Respiratory Stem Cells and Progenitors: Overview, Derivation, Differentiation, Carcinogenesis, Regeneration and Therapeutic Application

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Abstract: Recently, research of stem cells has garnered great attention and has shown promise by changing the view of traditional therapeutics, with broad impact on gene therapy, carcinogenesis, organ development, tissue injury, regeneration and almost all aspects of the life cycle and all living systems. A century's scientific progress has significantly improved controls for infectious diseases and many other disorders. However, many remaining problems (i.e. cancer, AIDS, diabetes, Parkinson's disease and Marburg infection) appear to be even harder than those that have already been solved. In particular, respiratory stem cell research has been less active and has moved more slowly than that of many other organs. This is probably due to the complexity of the lung and airway system, particularly owing to the many types of cells (>40), unique structures and functions, and technical difficulty in analyzing this system at the genetic, biochemical, molecular and cellular level. Compared with other epithelial cells (i.e., gastrointestinal epithelium), respiratory epithelia have a very low turnover rate and minimal regenerative activity. This review will discuss the current state of pulmonary stem cells, their origin, development, differentiation, and regenerative application, with a particular focus on potential impact on cancer development and lung injury repair.

OVERVIEW

Currently, research of stem cells is one of the most exciting research fields and talking points as politicians, scientists, and the public all are watching its development. This area is embedded in both promise and uncertainty as Laws that govern stem cell research vary from country to country - and even from state to state. In the third annual meeting of the International Society for Stem Cell Research in San Francisco (June 2005), scientists and doctors discussed everything from the clinical use of adult and embryonic stem cells. Three important issues were noticeable: 1) Should we use critical and sensitive words such as "embryo cloning" or more technical words such as "nuclear transfer", 2) which is more ethical: adult stem cells or embryo stem cells, 3) should the US government loosen the restrictions and fund embryo stem cell research? Stem cells include embryo stem cells (ES) and adult cells, the latter covering hematopoietic stem cells or bone marrow derived stem cells (HSC), mesenchymal stem (stromal) cells (MSC) as well as the cells residing in many organ systems, e.g., progenitor cells. Typical characteristics of stem cells are self-renewal and multipotent differentiation. The ability to differentiate into diverse tissue cells varies and can be defined as totipotent, pluripotent (Blastocyst, ES cells) and multipotent (HSC) (Fig. **1A**). ES are potent to develop into tissue specific precursor or progenitor cells. ES is also potentially useful for regeneration and repair of damaged tissues in various diseases. However, research of ES cells has been restricted in existing cell lines in the US by federal funds. It is relatively less constrained in other countries in Europe or in Asia. The widely expected applications of stem cell therapy include treatment of Parkinson's disease and diabetes. Although not a major disease requiring stem cells' therapy, spinal cord injury is probably more accessible and therapeutic testing of stem cells already demonstrated potential effect in animal models [1]. Therefore, human embryo stem cells could hit the clinical trials for spinal cord injury as soon as next year, to be conducted by Geron Inc (CA). In contrast, research of stem cells in the airway has much less flare. However, it is still very important to continue to research into the differentiation and application in lung stem cells that could afford potential new therapeutics for many respiratory diseases, such as, Cystic Fibrosis, pulmonary fibrosis, asthma, emphysema, bronchiolitis obliterans, lung cancer and acute respiratory distress syndrome (ARDS). However, only limited information is available about differentiation, regulatory mechanism and therapeutic value of respiratory stem cells.

The lung possesses a complex architecture, with cells of multiple germinal lineages that interact both during morphogenesis and adult lung function. The endoderm derived epithelium can be subdivided into at least four types, including pseudostratified epithelium, columnar epithelium and alveolar epithelium, whereas alveolar epithelium has two subtypes, type I as mature cells and type II as progenitor lung cells (for derivatives of lung cell types, see Fig. **1B**). The well-orchestrated interaction of multiple cell types, including alveolar epithelium, interstitial fibroblasts, myofibroblasts and pulmonary endothelium, is a key to developing alveolar septa in early morphogenesis and maintaining the function of adult lungs. These complex structures and the unique function of air-blood exchange generate specific difficulties in assessing lung cell populations and stem cell differentiation. Furthermore, lung epithelial progenitors have low turnover rates and minimal regeneration potential.

In contrast, liver or other gastrointestinal cells have much stronger regeneration ability. It has been shown that flk1

