ANTIOXIDANTS & REDOX SIGNALING
Volume 17, Number 7, 2012

DOI: 10.1089/ars.2011.4267

COMPREHENSIVE INVITED REVIEW

Lung Injury in Preterm Neonates: The Role and Therapeutic Potential of Stem Cells

Rajesh S. Alphonse,[1] Saima Rajabali,[1] and Bernard Thébaud[1–3]

Abstract

Continuous improvements in perinatal care have allowed the survival of ever more premature infants, making the task of protecting the extremely immature lung from injury increasingly challenging. Premature infants at risk of developing chronic lung disease or bronchopulmonary dysplasia (BPD) are now born at the late canalicular stage of lung development, just when the airways become juxtaposed to the lung vasculature and when gas-exchange becomes possible. Readily available strategies, including improved antenatal management (education, regionalization, steroids, and antibiotics), together with exogenous surfactant and exclusive/early noninvasive ventilatory support, will likely decrease the incidence/severity of BPD over the next few years. Nonetheless, because of the extreme immaturity of the developing lung, the extent to which disruption of lung growth after prematurity and neonatal management lead to an earlier or more aggravated decline in respiratory function in later life is a matter of concern. Consequently, much more needs to be learned about the mechanisms of lung development, injury, and repair. Recent insight into stem cell biology has sparked interest for stem cells to repair damaged organs. This review summarizes the exciting potential of stem cell-based therapies for lung diseases in general and BPD in particular. *Antioxid. Redox Signal*. 17, 1013–1040.

Reviewing Editors: *Alessandro Borghesi, Christiane Dammann, Paul Hyslop, Heber Nielsen, and Robin Steinhorn*

[1]Department of Pediatrics and Women and Children Health Research Institute, [2]Cardiovascular Research Center, and [3]Department of Physiology, University of Alberta, Edmonton, Alberta, Canada.

I. Bronchopulmonary Dysplasia: Chronic Lung Disease for a Life Time?

Preterm delivery is a major health-care problem affecting more than 12% of all births and accounting for more than 85% of all perinatal complications and death (www.iom.edu/Reports/2006/Preterm-Birth-Causes-Consequences-and-Prevention.aspx). Improvements in perinatal care have allowed the survival of extremely premature infants born at less than 28 weeks of gestation. However, these infants are at a high risk of developing bronchopulmonary dysplasia (BPD), a chronic lung disease that follows ventilator and oxygen (O_2) therapy for acute respiratory failure after premature birth (129). Arrested alveolar growth and lung vascular growth are histological hallmarks of BPD (60). These anomalies may persist beyond childhood, compromising lung structure and function (25, 258). Emerging reports of arrested alveolar growth in older children (81), early onset emphysema, and pulmonary hypertension in young adults who had BPD and adverse neurodevelopment raise concerns about the long-term outcome of infants born extremely preterm. Therefore, understanding how alveoli and the underlying capillary network develop and how these mechanisms are disrupted in disease are critical for developing therapies for lung diseases characterized by impaired alveolarization.

Recent advances in stem cell biology offer the possibility for cell-based treatment strategies to repair damaged organs. This review (i) summarizes the pathophysiology of lung injury in preterm neonates that leads to the development of BPD and (ii) analyzes the role of stem cells in lung development, injury, and repair.

II. Pathophysiology of Lung Injury in Preterm Neonates

The development of the lung can arbitrarily be divided into two phases: (i) the initial creation of an air-conducting system

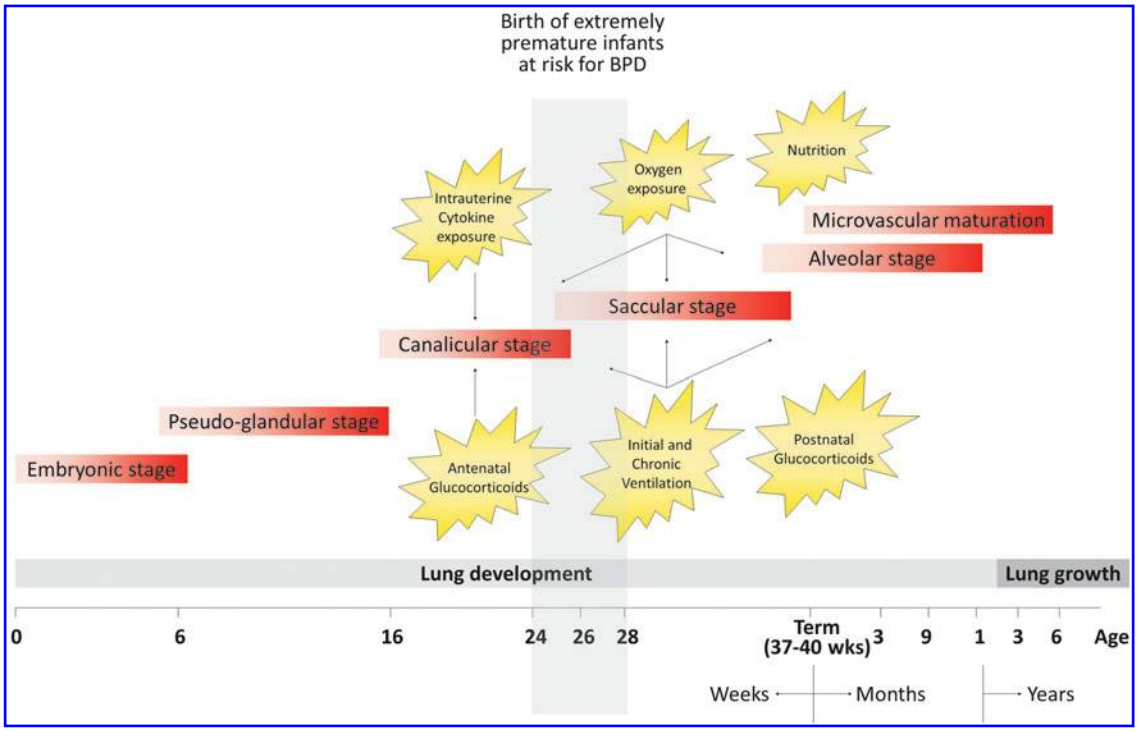

FIG. 1. Normal lung development and pathophysiological events contributing to BPD and decreased alveolarization in preterm infants. Schematic depicting the classical stages of histological lung development. It shows that preterm infants at risk of developing BPD are born at the late canalicular or early saccular phase of lung development. Pre-, peri-, and postnatal adverse events contribute to interrupt the normal sequence of lung development, leading to the arrest in lung growth observed in these infants. BPD, bronchopulmonary dysplasia. (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

followed by (ii) the formation of the gas-exchange surface area of the lung.

A. Stages of lung organogenesis

A generally accepted view of how the lung develops is summarized in Figure 1. In the embryonic stage, the lung appears as an outpouching of the primitive foregut on day 28. This bud bifurcates into the two main stem bronchi. During the pseudo-glandular stage, rudimentary bronchi divide by a structured branching program that was recently revisited by Metzger *et al.* These authors suggest that three geometrically distinct local modes of branching (domain branching, planar bifurcation, and orthogonal bifurcation), coupled in three different sequences (168), culminate in the first terminal sacs. These tubular structures are lined by columnar epithelium surrounded by mesenchymal tissue. The canalicular stage is characterized by the bifurcation of the last generations of distal bronchi. In this stage there is also capillary invasion and differentiation of the air space epithelium into alveolar type II (AT2) cells (responsible for surfactant production) and type I (AT1) cells (which primarily form the thin air–blood barriers). During the saccular stage the peripheral air spaces enlarge at the expense of the intervening mesenchyme, forming saccules. This is followed by the final alveolar stage when alveoli are generated *via* subdivision (septation) of the distal lung saccules (42). This process of alveologenesis begins at 36 weeks of gestation and extends into the first 2 years of postnatal life.

B. BPD: the impact of injury in an immature lung

BPD was described by Northway in 1967, shortly after mechanical ventilation for neonates became routine. At this time, BPD occurred in relatively larger preterm infants (around 34 weeks of gestation, 2200 g birth weight) (178) ventilated for neonatal respiratory distress. The pathology included mucosal metaplasia of airways, emphysema, and interstitial fibrosis (old BPD) (53, 178). Advances in neonatal intensive care have allowed the survival of ever more immature infants. Infants at greatest risk for BPD are now born <28 weeks of gestation (42) when their lungs are in the late canalicular or early saccular phase of development, when airspaces become juxtaposed to the capillaries and when gas-exchange becomes feasible. The hallmark of the new BPD is characterized by alveolar growth arrest and perturbed lung vascular growth (60, 205), consistent findings in experimental BPD and in humans (6, 15, 59, 61, 140) (Fig. 2) (15, 221). Alveologenesis involves budding of septal crests, which is followed by elongation of the septal walls to form individual alveoli. Septation increases the gas-exchange surface area, without a proportionate increase of lung volume (*i.e.*, alveoli have a larger surface/volume ratio than saccules). This process of septation is disrupted in BPD, resulting in alveolar simplification and enlargement (59). Alveolar angiogenesis is intricately interconnected with the process of alveologenesis (221, 232). Studies in human fetal lung suggest that airways act as a template for pulmonary artery development. Endothelial tubes line up around the terminal buds of the airways, suggesting an inductive influence on the part of the epithelium (95). The proposed link between alveolarization and angiogenesis is also reiterated by the secondary abnormalities that occur in one process when the other is primarily affected (221).

C. The multifactorial pathogenesis of BPD

BPD is a multifactorial disease with several pre- and postnatal factors contributing to its pathogenesis. Postnatal O_2

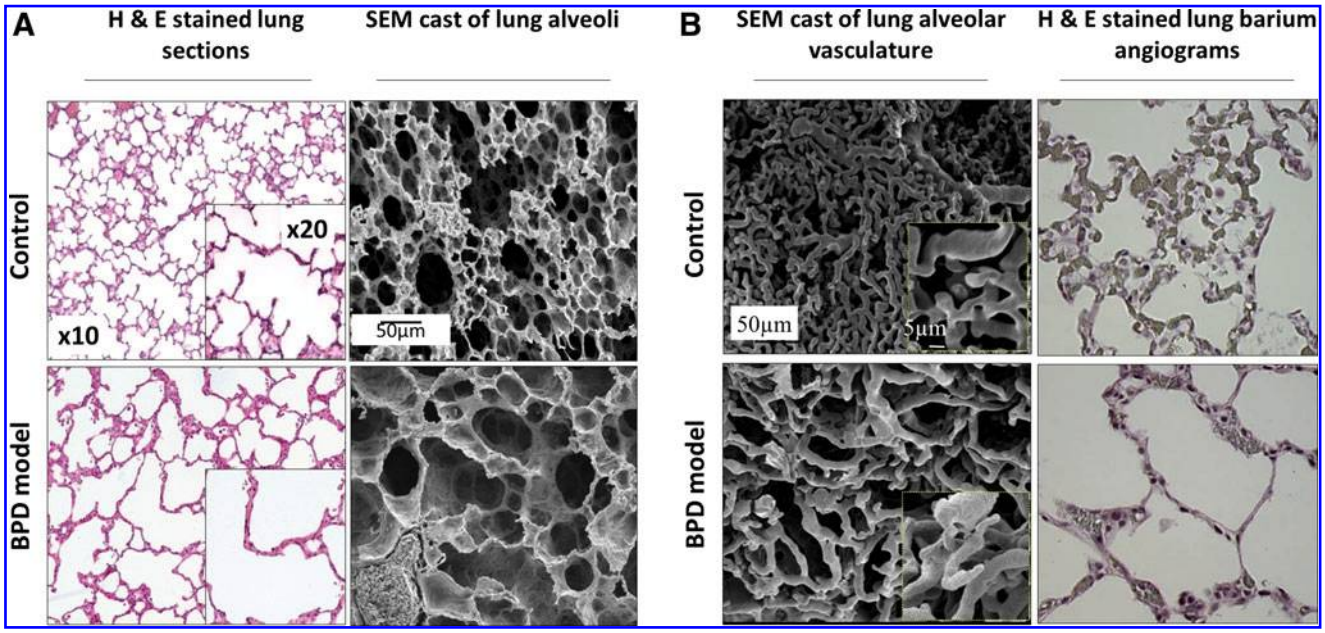

FIG. 2. Experimental O_2-induced BPD. Rat pups exposed to 95% O_2 from birth to P14 have decreased alveolar and vascular growth. In the O_2-injured lung, the alveolar spaces are enlarged with fewer secondary crests **(A)**, and there is a marked decrease in the density of lung capillaries **(B)**. These changes recapitulate the histological anomalies observed in human BPD. O_2, oxygen. (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

Table 1. A Representative (Not Exhaustive) List of Existing Experimental Models of Bronchopulmonary Dysplasia

	Model/mechanism	*Species*	*Reference*
1.	*In utero* ventilation	Sheep	Allison *et al.* (7)
2.	Prematurity, mechanical ventilation, O_2	Baboon	Altiok *et al.* (9)
			McCurnin *et al.* (162)
		Sheep	Pierce *et al.* (186a)
		Mouse	Hilgendorff *et al.* (105)
3.	LPS (intra-amniotic) administration and postnatal hyperoxia	Rat	Kim *et al.* (127)
			Lee *et al.* (142)
4.	Bleomycin (intraperitoneal)	Rat	Tourneux *et al.* (241)
5.	Hyperoxia exposure		
	80%–100% O_2	Mouse	Ohki *et al.* (179)
		Rat	ter Horst *et al.* (231)
	60% O_2	Rat	Masood *et al.* (159a)
6.	Transgenic BPD Models (Examples)		
	Perinatal expression of IL-1B	Mouse	Bry *et al.* (46)
	Triple transgenic construct overexpressing TGF-B1	Mouse	Vicencio *et al.* (249)
	Lung specific Fas-ligand overexpression	Mouse	De Paepe *et al.* (72)
	ErbB4 deletion	Mouse	Purevdorj *et al.* (192)
	ErbB4 deletion and antenatal intra-amniotic LPS administration	Mouse	Schmiedl *et al.* (211)

BPD-like changes were inflicted in the lungs of these animal models by a variety of methods representing the major injurious stimuli commonly associated with the development of human BPD.

BPD, bronchopulmonary dysplasia; O_2, oxygen; TGF-β, transforming growth factor-beta.

exposure in the setting of decreased host antioxidant defenses, barotrauma/volutrauma on a very immature lung, chorioamnionitis, and pre-eclampsia contribute to BPD and have been extensively reviewed elsewhere (42, 91, 97, 119). In addition, the susceptibility to BPD is further modulated by the existence of gene polymorphisms (91).

Most of our current understanding of the pathogenesis of BPD has come from research in small and large animal models of experimental BPD (Table 1), and a few human studies, mostly limited to exploring biomarkers in the tracheal aspirate of ventilated newborns. Lung alveolar development and maturation is a complex process involving well-coordinated interactions between mesenchymal, epithelial, and microvascular lung components. On this basis, the pathophysiological processes underlying the development and progression of BPD can be broadly compartmentalized into three interdependent mechanisms (Fig. 3).

1. Defective elastogenesis and extracellular matrix remodeling. Collagen, fibronectin, and elastin are the primary components of extracellular matrix (ECM), which constitutes the complex architectural framework of the lung and provides a medium for the intricate cell–cell and cell–matrix interactions crucial for lung development and maintenance (42, 50).

Elastin deposition in the saccular walls of the developing alveoli plays a spatially instructive role in the budding of secondary septa and formation of mature alveoli. Lungs of mice devoid of the elastin gene fail to progress beyond the saccular stage and usually die soon after birth from cardiorespiratory failure resulting from fewer, dilated distal air sacs with attenuated septa (257) and an overgrowth of smooth muscle cells in the pulmonary arteries (146). Exposure of newborn mice to mechanical ventilation in conjunction with hyperoxia, mimicking intensive care interventions in the NICU, increases lung elastase activity, causing elastin degradation and redistribution of elastic fibers from septal tips to alveolar walls (35). These BPD-like changes are significantly attenuated by blocking lung protease activity with intrapulmonary injections of recombinant human elafin (105).

Synthesis and deposition of septal elastin is controlled by peptide factors such as fibroblast growth factor (FGF)-2 and −18 (43, 54), platelet-derived growth factor-alpha (PDGFα) (41), and transforming growth factor-beta 1 (TGF-β1) (89, 131) *via* orchestration of fibroblast proliferation, migration, and differentiation. Overexpression of TGF-β1 in the lungs of newborn rodents results in histologic alterations consistent with the pathologic descriptions of BPD (89, 249). Earlier observations by Kotecha *et al.* report elevated concentrations of active and total TGF-β1 in the bronchoalveolar lavage fluid of infants who subsequently develop BPD (131). Conversely, TGF-β1 inhibition attenuates O_2-induced arrested alveolar growth in neonatal mice (31). Failure of alveolar septation is observed in postnatally surviving PDGF-A$^{-/-}$ mice (41), and this is associated with a lack of distal spreading of alveolar smooth muscle cell progenitors (150). In another study, elevated levels of basic FGF in tracheal aspirates of infants in their first day of life are shown to predict an outcome of BPD/death and correlate positively with elevated lung cell injury and apoptosis (10). Disrupted ECM remodeling attributable to dysregulation in the levels and activity of gelatinases such as matrix metalloproteinase (MMP)-2 and −9 is another suggested mechanism of impaired lung development in BPD (48, 67, 79).

2. Altered alveolar epithelial–mesenchymal interactions. The epithelial lining of the alveolar compartment is constituted by AT1 and AT2 pneumocytes. AT1 cells are large flat cells covering about 98% of the alveolar surface, although comprising only 10% or less of all cells in the peripheral lung. The relatively smaller and cuboidal AT2 cells contribute to only ~2% of the alveolar surface (224). Conventionally, AT1 cells were believed to serve as passive barriers lining the air–blood

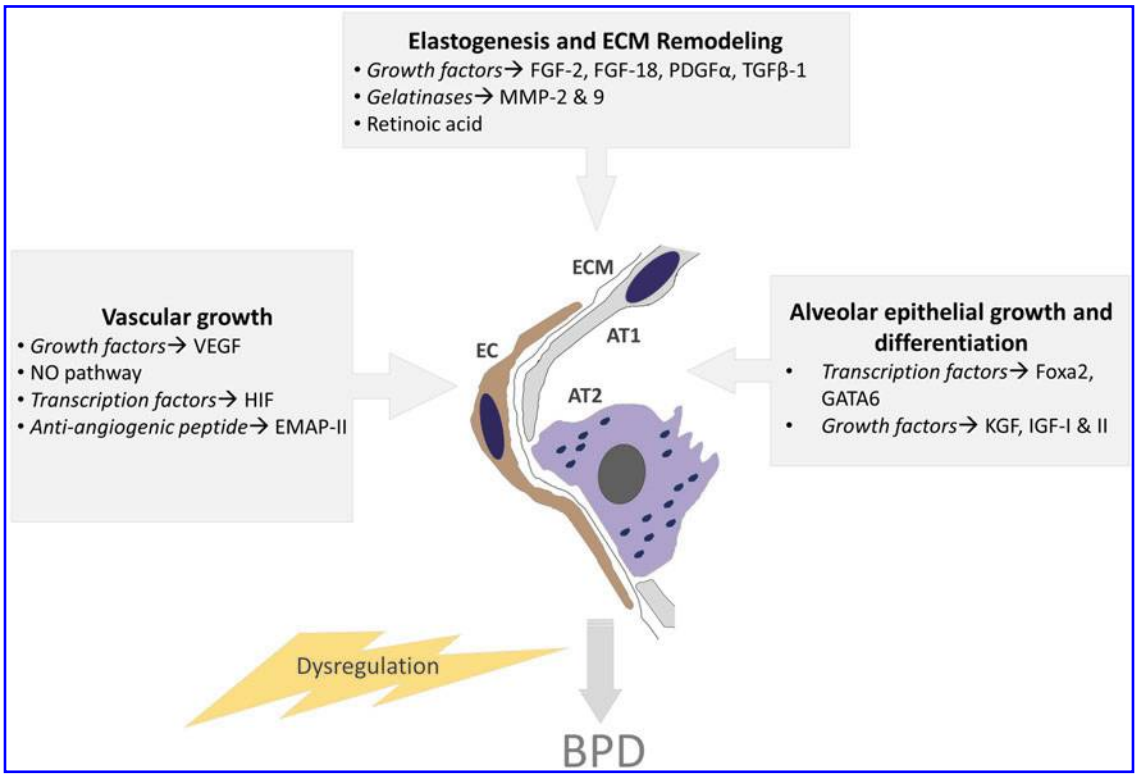

FIG. 3. Outline of the multifactorial pathogenesis of BPD. This simplified schematic outlines three major mechanistic compartments that underlie the pathogenesis of BPD. ECM, extracellular matrix; FGF, fibroblast growth factor; PDGFα, platelet-derived growth factor-α; TGF-β1, transforming growth factor-beta 1; MMP, matrix metalloproteinase; NO, nitric oxide; HIF-2α, hypoxia inducible factor 2α; EMAP-II, endothelial monocyte activating polypeptide-II; KGF, keratinocyte growth factor; VEGF, vascular endothelial growth factor; IGF, insulin-like growth factor. See text for description and references. (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

gas exchange interface. AT2 cells, on the other hand, are known to synthesize, secrete, and recycle surfactant components, transport ions, participate in lung immune responses, and function as progenitor cells in response to lung injury (82, 159, 198). However, the fairly recent demonstration of the ability of AT1 cells to proliferate and exhibit phenotypic plasticity (92) suggests that these cells play a role in lung development and lung repair after injury (75). The recent development of methods to isolate AT1 cells will allow further exploration of the contribution of these cells to lung homeostasis.

