airway epithelial cell types are able to give rise to differentiated secretory and ciliated epithelial progeny

Am J Respir Cell Mol Biol 2006;34:151–157

- In gas exchanging distal air sacs (alveoli), the cuboidal type II cell thought to function as the progenitor of the alveolar epithelium based on a capacity to replenish itself and to give rise to terminally differentiated flat type I cells
- In vitro study,type I cell phenotype arise during the culture of primary type II cells
- Despite these observations, uncertainty remains as to whether subtypes of type II cells occur with differing progenitor or other functional capacities.

Endogenous stem cell candidates for lung epithelium

- With immunofluorescence microscopy,cells at BADJ co-expressing both alveolar type II cell marker surfactant protein-C (SPC) and the Clara cell marker CC10,identified
- Following naphthalene exposure, these cells resist injury and begin to proliferate suggesting a role in repair
- Termed “broncho-alveolar stem cells” (BASCs) for properties of self-renewal and multipotent differentiation into cells expressing markers of airway and alveolar epithelium
- Using activated K-ras, BASCs hypothesized to be the cell of origin for some types of lung cancer
- BASCs relationship to the BADJ variant-Clara cells unclear

Cell 2005; 121:823–835

Controversies : non-local cells in lung repair

- Although some studies suggests that manipulated marrow cells may assume some aspects of a distal lung epithelial phenotype, it in no way supports the concept that lung epithelial cells normally arise from recruited bone marrow cells.
- In transplantation experiment using parabiotic mouse model,there are conflicting data of lung engraftment by cells resembling fibroblasts and type I alveolar epithelia cells derived from the circulation of the parabiotic partner

Development 2001;128:5181–5188

Science 2002;297:2256–2259

Controversies : non-local cell in lung repair,cont.

- The tracheal xenograft model used to test the potential of bone marrow or circulating cells to contribute to repopulation of the tracheobronchial epithelia also gives contrasting data
- Conflicting reports regarding whether cells from the bone marrow contribute structurally to the lung epithelium
- The picture from sophisticated techniques is that local cells within the lung are primarily responsible for maintaining or reconstituting the lung epithelium, and bone-marrow-derived cells conribute few, if any, cells directly to the structure of the airway or alveolar epithelium

J Cell Sci2005;118:2441–2450

J Immunol2006;176:1916–1927

Am J Respir Crit Care Med 2006 173:171–179

Controversies

Which cells engraft?

The ideal bone marrow stem cell has not yet been defined and effects of different types, *eg* MSC,HSC,MAPC alter

Is the engraftment real?

The engraftment may be due to artifact or happens at too low a rate, *eg* 0.01%, to have effect

Will cells form tumors?

Some stem cell characteristics similar to cancer cells and evidence of in vitro passage leading to karyotype abnormalities[43]

Will cells be rejected?

Thought to be less of a problem with autologous marrow

Could cells cause damage?

Exogenous stem cells may contribute to fibrosis, *eg* asthma or fibrosis

Potential Therapies

Genetic Manipulation

Use homing capacity of stem cells to deliver wild type gene in recessive disorders, *eg* CF