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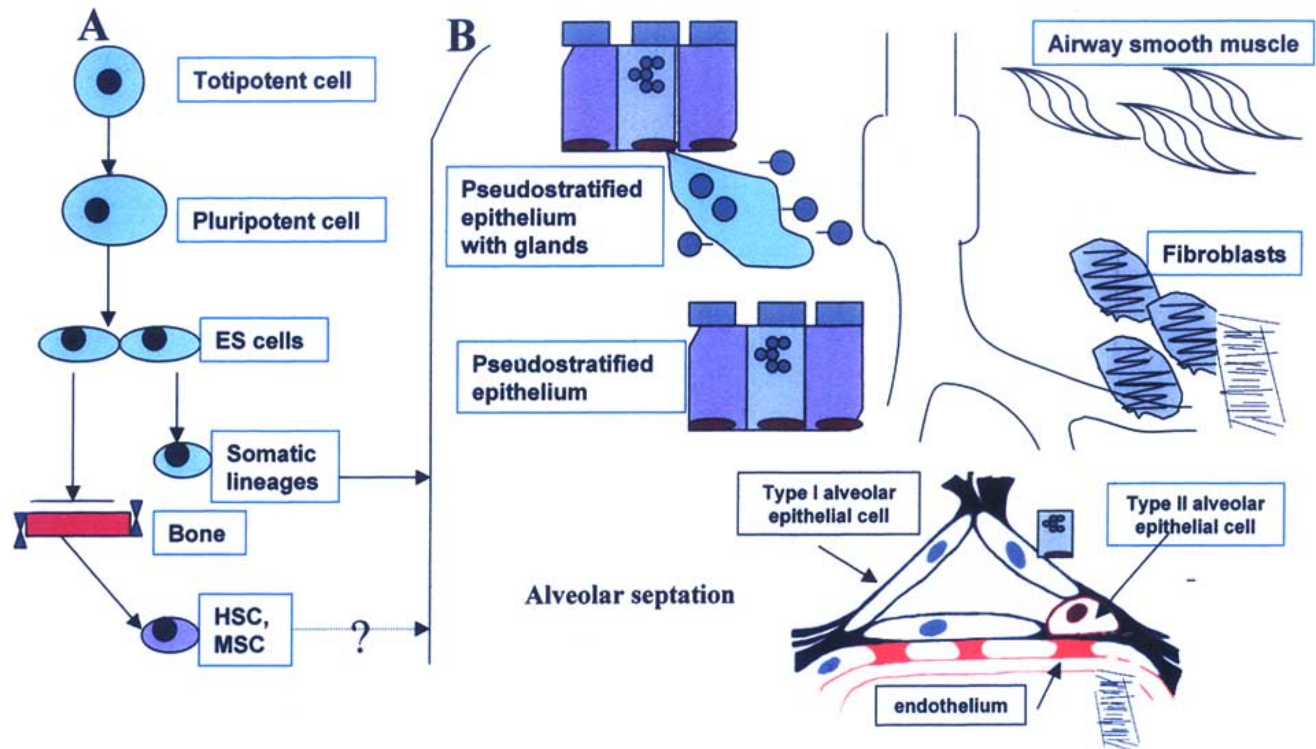

Fig. (1). (**A**). Cell lineage determination and the origins of respiratory cells. The three primary germ layers form during normal development or from inner cell mass to give rise the appropriate cell lineages. Endoderm gives rise to lung or gastrointestinal epithelial cells; mesoderm gives rise to cardiovascular cells; and ectoderm gives rise to nerves or epidermis. While no doubt for ES to differentiate many lineages of lung cells, HSC and MSC are debatable to give rise to new lung precursor or mature cells.
(**B**). Cell classification and lineage derivation. The endoderm derived epithelium can be subdivided into at least four types. The well-orchestrated interaction of multiple cell types, including alveolar epithelium, interstitial fibroblasts, myofibroblasts and pulmonary endothelium, is a key to develop alveolar septa in early development and to maintain the function of adult lungs.

MSC can significantly reduce carbon tetrachloride-induced liver fibrosis in mice [2]. Thus, the research of gastrointestinal stem cells has made admiral progress. An exemplified advance published in a recent issue of Nature (June, 2005) reveals that Notch/ -secretase are essential for maintaining undifferentiated progenitor cells in crypts and adenomas, and inhibition of Notch by -secretase inhibitor significantly altered the balance and turned proliferative cells into post-mitotic goblet cells [3]. Despite recent progress in the research field, one should note that application of stem cells for therapy in any organ system is still rudimentary and there is no certainty about whether and when this technology will be readily applicable for treatment of diseases. For example, in 2001, a publication by Orlic *et al.* [4] showed that injection of HSC transdifferentiated into cardiac myocytes. A new study by Murry *et al.* in 2004 demonstrated no cardiac myocyte transdifferentiation by a variety of methods to inject HSC cells following infarcts [5], although adopted the same approach as used in Orlic's report. The reason for this discrepancy is not clear.

DERIVATION OF LUNG PROGENITOR CELLS (EARLIER DATA)

The derivation of pulmonary stem cells is a complex subject. ES may differentiate into lung cells, while derivation or transdifferentiation of HSC/MSC into pulmonary progenitor cells is yet to be proved. Human embryonic stem cells (hES) were initially prepared by James Thomson of University of Wisconsin in 1998 [6]. The famous Korean group has recently made 13 new cell lines of hES cells, which can derive into almost any organ types including lungs [7]. In spite of noticeable progress, fundamental biology information about earlier established cells or more recently created embryo cell lines is still limited. In general, hES may possess the following traits: 1) are isolated from inner cell mass (ICM) of the blastocyst, 2) proliferate extensively *in vitro*, 3) maintain a normal euploid karyotype over extended culture period, 4) differentiate into derivatives of all three germ layers, 5) express high levels of Oct4 and 6) show telomerase activity [8]. hES may possess other stem cell markers including AC133, ckit (CD117), flt3 (CD135), Sca1 and CD9. hES can be maintained by mouse feeder layers, human feeders on Matrigel or incubated in serum free medium containing a combination of growth factors, TGF-1 , basic fibroblast growth factor (bFGF) and/or leukemia inhibitory factor (LIF). A number of transcription factors such as Oct3/4 are thought to play critical roles in stem cell self-renewal. Several target genes for Oct3/4 have been identified including Utf-1, Rex-1 PDGFaR, Otx-2, Lefty-1 and Nanog, but the roles of these factors are unclear [9]. Also, Wnt/beta-catenin and Notch