The importance of signals originating from differentiated epithelial cells in alveolar septation is evidenced by the need for optimal expression of transcription factors Foxa2 (254), GATA6 (152), and the AT1 cell differentiation marker, T1α (194). Keratinocyte growth factor (KGF), one of the key regulators of alveolar maturation, is released by alveolar fibroblasts (243). Paucity in KGF levels has been directly correlated with susceptibility for BPD (42). Another instance suggesting the importance of epithelial–interstitial cell interactions in alveologenesis is the strong correlation between the levels of insulin-like growth factor-I (IGF-I) and IGF-II and alveolar development (51, 153). However, expression of IGF-I and its receptor is increased in the lungs of patients with BPD (56), inviting more studies to understand the role of IGFs in lung development. Stretch-mediated surfactant synthesis in the developing lung is also regulated by a parathyroid hormone-related, peptide-dependent paracrine loop between AT2 cells and alveolar lipofibroblasts (213). Sustaining this paracrine pathway using peroxisome proliferator activated receptor-gamma agonists has been effective in preventing O_2, volutrauma, or infection-mediated injury in the developing lung (240). In addition, there is epithelial–mesenchymal crosstalk mediated by growth factors such as neuregulin (66) and epidermal growth factor (176), regulating surfactant synthesis and alveolar maturation. Dysregulated signaling by these growth factors during late gestational lung development is another likely mechanism contributing to BPD.

3. Impaired development of lung microvasculature. In addition to alveolar growth arrest, BPD is also characterized by a pronounced paucity of the distal lung microvasculature (31, 91). The notion that blood vessels passively follow the developing airways during lung organogenesis (95) has been challenged over the past 10 years testifying the interdependence between alveolarization and angiogenesis (1, 232). Secondary abnormalities almost invariably occur in one process when the other is primarily affected (113, 163). It appears plausible that a vascular component, at least in part, underlies the pathobiology of BPD (221).

When exposed to hyperoxia or prolonged mechanical ventilation, the developing lung exhibits varying degrees of microvascular disruption in conjunction with alveolar damage. It is very likely that alterations in angiogenic growth factors might underlie this process (64). Development of lung vasculature is controlled by a well-coordinated interaction of angiogenic growth factors and their receptors in concert with

the ECM. Expression of proangiogenic growth factors such as vascular endothelial growth factor (VEGF) and angiopoietin (Ang)-1 and −2 are observed very early during lung organogenesis (96, 99). VEGF signaling plays a crucial role in the development of lung microvasculature and in the postnatal growth and maintenance of alveolar structure (232). Genetic or pharmacologic VEGF inhibition during the critical period of lung development in newborn rodents results in fewer and larger alveoli and capillary rarefaction in distal lungs (88, 113, 163, 233). Conversely, strategies that enhance microvascular integrity *via* VEGF gene therapy or pharmacological treatment attenuate O_2-induced arrested alveolar growth (137, 233). Combined Ang-1 and VEGF gene therapy enhance the response by promoting vascular stabilization and maturation (234). It is believed that the regulation of Tie2 signaling through a balance in the expression of Ang-1 and Ang-2 is crucial for normal lung vascular development (99, 245). Studies also indicate that Ang-1 contributes to lung protection *via* attenuating inflammation (102, 161) and promoting pulmonary vascular homeostasis (135). In addition, transcription factors such as hypoxia inducible factor (HIF) 1/2, activation protein-1, and specificity protein-1 play a role in regulating angiogenic growth factor expression (120). Mice deficient in HIF-2α exhibit defective lung morphogenesis accompanied by neonatal respiratory distress due to a selective defect in pneumocyte maturation and surfactant production (62).

Nitric oxide (NO) is a downstream effector of VEGF. Consistent with the findings above, NO also plays a crucial role during normal and impaired lung vascular growth (96). Constitutive expression of endothelial nitric oxide synthase (eNOS) and neuronal NOS at strategic locations in the developing lung suggests a contributory role of these enzymes in airway branching morphogenesis (264). Mice deficient in eNOS demonstrate severe impaired compensatory lung growth postpneumonectomy, indicating that eNOS is probably critical for alveolar regeneration and maintenance (144). Even mild exposure to hypoxia during the early postnatal lung development impairs alveolarization and reduces vessel density in these mice (22). The primary function of NOS enzymes is the time-dependent synthesis and release of NO, a critical downstream mediator of several potent angiogenic growth factors (96, 215). It is also believed that NO might act upstream of angiogenic growth factors *via* transcriptional control of their expression (78). Preterm baboons with chronic ventilation-induced lung injury demonstrate marked decline in pulmonary NOS expression indicative of an attenuated capacity for endogenous NO production in this experimental BPD model (3). Accordingly, evidence exists that exogenous supplementation of NO preserves alveolar structure and lung vascular remodeling to varying extents in experimental models of hyperoxia (149), bleomycin (241) and mechanical ventilation-induced BPD (34). Given that VEGF-induced angiogenesis is in part mediated by NO (182), alveolar arrest and pulmonary hypertension induced in neonatal rats by VEGF receptor blockade are effectively reversed by inhaled NO treatment (230). Potentiating the effect of endogenous NO *via* prolonging the half-life of its downstream mediator, cyclic guanosine monophosphate, also promotes lung angiogenesis and attenuates O_2-induced lung injury (138). Collectively, these findings formed the rationale for testing the potential benefit of early, low-dose, inhaled NO in premature infants at risk for BPD. These randomized trials showed partial (24, 128, 212) or no benefit (167). Further studies are required to determine the best indication of inhaled NO in this patient population (12).

Consistent with the above findings, antiangiogenic factors are likely candidates contributing to the pathophysiology of BPD (193). Endothelial monocyte activating polypeptide-II (EMAP-II) is one such negative regulator of vessel formation (28). Its expression is negatively correlated with vascularization in the developing lung (214). EMAP-II expression is elevated in the lung tissue of infants with BPD as well as in the premature baboon model (193), suggesting that this protein may contribute to the disruption of vascular development in BPD. Endostatin is another endogenous antiangiogenic protein known to inhibit endothelial proliferation and migration in tumor tissue, in addition to promoting apoptosis and predisposing to G1 arrest of endothelial cells. Endostatin also antagonizes VEGR receptor 2 activation and interferes with cell–matrix interactions that are necessary during angiogenesis (202). Interestingly, there is a positive correlation between high concentrations of endostatin in cord plasma of preterm neonates weighing less than 1500 g and the development of BPD (116). More interestingly, ventilated preterm infants who developed BPD or died had a decreased Ang-1/endostatin ratio on the 1st, 3rd, and 15th days of life, suggesting an imbalance favoring the inhibition of lung angiogenesis (115, 235).

In summary, it appears that arrested and dysmorphic lung vascular growth in BPD results at least partly from altered signaling of angiogenic factors. Exogenous VEGF, NO, or activation of HIF preserve alveolar development, suggesting new therapeutic avenues for preserving or enhancing alveolar growth. Given the potential risk of angiomas, abnormal vascular proliferation, formation of immature and leaky vessels (69), and the associated propensity for pulmonary hemorrhage or edema (141, 236), it will be crucial to determine the appropriate mode of delivery, dosing, and timing of angiogenesis stimulation to avoid this potential undesirable effects. At this juncture, cell therapy using proangiogenic precursor cells such as endothelial progenitor cells (EPCs) may offer an appealing alternative. The existing evidence for the role and therapeutic potential of EPCs in lung development and BPD has been elaborated in later parts of the review.

D. Oxidative stress: a distinct contributor to BPD evolution

In normal development, the fetal antioxidant capacity undergoes a marked elevation during the final phase of gestation. This elevation is a result of (i) upregulation of endogenous antioxidant enzymes and (ii) materno-fetal placental transfer of nonenzymatic antioxidants (70). These changes permit the safe transition from the relative hypoxia of intrauterine development to the O_2 richer extrauterine environment. Preterm infants, however, are born before the onset of these adaptive changes and this leaves them extremely susceptible to oxidative injury. Besides BPD, oxidative stress contributes to other complications of prematurity, including retinopathy of prematurity, necrotizing enterocolitis, and periventricular leucomalacia in preterm newborns (16, 185).

The primary mediators of oxidative damage in the preterm newborn are reactive oxygen species (ROS) generated in large quantities after exposure to hyperoxia, postasphyxial

reperfusion, inflammation, and tissue release of nonprotein bound iron (11, 209). Excessive ROS production in the setting of decreased bioavailability of antioxidants elevates oxidative stress as a major causative mechanism in the multifactorial pathogenesis of BPD. Indicators of oxidative injury such as pulmonary epithelial DNA oxidation (17, 158), lipid peroxidation (155), and protein oxidation (252) have all been demonstrated in experimental models of O_2-induced BPD. Also in human BPD, several studies strongly support a role for ROS-mediated damage. Markers of protein oxidation such as protein carbonyls (23) and advanced oxidation protein products (185) are elevated in premature newborns at highest risk of developing BPD. Increased oxidation and decreased expression of clara cell secretory protein (a protective lung protein with antioxidant, immunomodulatory, and anticarcinogenic properties) has been reported in infants who subsequently developed BPD (195). Furthermore, oxidative stress impacts a complex orchestra of genes involved in inflammation, ECM turnover, and cell cycle regulation that contribute to alveolar enlargement, vascular paucity, and other pathological changes observed in the immature lungs developing BPD (253). Given that O_2 is the most common therapy used in the care of very preterm infants, clinicians target lower O_2 saturations in this patient population and cohort studies suggested that this practice reduces the incidence of BPD (208, 238). Currently, however, the appropriate level of oxygenation for extremely preterm neonates to maximize the greatest chance of survival, without incurring significant morbidity, remains unknown despite recent randomized controlled trials (14, 100). Indeed, the most recent randomized controlled trials directly addressing this question show that targeting O_2 saturations of $<90\%$ in the first weeks of life increased risk of death, cerebral palsy, patent ductus arteriosus, pulmonary vascular resistance, and apnoea, while targeting O_2 saturations of $>90\%$ induced greater rates of morbidity including retinopathy of prematurity and BPD (84, 222). The first prospective meta-analysis from the Neonatal Oxygenation Prospective Meta-analysis Collaboration may provide more answers to the O_2 target dilemma (13).

Essential nutrients such as vitamins A, C, and E are known to inhibit ROS-induced lipid peroxidation and scavenge ROS (74, 220). Preterm infants have lower antioxidant vitamins in their serum compared with term controls (26). Vitamin A contributes to antioxidant defense *via* its action on retinol-binding protein and the retinoic acid receptor (74). In addition to its aforementioned role in promoting alveolar elastogenesis, antioxidant activity could be another major mechanism underlying the consistently observed protective effect of vitamin A supplementation in experimental O_2-induced (63, 114, 248) and human BPD (242). Vitamin E inhibits lipid peroxidation; hence, it prevents membrane damage and modification of low-density lipoproteins. It is regenerated by the water-soluble vitamin C (220). However, studies investigating supplementation of antioxidant vitamins have demonstrated that although vitamin concentrations can be increased in the serum of preterm animal models or infants, this has not resulted in a significant reduction in ROS-induced injury (29, 44). Conversely, caution should be exercised in maintaining serum vitamin A levels within normal physiological range, since supplementation at elevated doses adversely impacts redox balance and free radical status in the lungs that are frequently associated to severe lung dysfunction (184). Likewise, high levels of water-soluble antioxidant vitamin C in babies, particularly in premature babies, may exacerbate oxidant damage by inhibiting ferroxidase activity of ceruloplasmin (190). Vitamin C can also act as a pro-oxidant and may have adverse effects on the lungs, especially in combination with excess free iron, promoting oxidative stress (209).

Trace elements such as copper, zinc, iron, selenium, and manganese play a major role in the synthesis and normal functioning of antioxidant enzymes. Therapeutic supplementation of these micronutrients can potentially optimize total antioxidant capacity (32). Copper, zinc, and manganese participate in the synthesis of major antioxidant enzymes copper–zinc superoxide dismutase and manganese superoxide dismutase. Selenium, on the other hand, has been shown to be an integral part of glutathione peroxidase and acts synergistically with vitamin E in the prevention of peroxide formation. Selenium deficiency causes rapid metabolism of vitamin E. Experimental animals were more prone to have oxidative lung injury with low selenium levels (207). However, a meta-analysis of oral supplementation of selenium in preterm infants failed to reduce O_2 dependency at 28 days and total days of O_2 dependency (68).

Enzymatic antioxidants are critical in protecting against ROS-induced injury. It is noticeable that preterm neonates have decreased expression of these antioxidant enzymes relative to term newborns. Supplementation with enzymatic antioxidants or with agents promoting their expression and/or activity might confer protection against oxidative damage in preterm infants *via* scavenging excess ROS. Increased expression of antioxidant enzymes superoxide dismutase and glutathione peroxidase reversed pro-apoptotic and growth inhibitory effects of hyperoxia on lung epithelial cells (130). However, a clinical trial using intratracheal doses of recombinant human superoxide dismutase in premature neonates showed no difference in short-term endpoints, including incidence of BPD or mortality (71). Nonetheless, significant clinical improvement and decreased episodes of rehospitalization were registered at 1 year corrected age compared to controls. Melatonin, a pineal hormone, demonstrates potent antioxidant properties *via* promoting superoxide dismutase and glutathione peroxidase activity in addition to direct ROS scavenging (38). Daily intraperitoneal injections of melatonin reversed oxidant/antioxidant imbalance and improved hyperoxia-induced lung damage in neonatal rats (181). Another recent investigation describes the existence of a putative circulating antioxidant, paroxonase 3, that is systemically upregulated in late-gestation rat, sheep, and human fetuses (27). With more detailed research, these agents are likely to emerge as candidate antioxidants to protect preterm human infants against oxidative damage.

ROS are critical secondary messengers in various cell signaling pathways that control normal cellular function. In addition, even though antioxidant supplementation appears logical in bolstering defense mechanisms against oxidative damage in preterm neonates, several animal studies and clinical trials in neonates have yielded varied results. Therefore, caution needs to be exercised in therapeutic approaches involving agents promoting ROS depletion (117).

In summary, our knowledge about the mechanisms regulating normal lung growth has increased over recent years and with it, the realization of its complexity. The outgrowth of

secondary crests, the anatomical substratum of alveolar development, in particular remains a mystery. In contrast to the large amount of information on early lung branching morphogenesis, much less is known about the mechanisms that regulate septation. Much of our understanding about the genetic control of the dichotomous division of the conducting airways in mammals derives from studies of the respiratory system in *Drosophila* (169). Conversely, the mechanisms that regulate *alveolar* development remain poorly understood, mainly because of the lack of alveoli in *Drosophila*. Furthermore, the conducting airways grow into the surrounding mesenchyme, whereas alveolar septa evaginate inward into the air space (206). What determines septal outgrowth and what is the process' driving force? How do distal lung cells (*i.e.,* alveolar epithelial cells, endothelial cells, and myofibroblasts) interact during septal outgrowth? Much less is known about how these mechanisms are perturbed during BPD and how to use this knowledge to develop new therapeutic strategies to prevent lung injury, not only in the newborn.

Recent insight into stem cell biology has offered new hope to repair damaged organs. Over the past decade, lung biologists have capitalized on these new insights to better characterize resident lung stem/progenitor cells, understand their role during lung development and disease, and test the lung repair capabilities of a variety of exogenously administered stem cells in experimental lung disease (4, 226). More recently, the therapeutic potential for stem/progenitor cells for BPD (19, 20, 39) has been demonstrated in hyperoxia-induced lung injury in neonatal rodents. The following sections introduce basic concepts of stem cell biology and discuss the recent advances and challenges of stem cell-based therapies for lung diseases, with a particular emphasis on BPD management.

III. Stem Cells: Concepts and Applications

Stem cells are primitive cells capable of extensive self-renewal and have the potential to give rise to multiple differentiated cellular phenotypes (36). They are not only critical for organogenesis and growth during early stages of development, but also contribute to organ repair and regeneration throughout life.

A. Developmental potency of stem cells

Fundamental to understanding the function and therapeutic potential of different types of stem cells is the concept of developmental potency, which refers to the range of possible fates open to cells during differentiation. Stem cells exhibit varying differentiation potencies and are typically categorized into embryonic and adult (or somatic) stem cells (ASC). Even though fertilized eggs are *toti*potent (differentiate into all cell types that constitute the entire organism), they do not self-renew by simple cell division. This makes embryonic stem cells (ESC), derived from the early blastocyst, the most potent of stem cells. These cells are *pluri*potent (differentiate into cell types derived from all three germinal lineages of the developing embryo—endoderm, mesoderm, and ectoderm) and capable of indefinite self-renewal. In contrast, ASC are cells that have assumed increasing degrees of fate restriction as the embryo develops and are either *multi*potent (differentiate into a limited range of cell types) or *uni*potent (generate only one cell type) (223). Residual pools of such multi- or unipotent stem cells are hypothesized to reside in almost all adult organs, contributing to their ability to repair and regenerate after injury.

B. *Classical* versus *nonclassical stem cell hierarchies*

While stem cells are held critical for growth and development throughout childhood, residual pools of ASC 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 ASC that are organized in a classical hierarchy, to maintain homeostasis (225) (Fig. 4). In marked contrast, anatomically complex tissues that turn over more slowly (brain, heart, lung, and kidney) do not appear to support a classical stem cell hierarchy. Such tissues are believed to be maintained by stem/progenitor cell populations that are organized in a nonclassical hierarchy and recruited facultatively for regeneration after injury. For example, in the liver, mature hepatocytes are responsible for postresection tissue reconstitution. However, under certain conditions, cells from a facultative pool of ASC (termed "oval" cells), are also called into action (265). Very similarly, in the lung, several local epithelial cell types can function both as differentiated functional cells and as transit-amplifying progenitors that proliferate in response to airway or alveolar injuries (198). Nevertheless, recent research suggests that the adult lung also 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 (90, 121, 126, 165, 199).

C. Regulation of stem cell function

There are a lot of unanswered questions about the role and regulation of stem cells in tissue formation, organogenesis, and tissue repair after injury. However, it is becoming more and more evident that the capacity to behave as a stem cell (stemness) is, to a significant extent, a functional state rather than an intrinsic cellular characteristic. The decisions about cell fate and maintenance of stem cell populations are controlled by a multitude of variables that include genetic, epigenetic, and cellular microenvironmental factors (36, 256). One classical example highlighting the importance of these factors in stem cell fate determination is the ability of ASC residing in the bone marrow to cross lineage barriers and adopt the phenotype of target organs such as liver, lung, gastrointestinal tract, and skin epithelia (134). Another example is the engineering of pluripotency into somatic cells by ectopic expression of transcription factors linked to pluripotency (228). The resulting induced pluripotent stem cells (iPSC) are functional equivalents of ESC and represent a transformative milestone in our understanding of stem cell behavior and function.