Repair acute damage

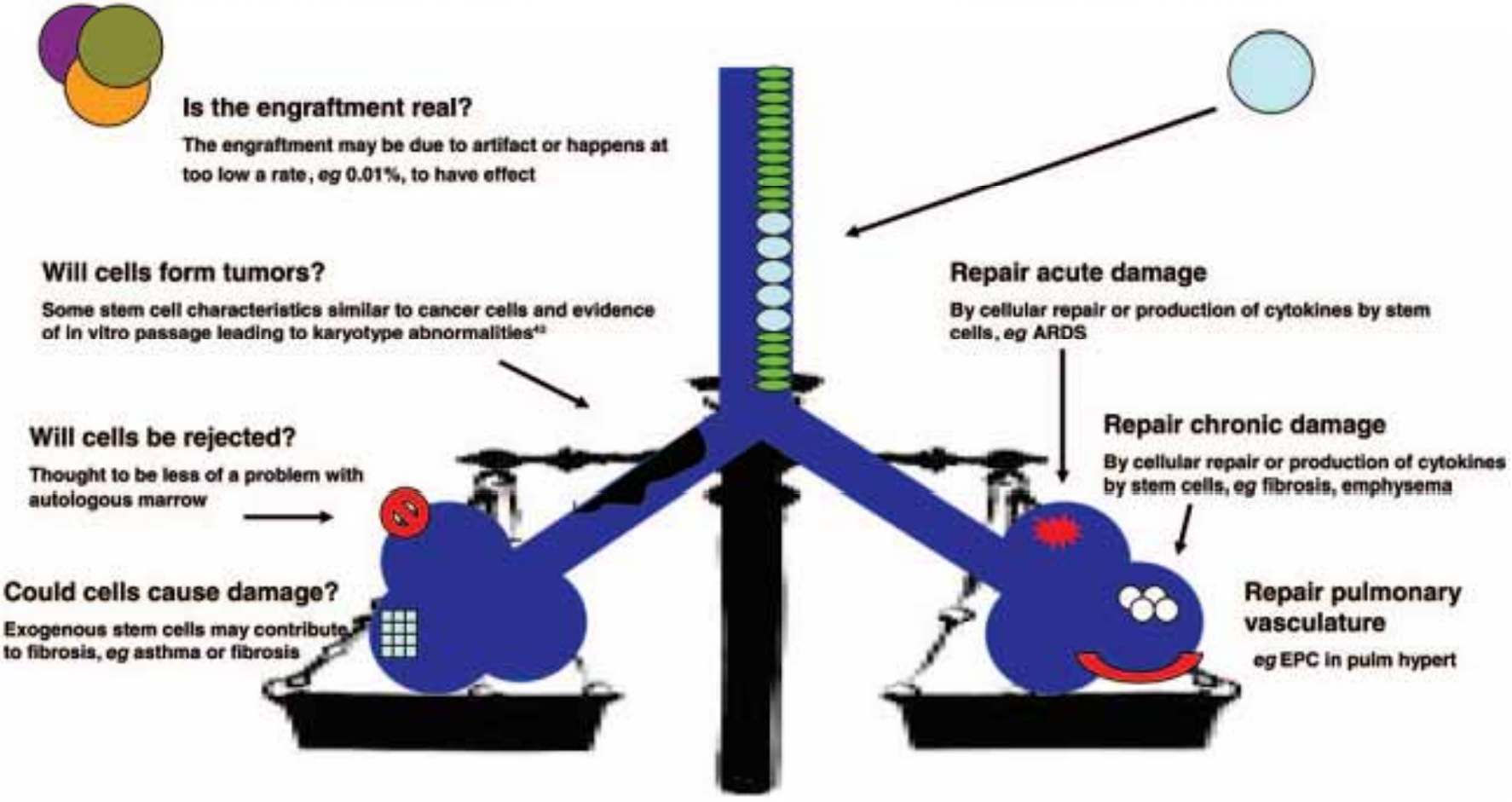
By cellular repair or production of cytokines by stem cells, *eg* ARDS

Repair chronic damage

By cellular repair or production of cytokines by stem cells, *eg* fibrosis, emphysema

Repair pulmonary vasculature

eg EPC in pulm hypert



CHEST 2007

Embryonic stem cells in lung epithelial differentiation

- ES cells derived from inner cell mass of mammalian blastocyst can be maintained,expanded in culture in a pluripotent state
- Transplanted into blastocyst-stage embryos, ES cells able to contribute to all somatic tissue lineages,including pulmonary epithelial cell types
- With specified inducing substances, ES cells able to generate a broad spectrum of differentiated cell lineages in culture
- With prolonged culture in proprietary “small airway growth medium”workers generate SPC expression in human ES cells
- Differentiation of mouse ES cells into cells expressing type I alveolar epithelial marker, aquaporin 5, was also reported

Curr Opin Cell Biol 1995;7:862–869

Tissue Eng 2006;12:867–875

- Workers successfully derive SPC+ cells by co-culturing ES cells with suspensions of digested fetal lung mesenchyme
- Activin A can stimulate nodal signaling in ES cells enabling for the first time, the efficient and robust derivation of definitive endoderm including lung both in vitro and in vivo
- Cultured with serum and puromycin antibiotic, ES cells differentiate, and antibiotic resistant cells can be selected until a highly pure (>99%) population of cells expressing a variety of type II alveolar epithelial markers are obtained

Tissue Eng 2005;11:1177–1187

Proc Natl Acad Sci USA 2007;104:4449–4454

Strategies to identify resident lung stem cells

- “stem cell antigens”, Sca-1, c-kit, or CD34 limited use as these do not in isolation specifically identify stem cells in lung tissue
- Sca-1 may be expressed on rare BASCs and throughout the endothelium of pulmonary arteries, veins, and capillaries
- c-kit and CD34 are similarly expressed on many cells in lung tissue, including subtypes of leukocytes and endothelial cells
- In humans,cells with MSC features retrieved by BAL found to be resident within the host lung tissue rather than being derived from the bone marrow or circulation
- Purified populations of these lung cells can be subjected to to ex vivo assays designed to test multipotency

Proc Natl Sci USA 2003;100:12313–12318

J Clin Invest 2007;117:989–996

Kim CF et al..2005..

