

Circulating Endothelial Progenitors and Bone Marrow Derived Cells as Biomarkers for Risk of Bronchopulmonary Dysplasia

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Abstract

Bronchopulmonary dysplasia (BPD), also known as neonatal chronic lung disease, is a multifactorial disease and its pathogenesis starts even before birth. Animal models of BPD and the study of infants with BPD suggest that impaired lung vascular development leads to the failure of alveolar development and strategies that promote vascular development result in improved alveolarization of the lung. Since 1997 when Asahara identified endothelial cell progenitors (EPCs) as blood cells having the ability to contribute to postnatal vasculogenesis, several studies have attempted to elucidate the role of EPCs in neonatal lung development and lung injury and repair. This review outlines the progress in defining early EPCs (believed to be of hematopoietic origin) and late EPCs or true EPCs (believed to be of endothelial origin) through the use of cell culture assays and flow cytometric characterization. Both animal and human studies have attempted to correlate the frequency of these specific populations with susceptibility to BPD. Animal studies use hyperoxia or endotoxin-induced lung injury as a model of BPD. Human studies use frequencies of specific cell populations as a prognostic index of BPD. Conflicting outcomes are likely the result of a lack of consistent definitions. Recently, there is increasing evidence that blood and bone marrow-derived stem cells exert a beneficial effect in models of chronic lung injury, not so much by engraftment and differentiation, but by a paracrine effect on the existing lung progenitor cells.

Keywords: Endothelial progenitor cell (EPC), Endothelial Colony Forming Cell (ECFC), Bronchopulmonary Dysplasia (BPD), Mesenchymal Stem Cell (MSC), angiogenesis, vasculogenesis, paracrine factors.

Abbreviations: DiI-Ac-LDL: Acetylated Low Density Lipoprotein labeled with 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate; AT2: Alveolar Epithelial Type 2 cell; BMDAC: Bone Marrow Derived Angiogenic Cell; BMPC: Bone Marrow Derived Progenitor Cells; BPD: Bronchopulmonary Dysplasia; ECFC: Endothelial Colony Forming Cell; ECFC-CM: Endothelial Colony Forming Cell-Conditioned Medium; eNOS: endothelial Nitric Oxide Synthase; EOC: Endothelial Outgrowth Cell; EPC: Endothelial progenitor cell; FiO₂: Fraction of Inspired Oxygen; KDR: Kinase Insert Domain Receptor; MSC: Mesenchymal Stem Cell; NO: Nitric Oxide; PBL: Peripheral Blood Lymphocytes; RVH: Right Ventricular Hypertrophy; UEA: Ulex europaeus agglutinin; VEGF: Vascular Endothelial Growth Factor; VEGFR-2: Vascular Endothelial Growth Factor Receptor.

Introduction

Premature deliveries account for 10% of live births in the US and the survival of premature infants has improved over the years with advances in perinatal and neonatal care. Nearly 25-30% of premature infants with birth weight below 1250 grams develop bronchopulmonary dysplasia (BPD) [1,2] characterized by abnormal alveolar and vascular development during a critical stage of postnatal lung development. Animal models of BPD suggest that abnormal lung microvascular development leads to the failure of alveolar development and strategies that promote vascular development result in improved alveolarization of the lung [3]. In addition, low levels of bone marrow-derived and circulating endothelial progenitors have been observed in the blood of hyperoxia exposed neonatal mice [4] that develop BPD, suggesting that bone marrow-derived endothelial progenitors may play a role in neonatal lung development. A human study reported lower levels of

endothelial colony forming cells (ECFC) in cord blood from preterm infants who developed BPD than in cord blood from preterm infants who did not develop BPD [5]. However, others have observed higher or equivalent numbers of ECFC counts in cord blood from preterm deliveries as compared to term cord blood [6,7]. Our group recently reported that circulating endothelial progenitor cell frequency in human neonates in the first three weeks of life may be influenced by postnatal clinical stress [8]. This review article discusses the pertinent literature on circulating endothelial progenitor cells and other cell populations and their association with bronchopulmonary dysplasia.