Table 1. Summary of Studies on Generation of Non-Hematopoietic Lung Cell Types from Circulating Progenitor Cells

Study Type	Model or Disease	Tissue Origin	Lung Cell Type Developed /Frequency	Detection Method	Reference
Animal, *in-vivo*	BMT	MSC	Undefined mesenchymal cells / occasional	PCR for collagen gene marker	[67]
Animal, *in-vivo*	Bleomycin fibrosis	MSC	Type I pneumocytes / rare	galactosidase protein	[13]
Animal, *in-vivo*	BMT	HSC enrichment	Type II pneumocytes / up to 20%, bronchial epithelium / 4%	Y chromosome FISH, surfactant B mRNA	[11]
Animal, *in-vivo*	Radiation pneumonitis	Whole bone marrow	Type II pneumocytes, bronchial epithelium / up to 20% of type II cells	Y chromosome FISH, surfactant B mRNA	[12]
Animal, *in-vivo*	BMT	Whole bone marrow/EGFP retrovirus	Type II pneumocytes / 1–7%	EGFP, keratin immunostain, surfactant protein B FISH	[68]
Animal, *in-vivo*	BMT and parabiotic animals	HSC	Hematopoietic chimerism but exceedingly rare lung cell types	EGFP	[23]
Animal, *in-vivo*	Bleomycin fibrosis	MSC	Type II pneumocytes / ~1%	Y chromosome FISH	[60]
Animal, *in-vivo*	Radiation fibrosis	MSC or whole bone marrow	Fibroblasts / common	EGFP, Y chromosome FISH, vimentin immunostain	[69]
Animal, *in-vivo*	BMT	Bone marrow, EGFP labeled	Fibroblasts, Type I pneumocyte / occasional to rare	Flow cytometry	[70]
Animal, *in-vitro* and *in-vivo*	Hypoxia-induced pulmonary hypertension	Circulating BM-derived c-kit positive	c-kit positive cells in pulmonary artery vessel wall; In hypoxia, circulating cells generate endothelial and smooth muscle cells *in-vitro*	Flow cytometry and immunohistochemistry	[71]
Animal, *in-vivo*	Ablative radiation and elastase induced emphysema	GFP + fetal liver	Alveolar epithelium and endothelium; frequency not reported but increased by G-CSF and retinoic acid	Immunohistochemistry for $CD45^-$, GFP^+ cells	[72]
Animal, *in-vivo*	Bleomycin fibrosis	Whole marrow GFP^+	GFP^+ type I collagen expressing	Flow cytometry and immunohistochemistry, RT-PCR	[61]
Human, *in-vitro*	Heat shock in cell culture	MSC and SAEC	Cell fusion / common	Immunostaining, microarray	[73]
Animal, *in-vivo* Human, *in-vivo*	OVA-sensitized mouse model Allergen – sensitized asthmatics	CD34 positive, collagen I expressing fibrocytes CD34 positive, collagen I expressing fibrocytes	Myofibroblasts / ? Myofibroblasts / ?	CD34-positive, collagen I, -smooth muscle actin CD34-positive, collagen I, -smooth muscle actin	[74]
Human, *in-vivo*	Human heart and lung transplant	Sex-mismatched donor lung or heart	No lung cell types of recipient origin	X and Y chromosome FISH, antibody stain for hematopoeitic cells	[75]
Human, *in-vivo*	Human lung transplant Human BMT	Sex-mismatched donor lung Sex-mismatched donor bone marrow	Bronchial epithelium, type II pneumocytes, glands of recipient origin / 9 – 24% No lung cell types of donor origin	Y chromosome FISH, short tandem repeat PCR Y chromosome FISH, short tandem repeat PCR	[76]
Human, *in-vivo*	Human BMT	Sex-mismatched donor bone marrow	Lung epithelium and endothelium of donor origin / up to 43%	X and Y chromosome FISH, keratin and PECAM immunostain	[77]
Human, *in-vivo*	Human BMT	Sex-mismatched donor bone marrow	No nasal epithelium of donor origin	Y chromosome FISH, cytokeratin immunostain	[78]
Animal, *in-vivo*	Mouse parabiotic	GFP transgenic-wt control	Lung epithelium type I and lung fibroblasts/5-20% EGFP	Immunostain	[21]
Human, *in-vitro*	Human MSC co-culture with AEC	MSC from CF patient	CF MSC turns into pseudostratified, occludin positive AEC/<10%	Occludin immunostain/CFTR channel	[62]
Animal *in-vivo*	Mouse HSC BMT	GFP transgenic-wt control	No alveolar epithelial cells of donor origin	EGFP, SPC, immunostain	[22]
Animal *in-vivo*	Mouse MSC transplant	Purified MSC to express EGFP by lenti-virus	Many cell types including lung epithelial cells/low frequency (3-5 cells per section)	EGFP, PCR/immunostain	[20]

BMT = bone marrow transplant (with prior ablation), CF=Cystic fibrosis, AEC= airway epithelial cells, MSC = mesenchymal stem cells (bone marrow stromal cells, adherent bone marrow cells), EGFP = enhanced green fluorescent protein, HSC = hematopoietic stem cells, FISH = fluoresence in situ hybridization, SAEC = small airway epithelial cells

signaling pathways play significant roles in controlling the fine balance between cell proliferation and differentiation in a variety of stem cells. Mutations in some of Wnt or Notch components irreversibly lead to carcinogenesis in humans and in mice [10].