D. Concept of stem cell replacement

One reason for studying stem cells is their usefulness in cell-replacement therapy. The self-renewal and regenerative capabilities of tissue-resident stem cell populations are naturally limited due to damage that accumulates with advancing age or disease. This may occur *via* simple exhaustion of the residual stem cell pools or as a consequence of genetic or microenvironmental changes that impede proper stem cell

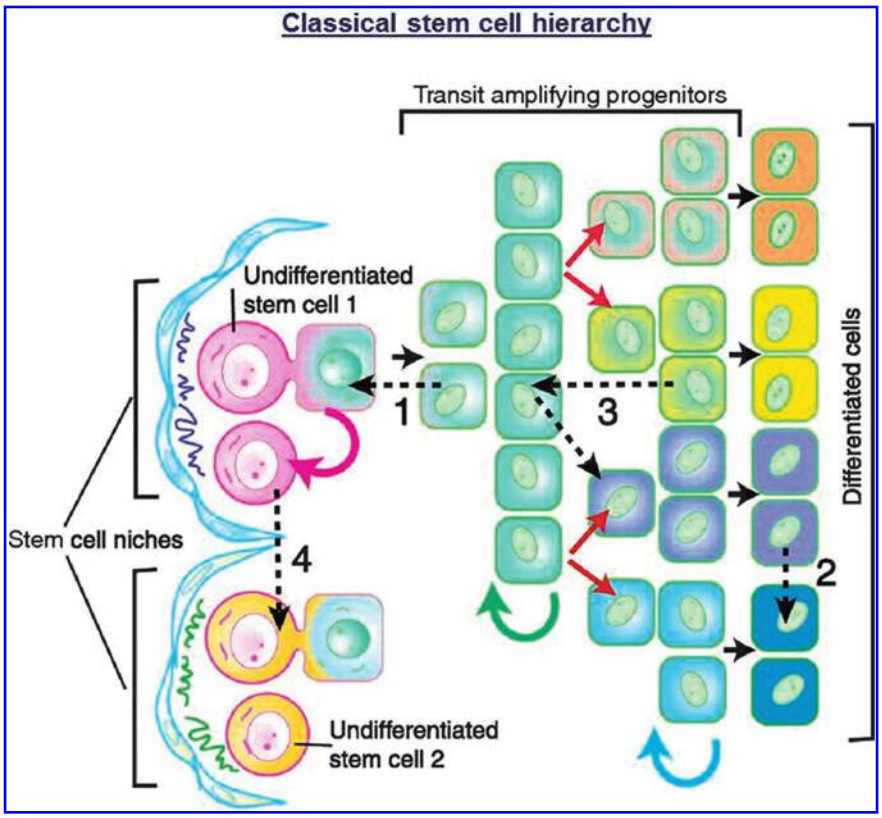

FIG. 4. Classical stem cell hierarchy. Model of the classical hierarchy of undifferentiated epithelial stem cells, TA progenitor cells, and mature postmitotic differentiated cells. Cell fate choices are indicated by *red arrows*. In this model, the stem cell in its niche and different TA cell subclasses can self-renew (*curved arrows*). Stem cells self-renew infrequently and TA cells more rapidly. Early TA cells may be able to replace stem cells if the niche is depleted (*dashed arrow 1*). The niche probably consists of several cell types and associated molecules, including blood vessels and nerves. Transdifferentiation of one well-defined differentiated cell type into another could occur directly, without cell division (*dashed arrow 2*) or might also involve reversion or de-differentiation between distinct TA progenitor populations (*dashed arrows 3*). Rarely, stem cells switch from one tissue-specific lineage to another (*dashed arrow 4*) in a process called metaplasia or transdetermination. Adapted with permission from Rawlins and Hogan (198). TA, transit amplifying. (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

function. These changes can potentially be reversed *via* stimulation of the endogenous stem cell pools or their therapeutic replacement with stem cells derived from exogenous sources. Such cell-replacement therapies exist already for hematological disorders using bone marrow-derived stem cells (87) and similar approaches have started showing promise in other debilitating childhood and adult disorders (103, 108, 247).

Another potential approach in cell therapy is to administer functionally mature cells of required tissue specificity, generated by targeted differentiation of ESC. However, isolation of human ESC for research or therapeutic applications is entangled in biological and ethical constraints. iPSC offer an ethically sound alternative to model specific diseases *in vitro* as well as for autologous cell therapy.

In addition to their use in tissue replacement, certain stem cell types may have utility as adjuvant therapies by virtue of their increasingly recognized trophic and immunomodulatory effects (83, 139, 239). Evidence shows that stem cells could also find application as delivery vehicles for targeted gene therapy (172).

Along these lines, the quest for a deeper understanding of lung stem/progenitor cell biology and its therapeutic translation has encountered several advances and challenges in the recent years. These will be discussed below with emphasis on the role of stem cells in lung alveolar growth and maintenance.

IV. Lung Stem Cells

Lungs are complex organs constituted by 40 or more cell types derived from all three germ layers (125). 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 after injury (216).

A. Lung epithelial stem/progenitor cells

The epithelial lining of the lung, generated almost exclusively from the foregut endodermal cells, is crucial in maintaining organ function and homeostasis (93). This cellular compartment is subdivided into at least four distinct anatomical zones along the proximodistal axis (Table 2) with each characterized by unique cellular organization and repair mechanisms (33). Organizational complexity coupled with prolonged turnover times of the adult lung epithelial cells has hindered the identification of true lung epithelial stem cells. However, it has been observed that relatively differentiated airway and alveolar epithelial cell types are capable of proliferating in response to epithelial injury (198). This observation drew the focus of lung stem cell research into identifying and defining those epithelial cell subpopulations that appear to contribute to postinjury regeneration. Basal cells (106, 203), clara cells (225), bronchoalveolar stem cells (126), and AT2 cells (45) have all been shown to exhibit stem cell properties. A representative list of lung cell populations with putative progenitor characteristics is summarized in Table 2. However, not all meet the criteria of clonogenicity, multipotentiality, and self-renewal or are not truly undifferentiated to qualify as stem cells.

1. Progenitor cells of the alveolar epithelium. Cuboidal AT2 pneumocytes have long been considered as the progenitors of the alveolar epithelium based on their capacity to

Table 2. A Representative (Not Exhaustive) List of Candidate Endogenous Lung Progenitor Cells Described in Human and Rodent Lungs

Anatomic location	*Species*	*Candidate progenitor cell*	*Attributed phenotype*	*Niche*	*Defining characteristics*	*Ref.*
Proximal trachea	Mouse	Unknown cell type	Tracheal epithelial cells	Submucosal glands	BrdU labeling-retained cells after i.t. detergent or SO_2-mediated epithelial injury	(40)
Distal trachea and bronchi	Mouse	Basal cells	Tracheo bronchial epithelial cells	Intercartilaginous zones	Cytokeratin 14-expressing multipotent progenitor cells capable of restoring differentiated tracheal epithelium after naphthalene injury; associated with innervated NEBs	(107)
Bronchioles	Mouse	Variant Clara cells	Distal airway epithelium	NEBs	Express CCSP; survive and repopulate distal airway epithelium after naphthalene injury; divide infrequently during steady state	(106)
Bronchioles and alveoli	Mouse	Bronchioalveolar stem cells	Bronchioalveolar epithelial cells	Bronchioalveolar duct junction	Resistant to naphthalene injury and proliferate in response; coexpress CCSP and SP-C	(126)
	Mouse	Pulmonary Oct-4$^+$ stem/progenitor cells	Alveolar type-I and -II pneumocytes	Bronchioalveolar duct junction	Oct-4$^+$, SSEA-1$^+$, Sca-1$^+$, cytokeratin-7+ cells; serially passaged, differentiate terminally into type-II and -I pneumocytes; susceptible to SARS-CoV infection	(151)
	Mouse	Multipotent lung epithelial progenitors	Airway and alveolar epithelium	Intrapulmonary airways and alveoli (not localized)	EpCAMhi, CD49f$^+$, CD104$^+$, CD24lo, Sca-1$^-$, CD45$^-$, CD31$^-$ lung epithelial CFUs, form colonies in Matrigel, serially passaged and retain multipotent potential	(165)
	Human	Self-renewing, multipotent, clonogenic cells	Airway and alveolar epithelium and vascular endothelium	Distal airways	C-kit+population	(121)
Alveoli	Mouse	Alveolar type-II pneumocytes	Alveolar type-I pneumocytes	Alveolar surface	All alveolar type-II pneumocytes	(2)
	Rat	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 O_2-induced injury	(200)

BrdU, 5-bromo-2-deoxyuridine; CCSP, Clara cell secretory protein; CFU, colony-forming unit; NEB, neuroendocrine bodies; SARS-Co V, severe acute respiratory syndrome coronavirus; SP-C, surfactant protein C; SSEA-1: stage specific embryonic antigen-1.

replenish themselves and to generate terminally differentiated AT1 cells pneumocytes (2, 45). Since then, AT2 cells are speculated to contain a subpopulation of progenitors or cells that can undergo reactivation into a progenitor-like state in response to injury. Using an acute model of O_2-induced injury, Driscoll *et al.* demonstrate the existence of a telomerase-positive subpopulation within the general AT2 cell population, during the recovery phase (77). These cells can be further characterized based on E-cadherin-positive and -negative fractions with heightened telomerase activity and injury resistance in the latter subset (200).

More recent research suggests the existence of multipotent stem cells in the distal lung that are capable of differentiating into epithelial cells specific to the airway and the alveoli. Kim *et al.* demonstrate the existence of dual-lineage bronchioalveolar stem cells at the airway–alveolar junction that express both airway (CC10) and alveolar (surfactant protein C [SP-C]) markers (126) and proliferate in response to airway and alveolar injury (126, 177). However, based on the employed techniques, there has been some ambiguity about the lineage potential (165, 219) and contribution of these cells to alveolar repair (197). Another population of Oct-4^+ stem/progenitor cells has been described by Ling *et al*. to specifically exist at the bronchoalveolar junction. These cells coexpress other stem cell markers such as SSEA-1, Sca-1, and cytokeratin 7 and do not express c-kit, CD34 or p63. They require mesenchymal stromal support for growth and maintenance and have the potential to differentiate into AT1 and AT2 cells. Interestingly, this Oct-4^+ cell subpopulation is particularly susceptible to SARS-coronavirus infection resulting in the loss of lung repair capability (151).

McQualter *et al.* applied techniques used for many years in the hematopoietic stem cell field to isolate a population of self-renewing $EpCAM^{hi}CD49f^{pos}CD104^{pos}CD24^{low}$ lung epithelial colony-forming units (CFUs). These epithelial CFUs form colonies when cocultured with $EpCAM^{neg}$ $Sca\text{-}1^{pos}$ lung mesenchymal stem cells (MSC), showing that the epithelial–mesenchymal interaction is essential for the formation of colonies by the epithelial colony forming units. In addition, epithelial growth factors, HGF and FGF-10, support the generation of epithelial colonies and could replace the mesenchymal support (165). More recently, a novel population of c-kit-positive, self-renewing, multipotent, clonogenic cells in the distal airways of the human lung has been identified (121). These undifferentiated human lung cells form bronchioles, alveoli, and pulmonary vessels in the cryoinjured lung. This study, however, does not demonstrate the tissue regenerative ability of these c-kit-positive stem cells in a lung disease model.

B. Lung mesenchymal progenitors

Additional lung cell types, including airway smooth muscle, fibroblasts, and the vasculature, are derived from the mesoderm. Interaction between the epithelial cells, mesenchymal microenvironment (including ECM proteins and growth factors), and the adjacent pulmonary vasculature regulates the structural and functional maturation of the developing lung (216). Our knowledge on lung mesenchymal precursors is very limited. Evidence, however, indicates the existence of small populations of resident lung cells expressing certain phenotypic characteristics of mesenchymal cells with progenitor capacity. For example, resident lung side population (SP) cells, which appear to have both mesenchymal and epithelial potential, have been isolated based on their capacity to efflux the Hoescht dye (156, 201, 227). Another population of Sca-1^+, $CD31^+$ endogenous clonogenic progenitors has been described in the adult murine lung (164). These cells do not express CD31 and CD45 and are suggested to be fibroblast precursors harbored in the lung mesenchyme. The precise role of these endogenous mesenchymal progenitors in lung repair, however, is not fully understood.

After the discovery of the plasticity characteristics of ASC that allow them to cross lineage barriers and adopt functional phenotypes of other tissues, a lot of interest has been diverted to understanding their role in repair and maintenance of the lungs (104). Experimental evidence indicates that the injured lung stimulates the release and preferential homing of MSC, a population of ASC derived from the bone marrow (147, 204). The mechanism by which exogenous progenitors such as bone marrow MSC assume lung phenotype and its clinical significance, however, remains unclear (175). In addition, by virtue of their ubiquitous presence and importance in organogenesis and tissue repair, EPCs, a population of circulating and resident vascular precursor cells, have also recently received widespread attention in the context of lung development and regeneration (132). Interesting findings indicating the therapeutic potential of these mesenchymal progenitor populations, and the need for further investigations in elucidating more precisely their role in lung regeneration, are highlighted in the next sections.

The recent surge in our knowledge of stem cell biology and the availability of advanced research tools in this field has motivated researchers into exploring the role of lung stem cells in the pathogenesis of chronic lung diseases that lack effective therapies. Indeed, several major lung diseases likely involve dysregulation in the numbers and/or the function of resident lung stem/progenitor cells (175). For instance, depletion or functional impairment of alveolar epithelial and/or endothelial progenitor cells 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. However, the precise identity and role of the various lung stem/progenitor cell populations should be established before realizing this therapeutic objective.

V. Therapeutic Potential of Stem Cells to Prevent/Repair Lung Damage

Numerous preclinical studies have provided compelling evidence for the beneficial effects of cell therapy using exogenous stem cells for a large variety of lung diseases (Table 3). Animal models mimicking major lung diseases such as acute lung injury (ALI)/acute respiratory distress syndrome, idiopathic pulmonary fibrosis, chronic obstructive pulmonary disease (COPD), cystic fibrosis, asthma, pulmonary hypertension, and BPD have been employed in these studies (4, 33, 226).

A. Mesenchymal stem cells

MSC were originally described by Friedenstein *et al.* as fibroblastoid cells that could be flushed out from the adult bone

Table 3. Studies Testing the Therapeutic Effect of Stem Cells in Various Experimental Lung Disease Models, Classified by the Stem/Progenitor Cell Type and Disease Model

Disease or experimental model	*Therapeutic cell or product*	*Therapeutic outcomes*	*Suggested mechanisms*	*Ref.*
Embryonic stem cells				
Bleomycin (i.t.)-induced ALI	Human ESC-derived cells with AT2 epithelial phenotype (i.t.)	Improved body weight and survival Improved arterial O_2 saturation Decreased collagen deposition	Structural engraftment & AT1 differentiation Paracrine-mediated mechanisms	(255)
Adult stem cells				
Exogenous stem or progenitor cells				
Bleomycin (i.t.)-induced LI/fibrosis	Bone marrow-derived MSC	Reduced fibrosis Reduced inflammation	IL-1 receptor antagonism Decrease of NO metabolites, proinflammatory & angiogenic cytokines	(136, 180, 204)
	Wharton's jelly-derived MSC	Reduced fibrosis	Decreased TGF-β and TIMP activity Increased MMP-2 activity	(170)
	Bone marrow-derived HSC with or without KGF overexpression (i.v.)	Reduced fibrosis	KGF-induced endogenous AT2 cell proliferation	(5)
Asbestos-induced LI/fibrosis	Allogenic whole bone marrow (i.v.)	Abrogation of inflammation and fibrosis		(145)
Escherichia coli endotoxin (i.p.)-induced systemic inflammation and LI	Bone marrow-derived MSC (i.v. or i.t.)	Decreased systemic and local inflammation Improved survival	Cell–cell interactions Paracrine mechanisms Decreased proinflammatory and increased antiinflammatory cytokines Antioxidant mechanisms	(260) (94) (112)
E. coli pneumonia	Bone marrow-derived MSC (i.t.) and MSC CdM	Reduced bacterial in the lung homogenates and in the bronchoalveolar lavage fluid	Secretion of human cathelicidin hCAP-18/LL-37	(133)
E. coli endotoxin (i.t.)-induced LI	Bone marrow-derived MSC overexpressing Ang-1 (i.v. or i.t.)	Decreased inflammation Decreased alveolar permeability	Decreasing inflammatory cytokines Ang-1-mediated effects	(166) (259)
Burn injury-mediated ALI	Bone marrow-derived MSC (i.m.)	Decreased inflammation and apoptosis	Decreased activation of epithelial NF-kB	(261, 262)
CLP-induced sepsis and ALI	Bone marrow-derived MSC (i.v.)	Control of sepsis Improved survival	Prostaglandin E2-mediated macrophage IL-10 secretion	(174)
Hyperoxia-induced lung injury	Bone marrow-derived MSC (i.t.)	Prevention of alveolar and vascular growth arrest	Paracrine mechanisms Immunomodulatory factors	(246) (15)
	hUCB-derived MSC (i.t.)	Decreased inflammation Improved alveolarization	Epithelial differentiation Decreased pro-inflammatory and fibrotic cytokines	(55)
Lung-specific Fas ligand overexpression	hUCB-derived $CD34^+$ hematopoietic progenitor cells (i.n.)	Pulmonary epithelial reconstitution	Long-term engraftment, functional differentiation, replication, and clonal expansion; no cell fusion observed	(72)
Papain-mediated emphysema	Bone marrow-derived MSC (i.v.)	Emphysema attenuation	Engraftment and AT2 differentiation of MSC Decreased alveolar epithelial apoptosis	(268)

(*continued*)

Table 3. (Continued)

Disease or experimental model	*Therapeutic cell or product*	*Therapeutic outcomes*	*Suggested mechanisms*	*Ref.*
Elastase-induced emphysema	Bone marrow-derived MSC (i.t.)	Reduced alveolar destruction	Paracrine effects: HGF, EGF, and secretory leukocyte protease inhibitor secretion	(123)
PPE-induced emphysema	Adipose tissue-derived MSC (i.v. or cultured on PGA scaffolds and transplanted after LVRS)	Gas exchange and exercise tolerance restored	Growth factor release (HGF, VEGF)	(217, 218)
Ragweed-mediated asthma	Bone marrow-derived MSC (i.v.)	Decreased asthma-specific allergic response	TGF-β production Regulatory T-cell recruitment	(173)
CFTR-KO mice with airway injury	Bone marrow-derived MSC (i.v.)	Detection of lung CFTR expression and activity	Cell engraftment and induction of CFTR expression	(154)
Monocrotaline-mediated PHT	Bone marrow-derived MSC with or without eNOS overexpression (i.v. or i.t.)	Improved RV pressure overload and function Improved lung structure Improved survival	ENOS-mediated vasodilatation VEGF-mediated enhanced microvasculature Paracrine effects	(18, 122, 196, 244)
	Bone marrow-derived EPC (i.v.)	Restored pulmonary hemodynamics Increased microvascular perfusion	eNOS-controlled vascular growth	(267)
	Peripheral blood-derived EPC (i.t.)	Improved cardiac function Improvement in small vessel medial thickness and lung neovascularization		(229)
Ovalbumin-mediated asthma/ allergic airway inflammation	Adipose tissue-derived MSC (i.v.)	Decreased local and systemic allergic response	Decreased Th2 activity	(57) (183)
	Bone marrow-derived MSC CdM	Reduced airway hyper-responsiveness and remodeling	Paracrine mechanisms	(109)
Endogenous stem or progenitor cells				
Elastase-induced emphysema	Lung-resident multilineage progenitors $Sca1^+CD45^-CD31^-$ (i.t.)	Attenuated elastase-induced alveolar damage Longer survival	Immunomodulation *via* paracrine mechanisms	(101)

Interestingly, most of the beneficial effects of stem cell therapy are, at least in part, accounted for by stem cell-derived paracrine mediators.

Ang-1, angiopoietin-1; ALI, acute lung injury; AT1, alveolar type 1; AT2, alveolar type 2; CdM, conditioned media; CFTR-KO, cystic fibrosis transmembrane regulator-knock out; CLP, cecal ligation and puncture; EGF, epidermal growth factor; eNOS, endothelial nitric oxide synthase; ESC, embryonic stem cell; EPC, endothelial progenitor cell; HGF, hepatocyte growth factor; HSC, hematopoietic stem cell; hUCB, human umbilical cord blood; i.t., intratracheal; i.p., intraperitoneal; i.v., intravenous; i.n., intranasal; IL, interleukin; KGF, keratinocyte growth factor; LI, lung injury; LVRS, lung volume reduction surgery; MMP, matrix metalloproteinases; MSC, mesenchymal stem cell; PHT, pulmonary hypertension; PPE, porcine pancreatic elastase; RV, right ventricle; Th2, helper T-cell type 2; TIMP, tissue inhibitor of metalloproteinases; VEGF, vascular endothelial growth factor.

marrow, form colonies on plastic and, when transplanted subcutaneously with appropriate carriers, could make bone and reconstitute a hematopoietic microenvironment (85). Ever since their discovery in 1970, they have by far been the most extensively studied adult stem cell type. Their remarkable differentiation plasticity, extraordinary immunomodulatory properties, as well as their ability to be recruited to sites of injury (210) have made them the most widely investigated stem cells in preclinical therapeutic studies.

In rodent models of ALI induced by *Escherichia coli* endotoxin administration, bleomycin, O_2 exposure, burn injury, or *via* cecal ligation and puncture-induced sepsis, MSC administration improved survival and lung inflammation (15, 55, 94, 112, 166, 174, 180, 204, 246, 259–262). In other models of alveolar damage such as papain or elastase-induced emphysema, MSC therapy attenuated alveolar destruction, improved gas exchange, and restored exercise capacity (123, 268).