Pathophysiology of BPD

Human fetal lung development progresses through five highly regulated stages with coordinated airway branching, expansion of the vascular capillary network, mesenchymal and epithelial cell interaction, proliferation and differentiation to form an effective alveolar capillary membrane capable of gas exchange that can support life after birth. The last stage of lung development or alveolar stage occurs in late gestation, at about 36 weeks and continues until two years of age with six-fold expansion in alveolar numbers from birth. 85-90% of

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all alveoli are formed in the first 6 months of life. During this stage there is active remodeling of the pulmonary capillary network and formation of secondary septation to increase alveolar surface area for gas exchange [9]. However many preterm infants are born during the saccular stage of lung development (24 to 36 weeks post conception) and are exposed to environmental factors that could interfere with the normal process of alveolarization and are at risk for the development of bronchopulmonary dysplasia.

BPD was first described by Northway in 1967 as pulmonary fibrosis in near term infants with acute respiratory distress at birth and who required mechanical ventilation and high oxygen concentrations [10]. With the use of prenatal steroids for fetal lung maturation, and the early use of continuous positive airway distending pressure (CPAP) in the delivery room, fewer infants require intubation and mechanical ventilation; however the rate of BPD continues to be high. The “new BPD” is characterized by arrested lung growth with impaired alveolar development resulting in large simplified alveolar structures and abnormal vasculature [11].

BPD is a clinical diagnosis defined as oxygen dependency for 28 days or more and assessed at 36 weeks postmenstrual age as either mild BPD: oxygen needed at 28 days but not at 36 weeks, moderate BPD: oxygen needed at 28 days and at 36 weeks with fraction of inspired oxygen (FiO₂) less than 30%, or severe BPD: requiring oxygen in excess of 30% FiO₂ or positive pressure at 36 weeks [12].

Factors that contribute to the pathogenesis of BPD include prematurity, intrauterine growth restriction and postnatal nutritional deficits, oxidative stress from oxygen therapy necessary to treat hypoxemia, ventilator induced barotrauma or volutrauma, infection or inflammation, and genetic predisposition [13,14]. Hyperoxia decreases vascular endothelial growth factor (VEGF) expression and alterations in VEGF signaling result in altered lung microvascular growth and alveolarization. Elevated pro-inflammatory cytokines in the settings of chorioamnionitis and inflammation, can disrupt Fibroblast growth factor 10 (FGF10) signaling and impair lung morphogenesis. Mechanical ventilation increases the production of pro-inflammatory cytokines with alteration of the balance between pro-angiogenic and anti-angiogenic gene expression and increases the risk for development of BPD. Prenatal steroids used for fetal lung maturation have increased the survival of extremely premature infants in the short term but while they mature the epithelial cells compartment, they may interfere with the lung microvascular development.

At the present time, there is no specific treatment for BPD. Animal models of BPD have demonstrated that bone marrow derived progenitor cells, specifically endothelial progenitor cells, play an important role in lung repair after injury. Recently considerable interest has been generated in testing the possibility of using cell based therapies for BPD [15,16], and in attempts to identify patients at high risk for BPD using quantitative measures of circulating endothelial progenitors of various phenotypes [17,18].

Traditional Classification of Endothelial Progenitor Cells

Endothelial Progenitor Cells are an important population of cells involved in the homeostasis, injury, and disease of the lining of the blood vessels. Circulating endothelial progenitor cells (EPCs) were first identified in peripheral blood by Asahara based on their ability to contribute to angiogenesis *in vivo* in animal models of hind limb ischemia [19]. Since then many groups have attempted to isolate and

characterize EPCs from blood using *in vitro* culture methods with functional assays to verify that the cultured cells possess the ability to form capillary tubes or integrate into existing or damaged blood vessels. Others have used flow cytometric characterization of cell surface markers to quantify the frequency of these putative EPCs in the blood of normal individuals and subjects with various acute and chronic illnesses.

