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Cell-Based Strategies to Reconstitute Lung Function in Infants with Severe Bronchopulmonary Dysplasia

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KEYWORDS

• Premature birth • Bronchopulmonary dysplasia • Oxygen • Lung injury • Stem cells • Regeneration • Cell therapy

KEY POINTS

- Various types of stem/progenitor cells have shown potential promise in preventing and/or repairing neonatal lung injury.
- Mesenchymal stem cells derived from both bone marrow and umbilical cord blood are being popularly studied and appear to function in a paracrine manner rather than through cell engraftment.
- Further knowledge and understanding in this novel and exciting area of research is necessary before safe clinical translation of cell-based therapies is warranted.
- Strong emphasis must be placed on developing and standardizing techniques for stem/progenitor cell definition, isolation, expansion, and therapeutic administration.
- Experimental studies also need to focus on the long-term outcomes of such therapies.
- By identifying the most appropriate "reparative cell(s)" and its source, combined with understanding alternative mechanisms of action beyond cell replacement, we can advance in the quest of providing therapeutic strategies to prevent/repair neonatal lung injury.

INTRODUCTION

Advances in perinatal care have led to improved survival following very preterm birth, with infants born as early as 23 to 24 weeks of gestation now being capable of survival. However, with this shift in the limit of viability toward a lower gestational age, the task of protecting the more immature lung from injury becomes increasingly challenging.

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Extreme prematurity is one of the major risk factors for the development of chronic lung disease of prematurity or bronchopulmonary dysplasia (BPD).[1] Preterm infants born between 24 and 28 weeks of gestation (ie, extremely preterm) have an immature pulmonary surfactant system, immature airway and vascular architecture, and an underdeveloped surface area for gas exchange (**Fig. 1**).[2] Many very preterm infants require prolonged respiratory support to ensure survival, which further increases their risk of developing BPD.

Recent evidence suggests that BPD may have long-term respiratory complications that reach beyond childhood. Numerous follow-up studies indicate that children and young adults who were born very preterm are at an increased risk of respiratory symptoms, poor lung function, and lower exercise capacity[3–7] this is especially apparent in infants who have developed BPD. More alarmingly, isolated case studies are surfacing of irreversible arrested alveolar development at adult age in former premature infants with BPD,[8,9] mirroring results from experimental models of BPD.[10]

Progress toward decreasing the incidence/severity of BPD over the next few years using currently available techniques and strategies is likely (ie, optimization of antenatal management combined with surfactant and early noninvasive ventilatory support targeting lower oxygen saturations).[11] However, further understanding of the mechanisms involved in lung development, injury, and repair are necessary to advance toward preventing lung injury and/or promoting lung development/regeneration in prematurely born infants. Exciting discoveries in stem cell biology in recent years may offer new insight into the pathogenesis of BPD and, more importantly, open new therapeutic avenues.

BASIC CONCEPTS OF STEM CELL BIOLOGY

Stem cells are primitive cells capable of extensive self-renewal with the potential to give rise to multiple differentiated cellular phenotypes.[12] These cells are not only critical for organogenesis and growth during the early stages of development but also contribute to organ repair and regeneration throughout life.

Developmental Potency of Stem Cells

The concept of developmental potency refers to the range of possible fates open to cells during differentiation. Stem cells exhibit varying differentiation potencies, and

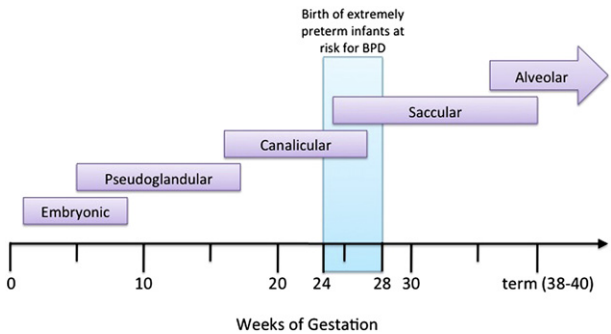

Fig. 1. Stages and gestational ages of normal lung development and preterm infants at risk of BPD. Schematic depicting stages of lung development in the human: embryonic (1–7 weeks), pseudoglandular (~5–16/17 weeks), canalicular (~16/17–24/26 weeks), saccular (~24–38 weeks), and alveolar (~36 weeks to postnatal) stages. Preterm infants at risk of developing BPD are born during the late canalicular to early saccular phase of lung development.

are typically categorized into embryonic and somatic stem cells. Embryonic stem cells (ESCs) are derived from the early blastocyst and represent the most potent of stem cells owing to their pluripotency (ie, ability to differentiate into cell types derived from all 3 germinal lineages: endoderm, mesoderm, or ectoderm) and their ability for indefinite self-renewal. By contrast, somatic stem cells (also termed adult stem cells [ASCs]) are cells that have assumed increasing degrees of fate restriction and are either multipotent (ie, can differentiate into a limited range of cell types) or unipotent (ie, can generate only one cell type).[13] Residual pools of such multipotent or unipotent stem cells are hypothesized to reside in almost all adult organs, and have the ability to contribute to tissue repair and regeneration via repopulation during growth, injury, or disease.

Classical Versus Nonclassical Stem Cell Hierarchies

While stem cells are essential for growth and development, residual pools of ASCs are considered important for tissue repair and maintenance through adulthood. Highly proliferative tissues, such as the intestinal epithelium or the hematopoietic compartment of the bone marrow, depend on a pool of ASCs that are organized in a "classical hierarchy" to maintain homeostasis.[14] By contrast, anatomically complex tissues that turn over more slowly (ie, brain, heart, lung, and kidney) do not appear to support a classical stem cell hierarchy. Such tissues are thought to be maintained by stem/progenitor cell populations that are organized in a nonclassical hierarchy and are recruited in a facultative manner for regeneration following injury.