In ovalbumin (57, 183) or ragweed-mediated murine asthma models (173), MSC therapy alleviates allergic reactions and airway remodeling *via* mechanisms involving decreased helper T-cell recruitment and activity. MSC restore arrested lung vascular growth and improve pulmonary hemodynamics in experimental models of lung vascular injury and pulmonary hypertension (18, 122, 196, 244). MSC have also been found to improve bleomycin (5, 136, 180, 204) and asbestos-induced fibrosis in mice (145). On the contrary, circulating fibrocytes, also originating from bone marrow stem cells, traffic into the lungs aggravating fibrosis (171, 186).

B. Endothelial progenitor cells

Clinical evidence indicates a reduction of circulating EPC in patients with chronic lung disease subject to prolonged hypoxia (80). This, in addition to observations correlating increased circulating EPC with improved outcomes in lung injury (49, 263), suggests EPC supplementation as a potential strategy to treat diseases involving lung damage. Several studies in newborn and adult experimental lung injury models have shown enhanced repair and improvement with therapeutic approaches such as mobilization of endogenous EPC from the bone marrow (111) or supplementation with exogenous EPC (21). EPCs derived from the bone marrow or peripheral blood attenuated pulmonary hypertension and improved microvascular circulation in monocrotaline-induced pulmonary hypertension (229, 267). The putative mechanisms suggested in these studies include eNOS-controlled vasodilatation and VEGF signaling induced vascular growth.

C. Embryonic stem cells

In addition to using ASC for cell therapy, targeted differentiation of ESC into alveolar epithelial progenitors could provide a novel approach to regenerate endogenous lung cells destroyed by injury and disease. There is at least one study demonstrating attenuated fibrosis, improved lung function, and survival after administration of ESC-derived AT2 cells, in a model of bleomycin-induced ALI (255). Research with ESC and more so its clinical translation are encumbered in ethical controversy, concerns of teratoma formation, and risks of immuno-incompatibility (4, 65). iPSC, on the other hand, are unique pluripotent cells of potential autologous origin that bypass ethical hurdles, and offer the promise of plausible translation into the clinic, although a recent report suggest some immunogenicity of iPSC (266). Judging by the interest channeled into these cells since their discovery, it would be interesting to know how effective can they be in treating lung disorders or if their role will be confined to understanding disease processes and drug testing.

D. Endogenous lung progenitors

While the therapeutic benefit of exogenous stem cells for lung diseases has been widely explored, few studies have sought to tap into the therapeutic potential of endogenous lung progenitors, in part due to our incomplete understanding of these cells (30) and the relative difficulty of obtaining those cells compared to other sources, such as bone marrow or cord blood for example. Hegab *et al.* have isolated a population of Sca-1^+CD45^-CD31^- endogenous lung multilineage progenitors capable of self-renewal and differentiation into pulmonary epithelial cells. These cells could attenuate alveolar damage and improve survival in a mouse model of elastase-induced emphysema (101). However, the authors suggest that their therapeutic benefit is primarily paracrine mediated. More stringent characterization of this cell population is, however, required before further therapeutic assessments.

E. Paracrine effect of stem cells

It is noteworthy that most of the beneficial effects of stem cell therapy are suggested to be due to cell-derived paracrine factors, rather than cell engraftment. In fact, the observed lung engraftment of stem cells is not more than 5% in the majority of studies highlighted in Table 3. Paracrine mechanisms of action have so far been widely studied in MSC. These pleiotropic cells dampen inflammation *via* decreasing pro-inflammatory mediators and active secretion of anti-inflammatory cytokines and promote tissue repair by the release of several growth factors. The identified paracrine factors that mediate the beneficial effect of MSC include interleukin (IL)-10, IL-1ra, KGF, prostaglandin E2, Tumour necrosis factor-α stimulated gene/protein 6, and stanniocalcin-1 (88, 99, 160). More recently, an interesting study reports that human bone marrow-derived MSC possess direct antimicrobial activity, which is mediated in part by the synthesis and release of antimicrobial peptide cathelicidin (133). Identification of these paracrine factors may lead to new therapies.

To date, a majority of the preclinical therapeutic studies have examined the potential of exogenous stem/progenitor cells to enhance lung repair or regeneration in adult lung diseases. Much less is known about their repair potential in BPD.

VI. BPD and Stem Cells

As described earlier, lung injury in BPD is complex involving epithelial surfaces, the lung matrix, and the microvasculature. Damage or depletion of epithelial and/or vascular progenitors in the developing lung is a likely contributor to its pathogenesis.

A. Fate of stem/progenitor cells in BPD

O_2 challenge is one of the most extensively studied injury mechanisms in BPD. Animal and human observations in

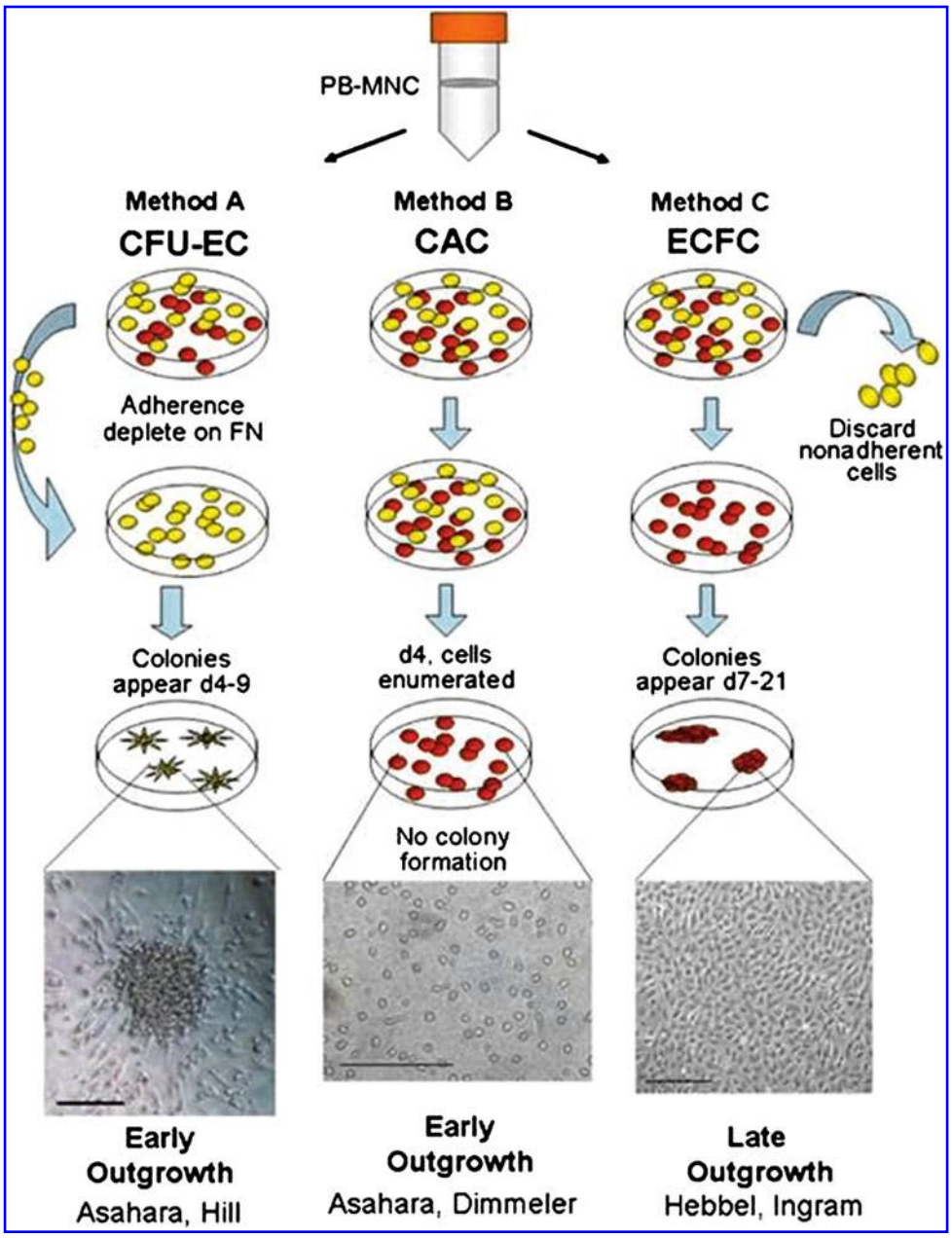

FIG. 5. Common methods of EPC culture. Culture of CFU-EC (Method A, scale bar = 100 μm) includes a 5-day process wherein non-adherent MNCs give rise to the EPC colony. CAC (Method B, scale bar = 200 μm) are the adherent mononuclear cells of a 4- to 7-day culture procedure. CAC cultures typically do not display colony formation. ECFCs (Method C, scale bar = 400 μm) are derived from adherent MNCs cultured for 7–21 days in endothelial conditions and colonies display a cobblestone morphology. ECFC emerge much later in culture as compared to both CFU-EC and CAC. Hence, ECFC have been called late outgrowth EPCs, while CFU-EC and CAC have been called early outgrowth EPC. Adapted with permission from Prater *et al.* (191). CAC, circulating angiogenic cells; CFU-EC, colony-forming unit-endothelial cells; ECFC, endothelial colony-forming cell; EPCs, endothelial progenitor cells. (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

recent years offer interesting clues about the behavior of vascular progenitor cells during oxidative stress. Baker *et al.* observed that cord blood of preterm infants yields higher numbers of endothelial colony-forming cells [ECFC; circulating progenitors of mature endothelial cells (191); defined in Figs. 5 and 6], compared to term infants (19). However, cells from the former group are relatively more sensitive to O_2 *in vitro*. Borghesi *et al.*, on the other hand, report that ECFC are lower in numbers in the cord blood of preterm infants who subsequently developed BPD (39). Interestingly, earlier reports have shown depletion of putative lung-resident EPC in experimental models of O_2-induced BPD. One study demonstrates decreased numbers of $CD45^-/Sca\text{-}1^+/CD133^+/VEGFR\text{-}2^+$ EPC in the peripheral blood, bone marrow, and lungs of O_2-challenged neonatal mice (20). Using the same animal model, another study shows a decrease in the number and endothelial differentiation potential of Hoechst33342 dye-effluxing multipotent lung SP cells (110). We have identified the presence of resident lung microvascular ECFCs in the developing rat lung; lung ECFCs isolated after 14 days of hyperoxia show compromised proliferative, clonogenic, and *in vitro* vessel-forming potential (8). These observations suggest an O_2-induced depletion of vessel-forming progenitors, and this could be a possible mechanism underlying arrested lung vascular growth in BPD. Sparing the endothelial progenitors, our knowledge on the fate of other stem cell populations within the BPD lung is still rudimentary and more extensive research is of critical importance.

For instance, the cells with myofibroblast phenotype crucial for alveolar septation and growth during normal lung development, under misguided signaling, could contribute to profibrotic changes and arrested alveolarization in BPD (189).

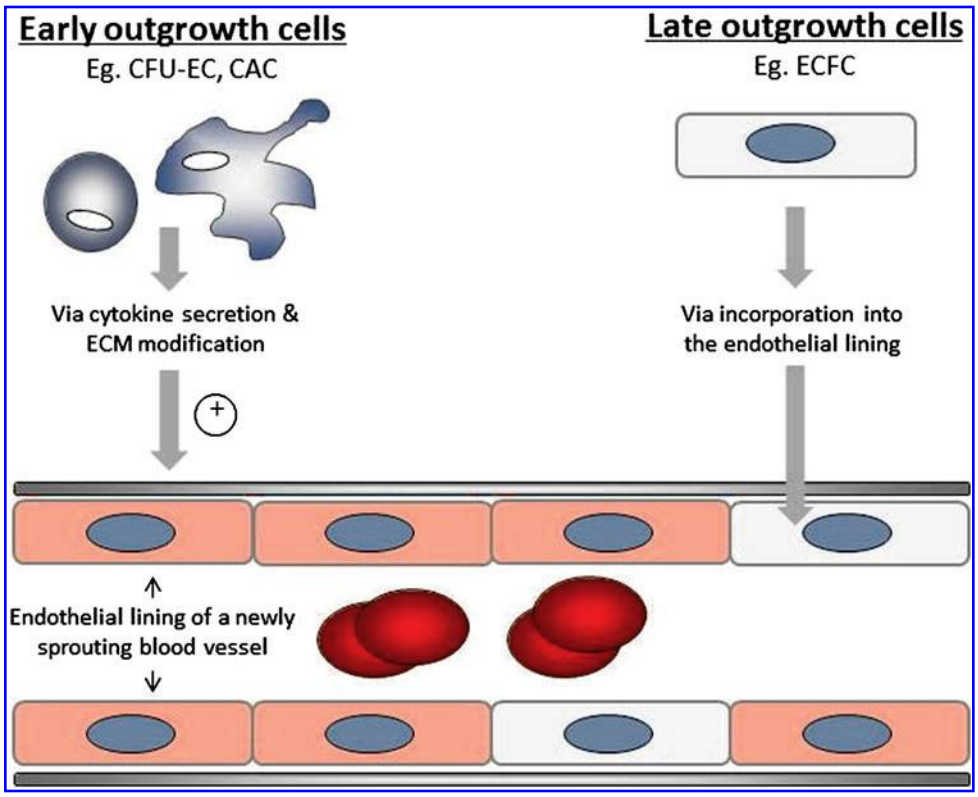

FIG. 6. Early and late outgrowth EPC in blood vessel formation. This figure depicts that ECFC (late-outgrowth EPC) get incorporated into and generate the endothelial lining of newly sprouting blood vessels. The circulating early-outgrowth EPC support new vessel formation *via* cytokine secretion and ECM modification. (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

In another recent study, Popova *et al.* provide interesting data connecting the presence of MSC in the tracheal aspirates of preterm infants and their propensity to develop BPD (188). With the growing interest to harness the therapeutic effects of MSC, these observations instruct caution and warrant closer investigation, since these cells could be forced into various phenotypes based on the intercellular microenvironmental cues they are exposed to (118, 187).

B. Cell therapy approaches for BPD

Lung damage in BPD could potentially involve a deficiency in the number and function of progenitors contributing to the growth and maintenance of the alveolar epithelial surfaces, lung matrix, and pulmonary microvasculature. This constitutes the rationale to use exogenous stem cells to replace the injured cells with multipotent stem cells that repopulate and grow the BPD lung. With this objective, several recent reports have started demonstrating therapeutic benefits with different stem cell types on rodent models of O_2-induced lung injury (Fig. 7).

1. Mesenchymal stem cells. Intravenous and airway delivery of bone marrow-derived MSC mitigates inflammation, prevents lung vascular and alveolar damage, and improves exercise tolerance and survival, in experimental O_2-induced BPD in newborn rodents (15, 246) (Fig. 8). Interestingly, both studies report that conditioned media from the MSC is as protective as the cells themselves since the observed therapeutic benefit is not in proportion to the degree of MSC engraftment.

MSC can be obtained from the bone marrow or from other sources such as umbilical cord blood, adipose tissue, Wharton's jelly, and the placenta (143). Among these, umbilical cord blood has started to receive a lot of attention in the recent years as a source of MSC and several other stem cell types for therapeutic use. It is a clinically relevant, viable, and readily available source of stem cells especially from the perspective of treating neonatal diseases. Chang *et al.* demonstrate that MSC from human cord blood prevent alveolar growth arrest and alleviate fibrotic changes in the lungs of O_2-challenged neonatal rats (55). This study also shows that intratracheal MSC transplantation is more effective than intraperitoneal transplantation in attenuating the hyperoxia-induced lung injury. In another interesting study, De Paepe *et al.* induced lung injury by conditional overexpression of lung-specific Fas ligand in transgenic newborn mice and treated them with an intranasal inoculation of UCB-derived $CD34^+$ hematopoietic progenitor cells. They report long-term pulmonary engraftment, replication, and clonal expansion of the injected $CD34^+$ cells followed by reconstitution of the injured respiratory epithelium by fusion-independent mechanisms (72). A prior report by the same group showed no epithelial reconstitution or therapeutic improvement with intranasal administration of unfractionated bone marrow-derived single-cell suspensions to newborn mice challenged with hyperoxia (86), while control mice exhibited impaired lung growth and alveolar remodeling.

2. Human amniotic fluid stem cells. Warburton and colleagues administered multipotent stem cells, derived from human amniotic fluid *via* c-kit selection, to adult nude mice injured with excessive O_2. They report that Human amniotic fluid stem cell (hAFSC) preferentially home and integrate into the injured lungs and a fraction of them show markers of epithelial and AT2 differentiation (52). A following study by

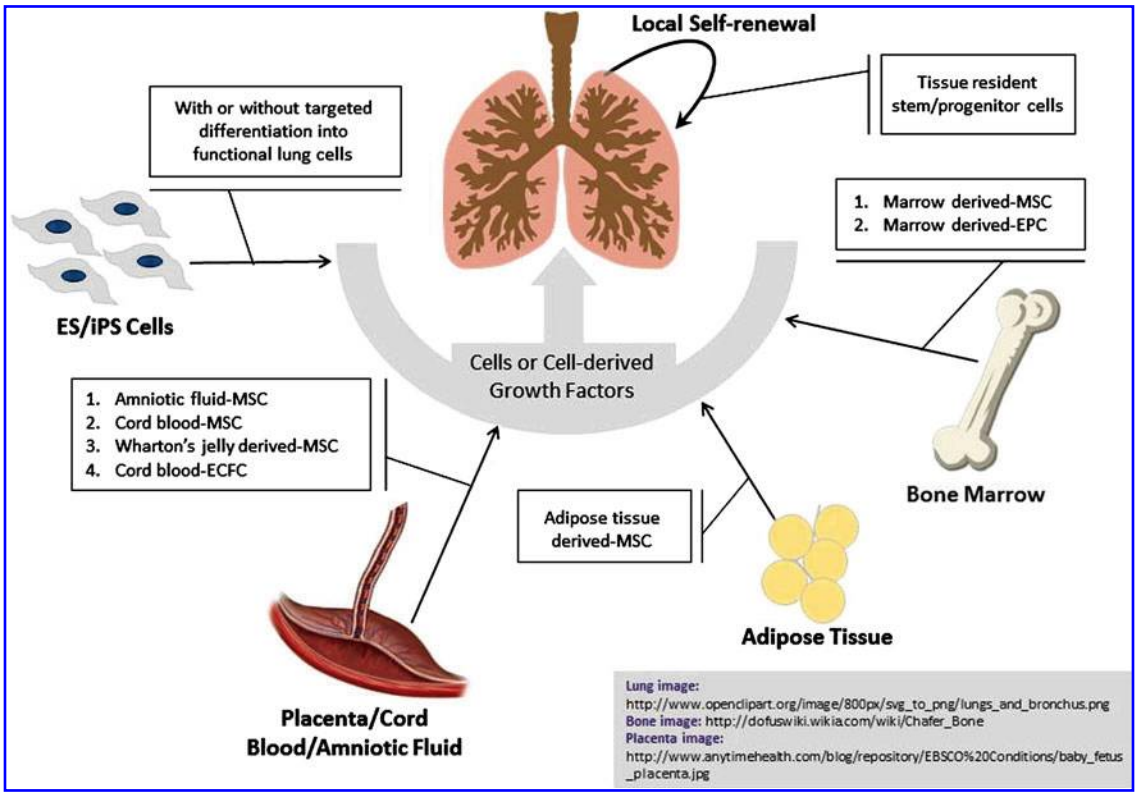

FIG. 7. Therapeutic opportunities with stem cells for lung regeneration. Several preclinical studies demonstrate evidence of the potential of stem cells (and stem cell-derived growth factors) to promote lung regeneration. (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

the same group reports that the microenvironment created by the damaged AT2 cells underlies the chemotactic recruitment and AT2 differentiation of uncommitted hAFSC (47). More detailed assessment of the mechanism of recruitment and differentiation of these cells is important for their translation into clinical and bioengineering applications.

3. Endothelial progenitor cells. The observed depletion of circulating and lung resident EPC in BPD (39) suggests a potential rescue from arrested alveolar and vascular growth after therapeutic supplementation with EPC of exogenous origin. In fact, O_2-exposed newborn mice treated intravenously with bone marrow-derived angiogenic cells (a population of bone marrow derived early outgrowth EPC; Fig. 5) shows almost complete restoration of lung structure that is indistinguishable from room air controls (21). Observations from our lab also show that human cord-blood derived ECFCs restore arrested alveolar growth and alleviate pulmonary hypertension in this newborn mouse model of BPD (8).