Early and Late EPCs are derived from *in vitro* culture

The historical definition and classification of “EPCs” includes different cell types that are not equivalent. The *in vitro* culture methods describe early EPCs and late EPCs, named for the time at which colonies arise from *in vitro* culture of blood cells.

Early EPCs emerge early during *in vitro* culture of blood cells—after 4 to 7 days. Early EPCs grow in a spindle shape and are angiogenic in that they can extend vasculature from pre-existing blood vessels. They have limited potential to form colonies *in vitro* or to be passaged serially [20]. Early EPCs arise from hematopoietic cells that may include monocytes, myeloid progenitor cells, and T lymphocytes. They are not true endothelial cells [20,21].

Late EPCs emerge at a late time point during *in vitro* culture of blood cells, after two to three weeks. Late EPCs form colonies with a cobblestone morphology and with high proliferative potential from single cells. Late EPCs are known as endothelial colony forming cells (ECFCs), or endothelial outgrowth cells (EOCs), or “true” EPCs [22]. Late EPCs are truly vasculogenic and can form *de novo* human-murine chimeric blood vessels when transplanted into immunodeficient mice [20]. Both early and late EPCs may both contribute to effective angiogenesis in the developing lung and in BPD [23]. Both the hematopoietic and endothelial lineages come from a common embryological ancestor, the hemangioblast, which may continue functioning as a common precursor into adulthood [22,24].

Functional *in vitro* assays show that both early and late EPC are capable of uptake of acetylated low-density lipoprotein (DiI-Ac-LDL), and the plant lectin Ulex europaeus agglutinin (UEA) [25]. However, only late EPCs are able to form capillary-like structures *in vitro* in Matrigel assays [25].

EPCs as defined by flow cytometric characterization

Cell surface molecules have been used as markers to identify EPC populations by flow cytometry, in place of, or in conjunction with functional assays. EPCs were first identified as CD34+VEGFR-2+. These antigens were selected as candidate markers because they are present on both hematopoietic stem cells and endothelial lineage cells, and it was thought that EPCs, as progenitor cells, would have these markers in common [19]. These CD34+VEGFR-2+ cells were later determined to be an early EPC population [26]. Hematopoietic stem cells lose expression of CD34 and VEGFR-2 as they differentiate [19], while endothelial cells retain VEGFR-2 expression.

Peichev introduced the hematopoietic stem cell marker CD133 in addition to the CD34+VEGFR-2+ phenotype for the identification of endothelial precursor cells. CD133 was selected because it is expressed on immature hematopoietic cells but is down regulated and absent on mature endothelial or differentiated hematopoietic cells [27]. Case et al. also confirmed that the CD34+CD133+VEGFR-2+ subpopulation of cells from blood was not ECFC because it did not form colonies under ECFC growth conditions, nor did it form capillary structures in Matrigel, but rather was a hematopoietic cell type that grew under hematopoietic growth conditions [28].

The same study showed that 99% of CD34+CD133+VEGFR-2+ cells were positive for CD45, the pan-leukocyte marker, and that only CD45 negative cells, a rare population, contained ECFCs [28]. Timmermans et al. concluded that true EPCs (EOCs) from human blood are CD34+CD45-CD133-VEGFR-2+ (both CD133 negative and CD45 negative), whereas CD34+CD45+CD133+VEGFR-2+ cells are early outgrowth EPCs of hematopoietic origin [25]. In a multi-color flow cytometry protocol for identifying EPC, Estes et al., distinguishes between early EPCs of hematopoietic lineage, which are CD45 positive, and true EPCs (referred to as “ECFCs”), which are CD45 negative [29].