Although unconvincing, evidence suggests that alveolar epithelial cells may be derived from bone marrow stem cells, such as HSC. Krouse *et al.* reported that a single HSC injection to mouse differentiated into multiple cell types in a variety of organs including lung epithelial type II cells, persisting for up to 11 months [11]. Follow-up studies demonstrated $CD34^+/Lin^-$ engraftment in the lung as alveolar type II cells were detectable 5 days after bone marrow transplantation and robust by 2 m [12]. However, using Lac Z expressing transgenic mice, Kotton *et al.* did not find differentiated type II cells from bone marrow origin but detected the transplanted cells in the alveoli with type I character [13]. More recently, a report demonstrated that type II cells of donor origin could be identified in recipient patients after allogeneic hematopoietic stem cell transplantation [14]. To date, only limited reports have shown, to different extents, that there is a potential transdifferentiation of HSC and MSC to lung progenitor cells (see below and Table **1**), thus HSC/MSC transdifferentiation in the lung has been far from conclusive. The variations in this regard may be due in part to differences in techniques and experimental models. Other factors, the niches of the lung and cytokines such as granulocyte-macrophage colony-stimulating factor (GM-CSF), may impact the transdifferentiation of HSC/MSC into lung progenitor cells [15, 16]. Many scientists consider that verification of stem cell transdifferentiation in the lung would be highly interesting and should be vigorously sought.

(TRANS)DIFFERENTIATION OF LUNG PROGENITOR CELLS (MORE RECENT DATA FROM 2004)

Development of stem cells in the lung is a controversial but important topic because it is relevant to the development of therapeutic cells. There is a potential that adult bone marrow stem cells can develop into alveolar type II cells or type I cells, but this remains to be confirmed. A good review discusses the evidence for or against circulating progenitor cell generation of non-hematopoietic lung cells [17], we have adapted their data and incorporated more recent studies relevant to stem cells becoming pulmonary progenitor cells (Table **1**).

If transdifferentiation from HSC is impossible, would local stem cells still be able to fulfill regeneration after tissue injury? We speculate that this is possible and will be discussed later in this review. Embryo stem cells are hardly tested in the lung system. In contrast, highflier diseases such as Parkinson's disease and diabetes are so prevalent and publicized that are widely expected to be treated by the technology of stem cells. Therefore, research in neural, gastrointestinal and cardiovascular systems has recorded striking progress over the last few years. Lung diseases have neither such accessibility to attenuate symptoms or to cure disease nor the great impact to generate public's interest. However, therapeutic potential of stem cells may be tested in some devastating lung diseases such as children or adult ARDS and cystic fibrosis.

Type II alveolar epithelial cells have been derived from murine ES cells, but the capacity of the latter to generate differentiated airway epithelial tissue has hardly been reported. A study recently showed that murine ES cells could differentiate into nonciliated secretory Clara cells in culture supplemented with type I collagen. Moreover, when cultured at the air-liquid interface, ES cells became a fully differentiated airway epithelium. Histological analysis demonstrates that the bioengineered epithelium is composed of basal, ciliated, intermediate, and Clara cells, similar to those of native tracheabronchial airway epithelium. The generated epithelium also has the ultrastructural features and secretory functions similar to airway epithelial tissue. These results provide a basis for ES cell therapy of injured epithelium in the upper airway diseases, such as cystic fibrosis [18].

ES cells can be induced to differentiate *in vitro* into epithelial type II pneumocytes using a serum-free medium designed for the maintenance of mature distal lung epithelial cells using small airway growth medium (SAGM). However, the resulting cell cultures are often heterogeneous. Directional induction of type II cells is a useful approach to therapeutic application of pulmonary stem cells. One report attempted to identify roles of medium components that enhance pneumocytes differentiation. The results suggested that most individual SAGM growth factors were inhibitory for type II pneumocyte differentiation, with the largest increases in SPC expression (approximately threefold) observed upon removal of retinoic acid and triiodothryonine. However, the authors noticed that large standard deviations occurred between replicates, illustrating a highly variable nature of ES cell differentiation [19].

Viral vectors including lentivirus were used to efficiently transduce an EGFP reporter gene in MSC/HSC cells. Lentivirus-transduced mesenchymal stem cells retained their *in vitro* ability to differentiate into adipocytes, osteocytes and chondrocytes as well as into myocyte- and astrocyte-like cells. EGFP-MSC were delivered systemically into mice with sub-lethal irradiation. Donor-derived hepatocytes, lung epithelial cells, myofibroblasts, myofibers and renal tubular cells were detected in some of the recipient mice [20]. This study indicates that phenotypically defined murine MSC may acquire tissue specific morphology and antigen expression, and thus contribute to different tissue cell-types *in vivo*.

In another recent study, involving parabiotic mice, tested whether HSC contributed to lung regeneration after injury [21]. The parabiotic mice were generated surgically by joining GFP transgenic mice and wild-type littermates. These mice demonstrate a common circulation (approximately 50% green cells in blood) after surgery. The wild-type mouse was injured by lethal irradiation or intratracheal elastase, or a combination of these two. Radiation or the combination of radiation with elastase significantly increased the proportion of bright green cells in the lungs of the wild-type mice. Type I alveolar epithelial cells and approximately 5 to 20% of lung fibroblasts primary cultured from injured wild-type mice were GFP positive cells, indicating their blood derivation from the

donor mouse. This study indicates that progenitor cells from blood might migrate and contribute to the repair of lung injury in irradiated mice.