4. Human amnion epithelial cells. More recently, Vosdoganes showed that human amnion epithelial cells significantly attenuate the fetal pulmonary inflammatory response in an intrauterine LPS-induced model of lung inflammation in fetal sheep (251). Cells were given before birth intravenous, intratracheal, or both. This treatment regimen was associated with improved lung structure and function.

These reports are promising starting points for recovery, *in vitro* expansion, and treatment with stem cells or their conditioned media in infants at risk of BPD. However, more thorough studies in other animal models of BPD are imperative before human clinical trials should be considered (157).

VII. Summary and Remaining Challenges

Recent insights into stem cell biology have allowed a better understanding of the role of resident and exogenous stem/progenitor cells in lung health and disease with the ultimate hope of offering new therapeutic options for life-threatening and debilitating lung diseases. Improved isolation and characterization techniques have allowed the identification of putative resident lung stem/progenitor cells in animal and in humans. While the characterization of resident stem/progenitor cells in the proximal airways is more advanced, it appears more difficult to identify distal lung progenitor cells with unequivocal stem cell characteristics including multipotency and clonogenicity. Identification of these cells could broaden our understanding about cancer stem cells and open new therapeutic avenues for other lung diseases by promoting the protection and/or the repair potential of these cells.

In parallel, the therapeutic potential of exogenous administration of a variety of stem cells—including ESCs, MSCs, and endothelial progenitor cells of various tissue origins—has been investigated in a variety of experimental lung diseases (Table 3). These promising findings offer multiple new avenues for the treatment of lung diseases. Many gaps remain to be filled before safe clinical translation of their therapeutic potential could be achieved (Table 4).

First, the choice of the appropriate reparative cell type should be considered. Based on the underlying pathophysiology of the lung disease, the appropriate stem cell type—

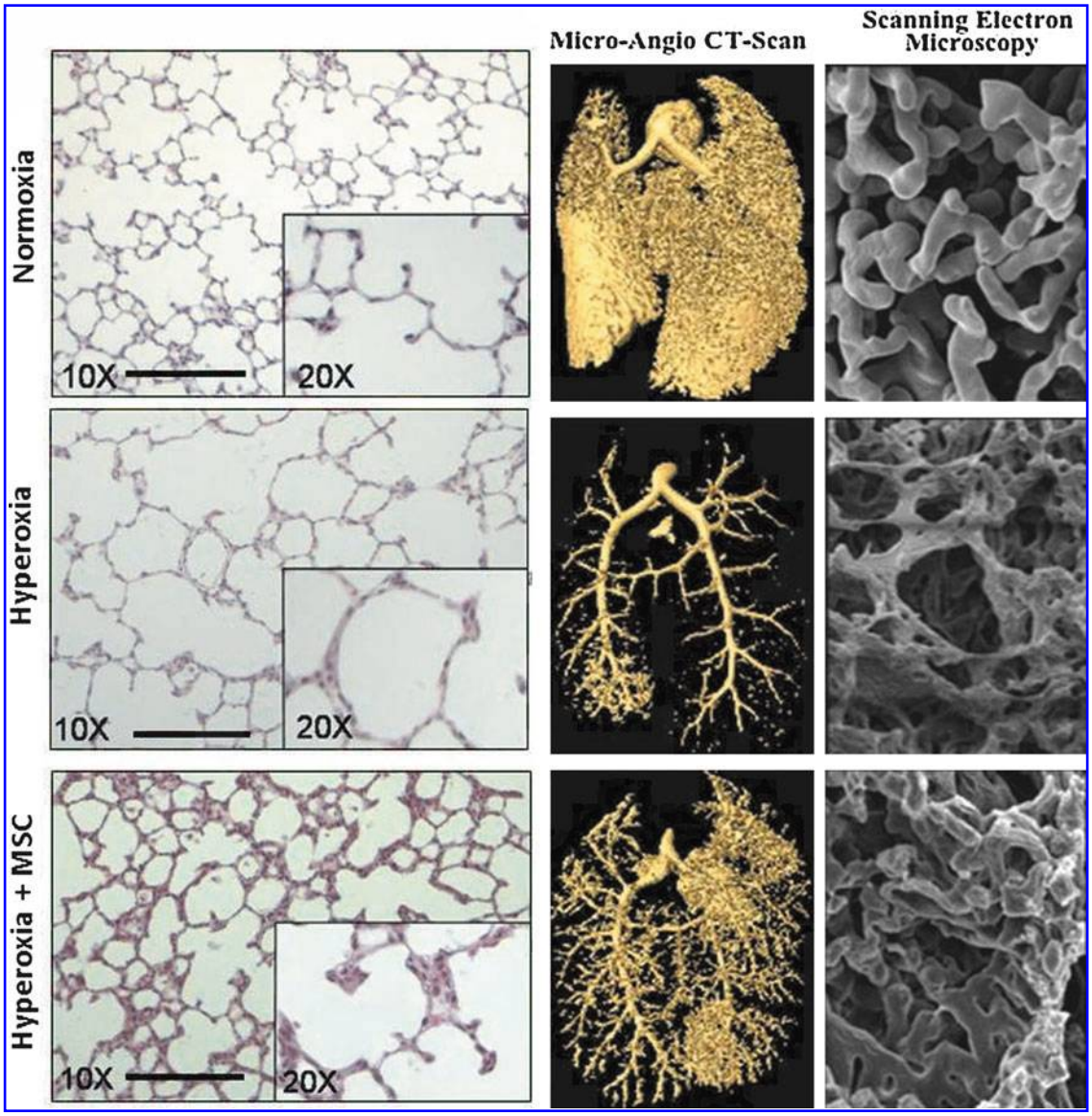

FIG. 8. Therapeutic effect of bone marrow MSC in experimental O_2-induced BPD in newborn rats. Airway delivery of bone marrow MSC prevents O_2-induced arrested alveolar and lung vascular growth in newborn rats as demonstrated by lung histology, micro-CT, and electron microscopy of the pulmonary vasculature. Adapted with permission from Van Haaften *et al.* (246). MSC, mesenchymal stem cell. (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

among the various stem cells with demonstrated therapeutic potential (Table 3)—should be chosen to derive the desired benefit in the specific target disease of interest.

Second, the appropriate mode of delivery needs to be defined, as most of the current approaches rely on the intrinsic capacity of stem cells to home into the injury sites and respond to the local demands, in contributing to recreation of the compromised tissue architecture and function. In this respect, the lungs offer the advantage of being accessible through direct airway delivery or the intravenous route, given that they receive the total cardiac output.

Third, the appropriate cell-based strategy needs to be determined. The indication that most of the therapeutic benefit of exogenous stem cells seems to relate to a paracrine effect opens additional therapeutic avenues. It suggests that rather than administering the cells, providing the paracrine product of stem cells may exert the same effect, eventually alleviating some of the concerns attributed to stem cell therapy.

Indeed, one of the major potential adverse effects associated with stem cell-based therapy is the propensity to form tumor or ectopic tissue. Each candidate cell type may harbor its own risk, as exemplified by teratoma formations with ESC implanted *in vivo* (37), putative stromal support for neoplastic cells by MSC and endothelial progenitors (124) and carcinogenic potential of bronchioalveolar stem cells (76, 126). Another concern includes the possibility of immunorejection of transplanted stem cells. This problem is important if using allogenic ESC or ASC, but less important when using stem cells from autologous sources such as bone marrow or cord blood. Likewise, MSCs do not seem to induce immunerejection as suggested by the administration of UCB-derived MSC to immunecompetent rodents and their use in graft *versus*

Table 4. Remaining Challenges in the Use of Stem Cells for Lung Diseases

What is the best reparative cell to treat a given lung disease?
ESCs, MSCs, endothelial progenitor cells, AFSCs, amnion epithelial cells?
What is the appropriate strategy for cell-based therapies?
Administration of exogenous stem cells: stem cells, stem cell-derived conditioned media, conditioned medium-derived factors?
Protection of endogenous lung progenitor cells?
What is the best mode of delivery of stem cells for lung diseases?
Intravenous
Intratracheal
What are the potential adverse effects of stem cell-based therapies?
Tumour formation?
Extopic tissue formation?
Immune rejection of the transplanted stem cells?

AFSC, amniotic fluid stem cell.

host disease. Moreover, harnessing the paracrine potential of stem cells could be an effective alternative to overcome this limitation (143).

Because of the promising results in the lab, MSC therapy in particular has already seen the light in the clinical setting for a variety of diseases, including myocardial infarct (98), graft *versus* host disease (148), and Crohn's disease (58). For lung diseases, there has been one clinical trial to date examining the feasibility and safety of allogeneic bone marrow-derived MSCs in patients with COPD (http://clinicaltrials.gov/ct2/show/NCT00683722). Two clinical trials on safety and efficacy of cord blood-derived MSCs for the treatment/prevention of BPD are currently ongoing (http://clinicaltrials.gov/ct2/results?term=bpd+and+stem+cells). The hope is that these clinical trials will yield valuable new knowledge that will answer some of the above-mentioned questions and allow fine tuning further experimental studies and clinical trials to translate this very promising therapy timely but safely into improved patient care.

VIII. Conclusion

Nearly 40 years after its original description, BPD still remains a major complication of premature birth. Over these years, BPD has evolved from marked lung fibrosis to a disease characterized by arrested alveolar growth. Despite improvements in our understanding of normal and impaired lung development, the multifactorial pathogenesis of BPD has made it problematic to identify efficacious treatments. Today, most of our efforts are targeted toward optimizing the use of currently available therapies (antenatal steroids and surfactant) and reducing the deleterious effects of other interventions (mechanical ventilation and O_2 therapy) (137, 161, 234, 245). To improve long-term outcomes, future treatment strategies will depend on the successful integration of basic research on fundamental mechanisms of lung development and the response to injury, targeted toward promoting lung growth and lung repair.

Half a century since the land mark discovery of the existence of stem cells by Till and McCulloch in 1961 (237), the therapeutic potential of these cells is being harnessed. On the basis of the promising studies in various animal models of lung diseases, stem cells may relieve clinicians of the vexing problem that modern clinical management remains largely devoid of therapies promoting lung repair. While the safety and efficacy of MSCs is already being tested in clinical trials, bench research continues to address some of the remaining challenges, such as the characterization of the reparative cells best suited for lung repair and regeneration. Strong emphasis must be placed on developing standardized techniques for stem/progenitor cell definition, isolation, expansion, and therapeutic administration. In addition, a better understanding of the role and regulation of endogenous lung stem cells may provide new therapeutic targets to promote lung growth and repair. Preclinical studies then need to include robust short- and long-term efficacy and safety data to accelerate and enhance the success of clinical trials.

Acknowledgments

B.T. is supported by the Canadian Institutes of Health Research (CIHR), Alberta Innovates Health Solutions/Alberta Heritage Foundation for Medical Research (AHFMR), the Canada Foundation for Innovation (CFI), and the Canada Research Chairs Program. R.S.A. is supported by a stipend from AHFMR

References

1. Abman SH. Bronchopulmonary dysplasia: "a vascular hypothesis". *Am J Respir Crit Care Med* 164: 1755–1756, 2001.
2. Adamson IY and Bowden DH. The type 2 cell as progenitor of alveolar epithelial regeneration. A cytodynamic study in mice after exposure to oxygen. *Lab Invest* 30: 35–42, 1974.
3. Afshar S, Gibson LL, Yuhanna IS, Sherman TS, Kerecman JD, Grubb PH, Yoder BA, McCurnin DC, and Shaul PW. Pulmonary NO synthase expression is attenuated in a fetal baboon model of chronic lung disease. *Am J Physiol Lung Cell Mol Physiol* 284: L749–L758, 2003.
4. Agostini C. Stem cell therapy for chronic lung diseases: hope and reality. *Respir Med* 104 Suppl 1: S86–S91, 2010.
5. Aguilar S, Scotton CJ, McNulty K, Nye E, Stamp G, Laurent G, Bonnet D, and Janes SM. Bone marrow stem cells expressing keratinocyte growth factor via an inducible lentivirus protects against bleomycin-induced pulmonary fibrosis. *PLoS One* 4: e8013, 2009.
6. Albertine KH, Jones GP, Starcher BC, Bohnsack JF, Davis PL, Cho SC, Carlton DP, and Bland RD. Chronic lung injury in preterm lambs. Disordered respiratory tract development. *Am J Respir Crit Care Med* 159: 945–958, 1999.
7. Allison BJ, Crossley KJ, Flecknoe SJ, Davis PG, Morley CJ, Harding R, and Hooper SB. Ventilation of the very immature lung *in utero* induces injury and BPD-like changes in lung structure in fetal sheep. *Pediatr Res* 64: 387–392, 2008.
8. Alphonse R, Vadivel A, Waszak P, Coltan L, Fung M, Eaton F, Yoder MC, and Thébaud B. Existence, functional impairment and therapeutic potential of endothelial colony forming cells (ECFCs) in oxygen-induced arrested alveolar growth. *Am J Respir Crit Care Med* 183: A1237, 2011.
9. Altiok O, Yasumatsu R, Bingol-Karakoc G, Riese RJ, Stahlman MT, Dwyer W, Pierce RA, Bromme D, Weber E, and Cataltepe S. Imbalance between cysteine proteases and inhibitors in a baboon model of bronchopulmonary dysplasia. *Am J Respir Crit Care Med* 173: 318–326, 2006.

10. Ambalavanan N and Novak ZE. Peptide growth factors in tracheal aspirates of mechanically ventilated preterm neonates. *Pediatr Res* 53: 240–244, 2003.
11. Asikainen TM and White CW. Pulmonary antioxidant defenses in the preterm newborn with respiratory distress and bronchopulmonary dysplasia in evolution: implications for antioxidant therapy. *Antioxid Redox Signal* 6: 155–167, 2004.
12. Askie LM, Ballard RA, Cutter GR, Dani C, Elbourne D, Field D, Hascoet JM, Hibbs AM, Kinsella JP, Mercier JC, Rich W, Schreiber MD, Wongsiridej PS, Subhedar NV, Van Meurs KP, Voysey M, Barrington K, Ehrenkranz RA, and Finer NN. Inhaled nitric oxide in preterm infants: an individual-patient data meta-analysis of randomized trials. *Pediatrics* 128: 729–739, 2011.
13. Askie LM, Brocklehurst P, Darlow BA, Finer N, Schmidt B, and Tarnow-Mordi W. NeOProM: Neonatal Oxygenation Prospective Meta-analysis Collaboration study protocol. *BMC Pediatr* 11: 6, 2011.
14. Askie LM, Henderson-Smart DJ, Irwig L, and Simpson JM. Oxygen-saturation targets and outcomes in extremely preterm infants. *N Engl J Med* 349: 959–967, 2003.
15. Aslam M, Baveja R, Liang OD, Fernandez-Gonzalez A, Lee C, Mitsialis SA, and Kourembanas S. Bone marrow stromal cells attenuate lung injury in a murine model of neonatal chronic lung disease. *Am J Respir Crit Care Med* 180: 1122–1130, 2009.
16. Auten RL and Davis JM. Oxygen toxicity and reactive oxygen species: the devil is in the details. *Pediatr Res* 66: 121–127, 2009.
17. Auten RL, Whorton MH, and Nicholas Mason S. Blocking neutrophil influx reduces DNA damage in hyperoxia-exposed newborn rat lung. *Am J Respir Cell Mol Biol* 26: 391–397, 2002.
18. Baber SR, Deng W, Master RG, Bunnell BA, Taylor BK, Murthy SN, Hyman AL, and Kadowitz PJ. Intratracheal mesenchymal stem cell administration attenuates monocrotaline-induced pulmonary hypertension and endothelial dysfunction. *Am J Physiol Heart Circ Physiol* 292: H1120–H1128, 2007.
19. Baker CD, Ryan SL, Ingram DA, Seedorf GJ, Abman SH, and Balasubramaniam V. Endothelial colony-forming cells from preterm infants are increased and more susceptible to hyperoxia. *Am J Respir Crit Care Med* 180: 454–461, 2009.
20. Balasubramaniam V, Mervis CF, Maxey AM, Markham NE, and Abman SH. Hyperoxia reduces bone marrow, circulating, and lung endothelial progenitor cells in the developing lung: implications for the pathogenesis of bronchopulmonary dysplasia. *Am J Physiol Lung Cell Mol Physiol* 292: L1073–L1084, 2007.
21. Balasubramaniam V, Ryan SL, Seedorf GJ, Roth EV, Heumann TR, Yoder MC, Ingram DA, Hogan CJ, Markham NE, and Abman SH. Bone marrow-derived angiogenic cells restore lung alveolar and vascular structure after neonatal hyperoxia in infant mice. *Am J Physiol Lung Cell Mol Physiol* 298: L315–L323, 2010.
22. Balasubramaniam V, Tang JR, Maxey A, Plopper CG, and Abman SH. Mild hypoxia impairs alveolarization in the endothelial nitric oxide synthase-deficient mouse. *Am J Physiol Lung Cell Mol Physiol* 284: L964–L971, 2003.
23. Ballard PL, Truog WE, Merrill JD, Gow A, Posencheg M, Golombek SG, Parton LA, Luan X, Cnaan A, and Ballard RA. Plasma biomarkers of oxidative stress: relationship to lung disease and inhaled nitric oxide therapy in premature infants. *Pediatrics* 121: 555–561, 2008.
24. Ballard RA, Truog WE, Cnaan A, Martin RJ, Ballard PL, Merrill JD, Walsh MC, Durand DJ, Mayock DE, Eichenwald EC, Null DR, Hudak ML, Puri AR, Golombek SG, Courtney SE, Stewart DL, Welty SE, Phibbs RH, Hibbs AM, Luan X, Wadlinger SR, Asselin JM, and Coburn CE. Inhaled nitric oxide in preterm infants undergoing mechanical ventilation. *N Engl J Med* 355: 343–353, 2006.
25. Baraldi E, Carraro S, and Filippone M. Bronchopulmonary dysplasia: definitions and long-term respiratory outcome. *Early Hum Dev* 85: S1–S3, 2009.
26. Baydas G, Karatas F, Gursu MF, Bozkurt HA, Ilhan N, Yasar A, and Canatan H. Antioxidant vitamin levels in term and preterm infants and their relation to maternal vitamin status. *Arch Med Res* 33: 276–280, 2002.
27. Belteki G, Kempster SL, Forhead AJ, Giussani DA, Fowden AL, Curley A, Charnock-Jones DS, and Smith GC. Paraoxonase-3, a putative circulating antioxidant, is systemically up-regulated in late gestation in the fetal rat, sheep, and human. *J Clin Endocrinol Metab* 95: 3798–3805, 2010.
28. Berger AC, Tang G, Alexander HR, and Libutti SK. Endothelial monocyte-activating polypeptide II, a tumor-derived cytokine that plays an important role in inflammation, apoptosis, and angiogenesis. *J Immunother* 23: 519–527, 2000.
29. Berger TM, Frei B, Rifai N, Avery ME, Suh J, Yoder BA, and Coalson JJ. Early high dose antioxidant vitamins do not prevent bronchopulmonary dysplasia in premature baboons exposed to prolonged hyperoxia: a pilot study. *Pediatr Res* 43: 719–726, 1998.
30. Bertoncello I and McQualter JL. Endogenous lung stem cells: what is their potential for use in regenerative medicine? *Expert Rev Respir Med* 4: 349–362, 2010.
31. Bhatt AJ, Pryhuber GS, Huyck H, Watkins RH, Metlay LA, and Maniscalco WM. Disrupted pulmonary vasculature and decreased vascular endothelial growth factor, Flt-1, and TIE-2 in human infants dying with bronchopulmonary dysplasia. *Am J Respir Crit Care Med* 164: 1971–1980, 2001.
32. Biniwale MA and Ehrenkranz RA. The role of nutrition in the prevention and management of bronchopulmonary dysplasia. *Semin Perinatol* 30: 200–208, 2006.
33. Blaisdell CJ, Gail DB, and Nabel EG. National Heart, Lung, and Blood Institute perspective: lung progenitor and stem cells—gaps in knowledge and future opportunities. *Stem Cells* 27: 2263–2270, 2009.
34. Bland RD, Albertine KH, Carlton DP, and MacRitchie AJ. Inhaled nitric oxide effects on lung structure and function in chronically ventilated preterm lambs. *Am J Respir Crit Care Med* 172: 899–906, 2005.
35. Bland RD, Ertsey R, Mokres LM, Xu L, Jacobson BE, Jiang S, Alvira CM, Rabinovitch M, Shinwell ES, and Dixit A. Mechanical ventilation uncouples synthesis and assembly of elastin and increases apoptosis in lungs of newborn mice. Prelude to defective alveolar septation during lung development? *Am J Physiol Lung Cell Mol Physiol* 294: L3–L14, 2008.
36. Blau HM, Brazelton TR, and Weimann JM. The evolving concept of a stem cell: entity or function? *Cell* 105: 829–841, 2001.
37. Blum B and Benvenisty N. The tumorigenicity of human embryonic stem cells. *Adv Cancer Res* 100: 133–158, 2008.
38. Bonnefont-Rousselot D and Collin F. Melatonin: action as antioxidant and potential applications in human disease and aging. *Toxicology* 278: 55–67, 2010.