In the lung, several local epithelial cell types function both as differentiated functional cells and as transit-amplifying progenitors that proliferate in response to airway or alveolar injuries.[15] Recent research suggests that the adult lung harbors rare populations of multipotent epithelial stem cells that are regulated by specific microenvironmental cellular niches, and are putatively recruited to repopulate the damaged epithelium.[16–19] With new insights into stem cell biology, other types of resident lung stem/progenitor cells have also been described over the past 5 years.

RESIDENT LUNG STEM/PROGENITOR CELLS

Lungs are complex organs constituted by more than 40 cell types derived from all 3 germ layers.[20] Normal lung morphogenesis involves spatiotemporally coordinated interactions between the stem/progenitor cells of different cellular compartments, which are later recapitulated during lung regeneration and repair following injury.[21] At present, the localization and properties of lung stem/progenitor cell niches and the type of cells within each niche are of major interest, yet also present controversy. Complexity of the lung architecture combined with an extensive diversity of cell types and niches has hindered the identification of true lung stem/progenitor cells. Because of the exceptionally low rate of epithelial cell turnover in the steady-state adult lung, the use of injury models has become necessary in unveiling the identity, fate, and specificity of resident lung stem/progenitor cells that contribute to homeostatic maintenance in the lung.[22] In doing so, it has been observed that relatively differentiated airway and alveolar epithelial cell types are capable of proliferating in response to epithelial injury.[15] This observation has drawn the focus of lung stem/progenitor cell research into identifying and defining those epithelial cell subpopulations that appear to contribute to postinjury regeneration.[15] Indeed, putative populations of such endogenous progenitor cells within the adult lung have been located in the basal layer of the

proximal conducting airways (trachea and major bronchi) and more distally (bronchioles and alveolar ducts) within or near neuroendocrine bodies (NEBs), in the bronchoalveolar duct junction (BADJ), and the alveolar surface.[23] A representative list of putative endogenous lung epithelial stem/progenitor cells is summarized in **Table 1**. While stem/progenitor cells in the proximal airways have been explored more extensively, the study of distal stem/progenitor cells remains more controversial.

Proximal Airway Stem/Progenitor Cells

Airway submucosal glands (SMGs) and SMG ducts are major secretory structures situated below the epithelium of the proximal trachea.[24] Studies have indicated that SMGs may serve as a protective niche for adult epithelial stem/progenitor cells of the proximal airways.[25,26] Using a model of epithelial damage induced by intratracheal detergent or SO_2 inhalation, Borthwick and colleagues[25] identified a population of 5-bromo-2′-deoxyuridine label–retaining cells that were localized to the gland ducts of the upper trachea and were able to reconstitute a surface-like tracheal epithelium. In support of these findings, SMG duct cells ($K5^+$, $K14^+$) survive severe hypoxic-ischemic injury and contain stem/progenitor cell populations for regenerating the pseudostratified surface epithelium, SMGs, and SMG ducts.[26]

An additional niche of stem/progenitor cells in the proximal airway is the K14-expressing tracheobronchial basal cells, which have been shown to repopulate the denuded airway epithelium, including columnar secretory and ciliated cells, following naphthalene-induced epithelial ablation.[27] This finding indicates that the K14-expressing basal cells are implicated as a stem/progenitor cell for this airway location. The potential contribution of these cells to the repair of the distal lung remains unknown.

Distal Lung Epithelial Stem/Progenitor Cells

With transition from the proximal to distal airways, it can be seen that the notion of multiple niches supporting different populations and their progenitors within the lung is evident. This idea is supported by studies using naphthalene to deplete the airways of Clara cells, revealing a subset of Clara cells that are $CCSP^+$, yet naphthalene resistant. These cells, termed variant Clara ($Clara^V$) cells, exhibit stem cell characteristics, including ability to efflux Hoechst dye and Sca-1 expression, and have been located either within NEBs or at the bronchoalveolar duct junctions (BADJs).[28,29] More recently, Volckaert and colleagues[30] also proposed that parabronchial smooth muscle cells (PSMCs) constitute a stem/progenitor cell niche for the $Clara^V$ cells. Activation of the $Clara^V$ cell for epithelial repair following naphthalene injury was shown to be dependent on the paracrine signaling of fibroblast growth factor 10 from the PSMC niche.[30]

Recently, multipotent stem cells in the distal lung capable of differentiating into epithelial cells specific to the bronchioles and the alveoli have been identified. Kim and colleagues[17] demonstrated the existence of dual-lineage bronchoalveolar stem cells (BASCs) at the BADJ that express both bronchiolar ($CCSP^+$) and alveolar ($SP\text{-}C^+$) markers, which proliferate in response to airway and alveolar injury. However, based on the techniques used, there has been some ambiguity regarding the lineage potential[23,31] and contribution of these cells to alveolar repair.[32] McQualter and colleagues[18] used a multiparameter cell separation strategy and an organotypic in vitro clonogenic assay to detect and characterize a rare population of multipotent adult lung epithelial stem cells that give rise to airway and alveolar epithelial lineages in vitro. More recently, p63-expressing cells in the bronchiolar epithelium have been

Table 1
A selective list of candidate endogenous lung stem/progenitor cells in the rodent lung

