Reduced platelet-derived growth factor receptor expression is a primary feature of human bronchopulmonary dysplasia

Antonia P. Popova,1 J. Kelley Bentley,1 Tracy X. Cui,1 Michelle N. Richardson,1 Marisa J. Linn,1 Jing Lei,1 Qiang Chen,1 Adam M. Goldsmith,1 Gloria S. Pryhuber,3 and Marc B. Hershenson1,2

1Department of Pediatrics and Communicable Diseases, University of Michigan, Ann Arbor, Michigan; 2Department of Molecular and Integrative Physiology, University of Michigan, Ann Arbor, Michigan; and 3Department of Pediatrics, University of Rochester, Rochester, New York

Submitted 25 November 2013; accepted in final form 3 June 2014

Popova AP, Bentley JK, Cui TX, Richardson MN, Linn MJ, Lei J, Chen Q, Goldsmith AM, Pryhuber GS, Hershenson MB. Reduced platelet-derived growth factor receptor expression is a primary feature of human bronchopulmonary dysplasia. Am J Physiol Lung Cell Mol Physiol 307: L231–L239, 2014. First published June 6, 2014; doi:10.1152/ajplung.00342.2013.—Animal studies have shown that platelet-derived growth factor (PDGF) signaling is required for normal alveolarization. Changes in PDGF receptor (PDGFR) expression in infants with bronchopulmonary dysplasia (BPD), a disease of hypoaalveolarization, have not been examined. We hypothesized that PDGFR expression is reduced in neonatal lung mesenchymal stromal cells (MSCs) from infants who develop BPD. MSCs from tracheal aspirates of premature infants requiring mechanical ventilation in the first week of life were studied. MSC migration was assessed in a Boyden chamber. Human lung tissue was obtained from the University of Rochester Neonatal Lung Biorepository. Neonatal mice were exposed to air or 75% oxygen for 14 days. PDGFR expression was quantified by qPCR, immunoblotting, and stereology. MSCs were isolated from infants who develop BPD showed lower PDGFR-α and PDGFR-β mRNA and protein expression and decreased migration to PDGF isoforms. Lungs from infants dying with BPD show thickened alveolar walls and paucity of PDGFR-α-positive cells in the dysmorphic alveolar septa. Similarly, lungs from hyperoxia-exposed neonatal mice showed lower expression of PDGFR-α and PDGFR-β, with significant reductions in the volume of PDGFR-α-positive alveolar tips. In conclusion, MSCs from infants who develop BPD hold stable alterations in PDGFR gene expression that favor hypoaalveolarization. These data demonstrate that defective PDGFR signaling is a primary feature of human BPD.

OVER 25% OF PREMATURE INFANTS with birth weight less than 1,500 g develop bronchopulmonary dysplasia (BPD) (24). This translates to over 10,000 new cases of BPD annually (29). Survivors of BPD have abnormal lung structure and function even as adults (10, 15, 40), making BPD a leading cause of pediatric lung disease. The lungs of infants with BPD demonstrate fewer and larger alveoli, as well as poorly formed secondary crests (23), indicating interference with the normal ingrowth of secondary septa into larger precursor sacculles. Furthermore, alveolar septa are thickened with collagen and elastin-producing myofibroblasts appear in the alveolar septa as early as 4 days after birth (38). Within days to weeks, numerous myofibroblasts feature intense immunoreactivity for transforming growth factor (TGF)-β, a stimulus for myofibroblastic differentiation (38). Together, these results indicate that BPD may result in part from the abnormal migration and differentiation of mesenchymal progenitor cells within the interstitia of the terminal air spaces.

PDGFR isoforms (PDGF-AA, PDGF-BB, PDGF-AB) stimulate migration and proliferation of lung fibroblasts in vitro (6, 31). PDGF binds to two high-affinity receptors: an α-receptor that binds both A- and B-chains and a β-receptor with high affinity for PDGF-B chains (18). In the saccular and alveolar stage of lung development, Pdgfra-expressing cells migrate to the tips of secondary alveolar septa and differentiate into α-smooth muscle actin- and elastin-producing myofibroblasts required for alveogenesis (7, 8, 26, 30, 32, 33, 41). Pdgfra-deficient mice surviving beyond birth fail to form alveoli owing to the failure of PDGFR-α mesenchymal progenitor cells to migrate distally to the site of secondary crest formation (8). PDGF-B- and Pdgfrα-deficient embryos show reduced recruitment and proliferation of vascular smooth muscle cells and pericytes during blood vessel formation (19).