Many investigators have attempted to distinguish between EPCs from the circulation and bone marrow derived cells by flow cytometric characterization. de la Puente et al [30] characterize bone marrow derived EPCs as immature cells that express CD133+ CD34+ VEGFR-2+ VE-cadherin- and note that the expression of different markers change during the migration and differentiation of EPCs from bone marrow in the process of tumor neovascularization. While bone marrow derived EPCs resemble stem cells more closely, the circulating EPCs are closer in phenotype to mature endothelial cells. de la Puente et al define early peripheral blood EPCs as cells that express CD133+/-CD34+ VEGFR-2+ CD31+ CD146+ VE-cadherin-, eNOS-, vWF- and late peripheral blood derived EPCs as CD133- CD34+ VEGFR-2+ CD31+ CD146+ VE-cadherin+, eNOS+, vWF+

Circulating endothelial progenitor cells as biomarkers for susceptibility to BPD

Several studies have described the relationship between the

frequency of circulating EPCs and the development of BPD in human infants or animal models, with results depending on the definition of EPCs and the methods used to characterize them. Relevant studies in the field of EPCs and BPD are summarized in Tables 1 and 2, with a focus on ECFCs and antigenically defined EPC respectively.

Endothelial colony forming cells (ECFCs) and BPD

Two studies have shown that ECFCs are decreased in cord blood samples of preterm infants who subsequently develop BPD as compared to preterm infants with mild or no BPD [5,23]. These assays were done using cord blood taken at birth before exposure of the infants to postnatal stress and before they developed BPD and so represent a predisposition to illness as opposed to a reaction to a biological challenge.

There is conflicting data regarding the proliferative capacity of ECFCs from CB as a function of gestational age. In one study, ECFC colony count was decreased in infants with decreasing gestational age [5]. In another study, Baker et al. found the opposite – preterm cord blood had a higher ECFC colony count and growth rate compared to term cord blood [23]. There are likely many variables that influence the frequency of circulating EPC beyond gestational age and postnatal age. In a separate study Baker et al. point out that ECFCs are not a homogenous population, but instead are a mixture of lineage committed endothelial progenitors with high proliferative potential [6].

Several studies have used *ex vivo* hyperoxia to measure the effect on ECFCs isolated from cord blood. These experiments use cell culture to model the high amounts of oxygen premature infants may

Cell Characterization	Results	Reference
ECFC	Decreased in CB from preterm infant with subsequent BPD	[5]
Late-outgrowth ECFC	Decreased in CB from preterm infants who later developed moderate or severe BPD compared to infants with mild or no BPD	[23]
ECFC	Colony count decreased in infants with decreasing gestational age	[5]
ECFC	Colony count increased in preterm cord blood compared to term cord blood	[6]
ECFC – Conditioned Media	Administration decreased RVH in a Bleomycin-injected newborn rats model for BPD.	[38]
ECFC	Decreased in preterm human CB exposed to hyperoxia <i>in vitro</i>	[6]
ECFC	Decreased in preterm human CB exposed to hyperoxia <i>in vitro</i> . Impaired expression of VEGFR-2+ and eNOS. Low NO production.	[31]

Table 1: Summary of studies involving Endothelial Progenitor Cells as Endothelial Colony Forming Cells.