39. Borghesi A, Massa M, Campanelli R, Bollani L, Tzialla C, Figar TA, Ferrari G, Bonetti E, Chiesa G, de Silvestri A, Spinillo A, Rosti V, and Stronati M. Circulating endothelial progenitor cells in preterm infants with bronchopulmonary dysplasia. *Am J Respir Crit Care Med* 180: 540–546, 2009.
40. Borthwick DW, Shahbazian M, Krantz QT, Dorin JR, and Randell SH. Evidence for stem-cell niches in the tracheal epithelium. *Am J Respir Cell Mol Biol* 24: 662–670, 2001.
41. Bostrom H, Willetts K, Pekny M, Leveen P, Lindahl P, Hedstrand H, Pekna M, Hellstrom M, Gebre-Medhin S, Schalling M, Nilsson M, Kurland S, Tornell J, Heath JK, and Betsholtz C. PDGF-A signaling is a critical event in lung alveolar myofibroblast development and alveogenesis. *Cell* 85: 863–873, 1996.
42. Bourbon J, Boucherat O, Chailley-Heu B, and Delacourt C. Control mechanisms of lung alveolar development and their disorders in bronchopulmonary dysplasia. *Pediatr Res* 57: 38R–46R, 2005.
43. Brettell LM and McGowan SE. Basic fibroblast growth factor decreases elastin production by neonatal rat lung fibroblasts. *Am J Respir Cell Mol Biol* 10: 306–315, 1994.
44. Brion LP, Bell EF, and Raghuveer TS. Vitamin E supplementation for prevention of morbidity and mortality in preterm infants. *Cochrane Database Syst Rev* CD003665, 2003.
45. Brody JS and Williams MC. Pulmonary alveolar epithelial cell differentiation. *Annu Rev Physiol* 54: 351–371, 1992.
46. Bry K, Hogmalm A, and Backstrom E. Mechanisms of inflammatory lung injury in the neonate: lessons from a transgenic mouse model of bronchopulmonary dysplasia. *Semin Perinatol* 34: 211–221, 2010.
47. Buckley S, Shi W, Carraro G, Sedrakyan S, Da Sacco S, Driscoll BA, Perin L, de Filippo RE, and Warburton D. The milieu of damaged AEC2 stimulates alveolar wound repair by endogenous and exogenous progenitors. *Am J Respir Cell Mol Biol* 45: 1212–1221 , 2011.
48. Buckley S and Warburton D. Dynamics of metalloproteinase-2 and −9, TGF-beta, and uPA activities during normoxic vs. hyperoxic alveolarization. *Am J Physiol Lung Cell Mol Physiol* 283: L747–754, 2002.
49. Burnham EL, Taylor WR, Quyyumi AA, Rojas M, Brigham KL, and Moss M. Increased circulating endothelial progenitor cells are associated with survival in acute lung injury. *Am J Respir Crit Care Med* 172: 854–860, 2005.
50. Burri PH. Structural aspect of prenatal and postnatal development and growth of the lung. In: *Lung Growth and Development*, edited by McDonald JA. New York: Marcel Dekker, 1997, pp. 1–35.
51. Capoluongo E, Ameglio F, and Zuppi C. Insulin-like growth factor-I and complications of prematurity: a focus on bronchopulmonary dysplasia. *Clin Chem Lab Med* 46: 1061–1066, 2008.
52. Carraro G, Perin L, Sedrakyan S, Giuliani S, Tiozzo C, Lee J, Turcatel G, De Langhe SP, Driscoll B, Bellusci S, Minoo P, Atala A, De Filippo RE, and Warburton D. Human amniotic fluid stem cells can integrate and differentiate into epithelial lung lineages. *Stem Cells* 26: 2902–2911, 2008.
53. Cerny L, Torday JS, and Rehan VK. Prevention and treatment of bronchopulmonary dysplasia: contemporary status and future outlook. *Lung* 186: 75–89, 2008.
54. Chailley-Heu B, Boucherat O, Barlier-Mur A-M, and Bourbon JR. FGF-18 is upregulated in the postnatal rat lung and enhances elastogenesis in myofibroblasts. *Am J Physiol Lung Cell Mol Physiol* 288: L43–L51, 2005.
55. Chang YS, Oh W, Choi SJ, Sung DK, Kim SY, Choi EY, Kang S, Jin HJ, Yang YS, and Park WS. Human umbilical cord blood-derived mesenchymal stem cells attenuate hyperoxia-induced lung injury in neonatal rats. *Cell Transplant* 18: 869–886, 2009.
56. Chetty A, Andersson S, Lassus P, and Nielsen HC. Insulin-like growth factor-1 (IGF-1) and IGF-1 receptor (IGF-1R) expression in human lung in RDS and BPD. *Pediatr Pulmonol* 37: 128–136, 2004.
57. Cho K-S, Park H-K, Park H-Y, Jung JS, Jeon S-G, Kim Y-K, and Roh HJ. IFATS collection: immunomodulatory effects of adipose tissue-derived stem cells in an allergic rhinitis mouse model. *Stem Cells* 27: 259–265, 2009.
58. Ciccocioppo R, Bernardo ME, Sgarella A, Maccario R, Avanzini MA, Ubezio C, Minelli A, Alvisi C, Vanoli A, Calliada F, Dionigi P, Perotti C, Locatelli F, and Corazza GR. Autologous bone marrow-derived mesenchymal stromal cells in the treatment of fistulising Crohn's disease. *Gut* 60: 788–798, 2011.
59. Coalson JJ. Pathology of new bronchopulmonary dysplasia. *Semin Neonatol* 8: 73–81, 2003.
60. Coalson JJ. Pathology of bronchopulmonary dysplasia. *Semin Perinatol* 30: 179–184, 2006.
61. Coalson JJ, Winter VT, Siler-Khodr T, and Yoder BA. Neonatal chronic lung disease in extremely immature baboons. *Am J Respir Crit Care Med* 160: 1333–1346, 1999.
62. Compernolle V, Brusselmans K, Acker T, Hoet P, Tjwa M, Beck H, Plaisance S, Dor Y, Keshet E, Lupu F, Nemery B, Dewerchin M, Van Veldhoven P, Plate K, Moons L, Collen D, and Carmeliet P. Loss of HIF-2alpha and inhibition of VEGF impair fetal lung maturation, whereas treatment with VEGF prevents fatal respiratory distress in premature mice. *Nat Med* 8: 702–710, 2002.
63. Couroucli XI, Liang YW, Jiang W, Barrios R, and Moorthy B. Attenuation of oxygen-induced abnormal lung maturation in rats by retinoic acid: possible role of cytochrome P4501A enzymes. *J Pharmacol Exp Ther* 317: 946–954, 2006.
64. D'Angio CT and Maniscalco WM. The role of vascular growth factors in hyperoxia-induced injury to the developing lung. *Front Biosci* 7: d1609–d1623, 2002.
65. Daley GQ. Stem cells: roadmap to the clinic. *J Clin Invest* 120: 8–10, 2010.
66. Dammann CE, Nielsen HC, and Carraway KL, 3rd. Role of neuregulin-1 beta in the developing lung. *Am J Respir Crit Care Med* 167: 1711–1716, 2003.
67. Danan C, Jarreau P-H, Franco M-L, Dassieu G, Grillon C, Abd Alsamad I, Lafuma C, Harf A, and Delacourt C. Gelatinase activities in the airways of premature infants and development of bronchopulmonary dysplasia. *Am J Physiol Lung Cell Mol Physiol* 283: L1086–L1093, 2002.
68. Darlow BA and Austin NC. Selenium supplementation to prevent short-term morbidity in preterm neonates. *Cochrane Database Syst Rev* CD003312, 2003.
69. Davda J and Labhasetwar V. An update on angiogenesis therapy. *Crit Rev Eukaryot Gene Expr* 11: 1–21, 2001.
70. Davis JM and Auten RL. Maturation of the antioxidant system and the effects on preterm birth. *Semin Fetal Neonatal Med* 15: 191–195, 2010.
71. Davis JM, Parad RB, Michele T, Allred E, Price A, and Rosenfeld W. Pulmonary outcome at 1 year corrected age in premature infants treated at birth with recombinant human CuZn superoxide dismutase. *Pediatrics* 111: 469–476, 2003.

72. De Paepe ME, Mao Q, Ghanta S, Hovanesian V, and Padbury JF. Alveolar epithelial cell therapy with human cord blood-derived hematopoietic progenitor cells. *Am J Pathol* 178: 1329–1339, 2011.
73. This reference has been deleted.
74. Debier C and Larondelle Y. Vitamins A and E: metabolism, roles and transfer to offspring. *Br J Nutr* 93: 153–174, 2005.
75. Dobbs LG, Johnson MD, Vanderbilt J, Allen L, and Gonzalez R. The great big alveolar TI cell: evolving concepts and paradigms. *Cell Physiol Biochem* 25: 55–62, 2010.
76. Dovey JS, Zacharek SJ, Kim CF, and Lees JA. Bmi1 is critical for lung tumorigenesis and bronchioalveolar stem cell expansion. *Proc Natl Acad Sci U S A* 105: 11857–11862, 2008.
77. Driscoll B, Buckley S, Bui KC, Anderson KD, and Warburton D. Telomerase in alveolar epithelial development and repair. *Am J Physiol Lung Cell Mol Physiol* 279: L1191–L1198, 2000.
78. Dulak J and Jozkowicz A. Regulation of vascular endothelial growth factor synthesis by nitric oxide: facts and controversies. *Antioxid Redox Signal* 5: 123–132, 2003.
79. Ekekezie II, Thibeault DW, Simon SD, Norberg M, Merrill JD, Ballard RA, Ballard PL, and Truog WE. Low levels of tissue inhibitors of metalloproteinases with a high matrix metalloproteinase-9/tissue inhibitor of metalloproteinase-1 ratio are present in tracheal aspirate fluids of infants who develop chronic lung disease. *Pediatrics* 113: 1709–1714, 2004.
80. Fadini GP, Avogaro A, and Agostini C. Pathophysiology of circulating progenitor cells in pulmonary disease and parallels with cardiovascular disease. *Am J Respir Cell Mol Biol* 35: 403–404, 2006.
81. Fawke J, Lum S, Kirkby J, Hennessy E, Marlow N, Rowell V, Thomas S, and Stocks J. Lung function and respiratory symptoms at 11 years in children born extremely preterm: the EPICure study. *Am J Respir Crit Care Med* 182: 237–245, 2010.
82. Fehrenbach H. Alveolar epithelial type II cell: defender of the alveolus revisited. *Respir Res* 2: 33–46, 2001.
83. Figliuzzi M, Cornolti R, Perico N, Rota C, Morigi M, Remuzzi G, Remuzzi A, and Benigni A. Bone marrow-derived mesenchymal stem cells improve islet graft function in diabetic rats. *Transplant Proc* 41: 1797–1800, 2009.
84. Finer NN, Carlo WA, Walsh MC, Rich W, Gantz MG, Laptook AR, Yoder BA, Faix RG, Das A, Poole WK, Donovan EF, Newman NS, Ambalavanan N, Frantz ID, 3rd, Buchter S, Sanchez PJ, Kennedy KA, Laroia N, Poindexter BB, Cotten CM, Van Meurs KP, Duara S, Narendran V, Sood BG, O'Shea TM, Bell EF, Bhandari V, Watterberg KL, and Higgins RD. Early CPAP versus surfactant in extremely preterm infants. *N Engl J Med* 362: 1970–1979, 2010.
85. Friedenstein AJ, Chailakhjan RK, and Lalykina KS. The development of fibroblast colonies in monolayer cultures of guinea-pig bone marrow and spleen cells. *Cell Tissue Kinet* 3: 393–403, 1970.
86. Fritzell JA, Mao Q, Gundavarapu S, Pasquariello T, Aliotta JM, Ayala A, Padbury JF, and De Paepe ME. Fate and effects of adult bone marrow cells in lungs of normoxic and hyperoxic newborn mice. *Am J Respir Cell Mol Biol* 40: 575–587, 2009.
87. Gahrton G and Björkstrand B. Progress in haematopoietic stem cell transplantation for multiple myeloma. *J Intern Med* 248: 185–201, 2000.
88. Galambos C, Ng YS, Ali A, Noguchi A, Lovejoy S, D'Amore PA, and DeMello DE. Defective pulmonary development in the absence of heparin-binding vascular endothelial growth factor isoforms. *Am J Respir Cell Mol Biol* 27: 194–203, 2002.
89. Gauldie J, Galt T, Bonniaud P, Robbins C, Kelly M, and Warburton D. Transfer of the active form of transforming growth factor-beta 1 gene to newborn rat lung induces changes consistent with bronchopulmonary dysplasia. *Am J Pathol* 163: 2575–2584, 2003.
90. Giangreco A, Reynolds SD, and Stripp BR. Terminal bronchioles harbor a unique airway stem cell population that localizes to the bronchoalveolar duct junction. *Am J Pathol* 161: 173–182, 2002.
91. Gien J and Kinsella JP. Pathogenesis and treatment of bronchopulmonary dysplasia. *Curr Opin Pediatr* 23: 305–313, 2011.
92. Gonzalez RF, Allen L, and Dobbs LG. Rat alveolar type I cells proliferate, express OCT-4, and exhibit phenotypic plasticity *in vitro*. *Am J Physiol Lung Cell Mol Physiol* 297: L1045–L1055, 2009.
93. Griffiths MJ, Bonnet D, and Janes SM. Stem cells of the alveolar epithelium. *Lancet* 366: 249–260, 2005.
94. Gupta N, Su X, Popov B, Lee JW, Serikov V, and Matthay MA. Intrapulmonary delivery of bone marrow-derived mesenchymal stem cells improves survival and attenuates endotoxin-induced acute lung injury in mice. *J Immunol* 179: 1855–1863, 2007.
95. Hall SM, Hislop AA, Pierce CM, and Haworth SG. Prenatal origins of human intrapulmonary arteries: formation and smooth muscle maturation. *Am J Respir Cell Mol Biol* 23: 194–203, 2000.
96. Han RN and Stewart DJ. Defective lung vascular development in endothelial nitric oxide synthase-deficient mice. *Trends Cardiovasc Med* 16: 29–34, 2006.
97. Hansen AR, Barnes CM, Folkman J, and McElrath TF. Maternal preeclampsia predicts the development of bronchopulmonary dysplasia. *J Pediatr* 156: 532–536, 2010.
98. Hare JM, Traverse JH, Henry TD, Dib N, Strumpf RK, Schulman SP, Gerstenblith G, DeMaria AN, Denktas AE, Gammon RS, Hermiller JB, Jr., Reisman MA, Schaer GL, and Sherman W. A randomized, double-blind, placebo-controlled, dose-escalation study of intravenous adult human mesenchymal stem cells (prochymal) after acute myocardial infarction. *J Am Coll Cardiol* 54: 2277–2286, 2009.
99. Hato T, Kimura Y, Morisada T, Koh GY, Miyata K, Tabata M, Kadomatsu T, Endo M, Urano T, Arai F, Araki K, Suda T, Kobayashi K, and Oike Y. Angiopoietins contribute to lung development by regulating pulmonary vascular network formation. *Biochem Biophys Res Commun* 381: 218–223, 2009.
100. Hay WW, Jr. and Bell EF. Oxygen therapy, oxygen toxicity, and the STOP-ROP trial. *Pediatrics* 105: 424–425, 2000.
101. Hegab AE, Kubo H, Fujino N, Suzuki T, He M, Kato H, and Yamaya M. Isolation and characterization of murine multipotent lung stem cells. *Stem Cells Dev* 19: 523–536, 2010.
102. Hegeman MA, Hennus MP, van Meurs M, Cobelens PM, Kavelaars A, Jansen NJ, Schultz MJ, van Vught AJ, Molema G, and Heijnen CJ. Angiopoietin-1 treatment reduces inflammation but does not prevent ventilator-induced lung injury. *PLoS One* 5: e15653, 2010.
103. Helmy KY, Patel SA, Silverio K, Pliner L, and Rameshwar P. Stem cells and regenerative medicine: accomplishments to date and future promise. *Ther Deliv* 1: 693–705, 2010.
104. Herzog EL, Chai L, and Krause DS. Plasticity of marrow-derived stem cells. *Blood* 102: 3483–3493, 2003.

105. Hilgendorff A, Parai K, Ertsey R, Jain N, Navarro EF, Peterson JL, Tamosiuniene R, Nicolls MR, Starcher BC, Rabinovitch M, and Bland RD. Inhibiting lung elastase activity enables lung growth in mechanically ventilated newborn mice. *Am J Respir Crit Care Med* 184: 537–546, 2011.
106. Hong KU, Reynolds SD, Giangreco A, Hurley CM, and Stripp BR. Clara cell secretory protein-expressing cells of the airway neuroepithelial body microenvironment include a label-retaining subset and are critical for epithelial renewal after progenitor cell depletion. *Am J Respir Cell Mol Biol* 24: 671–681, 2001.
107. Hong KU, Reynolds SD, Watkins S, Fuchs E, and Stripp BR. Basal cells are a multipotent progenitor capable of renewing the bronchial epithelium. *Am J Pathol* 164: 577–588, 2004.
108. Horwitz EM, Prockop DJ, Fitzpatrick LA, Koo WW, Gordon PL, Neel M, Sussman M, Orchard P, Marx JC, Pyeritz RE, and Brenner MK. Transplantability and therapeutic effects of bone marrow-derived mesenchymal cells in children with osteogenesis imperfecta. *Nat Med* 5: 309–313, 1999.
109. Ionescu LI, Alphonse RS, Arizmendi N, Morgan B, Abel M, Eaton F, Duszyk M, Vliagoftis H, Aprahamian TR, Walsh K, and Thebaud B. Airway delivery of soluble factors from plastic-adherent bone marrow cells prevents murine asthma. *Am J Respir Cell Mol Biol* 46: 207–216, 2012.
110. Irwin D, Helm K, Campbell N, Imamura M, Fagan K, Harral J, Carr M, Young KA, Klemm D, Gebb S, Dempsey EC, West J, and Majka S. Neonatal lung side population cells demonstrate endothelial potential and are altered in response to hyperoxia-induced lung simplification. *Am J Physiol Lung Cell Mol Physiol* 293: L941–L951, 2007.
111. Ishizawa K, Kubo H, Yamada M, Kobayashi S, Suzuki T, Mizuno S, Nakamura T, and Sasaki H. Hepatocyte growth factor induces angiogenesis in injured lungs through mobilizing endothelial progenitor cells. *Biochem Biophys Res Commun* 324: 276–280, 2004.
112. Iyer SS, Torres-Gonzalez E, Neujahr DC, Kwon M, Brigham KL, Jones DP, Mora AL, and Rojas M. Effect of bone marrow-derived mesenchymal stem cells on endotoxin-induced oxidation of plasma cysteine and glutathione in mice. *Stem Cells Int* 2010: 868076, 2010.
113. Jakkula M, Le Cras TD, Gebb S, Hirth KP, Tuder RM, Voelkel NF, and Abman SH. Inhibition of angiogenesis decreases alveolarization in the developing rat lung. *Am J Physiol Lung Cell Mol Physiol* 279: L600–L607, 2000.
114. James ML, Ross AC, Bulger A, Philips JB, 3rd, and Ambalavanan N. Vitamin A and retinoic acid act synergistically to increase lung retinyl esters during normoxia and reduce hyperoxic lung injury in newborn mice. *Pediatr Res* 67: 591–597, 2010.
115. Janer J, Andersson S, Haglund C, and Lassus P. Pulmonary endostatin perinatally and in lung injury of the newborn infant. *Pediatrics* 119: e241–e246, 2007.
116. Janer J, Andersson S, Kajantie E, and Lassus P. Endostatin concentration in cord plasma predicts the development of bronchopulmonary dysplasia in very low birth weight infants. *Pediatrics* 123: 1142–1146, 2009.
117. Jankov RP, Negus A, and Tanswell AK. Antioxidants as therapy in the newborn: some words of caution. *Pediatr Res* 50: 681–687, 2001.
118. Jiang Y, Jahagirdar BN, Reinhardt RL, Schwartz RE, Keene CD, Ortiz-Gonzalez XR, Reyes M, Lenvik T, Lund T, Blackstad M, Du J, Aldrich S, Lisberg A, Low WC, Largaespada DA, and Verfaillie CM. Pluripotency of mesenchymal stem cells derived from adult marrow. *Nature* 418: 41–49, 2002.
119. Jobe AH. Antenatal factors and the development of bronchopulmonary dysplasia. *Semin Neonatol* 8: 9–17, 2003.
120. Josko J and Mazurek M. Transcription factors having impact on vascular endothelial growth factor (VEGF) gene expression in angiogenesis. *Med Sci Monit* 10: RA89–RA98, 2004.
121. Kajstura J, Rota M, Hall SR, Hosoda T, D'Amario D, Sanada F, Zheng H, Ogorek B, Rondon-Clavo C, Ferreira-Martins J, Matsuda A, Arranto C, Goichberg P, Giordano G, Haley KJ, Bardelli S, Rayatzadeh H, Liu X, Quaini F, Liao R, Leri A, Perrella MA, Loscalzo J, and Anversa P. Evidence for human lung stem cells. *N Engl J Med* 364: 1795–1806, 2011.
122. Kanki-Horimoto S, Horimoto H, Mieno S, Kishida K, Watanabe F, Furuya E, and Katsumata T. Implantation of mesenchymal stem cells overexpressing endothelial nitric oxide synthase improves right ventricular impairments caused by pulmonary hypertension. *Circulation* 114: I181–I185, 2006.
123. Katsha AM, Ohkouchi S, Xin H, Kanehira M, Sun R, Nukiwa T, and Saijo Y. Paracrine factors of multipotent stromal cells ameliorate lung injury in an elastase-induced emphysema model. *Mol Ther J Am Soc Gene Ther* 19: 196–203, 2011.
124. Kidd S, Spaeth E, Klopp A, Andreeff M, Hall B, and Marini FC. The (in) auspicious role of mesenchymal stromal cells in cancer: be it friend or foe. *Cytotherapy* 10: 657–667, 2008.
125. Kim CB. Advancing the field of lung stem cell biology. *Front Biosci* 12: 3117–3124, 2007.
126. Kim CFB, Jackson EL, Woolfenden AE, Lawrence S, Babar I, Vogel S, Crowley D, Bronson RT, and Jacks T. Identification of bronchioalveolar stem cells in normal lung and lung cancer. *Cell* 121: 823–835, 2005.
127. Kim DH, Choi CW, Kim EK, Kim HS, Kim BI, Choi JH, Lee MJ, and Yang EG. Association of increased pulmonary interleukin-6 with the priming effect of intra-amniotic lipopolysaccharide on hyperoxic lung injury in a rat model of bronchopulmonary dysplasia. *Neonatology* 98: 23–32, 2010.
128. Kinsella JP, Cutter GR, Walsh WF, Gerstmann DR, Bose CL, Hart C, Sekar KC, Auten RL, Bhutani VK, Gerdes JS, George TN, Southgate WM, Carriedo H, Couser RJ, Mammel MC, Hall DC, Pappagallo M, Sardesai S, Strain JD, Baier M, and Abman SH. Early inhaled nitric oxide therapy in premature newborns with respiratory failure. *N Engl J Med* 355: 354–364, 2006.
129. Kinsella JP, Greenough A, and Abman SH. Bronchopulmonary dysplasia. *Lancet* 367: 1421–1431, 2006.
130. Koo HC, Davis JM, Li Y, Hatzis D, Opsimos H, Pollack S, Strayer MS, Ballard PL, and Kazzaz JA. Effects of transgene expression of superoxide dismutase and glutathione peroxidase on pulmonary epithelial cell growth in hyperoxia. *Am J Physiol Lung Cell Mol Physiol* 288: L718–L726, 2005.
131. Kotecha S, Wangoo A, Silverman M, and Shaw RJ. Increase in the concentration of transforming growth factor beta-1 in bronchoalveolar lavage fluid before development of chronic lung disease of prematurity. *J Pediatr* 128: 464–469, 1996.
132. Kovacic JC and Boehm M. Resident vascular progenitor cells: an emerging role for non-terminally differentiated