Not all recent reports are encouraging, the authors have heard some less desirable presentations in the 100th American Thoracic Society International Conference (May 2005, San Diego). An Epub report shows that immunofluorescence microscopy, as has been used in previous reports, can not reliably identify rare engrafted cells in lung tissue sections after transplantation of bone marrow cells or purified HSC tracked with ubiquitous labels [22]. A lineage-specific reporter system was employed based on transgenic mice that express the GFP reporter gene only in lung epithelial cells (surfactant protein-C-GFP) to detect engrafted cells by flow cytometry. By evaluating transplant recipients, including the bleomycin-induced lung injury model, this study demonstrates that when autofluorescence, dead cells, and contaminating blood cells are excluded from analysis, there is no detectable reconstitution of lung alveolar epithelial cells by unfractionated bone marrow cells or purified HSCs. This latest study indeed indicates that study of lung stem cell transdifferentiation is extremely painstaking.

PLASTICITY OF STEM CELLS: TRUE OR FALSE?

The traditional view is that tissues are maintained solely by stem cells in a specific organ. Evidence now challenges this notion and shows that adult stem cells from different origins are able to generate not only their own lineages, but also those of other tissues, even across the boundary that was previously considered impenetrable. However, this is not without controversy, because some reports have failed to be reproduced by other investigators and sometimes by the same researchers. One explanation is the limitations with the methodology that is currently available - for example, the Y chromosome, in sex-mismatched transplantation, or genetically engineered marker (EGFP) in mouse experiments and protein markers of the differentiated cell type in the lung (keratins in alveolar epithelium). Pre exposure to drugs or radiation is often used to boost stem cell homing to the lung. Varying injury extents in the lung may also increase the uncertainty and the different degrees of transdifferentiation and engraftment. Data of stem cell transdifferentiation into respiratory epithelial cells have been complicated by observations that *in vitro* coculture of embryonic stem cells and somatic cells can result in spontaneous cell fusion, giving rise to cells of mixed phenotype and genotype. The *in vivo* appearance of marrow derived hepatocytes, cardiomyocytes, and Purkinje cells is due, at least in part, to fusion of HSC with the corresponding local cells. In 2002, Wagers *et al.* used single GFP HSC cell to probe the plasticity and concluded that the cells only migrate to cerebellar Purkinje cells and hepatocytes [23]. It is argued that these types of observations can be accounted for by major variations including differing cell purification methods, tissue injury application and techniques to evaluate the engraftment. However, because some of these cell types are known to form heterokaryons in settings of profound tissue injury, the incidence of this process should be better examined in nonfusogenic organs under physiological conditions.

It is necessary to thoroughly evaluate the fusion events, including those that may have been masked by reductive division. In 2004, Krause and colleagues did this using the Cre/lox recombinase system to examine whether fusion occurred between HSC and host cells after bone marrow (BM) transplantation [24]. They used mice of the Z/EG Cre-reporter strain as marrow donors for transplantation into mice that ubiquitously expressed Cre. In this model, any cell resulting from fusion of a HSC with a host cell is expected to express EGFP. This group concluded that there was no fusion in the experiments. However, this report invoked arguments that the methods employed by EGFP expression can not completely rule out the possibility that cells in the lung and liver fused with the transplanted HSC cells but did not express EGFP [25]. Since the methods are inherently risky, other independent evidence is required to assess fusion of stem cells with resident cells. In addition, more innovative methods must be developed to determine whether there is fusion and how frequently this can occur. Fusion of a stem cell with an adult cell may produce a new phenotype that can be exploited for biological applications. Kevin Eggan of the Harvard Stem Cell Institute told an international stem cell scientific meeting that his lab has fused a human embryonic stem cell to an adult skin cell. The embryonic stem cell “reprogrammed” the skin cell's nucleus, causing the skin cell to start behaving like a youthful stem cell. This work raises the possibility that human embryonic stem-cell lines may be tailored to individual patients without first having to create an embryo clone of the patient. The challenge is that the fused cell contains twice the amount of DNA found in normal cells, and so can't be used for therapy yet.

Besides intrinsic nature of transdifferentiation of stem cells, other external factors may also play a role in this process and may be targets worthy studying. Nmyc was recently reported to be essential for lung development and progenitor differentiation [26]. This study indicates that certain factors might be applied to direct the stem cells proliferate into type II cells. Additional studies demonstrate that TGF- can be involved in epithelial mesenchymal transition [27, 28], a process that type II cells transform into mesenchymal cell types, such as fibroblasts.

ANALYSIS OF LUNG PROGENITOR CELLS

Isolation and study of the alveolar epithelial type II (AECII) from animals (either mice or rats) are major tools for investigating the characteristics and functions of lung progenitor cells [29, 30]. With special care, the methods of isolating both mouse and rat AECII are relatively reproducible and well used; however, the methods of culturing the cells are still unstable because of the intrinsic difficulty in slowing down or preventing the differentiation and dedifferentiation of primary culture of AECII [31]. Various approaches used to improve the culture have resulted in short term benefits, but there is a difficulty in maintaining the isolated cells for a long term and remaining the “true” characteristics of AECII [9]. This severely inhibits the research progress of progenitor cells in respiratory therapeutic application. Therefore, further research in this direction is required.