Anatomic Location	Candidate Stem/ Progenitor Cell	Attributed Differentiated Phenotype	Niche	Defining Characteristics	References
Proximal trachea	SMG duct cells	Tracheal epithelial cells, SMGs, SMG ducts	SMGs	Express cytokeratin-14 and -5; survives and repopulates tracheal epithelium following hypoxic-ischemic injury; BrdU labeling–retained cells following i.t. detergent or SO_2-mediated epithelial injury	25,26
Distal trachea and bronchi	Basal cells	Tracheobronchial epithelial cells	Intercartilaginous zone	Cytokeratin-14–expressing multipotent progenitor cells capable of restoring differentiated tracheal epithelium following naphthalene injury; associated with innervated NEBs	27
Bronchioles	$Clara^V$ cells	Distal airway epithelium	BADJs, NEBs, and PSMCs	Express CCSP; survives and repopulates distal airway epithelium following naphthalene injury; dependent on paracrine signaling of Fgf10	29,30
Bronchioles and alveoli	BASCs	Bronchoalveolar epithelial cells	BADJs	Resistant to naphthalene injury and proliferate in response; coexpress CCSP and SP-C	17
	Pulmonary $Oct\text{-}4^+$ stem/progenitor cells	Alveolar type-I and -II pneumocytes	BADJs	$Oct4^+$, $SSEA\text{-}1^+$, $Sca\text{-}1^+$, cytokeratin-7^+ cells; serially passaged, differentiate terminally into type-II and -I pneumocytes; susceptible to SARS-CoV infection	78
	Multipotent lung epithelial progenitors	Airway and alveolar epithelium	Intrapulmonary airways and alveoli (not localized)	EpCAMhi, $CD49f^+$, $CD104^+$, CD24lo, $Sca\text{-}1^-$, $CD45^-$, $CD31^-$ lung epithelial colony-forming units, form colonies in Matrigel, serially passaged and retain multipotent potential	18
Alveoli	Alveolar type-II pneumocytes	Alveolar type-I pneumocytes	Alveolar surface	All alveolar type-II pneumocytes	34
	A subset of alveolar type-II pneumocytes	Alveolar type-I and mature type-II pneumocytes	Alveolar surface	E-cadherin negative subset of alveolar type-II cells, proliferative, high telomerase activity, resistant to oxygen-induced injury	37

Abbreviations: BADJ, bronchoalveolar duct junction; BASC, bronchoalveolar stem cell; BrdU, 5-bromo-2′-deoxyuridine; CCSP, Clara cell secretory protein; $Clara^V$, variant Clara; EpCAMhi, epithelial cell adhesion molecule Fgf10, fibroblast growth factor 10; NEBs, neuroendocrine bodies; Oct-4, octamer-binding transcription factor 4; PSMC, parabronchial smooth muscle cell; Sca-1, stem cell antigen 1; SMG, submucosal gland; SP-C, surfactant protein C; SSEA-1, stage-specific embryonic antigen-1; SARS-CoV, severe acute respiratory syndrome coronavirus.

shown to undergo rapid proliferation after H1N1 influenza virus infection and to radiate to interbronchiolar regions of alveolar ablation. These cells assemble into Krt5$^+$ pods and initiate expression of markers typical of alveoli. Gene-expression profiles of these pods suggest that they are intermediates in the reconstitution of the alveolar-capillary network.[33] The presence of such putative endogenous alveolar stem cell populations provides fresh hope of target-directed, regenerative therapies for alveolar diseases.

Cuboidal type-II pneumocytes have long been considered as progenitors of the alveolar epithelium, based on their capacity to replenish themselves and generate terminally differentiated type I pneumocytes.[34,35] Since then, type-II pneumocytes have been speculated to contain a subpopulation of progenitors cells that can undergo reactivation into a progenitor-like state in response to injury cues. Using an acute model of oxygen-induced injury, Driscoll and colleagues[36] demonstrated the existence of a telomerase-positive subpopulation within the general type-II cell population during the recovery phase. These findings were further strengthened by a later study in which Reddy and colleagues[37] classified type-II cells into E-cadherin–positive and -negative fractions, and showed heightened telomerase activity and injury resistance in the latter subset.

Lung Mesenchymal Stem Cells

Additional lung cell types, including airway smooth muscle, fibroblasts, and the vasculature, are derived from the mesoderm. Interactions between the epithelial cells, mesenchymal microenvironment (including extracellular matrix proteins and growth factors), and the adjacent pulmonary vasculature regulate the structural and functional maturation of the developing lung.[21] Current knowledge of lung mesenchymal precursors is limited; however, there is evidence that small populations of resident lung cells expressing certain phenotypic characteristics of mesenchymal cells with progenitor capacity exist within the lung.

Resident lung "side population" (SP) cells, which appear to have both mesenchymal and epithelial potential, have been isolated based on their capacity to efflux Hoechst dye.[38–40] These SP cells have been shown to be present at all levels of the airway tree, and regardless of which lung compartment they were derived from, exhibited a relatively uniform phenotype.[40] Although it has been demonstrated that these SP cells are a source of adult lung mesenchymal stem cells (MSCs),[39] the role of SP cells in endogenous lung repair is not completely understood.

Furthermore, McQualter and colleagues[41] described a population of endogenous fibroblastic progenitor cells with clonogenic potential in the adult lung, which are predominantly representative of mesenchymal cell lineages. The cell fraction defined by McQualter and colleagues[18] was of similar cell phenotype (CD45$^-$, CD31$^-$, Sca-1$^+$, CD43$^+$) to the cell fraction defined as BASCs; however, they coexpressed immunophenotypic markers definitive of lung fibroblastic rather than epithelial cells.[41] These findings highlight the need for alternative, specific markers to enable precise identification of endogenous stem/progenitor cell subpopulations within the lung.

Following the discovery of the plasticity characteristics of ASCs that allow them to cross lineage barriers and adopt functional phenotypes of other tissues, much interest has been diverted to understanding their role in repair and maintenance of the lungs.[42] Experimental evidence indicates that the injured lung stimulates the release and preferential homing of MSCs, a population of ASCs derived from the bone marrow.[43,44] However, the mechanism by which exogenous progenitors, such as bone marrow MSCs, assume lung phenotype remains unclear, as does its clinical significance.[45,46]