vessel-resident cells in vascular biology. *Stem Cell Res* 2: 2–15, 2009.
133. Krasnodembskaya A, Song Y, Fang X, Gupta N, Serikov V, Lee JW, and Matthay MA. Antibacterial effect of human mesenchymal stem cells is mediated in part from secretion of the antimicrobial peptide LL-37. *Stem Cells* 28: 2229–2238, 2010.
134. Krause DS, Theise ND, Collector MI, Henegariu O, Hwang S, Gardner R, Neutzel S, and Sharkis SJ. Multi-organ, multilineage engraftment by a single bone marrow-derived stem cell. *Cell* 105: 369–377, 2001.
135. Kugathasan L, Ray JB, Deng Y, Rezaei E, Dumont DJ, and Stewart DJ. The angiopietin-1-Tie2 pathway prevents rather than promotes pulmonary arterial hypertension in transgenic mice. *J Exp Med* 206: 2221–2234, 2009.
136. Kumamoto M, Nishiwaki T, Matsuo N, Kimura H, and Matsushima K. Minimally cultured bone marrow mesenchymal stem cells ameliorate fibrotic lung injury. *Eur Respir J* 34: 740–748, 2009.
137. Kunig AM, Balasubramaniam V, Markham NE, Morgan D, Montgomery G, Grover TR, and Abman SH. Recombinant human VEGF treatment enhances alveolarization after hyperoxic lung injury in neonatal rats. *Am J Physiol Lung Cell Mol Physiol* 289: L529–L535, 2005.
138. Ladha F, Bonnet S, Eaton F, Hashimoto K, Korbutt G, and Thebaud B. Sildenafil improves alveolar growth and pulmonary hypertension in hyperoxia-induced lung injury. *Am J Respir Crit Care Med* 172: 750–756, 2005.
139. Le Blanc K, Rasmusson I, Sundberg B, Götherström C, Hassan M, Uzunel M, and Ringdén O. Treatment of severe acute graft-versus-host disease with third party haploidentical mesenchymal stem cells. *Lancet* 363: 1439–1441, 2004.
140. Le Cras TD, Markham NE, Tuder RM, Voelkel NF, and Abman SH. Treatment of newborn rats with a VEGF receptor inhibitor causes pulmonary hypertension and abnormal lung structure. *Am J Physiol Lung Cell Mol Physiol* 283: L555–L562, 2002.
141. Le Cras TD, Spitzmiller RE, Albertine KH, Greenberg JM, Whitsett JA, and Akeson AL. VEGF causes pulmonary hemorrhage, hemosiderosis, and air space enlargement in neonatal mice. *Am J Physiol Lung Cell Mol Physiol* 287: L134–L142, 2004.
142. Lee HJ, Choi CW, Kim BI, Kim EK, Kim HS, Choi JH, Lee MJ, and Yang EG. Serial changes of lung morphology and biochemical profiles in a rat model of bronchopulmonary dysplasia induced by intra-amniotic lipopolysaccharide and postnatal hyperoxia. *J Perinat Med* 38: 675–681, 2010.
143. Lee JW, Gupta N, Serikov V, and Matthay MA. Potential application of mesenchymal stem cells in acute lung injury. *Expert Opin Biol Ther* 9: 1259–1270, 2009.
144. Leuwerke SM, Kaza AK, Tribble CG, Kron IL, and Laubach VE. Inhibition of compensatory lung growth in endothelial nitric oxide synthase-deficient mice. *Am J Physiol Lung Cell Mol Physiol* 282: L1272–L1278, 2002.
145. Levis J, Loi R, Butnor KJ, Vacek P, Steele C, Mossman BT, and Weiss DJ. Decreased asbestos-induced lung inflammation and fibrosis after radiation and bone marrow transplant. *Am J Respir Cell Mol Biol* 38: 16–25, 2008.
146. Li DY, Brooke B, Davis EC, Mecham RP, Sorensen LK, Boak BB, Eichwald E, and Keating MT. Elastin is an essential determinant of arterial morphogenesis. *Nature* 393: 276–280, 1998.
147. Liebler JM, Lutzko C, Banfalvi A, Senadheera D, Aghamohammadi N, Crandall ED, and Borok Z. Retention of human bone marrow-derived cells in murine lungs following bleomycin-induced lung injury. *Am J Physiol Lung Cell Mol Physiol* 295: L285–L292, 2008.
148. Lin Y and Hogan WJ. Clinical application of mesenchymal stem cells in the treatment and prevention of graft-versus-host disease. *Adv Hematol* 2011: 427863, 2011.
149. Lin YJ, Markham NE, Balasubramaniam V, Tang JR, Maxey A, Kinsella JP, and Abman SH. Inhaled nitric oxide enhances distal lung growth after exposure to hyperoxia in neonatal rats. *Pediatr Res* 58: 22–29, 2005.
150. Lindahl P, Karlsson L, Hellström M, Gebre-Medhin S, Willetts K, Heath JK, and Betsholtz C. Alveogenesis failure in PDGF-A-deficient mice is coupled to lack of distal spreading of alveolar smooth muscle cell progenitors during lung development. *Development* 124: 3943–3953, 1997.
151. Ling T-Y, Kuo M-D, Li C-L, Yu AL, Huang Y-H, Wu T-J, Lin Y-C, Chen S-H, and Yu J. Identification of pulmonary Oct-4+ stem/progenitor cells and demonstration of their susceptibility to SARS coronavirus (SARS-CoV) infection *in vitro*. *Proc Natl Acad Sci U S A* 103: 9530–9535, 2006.
152. Liu C, Morrisey EE, and Whitsett JA. GATA-6 is required for maturation of the lung in late gestation. *Am J Physiol Lung Cell Mol Physiol* 283: L468–L475, 2002.
153. Liu H, Chang L, Rong Z, Zhu H, Zhang Q, Chen H, and Li W. Association of insulin-like growth factors with lung development in neonatal rats. *J Huazhong Univ Sci Technol Med Sci* 24: 162–165, 2004.
154. Loi R, Beckett T, Goncz KK, Suratt BT, and Weiss DJ. Limited restoration of cystic fibrosis lung epithelium *in vivo* with adult bone marrow-derived cells. *Am J Respir Crit Care Med* 173: 171–179, 2006.
155. Luo X, Sedlackova L, Belcastro R, Cabacungan J, Lye SJ, and Tanswell AK. Effect of the 21-aminosteroid U74389G on oxygen-induced free radical production, lipid peroxidation, and inhibition of lung growth in neonatal rats. *Pediatr Res* 46: 215–223, 1999.
156. Majka SM, Beutz MA, Hagen M, Izzo AA, Voelkel N, and Helm KM. Identification of novel resident pulmonary stem cells: form and function of the lung side population. *Stem Cells* 23: 1073–1081, 2005.
157. Malgieri A, Kantzari E, Patrizi MP, and Gambardella S. Bone marrow and umbilical cord blood human mesenchymal stem cells: state of the art. *Int J Clin Exp Med* 3: 248–269, 2010.
158. Maniscalco WM, Watkins RH, Roper JM, Staversky R, and O'Reilly MA. Hyperoxic ventilated premature baboons have increased p53, oxidant DNA damage and decreased VEGF expression. *Pediatr Res* 58: 549–556, 2005.
159. Mason RJ. Biology of alveolar type II cells. *Respirology* 11 Suppl: S12–S15, 2006.
159a. Masood A, Yi M, Lau M, Belcastro R, Shek S, Pan J, Kantores C, McNamara PJ, Kavanagh BP, Belik J, Jankov RP, and Tanswell AK. Therapeutic effects of hypercapnia on chronic lung injury and vascular remodeling in neonatal rats. *Am J Physiol Lung Cell Mol Physiol* 297: L920–L930, 2009.
160. Matthay MA, Thompson BT, Read EJ, McKenna DH, Liu KD, Calfee CS, and Lee JW. Therapeutic potential of mesenchymal stem cells for severe acute lung injury. *Chest* 138: 965–972, 2010.
161. McCarter SD, Mei SH, Lai PF, Zhang QW, Parker CH, Suen RS, Hood RD, Zhao YD, Deng Y, Han RN, Dumont DJ, and Stewart DJ. Cell-based angiopoietin-1 gene therapy for acute lung injury. *Am J Respir Crit Care Med* 175: 1014–1026, 2007.
162. McCurnin DC, Pierce RA, Chang LY, Gibson LL, Osborne-Lawrence S, Yoder BA, Kerecman JD, Albertine KH, Winter VT, Coalson JJ, Crapo JD, Grubb PH, and Shaul PW. In-

haled NO improves early pulmonary function and modifies lung growth and elastin deposition in a baboon model of neonatal chronic lung disease. *Am J Physiol Lung Cell Mol Physiol* 288: L450–L459, 2005.
163. McGrath-Morrow SA, Cho C, Zhen L, Hicklin DJ, and Tuder RM. Vascular endothelial growth factor receptor 2 blockade disrupts postnatal lung development. *Am J Respir Cell Mol Biol* 32: 420–427, 2005.
164. McQualter JL, Brouard N, Williams B, Baird BN, Sims-Lucas S, Yuen K, Nilsson SK, Simmons PJ, and Bertoncello I. Endogenous fibroblastic progenitor cells in the adult mouse lung are highly enriched in the sca-1 positive cell fraction. *Stem Cells* 27: 623–633, 2009.
165. McQualter JL, Yuen K, Williams B, and Bertoncello I. Evidence of an epithelial stem/progenitor cell hierarchy in the adult mouse lung. *Proc Natl Acad Sci U S A* 107: 1414–1419, 2010.
166. Mei SHJ, McCarter SD, Deng Y, Parker CH, Liles WC, and Stewart DJ. Prevention of LPS-induced acute lung injury in mice by mesenchymal stem cells overexpressing angiopoietin 1. *PLoS Med* 4: e269, 2007.
167. Mercier JC, Hummler H, Durrmeyer X, Sanchez-Luna M, Carnielli V, Field D, Greenough A, Van Overmeire B, Jonsson B, Hallman M, and Baldassarre J. Inhaled nitric oxide for prevention of bronchopulmonary dysplasia in premature babies (EUNO): a randomised controlled trial. *Lancet* 376: 346–354, 2010.
168. Metzger RJ, Klein OD, Martin GR, and Krasnow MA. The branching programme of mouse lung development. *Nature* 453: 745–750, 2008.
169. Metzger RJ and Krasnow MA. Genetic control of branching morphogenesis. *Science* 284: 1635–1639, 1999.
170. Moodley Y, Atienza D, Manuelpillai U, Samuel CS, Tchongue J, Ilancheran S, Boyd R, and Trounson A. Human umbilical cord mesenchymal stem cells reduce fibrosis of bleomycin-induced lung injury. *Am J Pathol* 175: 303–313, 2009.
171. Moore BB, Kolodsick JE, Thannickal VJ, Cooke K, Moore TA, Hogaboam C, Wilke CA, and Toews GB. CCR2-mediated recruitment of fibrocytes to the alveolar space after fibrotic injury. *Am J Pathol* 166: 675–684, 2005.
172. Myers TJ, Granero-Molto F, Longobardi L, Li T, Yan Y, and Spagnoli A. Mesenchymal stem cells at the intersection of cell and gene therapy. *Expert Opin Biol Ther* 10: 1663–1679, 2010.
173. Nemeth K, Keane-Myers A, Brown JM, Metcalfe DD, Gorham JD, Gorham JD, Bundoc VG, Bundoc VG, Hodges MG, Jelinek I, Madala S, Karpati S, and Mezey E. Bone marrow stromal cells use TGF-beta to suppress allergic responses in a mouse model of ragweed-induced asthma. *Proc Natl Acad Sci U S A* 107: 5652–5657, 2010.
174. Németh K, Leelahavanichkul A, Yuen PST, Mayer B, Parmelee A, Doi K, Robey PG, Leelahavanichkul K, Koller BH, Brown JM, Hu X, Jelinek I, Star RA, and Mezey E. Bone marrow stromal cells attenuate sepsis via prostaglandin E (2)-dependent reprogramming of host macrophages to increase their interleukin-10 production. *Nat Med* 15: 42–49, 2009.
175. Neuringer IP and Randell SH. Stem cells and repair of lung injuries. *Respir Res* 5: 6, 2004.
176. Nielsen HC. Epidermal growth factor influences the developmental clock regulating maturation of the fetal lung fibroblast. *Biochim Biophys Acta* 1012: 201–206, 1989.
177. Nolen-Walston RD, Kim CF, Mazan MR, Ingenito EP, Gruntman AM, Tsai L, Boston R, Woolfenden AE, Jacks T, and Hoffman AM. Cellular kinetics and modeling of bronchioalveolar stem cell response during lung regeneration. *Am J Physiol Lung Cell Mol Physiol* 294: L1158–L1165, 2008.
178. Northway WH, Jr., Rosan RC, and Porter DY. Pulmonary disease following respirator therapy of hyaline-membrane disease. Bronchopulmonary dysplasia. *N Engl J Med* 276: 357–368, 1967.
179. Ohki Y, Mayuzumi H, Tokuyama K, Yoshizawa Y, Arakawa H, Mochizuki H, and Morikawa A. Hepatocyte growth factor treatment improves alveolarization in a newborn murine model of bronchopulmonary dysplasia. *Neonatology* 95: 332–338, 2009.
180. Ortiz LA, Dutreil M, Fattman C, Pandey AC, Torres G, Go K, and Phinney DG. Interleukin 1 receptor antagonist mediates the antiinflammatory and antifibrotic effect of mesenchymal stem cells during lung injury. *Proc Natl Acad Sci U S A* 104: 11002–11007, 2007.
181. Pan L, Fu JH, Xue XD, Xu W, Zhou P, and Wei B. Melatonin protects against oxidative damage in a neonatal rat model of bronchopulmonary dysplasia. *World J Pediatr* 5: 216–221, 2009.
182. Papapetropoulos A, Garcia-Cardena G, Madri JA, and Sessa WC. Nitric oxide production contributes to the angiogenic properties of vascular endothelial growth factor in human endothelial cells. *J Clin Invest* 100: 3131–3139, 1997.
183. Park H-K, Cho K-S, Park H-Y, Shin DH, Kim Y-K, Jung JS, Park SK, and Roh HJ. Adipose-derived stromal cells inhibit allergic airway inflammation in mice. *Stem Cells Dev* 19: 1811–1818, 2010.
184. Pasquali MA, Gelain DP, Oliveira MR, Behr GA, Motta LL, Rocha RF, Klamt F, and Moreira JC. Vitamin A supplementation induces oxidative stress and decreases the immunocontent of catalase and superoxide dismutase in rat lungs. *Exp Lung Res* 35: 427–438, 2009.
185. Perrone S, Tataranno ML, Negro S, Longini M, Marzocchi B, Proietti F, Iacoponi F, Capitani S, and Buonocore G. Early identification of the risk for free radical-related diseases in preterm newborns. *Early Hum Dev* 86: 241–244, 2010.
186. Phillips RJ, Burdick MD, Hong K, Lutz MA, Murray LA, Xue YY, Belperio JA, Keane MP, and Strieter RM. Circulating fibrocytes traffic to the lungs in response to CXCL12 and mediate fibrosis. *J Clin Invest* 114: 438–446, 2004.
186a. Pierce RA, Albertine KH, Starcher BC, Bohnsack JF, Carlton DP, and Bland RD. Chronic lung injury in preterm lambs: disordered pulmonary elastin deposition. *Am J Physiol* 272: L452–L460, 1997.
187. Pierro M and Thebaud B. Mesenchymal stem cells in chronic lung disease: culprit or savior? *Am J Physiol Lung Cell Mol Physiol* 298: L732–L734, 2010.
188. Popova AP, Bozyk PD, Bentley JK, Linn MJ, Goldsmith AM, Schumacher RE, Weiner GM, Filbrun AG, and Hershenson MB. Isolation of tracheal aspirate mesenchymal stromal cells predicts bronchopulmonary dysplasia. *Pediatrics* 126: e1127–e1133, 2010.
189. Popova AP, Bozyk PD, Goldsmith AM, Linn MJ, Lei J, Bentley JK, and Hershenson MB. Autocrine production of TGF-beta1 promotes myofibroblastic differentiation of neonatal lung mesenchymal stem cells. *Am J Physiol Lung Cell Mol Physiol* 298: L735–L743, 2010.
190. Powers HJ, Loban A, Silvers K, and Gibson AT. Vitamin C at concentrations observed in premature babies inhibits the ferroxidase activity of caeruloplasmin. *Free Radic Res* 22: 57–65, 1995.
191. Prater DN, Case J, Ingram DA, and Yoder MC. Working hypothesis to redefine endothelial progenitor cells. *Leukemia* 21: 1141–1149, 2007.