We have isolated mesenchymal stromal cells (MSCs) from tracheal aspirates of premature infants with respiratory distress (20). Using array-based analysis, we have shown that unstimulated MSCs show a gene expression pattern associated with alveolar septal fibroblasts (36). Isolation and characterization of neonatal lung MSCs therefore provides the opportunity to examine gene expression patterns of alveolar mesenchymal cells ex vivo.

In this study, we hypothesized that there are stable differences in the expression of PDGFR-α and -β between neonatal lung MSCs from infants who develop BPD and cells from infants who do not develop this disease. We also hypothesized that similar differences would be found in the lungs from infants with BPD, as well as the lungs of hyperoxia-exposed neonatal mice, a model of BPD.

METHODS

Patients. We examined tracheal aspirates from infants admitted to the Newborn Intensive Care Unit, as approved by the Institutional Review Board of the University of Michigan Medical School. Entry criteria included gestational age at birth ≤32 wk, mechanical ventilation for respiratory distress, and age ≤7 days.

Ethics statement. The animal study was performed in strict accordance with the NIH Guide for the Care and Use of Laboratory Animals recommendations. The protocol was approved by the University of Michigan Committee on Use and Care of Animals.
Cell culture. Neonatal lung MSCs were isolated as described previously (20, 36). Unstimulated passage two or three MSCs were plated for 24 h in 10% fetal bovine serum, then serum starved for 2 h prior to harvesting.

Animal model. Two- 3-day-old C57BL/6J mice (Jackson Laboratories, Bar Harbor, ME) were exposed to air or 75% oxygen for 14 days by use of a polypropylene chamber coupled to an oxygen controller and sensor (BioSpherix, Lacona, NY) (35).

Quantitative real-time PCR. MSC mRNA was extracted with RNeasy Plus Mini Kit (Qiagen, Valencia, CA). Mouse whole-lung RNA was prepared by use of TRizol (Invitrogen, Carlsbad, CA). Gene expression of human and mouse PDGFRα (Santa Cruz Biotechnology, Santa Cruz, CA) and PDGFR-β (Cell Signaling, Danvers, MA).

Immunoblotting. Lysates were resolved by SDS-PAGE, and transferred to a nitrocellulose membrane. Membranes were blocked in 5% milk for 1 h in room temperature and probed with antibodies against PDGFRα (Santa Cruz Biotechnology, Santa Cruz, CA) and PDGFR-β (Cell Signaling, Danvers, MA).

Immunohistochemistry of mouse lung tissue. Lungs were perfused with 5 mM EDTA, inflated to 30 cmH2O pressure with 4% paraformaldehyde, and fixed in formalin overnight. Slides were probed with anti-PDGFRα (Santa Cruz Biotechnology, Santa Cruz, CA) and stained with a biotinylated anti-rabbit IgG-avidin horseradish peroxidase and diaminobenzidine detection system (Vector Labs, Burlingame, CA). Immunoadsorption of the PDGFRα antibody was carried out before incubating the anti-PDGFRα antibody at a 1:2,000 dilution with five times the concentration of PDGFRα blocking peptide (Santa Cruz Biotechnology, sc-338 P) for 1 h with mixing at room temperature.

Mouse lung tissue preparation for fluorescence microscopy. Lungs were harvested as described above and placed in cassettes in random orientation to allow the systematic uniform random sampling required for stereological morphometry (14). From a random start, paraffin-embedded lungs were sectioned every 250 µm, and 5-µm-thick sections were probed with CY3-labeled mouse anti-smooth muscle actin antibody (clone 1A4, Sigma-Aldrich), AlexaFluor 488-conjugated rabbit anti-PDGFRα antibody (Santa Cruz Biotechnology), or isotype control antibodies. Nuclei were visualized with Hoechst 33342 (Sigma-Aldrich).

Stereological morphometry of mouse alveolar tip volumes. To quantify the volume of alveolar tips expressing PDGFRα, we combined fluorescence microscopy and stereological methods. The tips of secondary alveolar crests were identified by expression of α-smooth muscle actin. For each experimental section, rabbit IgG- and mouse IgG-stained slides were used to set the background staining to 0.