Lung Endothelial Progenitor Cells

Endothelial progenitor cells (EPCs), a population of vascular precursor cells, have also recently received attention in the context of lung development and regeneration. Indeed, given the importance of lung angiogenesis and vascular growth factors during lung growth and repair, vascular progenitor cells are appealing candidate cells likely to be involved in the same mechanisms.[47] However, assessment of the contribution of endogenous lung EPCs in lung vascular repair and lung regeneration and remodeling is impeded by their rarity, lack of distinguishing markers, and the inability to discriminate circulating EPCs and tissue EPCs.[22] Alvarez and colleagues[48] demonstrated that the lung microvasculature is enriched with a population of EPCs, termed resident microvascular endothelial progenitor cells, which were shown to be highly proliferative and capable of renewing the entire hierarchy of endothelial cell growth potentials. It has been demonstrated that both circulating and resident lung EPCs are likely to contribute to endothelial cell regeneration and repair in the lung.[49,50]

The recent surge in our knowledge of stem cell biology and the availability of advanced research tools in this field has motivated researchers in exploring the role of lung stem cells in the pathogenesis of lung diseases. Indeed, several major lung diseases likely involve dysregulation in the numbers and/or the function of resident lung stem/progenitor cells.[46] For instance, depletion or functional impairment of alveolar epithelial and/or EPCs could putatively underlie the pathogenesis of alveolar growth arrest or destruction observed in BPD and emphysema, respectively. In such a scenario, augmentation of stem cells is an appealing strategy to minimize lung injury, promote repair, or possibly regenerate lost tissue.

LUNG STEM/PROGENITOR CELLS: IMPLICATIONS FOR THE PATHOGENESIS OF BPD

Recent animal and human studies suggest that damage or depletion of epithelial and/or vascular stem/progenitor cells in the developing lung likely contributes to the pathogenesis of BPD.

Perturbation of Distal Lung Epithelial and Mesenchymal Stem Cells

Exposure of neonatal rodents to high levels of oxygen is extensively used as an injury model to investigate experimental BPD. Irwin and colleagues[51] showed a reduction in the number and endothelial differentiation potential of multipotent lung SP cells. Observations from the authors' own laboratory in an oxygen-challenged neonatal rat model of BPD have also shown decreased numbers of circulating and resident MSCs in the lungs.[52] This finding highlights the potential of stem cell supplementation for the prevention or repair of neonatal lung injury. Accordingly, systemic treatment of neonatal hyperoxia–exposed mice with MSCs significantly increases the number of BASCs compared with untreated controls. In addition, treatment of BASCs with MSC-derived conditioned media (CdM) in culture stimulated BASC growth efficiency, indicating a direct effect of MSCs on BASCs.[53]

In contrast to the aforementioned reports of depleted numbers of stem/progenitor cells, Popova and colleagues[54] demonstrated that the presence of MSCs in tracheal aspirates of preterm infants indicated an increased risk for developing BPD. Those cells isolated from the tracheal aspirates expressed the markers STRO-1, CD73, CD90, CD105, CD166, CCR2b, CD13, propyl-4-hydroxylase, and α-smooth muscle actin, and were negative for CD11b, CD31, CD34, and CD45.[55] Furthermore, these cells were shown to acquire a myofibroblast phenotype, which suggest that they could contribute to the profibrotic changes and arrested alveolarization in BPD.[56] However, in contrast to tracheal aspirate MSCs, human bone marrow–derived MSCs did not

undergo myofibroblastic differentiation in response to transforming growth factor β1, suggesting distinct properties between these 2 populations of MSCs.[56] Indeed, it is possible that these reported resident lung MSCs are perturbed in BPD, as their cell phenotype is not analogous to the endogenous MSCs described by McQualter and colleagues[41] in the absence of lung injury. Therefore, with the growing interest in harnessing the therapeutic effects of stem progenitor cells for neonatal lung injury, it is necessary to perform further thorough investigations to understand the behavior of MSCs from different populations (ie, lung, umbilical cord blood [UCB], bone marrow) in the presence and absence of lung injury, and how this could affect potential cell-based therapies for BPD.[57]

Perturbation of Lung and Circulating EPCs

Neonatal mice with oxygen-induced chronic lung injury have depleted numbers of putative lung-resident EPCs ($CD45^-/Sca\text{-}1^+/CD133^+/VEGFR\text{-}2^+$).[50] Baker and colleagues[58] demonstrated that UCB of preterm infants yielded a higher amount of endothelial colony-forming cells (ECFCs; a specific subset of EPCs) than from UCB of term infants. Preterm ECFCs had an increased susceptibility to in vitro oxygen exposure than term ECFCs.[58] Borghesi and colleagues[59] reported that the number of ECFCs was lower in UCB of preterm infants who subsequently developed BPD, compared with preterm infants who did not develop BPD. In contrast to the findings of Borghesi and colleagues,[59] Paviotti and colleagues[60] recently reported no association between the number of EPCs at birth and the subsequent development of BPD. The apparent discordance between studies reporting EPCs in preterm infants highlights the importance of appropriately defining an EPC and establishing criteria similar to the "minimal criteria" for characterizing MSCs.[61] Furthermore, assessing EPC function may be more revealing than assessing EPC number.

These observations suggest that the capacity of resident stem cell populations to undergo self-renewal and regeneration can be limited, because of the natural effect of increasing age and/or the presence of disease. This situation forms the rationale for the therapeutic potential of stem cell–based therapies, either through stimulation of endogenous stem cell pools or their therapeutic replacement with exogenous-derived stem cells. Such cell-replacement therapies already show promise in debilitating childhood and adult disorders.[62–64] In the laboratory, stem cell–based strategies have shown therapeutic benefit in experimental models of lung disease.

