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HOT TOPIC

Lung Stem Cell Update: Promise and Controversy

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ABSTRACT: ***Lung Stem Cell Update: Promise and Controversy. I.P. Neuringer, S.H. Randell.***

Currently, there is great enthusiasm about potential stem cell therapies for intractable diseases. We previously reviewed the topic of stem cells in lung injury and repair, including the role of endogenous, tissue (somatic) stem cells and the contribution of circulating cells to the lung parenchyma. Our purpose here is to provide a concise update in this fast-moving field. New information and ongoing debate focus attention on basic issues in lung stem cell biology and highlight the need for additional studies to establish the feasibility of cell therapies to prevent or treat lung diseases.

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The concept of transdifferentiation, here defined as the ability of a cell from one tissue type to generate differentiated cells of another tissue type, is highly controversial. Specifically, there is debate surrounding the ability of circulating bone marrow derived cells to generate lung-specific cell types. Such transdifferentiated cells can either contribute to the development of pathology, as in fibrosis, or may be able to serve a protective function, as in endothelial or epithelial regeneration. Publications highly relevant to lung stem and progenitor cells, postdating our prior review [1], are summarized in table 1. We apologize in advance for any studies omitted due to oversight or space limitations. Furthermore, we did not exhaustively critique technical aspects of many of the studies individually. It is important to remember that, where applicable, each article variably characterized the starting cell populations, that technical standards for determination of cell origin and phenotype by imaging methods are in flux, and that few if any articles have shown clonal expansion of transdifferentiated lung cells. Our general approach in this brief update, with some exceptions, is to state the take home message of the study. We recommend that critical analysis of each paper is necessary to decide how well the data supports the conclusions.

Lung somatic stem cells. There are new data regarding local cell renewal within distinct anatomic and cellular lung compartments. Kim *et al.* identified a regional bronchio-alveolar epithelial stem cell population, confined to the junction between airway and alveoli and expressing characteristic markers, which was capable of clonal multi-potential growth, including generation of adenocarcinoma [2]. The extent of the proximal airway and distal alveolar epithelium supported by this stem cell niche remains to be determined. Starting with mixed populations of dissociated tissue cells from 3 adult human bronchi, Sabatini *et al.* cultured fibroblasts that displayed markers common to bone marrow stromal cells (MSCs), that retained the ability to follow adipogenic, chondrogenic, or osteogenic lineages and may contribute to lung repair and remodeling [3]. Summer *et al.* analyzed embryonic mouse lung side population cells, capable of dye efflux analogous to bone marrow hematopoietic stem cells (HSCs), and characterized them as a complex mixture of both hematopoietic (CD 45+) and non-hematopoietic (CD 45-) progenitors, the latter containing both smooth muscle or endothelial progenitors that when co-cultured formed tubular, vessel-like structures [4]. The molecular characteristics and full differentiation potential of the CD 45- lung side population cells require further study.

Transdifferentiation. Bone marrow cells of traditional hematopoietic lineages normally migrate to the lungs, and this process is dramatically accelerated in lung injury and pathology. Leukocytes characteristic of acute and chronic inflammatory processes are undoubtedly physiologically important, but the ability of circulating cells to generate lung-specific cell types by fusion or transdifferentiation is hotly debated, fueled by both supportive and negative studies. In general, the contribution of bone marrow to lung is directly correlated with the degree of lung injury, typically ablative radiation prior to bone marrow transplant followed by experimental injury in animals, or injury accompanying bone marrow or lung transplantation in people. Abe *et al.* created para-

Table 1. - Recent studies addressing lung somatic stem cells and generation of lung cell types by exogenous progenitors or ES cells