Quantitative real-time PCR. Primers used for quantitative real-time PCR are listed in Table 1. Relative gene expression was determined by use of a polypropylene chamber coupled to an oxygen controller and sensor (BioSpherix, Lacona, NY) (35).

Immunohistochemistry of mouse lung tissue sections. Tissue was fixed for paraffin sectioning, with the first cut at a random starting point followed by 8–12 systematic-uniform slices at 500-µm intervals thereafter. To determine reference volume initially, we superimposed low-power (×5) images of each section at 500 µm onto a 0.645-cm2 grid (NIH ImageJ, Bethesda, MD) to determine total sectional area by the Cavalieri point-counting method (14). The Cavalieri lung volume, Vref, was calculated as Vref = \( \sum A \times t \times K \), where \( \sum A \) is the sum of the reference area on each section, t is the tissue thickness, and K is the sampling interval. Randomly chosen fields of each systematically cut 5-µm-thick tissue section were photographed at ×200. A systematic randomly oriented 8 × 10-point crossgrid (NIH ImageJ) was superimposed on each image, and both the total number of points falling on alveolar tips and the number of points falling on PDGFRα-containing alveolar tips were counted. The fractional object volume for the whole lung, Vobj, was calculated as Vobj = \( \sum (Po)/\sum(Pref) \) × Vref, where \( \sum (Po) \) is the sum of the points counted in a lung, \( \sum(Pref) \) is the sum of all reference points counted in a systematic sectioning of the lung, and Vref is the reference volume. Given the assumption that the proportion PDGFRα (+) tips is uniform through the lung, and since \( Vv = Vobj/Vref \), the volume density, Vv, was calculated as the sum of the points in the reference space, i.e., total points landing on PDGFRα (+) tips, divided by the total number of points counted (3). To calculate the total volume of PDGFRα-positive alveolar tips, we multiplied Vv by lung volume, as obtained by the water displacement method. Fifty-eight slides from six hyperoxia-exposed animals and 40 slides from five air-exposed animals were examined.

Immunohistochemistry of lung tissue sections from infants with BPD. Human lung tissue sections were obtained from the University of Rochester Lung Biorepository under a protocol approved by the Institutional Review Board of Strong Memorial Hospital (Rochester, NY). Specimens were obtained from infants who died in the intensive care nursery. The diagnosis of BPD was based on premature delivery, need for chronic respiratory support, requirement for supplemental oxygen after 36 wk gestation, chest radiographs, and pathological tissue diagnosis at autopsy. Specimens were also obtained from infants succumbing to nonpulmonary disorders. Samples were processed within 6 h of death. These specimens were not suitable for stereological analysis and therefore were used for comparative histology and immunohistochemistry. Immunohistochemistry was performed as described above, using anti-PDGFRα antibody.

Chemotaxis assay. Migration of cells to PDGF-AA (PeproTech, Rocky Hill, NY, cat. no. 100-13A) (30 ng/ml), PDGF-BB (PeproTech, cat. no. 100-14B) (10 ng/ml) or serum-free medium, as control, was assessed after 4 h incubation in a 12-well Boyden chamber (Neuroprobe, Gaithersburg, MD) by use of an 8-µm pore size membrane. For each experiment, MSCs from three patients who developed BPD and three who did not were used. Membranes were stained (toluidine blue stain) and cells on the distal side of the membrane were counted. Four random high-power fields (×200) for each sample and condition were examined. Chemotaxis index was calculated as the number of MSCs migrating toward the growth factor divided by the number of MSCs migrating toward control serum-free medium.