192. Purevdorj E, Zscheppang K, Hoymann HG, Braun A, von Mayersbach D, Brinkhaus MJ, Schmiedl A, and Dammann CE. ErbB4 deletion leads to changes in lung function and structure similar to bronchopulmonary dysplasia. *Am J Physiol Lung Cell Mol Physiol* 294: L516–L522, 2008.
193. Quintos-Alagheband ML, White CW, and Schwarz MA. Potential role for antiangiogenic proteins in the evolution of bronchopulmonary dysplasia. *Antioxid Redox Signal* 6: 137–145, 2004.
194. Ramirez MI, Millien G, Hinds A, Cao Y, Seldin DC, and Williams MC. T1alpha, a lung type I cell differentiation gene, is required for normal lung cell proliferation and alveolus formation at birth. *Dev Biol* 256: 61–72, 2003.
195. Ramsay PL, DeMayo FJ, Hegemier SE, Wearden ME, Smith CV, and Welty SE. Clara cell secretory protein oxidation and expression in premature infants who develop bronchopulmonary dysplasia. *Am J Respir Crit Care Med* 164: 155–161, 2001.
196. Raoul W, Wagner-Ballon O, Saber G, Hulin A, Marcos E, Giraudier S, Vainchenker W, Adnot S, Eddahibi S, and Maitre B. Effects of bone marrow-derived cells on monocrotaline- and hypoxia-induced pulmonary hypertension in mice. *Respir Res* 8: 8, 2007.
197. Rawlins EL, Clark CP, Xue Y, and Hogan BLM. The Id2+ distal tip lung epithelium contains individual multipotent embryonic progenitor cells. *Development* 136: 3741–3745, 2009.
198. Rawlins EL and Hogan BLM. Epithelial stem cells of the lung: privileged few or opportunities for many? *Development* 133: 2455–2465, 2006.
199. Rawlins EL, Okubo T, Xue Y, Brass DM, Auten RL, Hasegawa H, Wang F, and Hogan BLM. The role of Scgb1a1+ Clara cells in the long-term maintenance and repair of lung airway, but not alveolar, epithelium. *Cell Stem Cell* 4: 525–534, 2009.
200. Reddy R, Buckley S, Doerken M, Barsky L, Weinberg K, Anderson KD, Warburton D, and Driscoll B. Isolation of a putative progenitor subpopulation of alveolar epithelial type 2 cells. *Am J Physiol Lung Cell Mol Physiol* 286: L658–L667, 2004.
201. Reynolds SD, Shen H, Reynolds PR, Betsuyaku T, Pilewski JM, Gambelli F, Di Giuseppe M, Ortiz LA, and Stripp BR. Molecular and functional properties of lung SP cells. *Am J Physiol Lung Cell Mol Physiol* 292: L972–L983, 2007.
202. Ribatti D. Endogenous inhibitors of angiogenesis: a historical review. *Leuk Res* 33: 638–644, 2009.
203. Rock JR, Randell SH, and Hogan BLM. Airway basal stem cells: a perspective on their roles in epithelial homeostasis and remodeling. *Dis Model Mech* 3: 545–556, 2010.
204. Rojas M, Xu J, Woods CR, Mora AL, Spears W, Roman J, and Brigham KL. Bone marrow-derived mesenchymal stem cells in repair of the injured lung. *Am J Respir Cell Mol Biol* 33: 145–152, 2005.
205. Rojas MA, Gonzalez A, Bancalari E, Claure N, Poole C, and Silva-Neto G. Changing trends in the epidemiology and pathogenesis of neonatal chronic lung disease. *J Pediatr* 126: 605–610, 1995.
206. Roth-Kleiner M and Post M. Similarities and dissimilarities of branching and septation during lung development. *Pediatr Pulmonol* 40: 113–134, 2005.
207. Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, and Hoekstra WG. Selenium: biochemical role as a component of glutathione peroxidase. *Science* 179: 588–590, 1973.
208. Saugstad OD. Optimal oxygenation at birth and in the neonatal period. *Neonatology* 91: 319–322, 2007.
209. Saugstad OD. Oxygen and oxidative stress in bronchopulmonary dysplasia. *J Perinat Med* 38: 571–577, 2010.
210. Schipani E and Kronenberg HM. Adult mesenchymal stem cells. In: *StemBook*. Cambridge, MA: Harvard Stem Cell Institute, 2008. Available at www.ncbi.nlm.nih.gov/pubmed/20614616 [cited 2012 Mar 30].
211. Schmiedl A, Behrens J, Zscheppang K, Purevdorj E, von Mayersbach D, Liese A, and Dammann CE. Lipopolysaccharide-induced injury is more pronounced in fetal transgenic ErbB4-deleted lungs. *Am J Physiol Lung Cell Mol Physiol* 301: L490–L499, 2011.
212. Schreiber MD, Gin-Mestan K, Marks JD, Huo D, Lee G, and Srisuparp P. Inhaled nitric oxide in premature infants with the respiratory distress syndrome. *N Engl J Med* 349: 2099–2107, 2003.
213. Schultz CJ, Torres E, Londos C, and Torday JS. Role of adipocyte differentiation-related protein in surfactant phospholipid synthesis by type II cells. *Am J Physiol Lung Cell Mol Physiol* 283: L288–L296, 2002.
214. Schwarz M, Lee M, Zhang F, Zhao J, Jin Y, Smith S, Bhuva J, Stern D, Warburton D, and Starnes V. EMAP II: a modulator of neovascularization in the developing lung. *Am J Physiol* 276: L365–L375, 1999.
215. Sessa WC. Molecular control of blood flow and angiogenesis: role of nitric oxide. *J Thromb Haemost* 7 Suppl 1: 35–37, 2009.
216. Shi W, Xu J and Warburton D. Development, repair and fibrosis: what is common and why it matters. *Respirology* 14: 656–665, 2009.
217. Shigemura N, Okumura M, Mizuno S, Imanishi Y, Matsuyama A, Shiono H, Nakamura T, and Sawa Y. Lung tissue engineering technique with adipose stromal cells improves surgical outcome for pulmonary emphysema. *Am J Respir Crit Care Med* 174: 1199–1205, 2006.
218. Shigemura N, Okumura M, Mizuno S, Imanishi Y, Nakamura T, and Sawa Y. Autologous transplantation of adipose tissue-derived stromal cells ameliorates pulmonary emphysema. *Am J Transplant* 6: 2592–2600, 2006.
219. Snyder JC, Teisanu RM, and Stripp BR. Endogenous lung stem cells and contribution to disease. *J Pathol* 217: 254–264, 2009.
220. Stahl W and Sies H. Antioxidant defense: vitamins E and C and carotenoids. *Diabetes* 46 Suppl 2: S14–S18, 1997.
221. Stenmark KR and Abman SH. Lung vascular development: implications for the pathogenesis of bronchopulmonary dysplasia. *Annu Rev Physiol* 67: 623–661, 2005.
222. Stenson B, Brocklehurst P, and Tarnow-Mordi W. Increased 36-week survival with high oxygen saturation target in extremely preterm infants. *N Engl J Med* 364: 1680–1682, 2011.
223. Stevenson K, McGlynn L, and Shiels PG. Stem cells: outstanding potential and outstanding questions. *Scott Med J* 54: 35–37, 2009.
224. Stone KC, Mercer RR, Gehr P, Stockstill B, and Crapo JD. Allometric relationships of cell numbers and size in the mammalian lung. *Am J Respir Cell Mol Biol* 6: 235–243, 1992.
225. Stripp BR. Hierarchical organization of lung progenitor cells: is there an adult lung tissue stem cell? *Proc Am Thorac Soc* 5: 695–698, 2008.
226. Sueblinvong V and Weiss DJ. Stem cells and cell therapy approaches in lung biology and diseases. *Transl Res* 156: 188–205, 2010.
227. Summer R, Kotton DN, Sun X, Ma B, Fitzsimmons K, and Fine A. Side population cells and Bcrp1 expression in lung. *Am J Physiol Lung Cell Mol Physiol* 285: L97–L104, 2003.

228. Takahashi K and Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* 126: 663–676, 2006.
229. Takahashi M, Nakamura T, Toba T, Kajiwara N, Kato H, and Shimizu Y. Transplantation of endothelial progenitor cells into the lung to alleviate pulmonary hypertension in dogs. *Tissue Eng* 10: 771–779, 2004.
230. Tang JR, Markham NE, Lin YJ, McMurtry IF, Maxey A, Kinsella JP, and Abman SH. Inhaled nitric oxide attenuates pulmonary hypertension and improves lung growth in infant rats after neonatal treatment with a VEGF receptor inhibitor. *Am J Physiol Lung Cell Mol Physiol* 287: L344–L351, 2004.
231. ter Horst SA, Fijlstra M, Sengupta S, Walther FJ, and Wagenaar GT. Spatial and temporal expression of surfactant proteins in hyperoxia-induced neonatal rat lung injury. *BMC Pulm Med* 6: 8, 2006.
232. Thebaud B and Abman SH. Bronchopulmonary dysplasia: where have all the vessels gone? Roles of angiogenic growth factors in chronic lung disease. *Am J Respir Crit Care Med* 175: 978–985, 2007.
233. Thebaud B, Ladha F, Michelakis ED, Sawicka M, Thurston G, Eaton F, Hashimoto K, Harry G, Haromy A, Korbutt G, and Archer SL. Vascular endothelial growth factor gene therapy increases survival, promotes lung angiogenesis, and prevents alveolar damage in hyperoxia-induced lung injury: evidence that angiogenesis participates in alveolarization. *Circulation* 112: 2477–2486, 2005.
234. Thomas M and Augustin HG. The role of the Angiopoietins in vascular morphogenesis. *Angiogenesis* 12: 125–137, 2009.
235. Thomas W, Seidenspinner S, Kramer BW, Kawczynska-Leda N, Chmielnicka-Kopaczyk M, Marx A, Wirbelauer J, Szymankiewicz M, and Speer CP. Airway concentrations of angiopoietin-1 and endostatin in ventilated extremely premature infants are decreased after funisitis and unbalanced with bronchopulmonary dysplasia/death. *Pediatr Res* 65: 468–473, 2009.
236. Thurston G. Complementary actions of VEGF and angiopoietin-1 on blood vessel growth and leakage. *J Anat* 200: 575–580, 2002.
237. Till JE and Mcculloch EA. A direct measurement of the radiation sensitivity of normal mouse bone marrow cells. *Radiat Res* 14: 213–222, 1961.
238. Tin W. Optimal oxygen saturation for preterm babies. Do we really know? *Biol Neonate* 85: 319–325, 2004.
239. Tögel F, Hu Z, Weiss K, Isaac J, Lange C, and Westenfelder C. Administered mesenchymal stem cells protect against ischemic acute renal failure through differentiation-independent mechanisms. *Am J Physiol Renal Physiol* 289: F31–F42, 2005.
240. Torday JS and Rehan VK. Developmental cell/molecular biologic approach to the etiology and treatment of bronchopulmonary dysplasia. *Pediatr Res* 62: 2–7, 2007.
241. Tourneux P, Markham N, Seedorf G, Balasubramaniam V, and Abman SH. Inhaled nitric oxide improves lung structure and pulmonary hypertension in a model of bleomycin-induced bronchopulmonary dysplasia in neonatal rats. *Am J Physiol Lung Cell Mol Physiol* 297: L1103–L1111, 2009.
242. Tyson JE, Wright LL, Oh W, Kennedy KA, Mele L, Ehrenkranz RA, Stoll BJ, Lemons JA, Stevenson DK, Bauer CR, Korones SB, and Fanaroff AA. Vitamin A supplementation for extremely-low-birth-weight infants. National Institute of Child Health and Human Development Neonatal Research Network. *N Engl J Med* 340: 1962–1968, 1999.
243. Ulich TR, Yi ES, Longmuir K, Yin S, Biltz R, Morris CF, Housley RM, and Pierce GF. Keratinocyte growth factor is a growth factor for type II pneumocytes *in vivo*. *J Clin Invest* 93: 1298–1306, 1994.
244. Umar S, de Visser YP, Steendijk P, Schutte CI, Laghmani EH, Wagenaar GTM, Bax WH, Mantikou E, Pijnappels DA, Atsma DE, Schalij MJ, van der Wall EE, and van der Laarse A. Allogenic stem cell therapy improves right ventricular function by improving lung pathology in rats with pulmonary hypertension. *Am J Physiol Heart Circ Physiol* 297: H1606–H1616, 2009.
245. van der Heijden M, van Nieuw Amerongen GP, Chedamni S, van Hinsbergh VW, and Johan Groeneveld AB. The angiopoietin-Tie2 system as a therapeutic target in sepsis and acute lung injury. *Expert Opin Ther Targets* 13: 39–53, 2009.
246. van Haaften T, Byrne R, Bonnet S, Rochefort GY, Akabutu J, Bouchentouf M, Rey-Parra GJ, Galipeau J, Haromy A, Eaton F, Chen M, Hashimoto K, Abley D, Korbutt G, Archer SL, and Thébaud B. Airway delivery of mesenchymal stem cells prevents arrested alveolar growth in neonatal lung injury in rats. *Am J Respir Crit Care Med* 180: 1131–1142, 2009.
247. Vats A, Bielby RC, Tolley NS, Nerem R, and Polak JM. Stem cells. *Lancet* 366: 592–602, 2005.
248. Veness-Meehan KA, Bottone FG, Jr., and Stiles AD. Effects of retinoic acid on airspace development and lung collagen in hyperoxia-exposed newborn rats. *Pediatr Res* 48: 434–444, 2000.
249. Vicencio AG, Lee CG, Cho SJ, Eickelberg O, Chuu Y, Haddad GG, and Elias JA. Conditional overexpression of bioactive transforming growth factor-beta1 in neonatal mouse lung: a new model for bronchopulmonary dysplasia? *Am J Respir Cell Mol Biol* 31: 650–656, 2004.
250. This reference has been deleted.
251. Vosdoganes P, Hodges RJ, Lim R, Westover AJ, Acharya RY, Wallace EM, and Moss TJ. Human amnion epithelial cells as a treatment for inflammation-induced fetal lung injury in sheep. *Am J Obstet Gynecol* 205: 156 e26–156 e33, 2011.
252. Vozzelli MA, Mason SN, Whorton MH, and Auten RL, Jr. Antimacrophage chemokine treatment prevents neutrophil and macrophage influx in hyperoxia-exposed newborn rat lung. *Am J Physiol Lung Cell Mol Physiol* 286: L488–L493, 2004.
253. Wagenaar GT, ter Horst SA, van Gastelen MA, Leijser LM, Mauad T, van der Velden PA, de Heer E, Hiemstra PS, Poorthuis BJ, and Walther FJ. Gene expression profile and histopathology of experimental bronchopulmonary dysplasia induced by prolonged oxidative stress. *Free Radic Biol Med* 36: 782–801, 2004.
254. Wan H, Xu Y, Ikegami M, Stahlman MT, Kaestner KH, Ang S-L, and Whitsett JA. Foxa2 is required for transition to air breathing at birth. *Proc Natl Acad Sci U S A* 101: 14449–14454, 2004.
255. Wang D, Morales JE, Calame DG, Alcorn JL, and Wetsel RA. Transplantation of human embryonic stem cell-derived alveolar epithelial type II cells abrogates acute lung injury in mice. *Mol Ther* 18: 625–634, 2010.
256. Watt FM and Hogan BL. Out of Eden: stem cells and their niches. *Science* 287: 1427–1430, 2000.
257. Wendel DP, Taylor DG, Albertine KH, Keating MT, and Li DY. Impaired distal airway development in mice lacking elastin. *Am J Respir Cell Mol Biol* 23: 320–326, 2000.

258. Wong PM, Lees AN, Louw J, Lee FY, French N, Gain K, Murray CP, Wilson A, and Chambers DC. Emphysema in young adult survivors of moderate-to-severe bronchopulmonary dysplasia. *Eur Respir J* 32: 321–328, 2008.
259. Xu J, Qu J, Cao L, Sai Y, Chen C, He L, and Yu L. Mesenchymal stem cell-based angiopoietin-1 gene therapy for acute lung injury induced by lipopolysaccharide in mice. *J Pathol* 214: 472–481, 2008.
260. Xu J, Woods CR, Mora AL, Joodi R, Brigham KL, Iyer S, and Rojas M. Prevention of endotoxin-induced systemic response by bone marrow-derived mesenchymal stem cells in mice. *Am J Physiol Lung Cell Mol Physiol* 293: L131–L141, 2007.
261. Yagi H, Soto-Gutierrez A, Kitagawa Y, Tilles AW, Tompkins RG, and Yarmush ML. Bone marrow mesenchymal stromal cells attenuate organ injury induced by LPS and burn. *Cell Transplant* 19: 823–830, 2010.
262. Yagi H, Soto-Gutierrez A, Navarro-Alvarez N, Nahmias Y, Goldwasser Y, Kitagawa Y, Tilles AW, Tompkins RG, Parekkadan B, and Yarmush ML. Reactive bone marrow stromal cells attenuate systemic inflammation via sTNFR1. *Mol Ther* 18: 1857–1864, 2010.
263. Yamada M, Kubo H, Ishizawa K, Kobayashi S, Shinkawa M, and Sasaki H. Increased circulating endothelial progenitor cells in patients with bacterial pneumonia: evidence that bone marrow derived cells contribute to lung repair. *Thorax* 60: 410–413, 2005.
264. Young SL, Evans K, and Eu JP. Nitric oxide modulates branching morphogenesis in fetal rat lung explants. *Am J Physiol Lung Cell Mol Physiol* 282: L379–L385, 2002.
265. Zaret KS and Grompe M. Generation and regeneration of cells of the liver and pancreas. *Science* 322: 1490–1494, 2008.
266. Zhao T, Zhang ZN, Rong Z, and Xu Y. Immunogenicity of induced pluripotent stem cells. *Nature* 474: 212–215, 2011.
267. Zhao YD, Courtman DW, Deng Y, Kugathasan L, Zhang Q, and Stewart DJ. Rescue of monocrotaline-induced pulmonary arterial hypertension using bone marrow-derived endothelial-like progenitor cells: efficacy of combined cell and eNOS gene therapy in established disease. *Circ Res* 96: 442–450, 2005.
268. Zhen G, Liu H, Gu N, Zhang H, Xu Y, and Zhang Z. Mesenchymal stem cells transplantation protects against rat pulmonary emphysema. *Front Biosci* 13: 3415–3422, 2008.

Address correspondence to:
Dr. Bernard Thébaud
Department of Pediatrics
University of Alberta
3020 Katz Centre
Edmonton
Alberta T6G 2S2
Canada

E-mail: bthebaud@ualberta.ca

Date of first submission to ARS Central, September 5, 2011; date of final revised submission, March 1, 2012; date of acceptance, March 1, 2012.

Abbreviations Used

ALI = acute lung injury
Ang-1 = angiopoietin-1
ASC = adult (or somatic) stem cells
AT1 = alveolar type 1
AT2 = alveolar type 2
BPD = bronchopulmonary dysplasia
BrdU = 5-bromo-2-deoxyuridine
CAC = circulating angiogenic cells
CCSP = Clara cell secretory protein
CdM = conditioned media
CFTR-KO = cystic fibrosis transmembrane regulator-knock out
CFU = colony-forming unit
CLP = cecal ligation and puncture
COPD = chronic obstructive pulmonary disease
ECFC = endothelial colony-forming cell
ECM = extracellular matrix
EGF = epidermal growth factor
EMAP-II = endothelial monocyte activating polypeptide-II
eNOS = endothelial nitric oxide synthase
EPC = endothelial progenitor cell
ESC = embryonic stem cell
FGF = fibroblast growth factor
hAFSC = human amniotic fluid stem cells
HGF = hepatocyte growth factor
HIF = hypoxia inducible factor
HSC = hematopoietic stem cell
hUCB = human umbilical cord blood
i.n. = intranasal
i.p. = intraperitoneal
i.t. = intratracheal
i.v. = intravenous
IGF = insulin-like growth factor
IL = interleukin
iPSC = induced pluripotent stem cell
KGF = keratinocyte growth factor
LI = lung injury
LVRS = lung volume reduction surgery
MMP = matrix metalloproteinases
MSC = mesenchymal stem cell
NEB = neuroendocrine bodies
NO = nitric oxide
O_2 = oxygen
PDGFα = platelet-derived growth factor-α
PHT = pulmonary hypertension
PPE = porcine pancreatic elastase
ROS = reactive oxygen species
RV = right ventricle
SARS-Co V = severe acute respiratory syndrome coronavirus
SP = side population
SP-C = surfactant protein C
SSEA-1 = stage specific embryonic antigen-1
TGF-β = transforming growth factor-beta
Th2 = helper T-cell type 2
TIMP = tissue inhibitor of metalloproteinases
VEGF = vascular endothelial growth factor