As above, it may be still possible that HSC transdifferentiate into progenitor cells in various organs, but these studies require additional evidence for successful engraftment and transdifferentiation into specific lung type II cells or even a new phenotype of stem cells with some respiratory cell properties. The new generated cells possess the characteristics of progenitor cells that may be useful for therapeutic supplement/replacement during oxidation, lung injury and other genetic or acquired disease.

Recent advances have shown that small populations of resident cells within solid organs display phenotypic features of known marrow stem cells. Taking this into account, investigations have attempted to isolate and identify tissue stem cells by analyzing their specific stem cell phenotypes. There is a paucity of information on studies of this aspect.

Two general strategies are used to determine properties of stem/progenitor cells: common markers and local specific progenitor markers, although neither of these is absolutely specific. For lung progenitor type II cells, surfactant protein C (SP-C), lamellar bodies, cytokeratin 19 are widely accepted markers. Several common HSC markers have recently been tested in the lung stem cells: immunodepletion of linage markers (lin^-), exclusion of Hoechst dyes or side population and CD34, Sca-1 and c-kit [32]. Sca-1 or c-kit is lack of specificity for these antigens that limits their potential, as Sca-1 is expressed in endothelium of arteries, veins and capillaries in the lung [33]. Another strategy is based upon the observed capacity of HSCs to efflux Hoechst dye, a process mediated by the ABC half transporter Bcrp-1 (breast cancer resistant protein) [34]. Such cells, termed side population (SP) cells, have been isolated by dual wavelength flow cytometry due to the absence of staining with Hoechst dye [35]. Recently, Giangreco and colleagues found that a $CD45^-$, $Sca1^+$ SP from mouse lungs have a molecular phenotype similar to neuroepithelial body-associated variant Clara cells. The Clara cells are a label-retaining cell of multipotent differentiation capacity and are pollutant resistant (see below).

RESIDENT LUNG PROGENITOR CELLS

Increasing studies have further confirmed the complexity of lung and airway progenitor phenotypes and their roles. It must be understood that the lung is a unique organ that has a huge surface area that may be damaged by various oxidants, air-pollutants, infectious organisms and administered drugs. As such, the lung has developed vastly diverse cell populations with various typical phenotypes on the airway surface. The lung also is very idle in terms of regenerative activity. These unique characteristics of lung structure, cell compositions and technical difficulties are all obstacles to research of respiratory stem cells. Bearing this in mind, one must be particular of the data and reports from different populations of lung cells, which may vary with isolation methods, culture approaches and various animals.

Alveolar epithelial type II cells are cuboidal structures aligned in gas exchange distal air sacs, and are traditionally thought to be the progenitor lung cells based on their capacity to replenish themselves and give rise to terminally differentiated flat type I cells. Recent advances have strengthened the concept of type II cells as progenitors by demonstrating that the cell types can produce TNF- and IL1- TiO_2 particle stimulation [36]. Additionally, Kasper *et al.* reported that Ype II cells display slice variant CD44 molecules, which can mediate cell matrix adhesion in the lung (serving as niches for bone marrow cells) [37]. Technically, *in vitro* culture of lung cells is difficult, as is the identification of the histological evaluation. Analyzing isolated type II cells following *in vivo* exposure to hyperoxia, a recent study confirmed an observation that a fraction of lung type II cells is damage resistant and probably pluripotent, representing a heterogenous population of highly proliferative cells [38, 39]. We point out that handling of progenitor cells can cause additional trauma and damage to the cells. Although the techniques have inherent problems, these current studies at least demonstrate that lung progenitor cells are a complex population. As much of progenitor cell differentiation and its role are still unclear, further studies would be very useful.

RESIDENT AIRWAY PROGENITOR CELLS

The concept of airway progenitor cells is also similar to that of lung progenitor cells, which can respond to injury *via* a reparative process involving label-retaining stem cell-like precursors. Tracheal gland ductal cells were label-retaining after SO_2 inhalation damage for 4 wks. These label-retaining cells were capable of repopulating the tracheal surface after injury [40]. In the upper airway (trachea or bronchia) basal cells or epithelial cells on proximal conducting airways are considered the progenitor cells. Also, parabasal cells, by their CK5/14 positivity and tracheal submucosal gland stem cells may repopulate the basal cells or surface mucosa [41]. This concept is supported by two recent studies, as summarized below. Schoch *et al.* created bitransgenic mice *via* breeding of K5 promoter-GFP and Rosa26 (-galactosidase expression) as starting cells for a colony-forming efficiency assay in an air-liquid interface culture system and concluded that adult mouse proximal tracheal surface epithelial stem-like cells reside in basal cell compartments [42]. Likewise, Hong *et al.* revealed an increase in GSI-B4 reactive/K14 immunoreactive basal cells after secretory cell ablation with naphthalene in intact mice [43]. These authors identified the differentiation and clonogenic potential of this population by engineering a bitransgenic ligand-regulated Cre-lox P reporter mouse. The ubiquitous reporter (lacZ) was activated by K14-expressing progenitor cells during airway repair from naphthalene, and tagged cluster including basal, ciliated, and secretory cell types with pluripotent capability.