Statistical analysis. All data were described as means ± SE or median and interquartile range. An unpaired t-test or Mann-Whitney

Table 1. Primers used for quantitative real-time PCR

<table>
<thead>
<tr>
<th>Gene Name</th>
<th>Forward Primer (5′-3′)</th>
<th>Reverse Primer (5′-3′)</th>
</tr>
</thead>
<tbody>
<tr>
<td>hPDGFRα</td>
<td>GCTCCTCGAGACGCACTTCA</td>
<td>GACCTTGGAGGACATCTTGAT</td>
</tr>
<tr>
<td>hPDGFRB</td>
<td>GTGCTCAGCTGTCGATAGGCT</td>
<td>ACTGAATACGAGCTGTCTCT</td>
</tr>
<tr>
<td>hKi-67</td>
<td>AGAGATCTGTCGATAGGCT</td>
<td>GGTGAGGAGGAGGAGGAGGAG</td>
</tr>
<tr>
<td>hGAPDH</td>
<td>CGACACCTTTTGGCACAGCTC</td>
<td>AGCGACCTGATCTTACGAT</td>
</tr>
<tr>
<td>mPDGFRα</td>
<td>TGGAGTTGCTTCACGGATCTCAG</td>
<td>AAGCCACCTCCATATACGAGGTTGGAG</td>
</tr>
<tr>
<td>mPDGFRB</td>
<td>ACTGATCTCGAGAAAGGACGAGCT</td>
<td>TGTGAGACCTGATCTTACGAGGAGGAG</td>
</tr>
<tr>
<td>mGAPDH</td>
<td>TGGAGTTGCTTCACGGATCTCAG</td>
<td>AAGCCACCTCCATATACGAGGTTGGAG</td>
</tr>
</tbody>
</table>

L.232 PLATELET-DERIVED GROWTH FACTOR RECEPTOR EXPRESSION IN BPD
were immunostained after immunoadsorption of the primary antibody with an antigenic blocking peptide. Figure 2D demonstrates that PDGFR-α-positive staining at the tips of secondary crests is abolished following immunoadsorption with blocking peptide.

**Neonatal lung MSCs from infants who develop BPD show decreased migration to PDGF.** To further investigate the functional effect of decreased PDGFR expression, we assessed migration of the cells to PDGF-AA (30 ng/ml) and PDGF-BB (10 ng/ml) using a Boyden chamber. Compared with cells from infants not developing BPD, MSCs from infants developing BPD showed decreased migration to both PDGF-AA and PDGF-BB (Fig. 3, A–C).

**Effect of TGF-β1 treatment on MSC PDGFR-α expression.** Overexpression of TGF-β is sufficient for development of a BPD phenotype in neonatal mice, including proliferation of α-actin-positive cells within the alveolar septal walls and hypoaeroveolarization (17, 39). To examine the effects of TGF-β1 on PDGFR-α expression in vitro, we treated neonatal lung MSCs with TGF-β1 (1 ng/ml) in serum-free medium for 48 h. In contrast to MSCs from infants who do not develop BPD, MSCs from infants who develop BPD showed PDGFR-α downregulation in response to TGF-β1 (Fig. 4A). In addition, we examined the effects of TGF-β1 on MSC expression of the cell proliferation marker, Ki67. Following treatment with TGF-β1 (1 ng/ml), Ki67 was equally expressed in MSCs from infants who develop BPD and MSCs from infants who do not develop disease (Fig. 4B). These data suggest that the differences in PDGFR-α expression following treatment with TGF-β1 are not related to differences in proliferation.

**Lungs from hyperoxia-exposed neonatal mice show significantly lower expression of PDGFR.** We examined PDGFR-α and PDGFR-β expression in hyperoxia-exposed neonatal mice, a model of BPD-like hypoaeroveolarization. Two-to 3-day-old C57BL/6J mice were exposed to air or 75% oxygen for 14 days. Total lung mRNA and protein lysates were harvested. Compared with lungs from air-exposed mice, hyperoxic exposure caused decreased mRNA (Fig. 5A) and protein (Fig. 5B) expression of PDGFR-α and PDGFR-β.

To further define the effects of hyperoxia on PDGFR-α expressing cells in the distal lung, we again exposed 2- to 3-day-old mice to 14 days of 75% oxygen or air. Mouse lungs were harvested for morphology as described above. Compared with air exposure (Fig. 6, A–C), lungs from hyperoxia-exposed mice showed fewer and larger air spaces and thickened alveolar walls (Fig. 6, D–F). Hyperoxic exposure also decreased PDGFR-α immunoreactivity at the tips of secondary crests in

---

**RESULTS**

**Patient data.** Lung MSCs from 25 neonates were used in this study. Thirteen of the 25 babies (52%) required supplemental oxygen at 36 wk, a clinical definition of BPD (16, 24). Characteristics of the patients are described in Table 2. Gestational age and birth weight were significantly lower, and the number of surfactant doses, day of sampling, days requiring mechanical ventilation, and days on oxygen were significantly higher in the infants who developed BPD.