Study Type	Disease or Model	Tissue of Origin	Lung Cell Type Formed / Frequency	Method of Detection	Ref.
Animal, *in-vivo*	Not applicable	Not applicable	Putative bronchiole-alveolar epithelial stem cell, co-expresses CCSP and SPC, increased in oncogenic K-ras mouse tumor model	Immunohistochemistry, Cell culture of purified cells	[2]
Human *in-vivo* and *in-vitro*	Not applicable	Not applicable	MSC-like bronchial fibroblast that generates adipogenic, chondrogenic, or osteogenic lineages	Flow cytometry and RT-PCR	[3]
Animal, *in-vivo*	BMT	Embryonic mouse lung side population	HSCs, endothelial cells, and smooth muscle cells	Immunohistochemistry, FACS, RT-PCR, cell culture	[4]
Animal *in-vivo*	Parabiotic mice	Partner mouse	Hematopoeitic cells, epithelial cells, fibroblasts	FACS, immunohistochemistry, *in-vitro* culture	[5]
Animal, *in-vivo*	Bleomycin injury, myelosuppression	MSCs	Fibroblasts, type I and type II cells, endothelium	Immunohistochemistry	[6]
Animal, *in-vivo*	Polidoconol injury and BMT	Side-population HSCs	0.83% Y chromosome/cytokeratin+ epithelial cells	FACS, RT-PCR, FISH, immunohistochemistry	[7]
Animal, *in-vivo*	LPS-induced lung injury	Fetal liver, whole bone marrow	Alveolar epithelium and endothelium, ~10% total GFP+	Flow cytometry and immunohistochemistry	[8]
Human, *in-vivo*	Pneumonia	Bone marrow	Reduced circulating endothelial cells in patients developing fibrosis	Di-acLDL, lectin, VEGF2, and CD31 expression	[9]
Animal, *in-vivo*	Monocrotaline pulmonary arterial hypertension	Endothelial-like progenitor cells, +/- eNOS expression	Endothelium of arterioles, reversal of hypertension	Microangiography, RT-PCR	[10]
Human *in-vivo*	Human acute lung injury	Circulating endothelial cell progenitors	Increased progenitors associated with improved survival	Cell culture, Di-acLDL and lectin stain	[11]
Animal *in-vivo*	Elastase lung injury	Endotracheal GFP+ embryonic fibroblasts	GFP+ fibroblasts present for 20 days	Immunohistochemistry, Northern blot	[12]
Human, *in-vitro*	Co-culture	MSCs and airway epithelial cells	Cytokeratin 18 positive cells expressing CFTR from MSCs	Immunohistochemistry, RT-PCR, Cl^- efflux assay	[13]
Animal, *in-vivo*	BMT and naphthalene	Stroma and total marrow from wild type mice	0.01% of airway epithelial cells positive for CFTR	Y chromosome FISH, RT-PCR and FACS	[14]
Human, *in-vivo*	Human lung transplant	Sex-mismatched transplant	Bronchial ~1.4% and alveolar ~3.6% chimerism, no cell fusion	Y chromosome FISH, immunohistochemistry	[15]
Human, *in-vivo*	Human lung transplant	Sex-mismatched transplant	Type II cells, ~0.55%	Y chromosome FISH, immunohistochemistry	[16]
Human *in-vivo*	Human lung and BMT	Sex-mismatched transplant	Type II cells, macrophages, endothelium, qualitative	Y chromosome FISH, immunohistochemistry	[17]
Animal, *in-vivo*	BMT	Whole BM, HSC from SPC-GFP mice	No donor lung cell types expressing GFP	Flow cytometry, RT-PCR	[19]
Animal, *in-vivo*	BMT	Whole BM, HSC from SPC-GFP, and LacZ mice	No donor lung cell types expressing GFP or LacZ	Immunohistochemistry, deconvolution microscopy, flow cytometry	[20]
Animal, *in-vivo*	Endotoxin or NO2 lung injury	Sex-mismatched, GFP+ whole bone marrow	Alveolar epithelium ~0.5%	FISH for Y chromosome, immunohistochemistry	[21]
Animal, *in-vitro*	In-vitro differentiation	ES cells	Airway epithelium	Immunohistochemistry, RT-PCR, Western blotting	[22]
Animal, *in-vitro*	In-vitro differentiation	ES cells	Type II cells	RT-PCR	[23]
Animal, *in-vitro*	In-vitro differentiation	ES cells	Type II cells	Histology, RT-PCR	[24]
Animal, *in-vitro*	In-vitro differentiation	ES cells	Type II cells	Histology, RT-PCR	[25]