Bronchiolar progenitor cells are thought to be Clara cells, particularly the ones residing within neuroepithelial bodies or bronchoalveolar duct junctions [44]. This is relatively a new concept. In naphthalene treated mice, Clara-like cells appeared near the neuroepithelial body and appeared to be able to divide. Emura also demonstrated that Syrian hamster fetal lung epithelial M3E3/C3 cells could differentiate *in vitro* into Clara and type II cells in different media [45]. This is rare evidence for a bipolar stem cell in the lung that may relate to clinical findings of small cell lung cancer [46]. Pulmonary neuroendocrine cells and bodies (PNECs/NEBs) have been suggested to be capable of dividing, although further research is needed to confirm this notion [47, 48].

Nevertheless, recent reports suggest that these cells or a nearby, immediate precursor can take up thymidine or BrdU [48]. Although knowledge on airway progenitor cells has been updated extensively in the last several years, much is unknown about the differentiation and regulation during tissue damage and repair. A concerted effort is warranted to further dissect the molecular mechanism of reparative process by airway progenitor cells.

It is not surprising that recent evidence also suggests HSC can transdifferentiate into airway progenitor cells. The molecular mechanism of this differentiation is less clear. Previous studies suggest that Foxj1 forkhead box transcription factor could contribute to elements of terminal differentiation in the upper airway in ciliated epithelium [49, 50]. Targeted deletion of Foxj1 resulted in mice with absent cilia [51]. To further assess the molecular mechanism of differentiation, You and colleagues reported that knockdown of Foxj1 in airway cells promotes differentiation only in late-stage ciliogenesis, suggesting a role for this transcription factor in the postcentriologenesis, rather than commitment stage [52]. These assays suggested that, in addition to Foxj1 family members, other factors might be involved in airway stem cell differentiation.

CANCER STEM CELLS AND THEIR APPLICATION

Cancer stem cells are a subset of cancer cells that bear stem cell characteristics, i.e., self-renewal. In normal individuals, the genetic constraints on self-renewal restrict the expansion of stem cells. A breakdown in the regulation of self-renewal is likely a key event in the development of cancer as demonstrated by the fact that several pathways implicated in carcinogenesis also play a key role in normal stem cell self-renewal decisions. Thus malignant tumors may be considered as abnormal organs in which a minority population of tumorigenic cancer cells have escaped the normal constraints on self-renewal, giving rise to abnormally differentiated cancer cells that have lost the ability to form tumors. This concept emerges as a potential tool that impacts the designing of cancer therapy. A very recent study by Michor *et al.* proposed the first mathematic dynamic model of tumor differentiation based on investigation into chronic myogenous leukemia (CML). A small population of cancer stem cells may contribute to the resistance to Imatinib treatment (an efficient inhibitor of BCR-ABL enzyme) [53]. Development of mathematic models like this may help modulate efficient cancer treatment using the concept of cancer stem cells.

Although multiple factors are involved in tumor genesis, development of a vascular network plays a crucial role in tumor growth and metastasis. Understanding how tumors acquire their vasculature is vital for developing novel therapeutic approaches. However, the vascularisation of tumors is very complex, consisting of sprouting, vessel cooption, glomeruloid angiogenesis, mosaic vessel formation, vascular mimicry, and intussusceptive angiogenesis. Furthermore, there is emerging evidence that putative angioblasts, also known as endothelial progenitor cells (EPC), might persist in adult life and contribute to the vascularization of tumors. EPC derived from HSC may have many of the same functional properties as mature endothelial cells represented by human umbilical vein endothelial cells [54]. This suggests the importance of distinguishing EPC from mature cells using more than one early stem cell marker, such as different antibody combinations.

It is a controversial belief that bone marrow-derived cells may contribute to tumor neovascularization, claiming an exclusive role for sprouting angiogenesis in tumor blood vessel development. A new study shows that bone marrow-derived lin(-)c-kit(+)Sca-1+ stem cells can be recruited to subcutaneously implanted Lewis lung carcinoma in a syngeneic bone marrow transplantation model. However, the recruited stem cells do not appear to functionally contribute to tumor neovascularization [55]. Thus, the authors hypothesize that new vessel formation in carcinomas occurs primarily through endothelialization from adjacent and preexisting vasculature rather than the transplanted stem cells.

It is unclear which cell types give rise to lung cancers, although recent advancements have accumulated greater molecular evidence of mutations of lung cancer. Thus it's necessary to know whether tumors originate from stem cells, and if yes, to identify the cell types involved. One report showed that human adenocarcinomas and squamous cell carcinomas of the lung are positive in type II markers such as SP-C and proliferation marker Ki67 [46]. These studies indicate that stem cells can be used to investigate tumor development.

Stem cells may be useful for treatment of cancer by carrying and delivering specific genes and drugs to sites of malignancies. Wei *et al.* [56] used *ex vivo* approach to expand embryonic endothelial progenitor cells that are transfected with the suicide construct containing yeast cytosine deaminase (CD) gene fused to uracil phosphoribosyl transferase (UPRT, which shortcuts rate-limiting enzymatic steps). The fusion gene acts as a strong catalyst converting the harmless prodrug 5-fluorocytosine (5-FC) to the cytotoxic compound 5-fluorouracil (5-FU) and its active metabolites. The engineered embryonic cells were intravenously administered into tumor-bearing mice. Surprisingly, the cells preferentially localized to lung metastases, where they integrated in tumor stromal microenvironments. Upon treatment with 5-FC, protein expression of the suicide construct in the tumors exerted a bystander cytotoxic effect that affected metastatic sites and slightly prolonged the lifespan of treated tumor-bearing mice. This may rely on a pulmonary first pass effect, suggesting a model for pulmonary tumor or metastases of tumors of other organs.