**Neonatal lung MSCs from infants who develop BPD show lower mRNA and protein expression of PDGFR-α and PDGF-β.** We examined MSCs from 13 infants who developed BPD and MSCs from 12 infants who did not develop this disease. RT-PCR analysis demonstrated that MSCs from infants who develop BPD showed lower mRNA expression of PDGFR-α (Fig. 1A) and PDGFR-β (Fig. 1B). Immunoblots confirmed reduced PDGFR-α (Fig. 1, C and E) and PDGFR-β (Fig. 1, D and F) protein expression in MSCs from infants who developed BPD. We also examined the relationship between PDGFR-α and PDGFR-β mRNA expression and gestational age at birth. For the same gestational age PDGFR-α mRNA expression (Fig. 1G) was lower in MSCs from infants who develop BPD. A similar relationship was observed for PDGFR-β mRNA expression (Fig. 1H). Finally, we measured PDGF-AA and PDGF-BB levels in tracheal aspirate fluid. There was no difference in PDGF concentration between aspirates from babies who developed BPD compared with aspirates from babies who did not develop BPD (PDGF-AA, 114 ± 73 vs. 85 ± 46 pg/ml, means ± SD; PDGF-BB, 142 ± 71 vs. 227 ± 278 pg/ml, means ± SD).

**Lungs of infants with BPD show a paucity of PDGFR-α-positive mesenchymal cells in thickened dysmorphic alveolar walls.** Lung sections from age matched full-term infants (Fig. 2A) were immunostained and compared with lungs from infants dying of BPD (Fig. 2B). Immunostaining of the full-term infant lung showed PDGFR-α-positive cells at the tips of the alveolar septa. Immunostaining of lungs from infants dying of BPD showed distorted lung architecture with thickened alveolar walls, fewer and poorly formed alveolar secondary crests, and reduced PDGFR-α content at the alveolar septal tips. To confirm the specificity of the PDGFR-α-positive staining, lung sections from mice exposed to room air or hyperoxia were immunostained after immunoadsorption of the primary

---

**Table 2. Characteristics of patients with neonatal lung MSCs isolated from tracheal aspirates, used for the in vitro studies**

<table>
<thead>
<tr>
<th></th>
<th>BPD</th>
<th>Non-BPD</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>13</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Male gender, n (% of total)</td>
<td>9 (69%)</td>
<td>8 (67%)</td>
<td>1.00 (Fisher’s exact test)</td>
</tr>
<tr>
<td>Gestational age, mean ± SD, wk</td>
<td>26.3 ± 2.1</td>
<td>29.3 ± 1.9</td>
<td>&lt;0.005 ( t-test)</td>
</tr>
<tr>
<td>Birth weight, mean ± SD, g</td>
<td>1893 ± 23</td>
<td>1303 ± 26</td>
<td>&lt;0.001 ( t-test)</td>
</tr>
<tr>
<td>Surfactant dose, mean ± SD</td>
<td>2.4 ± 0.7</td>
<td>1.3 ± 0.5</td>
<td>&lt;0.0001 ( t-test)</td>
</tr>
<tr>
<td>Suspected chorioamnionitis, n (% of total)</td>
<td>3 (23%)</td>
<td>2 (17%)</td>
<td>1.00 (Fisher’s exact test)</td>
</tr>
<tr>
<td>Vent days, mean ± SD, days</td>
<td>35 ± 22</td>
<td>5 ± 4</td>
<td>&lt;0.0001 ( t-test)</td>
</tr>
<tr>
<td>O2 days, median (IQR)</td>
<td>188 (113)</td>
<td>27 (33)</td>
<td>&lt;0.0001 (Mann-Whitney test)</td>
</tr>
<tr>
<td>Died, n (% of total)</td>
<td>1 (8%)</td>
<td>0</td>
<td>1.00 (Fisher’s exact test)</td>
</tr>
<tr>
<td>Day of sampling, median (IQR)</td>
<td>4 ± 3</td>
<td>1 ± 1</td>
<td>&lt;0.01 (Mann-Whitney test)</td>
</tr>
</tbody>
</table>