Cell Characterization	Results	Reference
EPC as: CD45dim/-.Sca1+,CD133+,VEGFR-2+	Decreased in PB, BM, and lung of neonatal mice in hyperoxia model of BPD	[4]
EPC as: VEGFR-2+CD133+DiI-ac-LDL+UEA+	Autologous transplant improved endotoxin induced lung injury in rabbits	[33]
EPC as: CD34+CD45-	Rapidly decrease after birth in humans	[5]
EPC as: CD34+CD45-VEGFR-2+	No significant difference between term and preterm infants, PB samples	[8]
EPC as: CD34+CD45-VEGFR-2+	Persistently low for the first 3 weeks in the PB of preterm infant who later developed BPD	[8]
EPC as: CD34+KDR+,KDR+CD133+ and CD34+KDR+CD133+	Decreased at day 7 in infants later developing BPD, no difference at birth. Decreased day 7 in severe BPD	[18]
Ratio of angiogenic CPC (CD45-/dim CD34+ CD31+ AC133+) to non-angiogenic CPC (CD45-/dim CD34+ CD31+ AC133-)	Decreased in preterm human CB before BPD	[23]
EPC as: CD45-CD34+CD133+VEGFR-2+	Did not correlate with ECFCs in human preterm CB	[6]
EPC as: CD34+CD133+VEGFR-2+	Did not correlate with ECFCs in human preterm CB	[5]
EPC as: CD34+CD133+VEGFR-2+	Comparable in preterm human CB before BPD	[5]
CPC as: CD34+, CD133+, and CD34+CD133+ cells	Did not affect the risk of BPD in preterm human CB	[34]
EPC as: CD34+VEGFR-2+, CD133+VVEGFR-2+, and CD34+CD133+VEGFR-2+	Did not affect the risk of BPD in preterm human CB	[34]
Both Early EPC as: CD133+CD34+CD144+; and Late EPC as: CD133-CD34+CD144+	Increased in human preterm CB before BPD, not an independent predictor, related to low gestational age. Increased in preterm compared to full-term	[35]

Table 2: Summary of studies involving Endothelial Progenitor Cells by antigenic definition.

be exposed to postnatally. Preterm cord blood ECFCs had an increased susceptibility to hyperoxia compared with term ECFCs, as measured by impaired growth [6]. In another experiment studying ECFCs derived from preterm cord blood and exposed to hyperoxia, growth was similarly impaired, as was expression of VEGFR-2 and endothelial nitric oxide synthase (eNOS) as compared to preterm ECFCs kept in room air. Hyperoxia also lowered nitric oxide (NO) production and vascular endothelial growth factor (VEGF). NO rescued ECFC growth in this system [31]. These results suggest that interference in VEGF-NO signaling during hyperoxia may be a mechanism of impaired ECFC growth and part of the chemical response pathway of ECFCs to high oxygen resulting ultimately in the underdevelopment of lung vasculature.

Antigenically-defined EPCs and BPD

The role of EPCs, as defined by their antigenic profile, has been studied in several animal models of BPD, including hyperoxia models. In addition, observational studies have attempted to establish a relationship between circulating EPC in preterm human neonates and the risk of developing BPD.

High levels of oxygen or hyperoxia has been long recognized as being injurious to the lung and is used as an animal model of BPD. Balasubramaniam et al. showed that hyperoxia exposure decreased the number of circulating endothelial progenitor cells (CD45-Sca-1+CD133+VEGFR-2+) in the blood, bone marrow and lungs of neonatal mice. The authors surmised that since hyperoxia affects lung vascular and alveolar growth and since EPC are important for vascular growth, then hyperoxia might compromise EPC mobilization and homing to the lung. On the other hand, hyperoxia had an opposite effect in adult mice by increasing the numbers of EPCs in the bone marrow and lung, and did not alter the number of circulating EPCs. The authors attributed the reduction in lung vascular density and alveolar simplification to decreased vasculogenesis as a result of lower EPC levels in the neonate [4].

Whereas most sources of stem and progenitor cells that have been used to treat experimental models of lung injury have been allogeneic [32], a recent study by Cao et al. looked at the protective effect of autologous transplantation of EPC (7 day culture of peripheral blood lymphocytes (PBL), VEGFR-2+ CD133+, DiI-Ac-LDL positive, UEA positive cells) in a model of endotoxin induced lung injury in rabbits. The treated animals showed significant improvement in oxygenation and histological indices of lung injury, increased VEGF expression in lung tissue and decreased proinflammatory cytokines. The mechanism of action of the EPC is still not understood [33].