BM = bone marrow, BMT = bone marrow transplant (with prior ablation), CCSP = Clara cell specific protein, CFTR = cystic fibrosis transmembrane regulator protein, Di-acLDL = di-acetylated low density lipoprotein, eNOS = endothelial nitric oxide synthase, ES = embryonic stem cells, FISH = fluorescence in situ hybridization, FACS = fluorescence activated cell sorting, GFP = enhanced green fluorescent protein, HLA = human leukocyte antigen, HSC = hematopoietic stem cells, LacZ = gene for beta galactosidase, MSC = bone marrow stromal cells, adherent bone marrow cells, SCNT = somatic cell nuclear transfer (therapeutic cloning), SPC = surfactant protein C, VEGF2 = vascular endothelial growth factor 2.

biotic pairs of mice, one ubiquitously expressing green fluorescent protein (GFP) and the other, wild type [5]. After injuring the lungs of the wild type mice, they observed GFP-positive hematopoietic and lung parenchymal cell types. GFP-positive, fibroblast-like cells were cultured from the wild-type lungs, but their clonal growth or ability to be passaged was not assessed. Rojas and colleagues found that myelo-suppression increased homing and localization of a mixed, CD 45-depleted population of donor MSCs to bleomycin-injured lung, where they reportedly differentiated into fibroblasts, type I and type II alveolar epithelium, and endothelium [6]. After host irradiation and transplantation of bone marrow side population cells highly enriched for their stem cell potential, MacPerson *et al.* detected Y chromosome-positive donor cells expressing cytokeratin at a frequency of 0.8% in recipient lung tissue [7]. Yamada *et al.* found that LPS-induced lung inflammation recruited bone marrow derived progenitor cells, which reportedly differentiated into endothelial and epithelial cells and protected the lung [8]. In parallel, these investigators identified circulating endothelial cell progenitors in humans with bacterial pneumonia, which were reduced in number when fibrosis ensued, further suggesting a role for bone marrow derived cells in lung repair [9]. Work by the group of Dr. Stewart showed that bone marrow-derived endothelial-like progenitor cells reversed monocrotaline-induced pulmonary arterial hypertension in rats [10], and this preclinical data has supported a human trial of endothelial progenitor cell therapy for pulmonary hypertension (http://www.stemcellnetwork.ca/news/articles.php?id=889). A recent study suggests that increased numbers of circulating endothelial-like progenitor cells are associated with survival in human acute lung injury [11]. Thus, while the actual prospects remain unknown, pulmonary endothelial cell supplementation with putative circulating progenitors to treat or reverse vascular injury is at the forefront of potential lung cell therapies. Also of note regarding cell therapy is that embryonic mouse lung fibroblasts instilled down the trachea of elastase-treated mouse lungs apparently penetrated into the lung interstitium, where they persisted for 20 days [12]. The prospects for treatment of lung diseases characterized by epithelial dysfunction with bone marrow-derived stem cells are less certain, and hinge on the controversy surrounding the extent of transdifferentiation, again here defined as the generation of cells of one tissue type by cells of a different tissue type. Wang and colleagues procured MSCs from cystic fibrosis (CF) patients, introduced a correct copy of the *CFTR* gene, and co-cultured them with airway epithelial cells from CF patients [13]. The studies suggested that *CFTR* gene-corrected MSCs converted into airway epithelium and promoted chloride transport. However, the magnitude of the correction in comparison to normal chloride transport in non-CF epithelial cells was not determined. In fact, a very recent study in *CFTR* gene-deleted mice illustrated that transdifferentiation followed by CFTR protein expression was extremely rare, occurring in 0.01% of epithelial cells [14], arguing against the feasibility of bone marrow transplantation to treat CF.