THERAPEUTIC POTENTIAL OF STEM CELLS TO PREVENT OR REPAIR THE DAMAGED LUNG

Numerous studies in experimental animal models provide compelling evidence for the beneficial effects of stem cell therapy approaches for a wide variety of adult lung diseases (**Table 2**), including acute lung injury/acute respiratory distress syndrome, pulmonary hypertension, asthma, and chronic obstructive pulmonary disease (including emphysema).[65–67]

Of the many different stem/progenitor cell therapies that have been used in experimental models, MSCs appear to be the most extensively examined cell type. MSCs can be sourced from the bone marrow, UCB, Wharton jelly, the placenta, and adipose tissue.[68] As outlined in **Table 2** benefits of MSC therapy in experimental adult lung diseases include, but are not limited to, improvements in alveolar, airway, and vascular structure; attenuation of lung inflammation; decreased pulmonary fibrosis; reduced pulmonary edema, hemorrhage, and alveolar and endothelial permeability; and

Table 2
Studies testing the therapeutic effect of stem/progenitor cells in experimental adult lung disease models

Experimental Model	Therapeutic Cell of Product	Outcomes	Suggested Mechanisms	References
Bleomycin Lung Injury/Acute Respiratory Distress Syndrome				
Bleomycin-induced (i.t.)	Human ESC-derived cells with AT2 epithelial phenotype (i.t.)	Improved body weight and survival Improved arterial oxygen saturation Decreased collagen deposition	Engraftment and AT1 differentiation Paracrine mechanisms	79
	Bone marrow–derived MSCs (i.v.)	Reduced fibrosis and inflammation	IL-1 receptor antagonism Decrease in NO metabolites, proinflammatory, and angiogenic cytokines	44,80,81
	hUC Wharton jelly–derived MSCs (i.v.)	Reduced fibrosis	Decreased TGF-β and TIMP activity Increased MMP-2 activity	82
	Bone marrow–derived HSCs ± KGF overexpression (i.v.)	Reduced fibrosis	KGF-induced endogenous AT2 cell proliferation	83
Bleomycin-induced (i.n.)	hAECs (i.p.)	Reduced fibrosis and collagen deposition Improved lung function Modulated inflammatory response	Anti-inflammatory effects	84
Escherichia coli endotoxin-induced (i.p.)	Bone marrow–derived MSCs (i.v.; i.t.)	Improved survival Decreased systemic and local inflammation	Cell-cell interactions Paracrine mechanisms Decreased proinflammatory and increased anti-inflammatory cytokines Antioxidant mechanisms	85–87
E coli endotoxin-induced (i.t.)	iPS cells and CdM (i.v.)	Attenuated lung injury Reduced inflammation Reduced MPO and NF-κB activity Improved Pao_2 and lung function	Paracrine mechanisms Regulation of neutrophil activity Attenuating inflammatory cascade Immunomodulatory effects	88
	Bone marrow–derived MSCs overexpressing Ang-1 (i.v.; i.t.)	Decreased inflammation Decreased alveolar permeability	Decreased inflammatory cytokines Ang-1–mediated effects	89,90
	hUCB-derived MSCs (i.t.)	Increased survival Attenuated lung injury Reduced inflammation Increased MPO activity Inhibited bacterial growth	Down-modulating inflammatory process Enhancing bacterial clearance	91

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Table 2
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Experimental Model	Therapeutic Cell of Product	Outcomes	Suggested Mechanisms	References
LPS-induced (i.t.)	Human orbital fat–derived stem/stromal cells (i.v.)	Decreased systemic and local inflammation Decreased alveolar and endothelial permeability	Inhibition of macrophage and neutrophil-associated inflammatory responses	92
	EPCs (i.v.)	Improved Pao_2 and SaO_2 Preservation of alveolocapillary permeability Reduced interstitial edema, hyaline membrane formation, hemorrhage	Paracrine mechanisms Anti-inflammatory effects	93
	hUCB-derived MSCs (i.t.)	Increased survival Reduced edema, hemorrhage, alveolar and endothelial permeability Reduced inflammation	Paracrine mechanisms Anti-inflammatory effects	94
LPS-induced (i.v.)	EPCs (i.v.)	Reduced pulmonary edema, inflammation, hemorrhage, and hyaline membrane formation Decreased adhesion molecule expression Reduced endothelial and epithelial cell apoptosis	Engraftment of EPCs Re-endothelialization Downregulation of adhesion molecules Alleviation of inflammatory response Apoptosis prevention	95
Ventilator-induced	Bone marrow–derived MSCs and CdM (i.v.)	Improved lung function Modulated inflammation Restored lung structure	Paracrine mechanisms	96
Pulmonary Hypertension				
Monocrotaline-induced	Bone marrow–derived MSCs ± eNOS overexpression (i.v.; i.t.)	Improved survival Improved RV pressure overload and function Improved lung structure	eNOS-mediated vasodilation VEGF-mediated enhanced microvasculature Paracrine effects	97–100
	Bone marrow–derived EPCs (i.v.)	Restored pulmonary hemodynamics Increased microvascular perfusion	eNOS-mediated vascular growth	101
	Peripheral blood-derived EPCs (i.t.)	Improved cardiac function Improved vasculature thickness and lung neovascularization		102

Asthma/Allergic Airway Inflammation				
Ovalbumin-induced (i.p. and i.t.; nebulized)	Adipose tissue–derived MSCs (i.v.)	Decreased local and systemic allergic response	Decreased Th2 activity	103,104
	Bone marrow–derived MSCs (i.v.)	Reduced airway hyperresponsiveness and remodeling Reduced serum NO levels Reduced inflammatory cell infiltration and mast cell degranulation	Immunomodulatory effects Anti-inflammatory effects	105,106
	BMC-CdM	Prevented airway inflammation Reduced airway remodeling Prevented airway hyperresponsiveness	Paracrine mechanisms Anti-inflammatory effects of adipokine, APN	107
Ragweed-induced (i.p.)	Bone marrow–derived MSCs (i.v.)	Decreased asthma-specific allergic response	TGF-β production Regulatory T-cell recruitment	108
Chronic Obstructive Pulmonary Disease/Emphysema				
Cigarette smoke–induced	Bone marrow–derived MSCs, CdM, and BMCs (i.v.)	Restoration of alveolar structure Increased pulmonary vascularity Alleviation of pulmonary hypertension (by BMCs)	Paracrine mechanisms Recruitment of BMCs by donor cells	109
Papain-induced	Bone marrow–derived MSCs (i.v.)	Improved alveolar structure	Engraftment and AT2 differentiation Reduced alveolar epithelial apoptosis	110
Elastase-induced (i.t.)	Adipose tissue–derived MSCs (i.v. or cultured on PGA and transplanted after LVRS)	Restored gas exchange Improved exercise tolerance	Growth factor release (HGF, VEGF)	111,112
	Bone marrow–derived MSCs (i.t.)	Preservation of alveolar structure Reduced inflammation Upregulated growth factors	Paracrine mechanisms HGF, EGF, and secretory leukocyte protease inhibitor secretion	113
	Lung resident multilineage progenitors $Sca1^{+}CD45^{-}CD31^{-}$ (i.t.)	Improved survival Attenuated alveolar damage	Immunomodulatory effects Paracrine mechanisms	114