MSCs, mesenchymal stromal cells; BPD, bronchopulmonary dysplasia; IQR, interquartile range.
the alveolar septa, as identified by α-smooth muscle actin. Following hyperoxia exposure, there was a 2.7-fold decrease in the Vv of total alveolar tips and an 11-fold decrease in Vv of tips positive for PDGFR-α (Table 3). Despite significant increases in lung volume, hyperoxia-exposed lungs showed a 9-fold decrease in the PDGFR-α (h11001) alveolar tips volume per lung and a 3.4-fold decrease in the total volume of PDGFR-α-positive alveolar tips normalized to the total volume of alveolar tips per lung.

DISCUSSION

PDGF is a mesenchymal cell mitogen and chemoattractant (6, 7, 19, 26, 30, 31). During the pseudoglandular stage of lung development, PDGF-A is expressed by the branching lung epithelium, whereas PDGFR-α is expressed in the surrounding mesenchyme (8, 26). The requirement of PDGF for alveolarization has been established in animal studies (28). PDGF-A released from the growing bronchial epithelium promotes proliferation and disaggregation of PDGFR-α-positive progenitor cells, leading to their distal spread along the epithelial basement membrane to the tips of secondary alveolar septa. These cells then differentiate into α-smooth muscle actin- and elastin-producing myofibroblasts required for alveogenesis (8, 26, 30, 32, 33, 41). PDGF-A-deficient mice develop lung hypoalveolarization secondary to the loss of alveolar myofibroblasts and failure of alveolar septation (8, 26). Neonatal rats injected daily with the PDGF tyrosine kinase antagonist imatinib mesylate from day 1–7 of life show impaired alveologenesis as reflected by a decrease in secondary crests, increase in alveolar size, and decrease in alveolar number (25). Analogous to rodents undergoing PDGF-A blockade, mice undergoing hyperoxic exposure show lungs with arrested alveolar development that show a paucity of α-actin-positive myofibroblasts at the septal tips (21). Similarly, the lung pathology of surviving premature infants with BPD is characterized by fewer and larger alveoli, as well as poorly formed secondary crests, indicating interference with septation (23). However, changes in PDGFR-α expression in lung mesenchymal stromal cells (MSCs) from infants who develop bronchopulmonary dysplasia (BPD) show lower mRNA and protein expression of PDGFR-α and PDGFR-β. mRNA expression was assessed by RT-PCR and protein expression was assessed by immunoblotting. Unstimulated MSCs from infants who developed BPD showed significantly lower mRNA expression of PDGFRα (A) and PDGFRβ (B) (N = 12–13 for each group, *P < 0.05, Mann-Whitney test). Representative immunoblots confirm decreased protein expression of PDGFR-α (170 kDa, C) and PDGFR-β (190 kDa, D). MSCs from 3 infants who developed BPD and 3 infants who did not develop BPD are shown. E and F: densitometry analysis group mean data (N = 11–12 for each group, *P < 0.05, unpaired t-test). The relationships between PDGFRα (G) and PDGFRβ mRNA expression (H) and gestational age demonstrate that, for the same gestational age, PDGFRα and PDGFRβ mRNA expression is lower in MSCs from infants who develop BPD.
expression in infants with BPD, a disease of hypoalveolarization, have not previously been examined.

In this study, we examined the PDGFR expression of MSCs isolated from the tracheal aspirates of 25 premature infants with respiratory distress. These cells demonstrate a gene expression pattern related to alveolar septal fibroblasts (36) and offer a window on examining processes occurring in mesenchymal cells of the distal lung. We found that lung MSCs from premature infants who develop BPD expressed lower levels of PDGFR-α. The lower levels of PDGFR-α were not related to gestational age. In addition, MSCs from infants who developed BPD showed reduced migration to PDGF in vitro compared with cells isolated from infants who did not develop this disease. Thus MSCs from infants developing BPD appeared to be functionally distinct from control cells. Finally, tissue from infants with BPD showed similar reductions in PDGFR-α staining. Together, these data show that MSCs from infants developing BPD hold stable alterations in PDGFR-α gene expression that favor reduced alveolarization. These data demonstrate for the first time that defective PDGFR-α signaling is a primary feature of human BPD.