- Regional pulmonary stem cell population, termed bronchioalveolar stem cells (BASCs) identified at the bronchioalveolar duct junction(BADJ)
- Pointed out to be the putative cells of origin for adenocarcinoma of lung for the first time
- Using adult mouse model,identifying BASC as Sca-1+cd45-Pecam- cells employing FACS,IM technologies

Kajstura et al ..2011..

- Claimed to define for the first time phenotypic and functional properties of multipotent “human lung stem cell”,generating both endothelial and mesenchymal elements
- Stem-cell antigen c-kit,for identification & characterization
- Criteria for human lung stem cells were self-renewal, clonogenicity, and multipotentiality in vitro and in vivo
- After injection into damaged mouse lung in vivo, human lung stem cells form human bronchioles, alveoli, and pulmonary vessels integrated structurally and functionally with the lung
- The formation of a chimeric lung confirmed by detection of human transcripts for epithelial and vascular genes
- Self-renewal and long-term proliferation of human lung stem cells was shown in serial-transplantation assays

Applications of Stem Cell Biology in Clinical Medicine

- Normally an equilibrium is maintained in which endogenous stem cells intrinsic to the tissue replenish dying cells
- After tissue injury, stem cells in organs like liver and skin, have a remarkable ability to regenerate the organ, but those in the heart and brain, have a much more limited capability for self-repair
- Rarely circulating stem cells may contribute to regenerative responses by migrating into a tissue and differentiating into organ-specific cell types
- The goal of stem cell therapies is to promote cell replacement in organs that are damaged beyond their ability for self-repair

Disease-Specific Applications of Stem Cells

- Myocardial infarction, diabetes and Parkinson's diseases,some hematological malignancies becoming potentially curable
- Under study--skin,eye,cartilage,bone,kidney,lung, endometrium,vascular endothelium,smooth and striated ms
- Stem cell regeneration of organs & tissues--limitless potential
- Only hematopoietic stem cells adequately characterized by surface markers identified for reliable clinical applications
- Differentiating stem cells into specific phenotypes largely unknown,and ability to control migration of transplanted cells or predict their response to diseased environment, limited
- No way to image stem cells in vivo after transplantation
- But stem cells can be engineered before transplantation to contain a contrast agent that may make this feasible

Table 2: Major lung diseases potentially treatable by stem cell manipulation.

Disease Category	Injured, Depleted, or Deranged Cellular Compartment*	Therapeutic Goals
Congenital lung hypoplasia Chronic lung disease of prematurity Pulmonary emphysema	Alveolar epithelium, Interstitial fibroblast, Capillary endothelium,	Generate alveolar septa Restore complex three dimensional structure
Neonatal RDS Adult RDS	Alveolar epithelium, Capillary endothelium	Enhance surfactant production Reinforce endothelial and epithelial barriers
Pulmonary fibrosis	Alveolar epithelium, Interstitial fibroblast	Prevent alveolar epithelial loss Inhibit fibroblast proliferation
Asthma	Airway epithelium, Myofibroblasts, Airway smooth muscle	Create an anti-inflammatory environment Inhibit airway wall remodeling Inhibit smooth muscle hypertrophy and hyperplasia
Cystic fibrosis	Airway epithelium	Deliver functional CFTR
Bronchiolitis obliterans	Airway epithelium	Reinforce the epithelium against toxic, viral or immunologic injury
Lung cancer	Epithelium	Detection, monitoring or treatment based on molecular regulation of stem cell proliferation and differentiation

RDS = respiratory distress syndrome, CFTR= cystic fibrosis transmembrane conductance regulator *Each cell type listed in this column is affected in all of the specific conditions listed in the left hand column

Sources of Stem Cells for Tissue Repair

- Embryonic Stem Cells:

  - blastocysts from fertility clinics for new ES cell,somatic cell nuclear transfer (therapeutic cloning) for genetically similar ES

  -difficult to culture,grow slowly,potential to form teratomas, ethically controversial

- Induced Pluripotent Stem Cells(iPS):

  -somatic cells can generate iPS cells genetically identical to those of the patient,no ethical constraints unlike ES

- Umbilical Cord Stem Cells:

  - widely,easily available,less GVHD than marrow stem cells/ HLA restriction/herpesvirus contamination(quantity limiting)

- Organ-Specific Multipotent Stem Cells:

  - already specialized cells,easier inducement from patient & amplification in cell culture,avoids immune rejection, easier to harvest from BM,blood(but limited potentiality vs ES or iPS)

- **Strategies for transplantation of stem cells**

1. Undifferentiated or partially differentiated stem cells may be injected directly into the target organ or intravenously
2. Stem cells may be differentiated ex vivo before injection into the target organ
3. Growth factors or other drugs may be injected to stimulate endogenous stem cell populations.