Abbreviations: Ang-1, angiopoietin-1; APN, adiponectin; AT1, alveolar epithelial type 1; AT2, alveolar epithelial type 2; BMC, bone marrow–derived cells; CdM, conditioned media; EGF, epidermal growth factor; eNOS, endothelial nitric oxide synthase; EPC, endothelial progenitor cell; HGF, hepatocyte growth factor; HSC, hematopoietic stem cell; hAEC, human amnion epithelial cell; hUC, human umbilical cord; hUCB, human umbilical cord blood; IL, interleukin; i.n., intranasal; i.p., intraperitoneal; iPS, induced pluripotent stem; i.t., intratracheal; i.v., intravenous; KGF, keratinocyte growth factor; LPS, lipopolysaccharide; LVRS, lung volume reduction surgery; MMP-2, matrix metalloproteinase 2; MPO, myeloperoxidase; MSC, mesenchymal stem cell; NF-κB, nuclear factor kappa light-chain enhancer of activated B cells; NO, nitric oxide; Pao_2, partial pressure of oxygen in arterial blood; PGA, polyglycolic acid; RV, right ventricle; Sao_2, oxygen saturation; TGF-β, transforming growth factor β; Th2, helper T cell type 2; TIMP, tissue inhibitor of metalloproteinase; VEGF, vascular endothelial growth factor.

restoration of lung function and exercise capacity. Of importance, the beneficial therapeutic actions of MSCs appear to be mediated through paracrine mechanisms and immunomodulatory effects, rather than cell engraftment and direct actions in the lungs.[69]

The use of MSCs and other types of stem/progenitor cells are also being increasingly examined in experimental models of neonatal lung disease, in particular BPD. Given the perturbations of resident lung stem cells in BPD, the ideal therapeutic approach would involve replenishing the lung with healthy multipotent stem/progenitor cells that repopulate, repair, and regenerate the injured, developing lung. Indeed, several recent studies have demonstrated promising outcomes using different stem/progenitor cell types in animal models of BPD (**Fig. 2**, **Table 3**).

Mesenchymal Stem Cell Therapy in Experimental BPD

Administration of bone marrow–derived MSCs, either intratracheally, intravenously, or intraperitoneally, have ameliorated numerous aspects of neonatal lung injury, as evident by mitigation of lung inflammation, prevention of lung vascular damage and alveolar growth arrest, inhibition of lung fibrosis, and improvement in exercise tolerance (**Fig. 3**).[52,53,70,71] Low engraftment and differentiation of these MSCs into the injured neonatal lung suggest that the potential mechanisms through which MSCs exert their actions are paracrine mediated. These speculations are supported by in vitro and in vivo studies demonstrating that administration of CdM from MSCs has the ability to protect alveolar epithelial and lung microvascular endothelial cells from oxidative stress, prevent oxygen-induced alveolar growth arrest, and stimulate a subset of stem/progenitor cells, namely BASCs, to aid in lung repair.[52,53,70] Furthermore, the therapeutic benefits of MSC-CdM may surpass those of MSCs, with in vivo

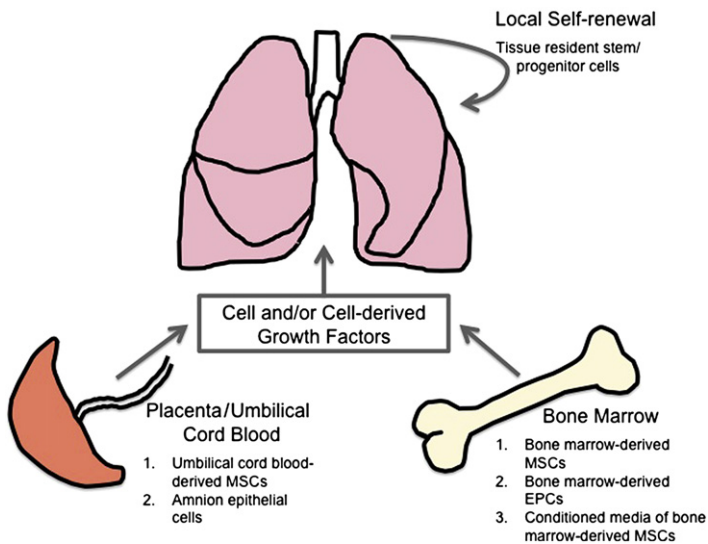

Fig. 2. Current sources of stem/progenitor cells for lung regeneration in experimental models of neonatal lung injury. Several studies have demonstrated the effects of stem/progenitor cells and stem/progenitor cell-derived growth factors (ie, conditioned media) to promote lung regeneration following neonatal lung injury in animal models of BPD. These cells were sourced from the bone marrow, umbilical cord blood, and placenta amnion.