Subsets of human EPCs, as identified by their surface antigen markers, have been the focus of several studies that compared gestational age (term versus preterm), and postnatal age and attempted to determine whether certain subtypes could predict BPD outcome in a population of preterm infants at risk. For example, one study reported that the EPCs (CD34+CD45-) rapidly decreased after birth [5], and another study reported that cell frequencies of EPC, identified using the CD34+CD45-VEGFR-2+ phenotype, from preterm neonates compared to term infants were not influenced by gestational or postnatal age [8]. However, serial measurements of CD34+CD45-VEGFR-2+ made in the peripheral blood of preterm infants at 1, 7, 14, and 21 days of life, found persistently low or undetectable EPCs in infants who later developed BPD, compared to infants without BPD [8]. A newer study which defined EPC sub-types as CD34+KDR+, KDR+CD133+ and CD34+KDR+CD133+, found that they were lower in the blood of 7

day old infants who later developed BPD compared to those without BPD, with no difference at birth, suggesting the importance of disease development during the first week of life (KDR refers to VEGFR-2). Also, patients with the most severe BPD had lower EPC levels at day 7 than patients with less severe cases [18]. Yet another study reported that the ratio of the EPC subtype, angiogenic circulating progenitor cell (CPC) (CD45-dim CD34+ CD31+ AC133+) to non-angiogenic CPC (CD45-dim CD34+ CD31+ AC133-) was lower in cord blood samples from preterm infants who subsequently developed moderate or severe BPD compared to those who had mild or no BPD [23]. Because BPD is a lung disease of prematurity, differing gestational age and frequency of EPCs at birth and early life is relevant in all these studies.

Whereas antigenically-defined EPCs as described above are decreased in mice [4] and preterm infants [8] who develop BPD, there is a body of evidence that show no such correlation. In ECFC experiments, CD45-CD34+CD133+VEGFR-2+ [6], and CD34+CD133+VEGFR-2+ [5] cell numbers from cord blood did not correlate with the clonogenicity of ECFCs, nor with the development of BPD. Other EPC phenotypes that also showed no predictive association with BPD were "CPCs" (CD34+, CD133+, and CD34+CD133+) "EPCs" (CD34+VEGFR-2+, CD133+VEGFR-2+, and CD34+CD133+VEGFR-2+) [34], and both "Early EPC" (CD133+CD34+CD144+) and "Late EPC" (CD133-CD34+CD144+) [35]. The antigenic phenotype used to describe EPC cells, therefore, does not always reflect the biological function of a putative EPC population and relate to the presence of the disease state in a useful way.

The role of bone marrow derived cell types and soluble factors in lung injury and BPD

More recently, there is increasing evidence that the blood and bone marrow-derived stem cells exert a beneficial effect in models of chronic lung injury, not so much by engraftment and differentiation, but by a paracrine effect on the existing lung progenitor cells [36].

The Yamada group was among the first to show the importance of bone marrow-derived progenitor cells (BMPC) in lung repair in a LPS-induced model of lung injury. BMPCs were found to mobilize from the bone marrow, participate in lung repair and to be incorporated into the alveolar walls as epithelial and endothelial cells [37]. However the question of whether this was a result of cell fusion with the existing parenchyma or due to cell differentiation was never resolved.

It has long been debated whether the EPC affect vascular repair by engraftment in the injured area and proliferation or through a paracrine effect or both. Studies using both term and preterm ECFC conditioned medium (ECFC-CM) from cord blood have shown that the conditioned medium contained soluble factors that promote the growth of fetal sheep pulmonary artery endothelial cells, fetal alveolar type 2 cells and angiogenesis in vitro [38].

When bleomycin treated newborn rats (showing impaired vascular and alveolar growth and secondary pulmonary hypertension, but no pulmonary fibrosis) were used as an experimental model of BPD, ECFC-CM reduced right ventricular hypertrophy (RVH), but did not increase alveolar septation [38]. It has long been debated whether the EPC affect vascular repair by engraftment in the injured area. Undeniably, the ECFC conditioned medium may contain therapeutic soluble factors and thus have a paracrine or autocrine effect.