Stem cell in COPD

- COPD results from abnormal inflammatory response, proteolytic and oxidant stress driven by the influx of inflammatory cells ie.neutrophils, macrophages (innate response), and lymphocytes (adaptive response)
- DNA damage, abnormal DNA repair, impairment of epigenetic modifications of DNA,telomere shortening, and free radical formation and protein damage.
- -Manageable with enhanced resident stem cell regeneration by Adrenomedullin,ATRA

  -Bioengineering of lung tissue epithelial cells and MSC

  -Mesenchymal stem cells and immune modulators

Stem cell in COPD contd..

- In 2008, Osiris therapeutics initiated a multi-center, double-blind, placebo-controlled Phase II clinical trial of Prochymal (allogenic MSC infusion)in cases of moderate to severe COPD
- At the six-month,the trial contained 62 patients (58% men), age range of the subjects was from 47 to 80 years, and 23 of the patients had moderate and 39 had severe disease
- Important interim report were that Prochymal was safe and significantly reduced systemic inflammation in these patients vs placebo as determined by circulating levels of CRP
- However Prochymal did not significantly alter lung function in these patients

Osiris. Osiris Therapeutics Reports interim data for COPD stem cell study 2009

Stem cell therapy in pulmonary fibrosis

- IPF reflect dysregulated healing in response to multiple sites of alveolar epithelial injury of unknown origin leading to fibroblast activation and exaggerated accumulation of extracellular matrix in lung parenchyma
- MSCs reported to be pleiotropic cells exerting properties including differentiation,regenerative and migratory capacity, immunomodulation and paracrine activity with the secretion of angiogenic, antiapoptotic and anti-inflammatory factors

Am J Respir Crit Care Med 2011; 183:788

Cell Tissue Res 2008; 331:145–156

Stem cell therapy in pulmonary fibrosis,contd..

- A nonrandomized unicentric, dose-ranging safety study in IPF patients with moderate disease(FVC >50%,DLCO>35%)pattern
- Primary endpoint—acute exacerbation, infections or death, minor (fever, allergic reactions),with 3 trials of monthly dose
- 2ndary endpoint-- functional and radiological parameters
- PRP activated autologous ADSCs incubated with Tc99m and instilled endobronchially using FOB to both lung lower lobes
- Tc99m lung scan at 6 and 24 h post infusion to visualize cells
- No significant allergic reactions, disease exacerbation, infection,ectopic tissue or tumor formation(whole-body CT)
- Improvement in 6-min walking distance test over baseline,at 6 months after the first infusion was reported

Current Opinion in Pulmonary Medicine 2011,17:368–373

Stem cell in carcinoma lung

- Although the identity of tissue stem cells in the lung is controversial, BASCs drive the tumorigenic process in several mouse models of lung Adenocarcinomas(comprising 40% of all lung cancers)
- Transformed BASC-like cells whether fulfill all criteria of being CSCs unproven,as no evidence of a sub-population with the BASC phenotype exist to establish a histophenocopy of the initial tumor in secondary and tertiary hosts
- As CSCs share several resistance mechanisms with normal tissue stem cells, there is a high risk to hit normal stem cells by a targeted anti-CSC therapy with disastrous consequences for the patient

Selective hit to CSC-specific pathways

- Elimination of CSCs with selective targeting or sensitization to conventional chemotherapy and differentiation therapies
- Targeted inhibition--signal transduction pathway, transcription factors active in CSCs,like embryonic and hedgehog pathways
- Expression of CSC-associated markers ALDH1 or Sox2 correlated with higher stage,grade in different lung tumors
- Potentially useful CSC markers for lung cancers are CD133, ALDH and nuclear beta-catenin
- CSC-considering diagnoses likely the starting point for improved and tailored lung cancer treatments as therapeutic choice has to be strictly linked to the type,stage of the tumor

Bioessays 2009;31: 1038–1049

Mol Cancer Res 2009;7: 330–338.

Summary:human lung stem cell

- Controversies,uncertainties persist
- Limitless possible therapeutic applications
- Recent publication by Kajstura et al(2011 May) about "evidence for human lung stem cell" rekindle both hope and criticism
- Encouraging preliminary results in

  -COPD

  -IPF

  -LUNG CANCER