Table 3
Studies testing the therapeutic effect of stem/progenitor cells in experimental models of neonatal chronic lung disease

Experimental Model	Therapeutic Cell or Product	Outcomes	Suggested Mechanism	References
Hyperoxia-induced lung injury (mice, rats)	Bone marrow–derived MSCs (i.t.)	Improved survival Improved alveolar structure/ prevented alveolar arrest Prevented vascular growth arrest Improved exercise capacity Reduced pulmonary hypertension	Engraftment as AT2 Paracrine mechanisms	52
	Bone marrow–derived MSCs or CdM (i.v.)	Improved alveolar structure/prevented alveolar arrest Attenuated inflammation Prevented vascular growth arrest Prevented pulmonary hypertension	Paracrine mechanisms Immunomodulatory effects	70
	Bone marrow–derived MSCs or CdM (i.v.)	Increased number of BASCs Improved alveolar structure/prevented alveolar arrest	Stimulation of BASCs Paracrine mechanisms	53
	Bone marrow–derived MSCs (i.p.)	Improved survival Improved alveolar structure/prevented alveolar arrest Attenuated inflammation Inhibited lung fibrosis	Engraftment as AT2 Reduction in ECM remodeling and fibrosis gene expression (TGF-β1, collagen 1α, TIMP-1) Anti-inflammatory effects	71
	hUCB-derived MSCs (i.t.)	Improved survival and growth restriction Improved alveolar structure Attenuated lung fibrosis, inflammation, and ROS activity	Paracrine anti-inflammatory, antifibrotic, and antioxidative effects	72,73
	BMDACs (i.v.)	Improved alveolar structure Improved vascular growth	Paracrine mechanisms	74
LPS-induced (i.a.) lung injury (sheep)	hAECs (i.t.; i.v.)	Improved alveolar structure Increased surfactant protein expression Attenuated inflammation	Immunomodulatory effects	75

Abbreviations: AT2, alveolar epithelial type 2; BASC, bronchoalveolar stem cell; BMDAC, bone marrow–derived angiogenic cell; CdM, conditioned media; ECM, extracellular matrix; hAEC, human amnion epithelial cell; hUCB, human umbilical cord blood; i.a., intra-amniotic, i.p., intraperitoneal; i.t., intratracheal; i.v., intravenous; LPS, lipopolysaccharide; MSC, mesenchymal stem cell; ROS, reactive oxygen species; TGF-β1, transforming growth factor β1; TIMP-1, tissue inhibitor of metalloproteinase 1.

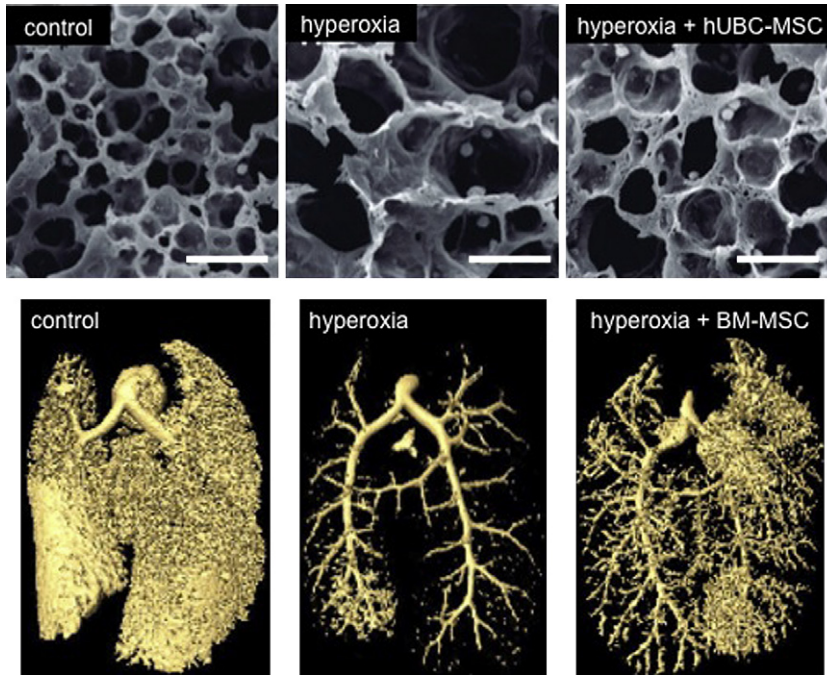

Fig. 3. Therapeutic effects of bone marrow–derived MSCs and human umbilical cord (hUBC) blood-derived MSCs in experimental oxygen-induced BPD. Intratracheal delivery of MSCs derived from hUBC and from bone marrow (BM) improves hyperoxia-induced alveolar and lung vascular growth in neonatal rats, as demonstrated by electron microscopy (*top panels*) and micro–computed tomography (*bottom panels*) of the alveolar structure and pulmonary vasculature, respectively. (*Adapted from* Chang YS, Oh W, Choi SJ, et al. Human umbilical cord blood-derived mesenchymal stem cells attenuate hyperoxia-induced lung injury in neonatal rats. Cell Transplant 2009;18:869–86; with permission; and van Haaften T, Byrne R, Bonnet S, et al. Airway delivery of mesenchymal stem cells prevents arrested alveolar growth in neonatal lung injury in rats. Am J Respir Crit Care Med 2009;180:1131–42; with permission.)

findings indicating a more profound therapeutic effect of MSC-CdM in preventing and repairing lung injury than that of MSCs.[70]

UCB also represents an appealing source of MSCs for therapeutic use in the newborn because of its clinically relevant, easily accessible, ethically viable, and readily available source of stem/progenitor cells. Chang and colleagues[72,73] demonstrated that MSCs obtained from human UCB prevent hyperoxia-induced alveolar growth arrest and alleviate fibrotic changes in the neonatal rat lung (see **Fig. 3**). Chang and colleagues[73] also show that the route of administration may alter the outcome, with intratracheal transplantation resulting in a more prominent attenuation of hyperoxia-induced lung injury than intraperitoneal transplantation. Furthermore, Chang and colleagues[72] recently demonstrated the dose-dependent effects of human UCB-derived MSCs in the oxygen-challenged neonatal rat lung. This study indicated that intratracheal delivery of a minimum of 5×10^4 cells is required to exhibit efficient anti-inflammatory, antifibrotic, and antioxidative effects following hyperoxia-induced lung injury in neonatal rats.[72] In light of these findings, further studies determining

the optimal dose of MSCs for potential clinical benefit in human neonates are anticipated.