Sex-mismatched human bone marrow and lung transplantation provides an opportunity to assess transdifferentiation, and recent studies again yielded conflicting results. Spencer *et al.* examined serial biopsies from 2 boys receiving female lung allografts and suggested significant tissue chimerism, namely, 1.6% and 3.6% of airway and alveolar Y chromosome-positive epithelial cells, respectively [15]. On the other hand, Zander *et al.*, using a similar approach, typically found only one Y chromosome-positive, cytokeratin-positive alveolar epithelial cell, estimated to be 0.5% of alveolar epithelium, in 9 of 25 specimens [16]. Albera *et al.* studied 8 lungs from female donors transplanted into male recipients, then explanted at re-transplantation for chronic rejection, and also 3 lungs at autopsy from females receiving male bone marrow transplants [17]. Their results were completely qualitative, but suggested the possibility of exogenous cells contributing to lung epithelium. Common to many investigations in this field, these studies all rely on finicky and somewhat subjective staining and imaging techniques, in this case made even more difficult by variable tissue sampling, post mortem changes, and/or the length of fixation.

Recent studies have revisited earlier animal experiments that initially supported a bone marrow contribution to lung parenchymal cells. Kotton *et al.* used mice expressing GFP driven by the surfactant protein C (SPC) promoter, which is a specific reporter system for alveolar type II cells [18]. They transplanted bone marrow cells from these mice into lethally irradiated adult mice, which were then treated with bleomycin [19]. Similarly, Chang *et al.* studied neonatal mice following bone marrow transplantation from donors ubiquitously expressing marker genes or from SPC-GFP mice [20]. The investigators carefully employed flow cytometry, deconvolution microscopy, and SPC gene expression and reported the absence of alveolar epithelial reconstitution by whole bone marrow or HSCs. The results from both studies suggest that artifactual co-localization of markers, in the face of a flood of phagocytic hematopoietic cell types, many dead cells, and abundant autofluoresence in lung tissue, is more likely than actual transdifferentiation. Another recent study in mice reports absence of significant parenchymal engraftment of marrow-derived cells after endotoxin or NO_2 lung injury [21].

The literature to date highlights the technical difficulty of definitively establishing transdifferentiation of bone marrow-derived stem cells into lung parenchymal cell types. We suggest that further studies solely relying on imaging will not help to resolve the controversy, and that additional approaches such as those illustrated in figure 1 will be necessary. The ability to culture parenchymal cell types of exogenous derivation from recipient lung, as illustrated in figure 1A, would be supportive of transdifferentiation. Since typical hematopoietic lineage cell types can persist for many days in cul-