A novel murine myeloid angiogenic bone marrow-derived progenitor (BMDAC) (Tie2+ CD45+ CD34- CD38+ VEGFR-2- cKit + CXCR4+ CD44+ CD11b- Sca1- CD133- F4/480+ Gr1- CX3CR1-),

when injected into the pulmonary circulation, has been shown to restore alveolar and vascular structure in hyperoxia exposed neonatal mice. While these cells engrafted close to alveolar epithelial progenitor type 2 (AT2) cells, they did not differentiate into AT2 cells. The study concluded that the therapeutic effect of these cells was possibly due to a paracrine effect [39].

Mesenchymal stem cells (MSCs) are adult stem cells that are found in the bone marrow [40], cord blood [41] and peripheral blood of human infants with severe acute life threatening respiratory failure who require extracorporeal life support [42]. Intra-pulmonary administration of bone marrow MSCs noticeably decrease inflammatory responses and improve survival in an endotoxin model of lung injury in mice [43]. Co-culture experiments with alveolar macrophages further showed that the modulatory effect of MSC on inflammation is paracrine, and is not cell contact dependent [43]. In a chronic hyperoxia induced BPD model in newborn rats, Van Haaften et al. show that intratracheally delivered MSC have a protective effect on the lungs. While alveolar and vascular injury was minimized, engraftment levels of the intratracheally delivered MSCs were sufficiently low to suggest that the protective effects are a result of paracrine mechanisms [44].

In a similar hyperoxia-induced BPD model in newborn rats, Pierro et al. found that airway delivery of MSCs isolated from cord blood successfully prevented and reversed arrested alveolar growth. In keeping with the findings of other investigators they also found that paracrine mechanisms are likely to be responsible, since cell-free conditioned media from human umbilical cord blood MSCs and pericytes also had a therapeutic effect on tissue repair [41].

Cord blood mononuclear cells were tested for their therapeutic potential in a double-hit model of BPD in neonatal mice. Cord blood MNC's did not offer as much of a protective effect as MSCs, but did improve lung structure and normalized *Mtor* expression to levels seen in the normoxic controls [45]. Studies by Fielhaber et al. suggest that inhibition of mTOR increases proapoptotic responses in a lipopolysaccharide-induced lung injury model of BPD in mice [46].

The evidence of repair of alveolar and vascular structure in the experimental models of BPD by exogenous administration of blood and bone marrow-derived cells and conditioned media derived from these cell types is exciting proof of principle that diverse cell types can have a beneficial effect.

Concluding remarks

The term "EPC" has been used to refer to diverse populations of cells involved in normal function and diseased states of the vasculature. EPC are defined by their surface markers as well as clonogenic and functional properties. Whereas many experiments refer to EPCs in common, each group of researchers chooses how to define the term EPCs in any given study and these frequently do not share an exact definition with other experiments. Therefore, these experiments actually describe non-equivalent cells. EPCs share in common their ability to contribute to angiogenesis and the formation of vasculature from existing blood vessels. However, only late EPCs are truly vasculogenic and capable of forming blood vessels *de novo*, indicating that the term "EPCs" does not describe a homogeneous group of cells. While several studies have suggested that ECFCs and some antigenic phenotypes of EPC may be biomarkers for BPD risk in preterm infants and have implicated them in animal models of chronic lung disease, there are also other antigenically defined EPC phenotypes that show no relationship to BPD. This variability is likely attributable to differing definitions of

EPC. The protective effect of bone marrow-derived progenitor cells in lung injury and vascular repair may result from paracrine effects, more than actual cell engraftment and differentiation.

This review reflects the limited scope of knowledge about EPC in newborn infants and in animal models by different groups. More studies are needed to fully define and characterize different cell populations and their contribution to BPD.

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