Another possibility for cancer therapy uses MSC as delivery vehicles for delivering IFN- [57], as IFN- was previously shown to inhibit malignant cell growth *in vitro*. However, the therapeutic utility of IFN- *in vivo* is limited by its excessive toxicity when administered systemically at high doses. The authors tested whether MSC can deliver IFN- to tumors with lowered toxicity. Human MSC were transduced with an adenoviral expression vector carrying the human IFN- gene (MSC-IFN- cells). Injected MSC-IFN- cells suppressed the growth of pulmonary metastases, presumably through the local production of IFN- in the tumor microenvironment. Therefore, this provides another evidence that MSC may be useful for the targeted delivery of various therapeutic proteins to cancer sites.

APPLICATION IN THERAPY OR BIOMEDICAL RESEARCH

In the last section, we briefly discussed the possible use of stem cells in cancer therapy. The following provides comments on other applications of stem cells in respiratory diseases. Although HSC were able to migrate into various organs, Krause *et al.* did not observe functional differentiation [11]. In contrast, the study by Grant *et al.* found functional differentiation as blood flow was restored in the damaged retina [58]. Other studies, such as that by Clarke *et al.* could not prove the controversies [59]. Whether stem cells can provide therapeutic benefit in lung disease is unclear, but this question is being continuously investigated. Ortiz *et al.* have reported that bone marrow derived stem cells may be able to alleviate bleomycin-induced fibrosis; this is the first study that has shown noticeable effect in stem cell derived cell therapy for pulmonary disease [60].

Application of stem cells to the body may cause pathogenesis such as forming tumors or participating inflammation in the regions of injury. This has been recently confirmed by Hashimoto *et al.* who transplanted EGFP expressing MSC/HSC into mice that received bleomycin [61]. Induction of pulmonary fibrosis in such chimera mice by intratracheal bleomycin injection caused large numbers of GFP cells to appear in active fibrotic lesions, while only a few GFP cells could be identified in control lungs. This indicates that in the fibrosis model, most fibroblasts are derived from BM precursor cells, which may be regulated by chemokines such as SDF1a and SLC. This finding directly demonstrates for the first time that BM-derived fibroblasts may play a role in the pathogenesis of pulmonary fibrosis. Therefore, scientists may have to revise the current thinking and approaches to the treatment and management of pulmonary fibrosis, and perhaps fibrosis in other organs.

One example for airway chronic disease is to test therapeutic role of adult stem cells (MSC) in CF. MSC from CF patients are amenable to CFTR gene correction, and expression of CFTR does not influence the pluripotency of MSC. Moreover, the CFTR-corrected MSC from CF patients are able to contribute to apical Cl^- secretion in response to cAMP agonist stimulation, suggesting the possibility of developing cell-based therapy for CF. The *ex vivo* coculture system may afford an approach to selection of stem-cell populations [62].

In house, we have been testing whether alveolar type II cells can be used as vehicles to deliver DNA repair genes to attenuate the injury of chemotherapeutics or other oxidative reagents [63-65]. A series of assays *in vitro* transfection have indicated that this strategy indeed has effect in reducing oxidative damage in lung cells. Although this work needs to be tested *in vivo*, we anticipate that type II cells or adult HSC may prove to be useful for gene delivery to reduce oxidative toxicity. Another interesting subject is assessment of the molecular mechanism that acts when type II cells have encountered the bacterial infection. We proposed that the infectious mechanism of *Pseudomonas aeruginosa* is different between normal adult lung cells and progenitor lung cells. However, this mechanism is not clear. Understanding of this mechanism will significantly broaden our knowledge to better regulate the progenitor cells and develop new therapeutic methods of controlling this infectious disease. In a recent study, we showed that *Pseudomonas aeruginosa* invades type II cells through lipid raft-mediated mechanism and in which the src like tyrosine kinase, Lyn, plays a pivotal role [66]. One obvious advantage of type II cells is their defined phenotype and genotype, which makes these cells more appropriate candidates for repair of injury and replenishing of infected cells. In addition, the local progenitors are theoretically safer than ES cells with much-reduced possibility of generating malignant cells.

PERSPECTIVES

It has been more than two decades since the first isolation of animal stem cells. It may be not much longer until the first clinical trial with ES cells in patients. It is extremely important to establish safety data with the use of ES cells in humans. The original cells established by Thomson may have contained some animal components that may be risky in humans. However, the cells are well characterized and relatively safer than newer cells that may contain no animal feeders. GDP is a critical factor for new product in therapeutic production. However, cell therapy is complex and there is no prior example to follow. FDA (Office of Cellular, Tissue and Gene Therapy) is now assessing and establishing the standards for stem cell therapy products. The potential of circulating stem cells homing to the lung may be meaningful for experimental and clinical repair of injured or diseased respiratory system. Although much evidence is needed to confirm the notion of homing HSC to the local respiratory progenitors, study of this interesting topic will provide excitement and new insight into our knowledge of stem cell differentiation. Another possibility is the induction of local progenitor cells for gene delivery or transfection for therapy. The unique structures and technical difficulties involved in the study of pulmonary stem cells present unusually stiff challenges. Hopefully, more groundbreaking findings will come out in the lung research community and the extensive potential of respiratory stem cell therapy will be realized.

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