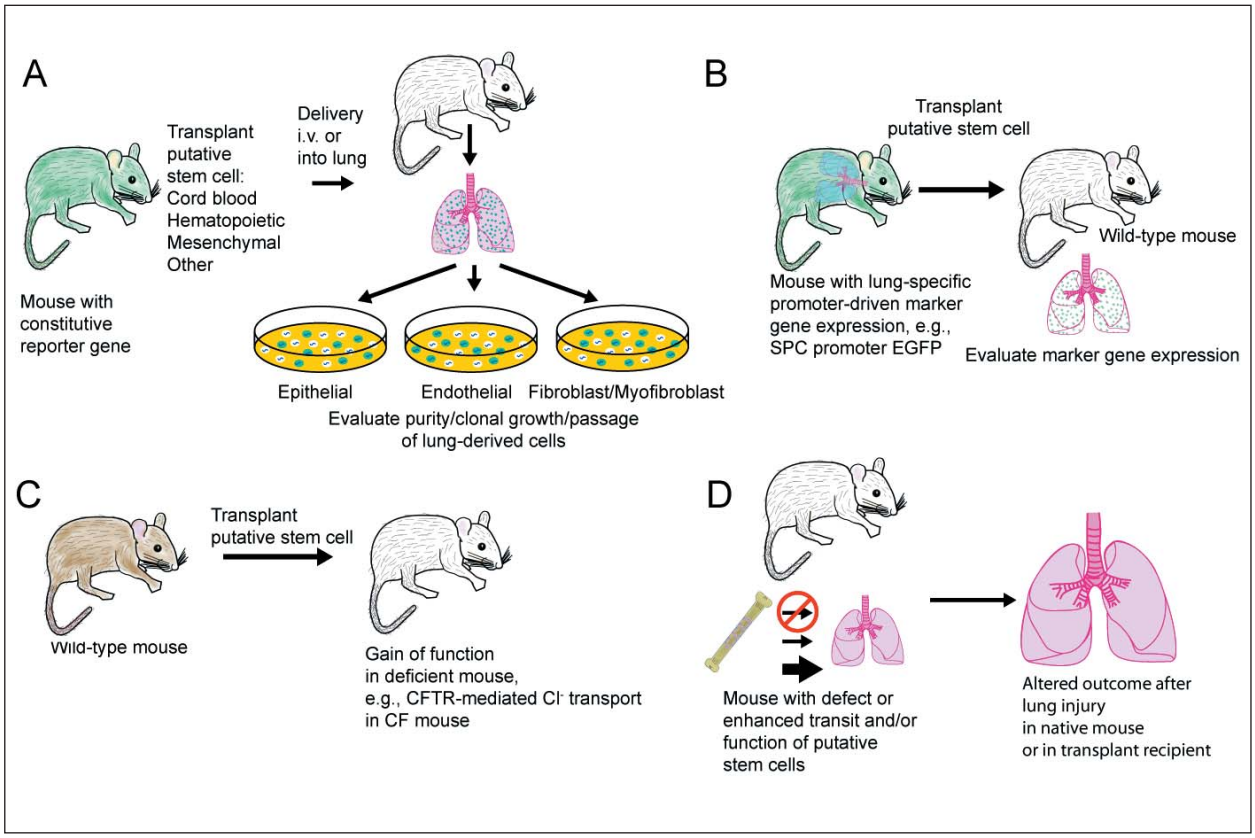

Fig. 1. - Functional approaches to determine the contribution of exogenous, circulating cells to the lung parenchyma. See text for further explanation.

ture, clonal growth and sub-passaging comparable to endogenous lung cells would be stronger proof. Furthermore, this method can theoretically be applied to human transplant tissues. The approach of using donor cells with a reporter driven by a lung specific promoter (figure 1B) has already been tried, but with negative results [19, 20]. However, false negatives are possible, and additional studies using different constructs, donor populations, injuries, etc. are warranted. Unequivocal gain of function in a deficient mouse (figure 1C), for example CFTR mediated chloride transport in CF mice, would obviously be precedent-setting. Finally, generation of mice with enhanced or depleted function of putative exogenously-derived lung cells, and demonstration of an altered outcome after lung injury in either the native lung or in recipients of bone marrow transplants from the mutant mice, would provide functional evidence (figure 1D). It is important to note that, whether transdifferentiation of exogenous cells to lung cell types is ultimately deemed fact or fancy, circulating cells are likely integral, if not vital, to healing and strongly contribute to lung remodeling.

Embryonic stem cell (ES) generation of lung cells. Pluripotent ES cells offer promise as a potential source of lung endoderm, mesoderm, and ectoderm cell types. Murine embryonic stem cells grown as embryoid bodies, and then on collagen coated surfaces with growth factors, followed by replating at an air-liquid-interface developed into an epithelium with Clara, ciliated, basal, and intermediate cells [22]. Additional studies explored conditions promoting mouse ES cell expression of Type II cell genes [23-25]. Similar studies with human ES cells are likely imminent. Although an intial report of somatic cell nuclear transfer (SCNT, therapeutic cloning) to create human ES cells that were pluripotent, chromosomally normal, and with DNA and immunological haplotype identical to the donor specimen has now been discredited [26], it is likely that this technique will ultimately be applied to humans. Respiratory epithelium is commonly observed in teratomas derived from human ES cells, and the ultimate success of SCNT with human cells will enable study and potentially therapeutic employment of a patient's own cells. Recent advances and setbacks highlight opportunities for novel lung research using ES cells and the need for more studies to determine the practical relevance and safety of using ES cells for cell therapy.

In summary, many challenges remain to better understand endogenous lung stem cells, to determine the role of exogenous cells in lung injury, repair and pathology, and to explore the potential uses of ES cells. Developing rational strategies for lung protective or regenerative cell therapy will require an intensive and scientifically rigorous effort.

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