Endothelial Progenitor Cell Therapy in Experimental BPD

The therapeutic potential of EPCs in neonatal lung injury has been effectively demonstrated in an oxygen-induced BPD mouse model.[74] Treatment of neonatal mice exposed to hyperoxia with intravenously administered bone marrow–derived angiogenic cells (a population of bone marrow myeloid-like precursor cells) showed restoration of the alveolar structure and vessel density to that of control (room air–exposed) levels.[74]

Amnion Epithelial Cell Therapy in Experimental BPD

The therapeutic potential of human amnion epithelial cells (hAECs) has recently been investigated in a sheep model of neonatal lung injury, induced by lipopolysaccharide (LPS) administration in fetal sheep.[75] Because hAECs are sourced from placentae, which are normally discarded after birth, they present an easily accessible and ethically viable candidate for cell therapy. Administration of hAECs to fetal sheep exposed to LPS attenuated inflammation-induced changes in lung function and structure, and reduced pulmonary inflammation.[75] Of particular interest is the ability of hAECs to significantly increase the expression levels of SP-A and SP-C. The low engraftment into the lungs indicates that these hAECs act via immune modulation rather than cell engraftment and differentiation. More detailed assessment of the therapeutic potential of these cells in other models of neonatal lung injury will be of interest.

In summary, findings from several exciting studies indicate that a variety of stem/progenitor cells can prevent and/or regenerate neonatal lung injury in experimental models. Additional studies in different animal models of BPD are necessary to broaden the current knowledge and understanding of the therapeutic potential of stem/progenitor cells. In doing so, further evidence for creating a strong rationale for transitioning this potential breakthrough into clinic can be generated.

REMAINING CHALLENGES

Although stem/progenitor cell therapies present potential promise in preventing and/or repairing lung injury, many gaps in our knowledge and understanding of stem cell biology in health and disease are yet to be filled (**Box 1**). In part, it is important to more precisely define putative reparative cells. One of the obstacles is the lack of biomarkers available for the characterization of candidate stem/progenitor cells in different species[22] and the inability to easily study these cells in vivo.

Current studies elegantly detail the short-term effects of stem/progenitor cell therapies in animal models of neonatal lung injury.[52,53,70–75] However, few of these studies have reported the long-term outcomes (ie, in mid-adult or aged lung) of such stem/progenitor cell therapies,[52,71] which is a vital and clinically important area of research that needs to be understood to warrant safe clinical translation.

In addition, it would be valuable to understand the effects of such stem/progenitor cell therapies in other animal models of neonatal lung injury closely mimicking the clinical setting (ie, ventilator-induced, fetal/neonatal inflammation-induced), rather than the frequently used hyperoxia-induced model; indeed, this is already being used by some.[75]

Current studies highlight the beneficial effects of stem/progenitor cell therapy on attenuating structural and/or molecular alterations to the injured developing lung, yet the effects on lung function are infrequently reported.[52] This aspect of experimental studies requires further investigation and thorough documentation, because

Box 1
Future directions and questions for the use of stem/progenitor cells in neonatal lung injury

Determine Optimal Stem Cell-Based Strategy for Lung Diseases

- What is the best reparative cell to treat a given lung disease?
  - ESCs, MSCs, EPCs, amniotic fluid stem cells, amnion epithelial cells
- What is the appropriate strategy for cell-based therapies?
  - Administration of exogenous stem cells: stem cells, stem cell–derived CdM, CdM-derived factors
  - Protection of endogenous lung progenitor cells
- What is the best mode of delivery of stem cells for lung diseases?
  - Intravenous
  - Intratracheal

Long-Term Outcomes of Cell Therapy in BPD Models

- Can stem/progenitor cell therapies permanently prevent/repair neonatal lung injury, or will continuous treatment be required for life?
- Would additional later in life "insults" reduce the protective effects of stem/progenitor cell therapies?
  - Lung infection
  - Smoking
- What are the effects of stem/progenitor cell therapies on short-term and long-term lung function outcomes?
  - Baseline lung function
  - Challenged lung function: exercise-induced, asthma/allergy-induced
  - Age-induced decline in lung function
- What are the potential adverse effects of stem cell–based therapies?
  - Tumor formation
  - Ectopic tissue formation
  - Immune rejection of the transplanted stem cells

Cell Therapy in Other Experimental Models of BPD

- What are the effects of stem/progenitor cell therapies in other relevant animal models of neonatal lung injury?
  - Type of lung injury: ventilator-induced, inflammation/infection-induced
  - Type of animal model: rodent, sheep, baboon

the overall aim of treating neonatal lung injury with stem/progenitor cells is to reduce and/or prevent lung dysfunction.

SUMMARY

Half a century since the landmark discovery of stem cells by the Canadian researchers Till and McCulloch in 1961,[76] their therapeutic potential in regenerative medicine is now being harnessed for treatment of neonatal lung injury, almost half a century since Northway and colleagues[77] described BPD. Various types of stem/progenitor cells

have shown benefit in experimental models of neonatal lung injury, with MSCs derived from both bone marrow and UCB being popularly studied, as well as CdM from these cells. However, before safe clinical translation of cell-based therapies is warranted, we must broaden our knowledge and understanding in this novel and exciting area of research. Strong emphasis must be placed on developing and standardizing techniques for stem/progenitor cell definition, isolation, expansion, and therapeutic administration. Experimental studies also need to focus on the long-term outcomes of such therapies. By identifying the most appropriate reparative cell(s) and its source, combined with understanding alternative mechanisms of action beyond cell replacement and assessing the short-term and long-term efficacy and safety, we can advance in the quest of providing therapeutic strategies to prevent and repair neonatal lung injury.

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