PDGF-B and its receptor PDGFR-β play a role in recruitment of vascular smooth muscle cells and pericytes during blood vessel formation (19). Given the importance of angiogenesis in the development of mature alveoli (37), one might expect a correlation between alveolarization and

Fig. 2. Lungs of infants dying with BPD show a paucity of PDGFR-α-positive mesenchymal cells in the thickened alveolar walls. Compared with the lung of an age matched full-term infant dying of a nonpulmonary cause (A and C), immunostained lung sections from infants dying with BPD (B) show widened alveolar spaces and thickened alveolar walls with fewer alveolar secondary crests and a paucity of brown-staining PDGFR-α-positive cells at the tips of the dysmorphic alveolar septa (original magnification, ×400; insets, ×1,000 magnification). C shows normal lung stained with a control antibody. In D, lungs from air-exposed mice show brown PDGFR-α staining at the tips of secondary crests. The lungs of hyperoxia-exposed mice show reduced number of PDGFR-α-positive tips. Staining is decreased when lung sections were probed in the presence of antigenic peptide. (Original magnification, ×400).
PDGFR-β in the lungs of infants with BPD. Indeed, we found reduced PDGFR-β mRNA and protein expression in MSCs from infants developing BPD. During early postnatal lung growth in rats, neutralizing antibodies to either PDGF-B or a truncated soluble PDGFR-β each cause a significant reduction of DNA synthesis (9). Together, these results suggest that reduced PDGFR-β contributes to hypoalveolarization in BPD.

One important question is whether reduced PDGFR expression in MSCs from infants with BPD is related to the maturity of the infants studied. In the cohort studied, premature infants developing BPD tended to have a lower gestational age at birth and lower birthweight than premature infants who did not develop BPD. To test this, we examined the relationship...
between gestational age at birth and PDGF mRNA expression, normalized to GAPDH. For the same gestational age, PDGFRα and PDGFRβ mRNA expression tended to be lower in MSCs from infants who developed BPD, consistent with the notion that reduced expression was indeed defective in BPD. On the other hand, there appeared to be a positive relationship between gestational age and PDGFRα, but not PDGFRβ, expression, suggesting that PDGFR expression increases with fetal maturation. This being the case, the later day of sampling in BPD infants should not have contributed to the observed reduction in PDGFR expression. Also, we found no differences in tracheal aspirate PDGF-AA and PDGF-BB levels between aspirates from babies who develop BPD and aspirates from babies who do not develop BPD. Our results for PDGF-BB confirm a previous report showing no significant association between BPD and bronchoalveolar lavage fluid concentration of PDGF-BB in premature infants (2) and are consistent with the notion that differences in PDGFR expression appear to be the primary PDGF pathway defect associated with the development of BPD.

To further characterize this process in vivo, we examined the lungs of neonatal mice exposed to hyperoxia, which show arrested alveolar development and thickened alveolar walls analogous to BPD. Hyperoxic exposure was associated with decreased lung mRNA and protein expression of PDGFR-α. Previous studies examining the effects of hyperoxia on lung mRNA and protein expression have showed mixed results. Hyperoxia-exposed neonatal rats show increased PDGFR-α mRNA expression but decreased protein expression (9). Furthermore, in normoxic mice, PDGFR-α protein was localized primarily to the airway epithelium. In piglets, hyperoxia has no effect on the protein expression of PDGFR-α in the airway epithelium or alveolar septum. More recently, it was shown that mechanical ventilation of preterm lambs reduced lung PDGFR-α mRNA and protein expression in the distal lung (5). However, no studies have focused on PDGFR-α expression at the tips of secondary alveolar septa, which is required for alveogenesis. We therefore performed immunofluorescent staining and stereological analysis on neonatal mice undergoing hyperoxic exposure. We found that hyperoxia decreased the volume fraction of PDGFR-α-expressing cells located at

Table 3. **Hyperoxia exposure decreases the total volume of PDGFR-α positive cells at the alveolar tips**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Room Air</th>
<th>Hyperoxia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung volume, ml</td>
<td>0.25 ± 0.02</td>
<td>0.48 ± 0.03*</td>
</tr>
<tr>
<td>Alveolar tips Vv</td>
<td>0.040 ± 0.0065</td>
<td>0.015 ± 0.0024†</td>
</tr>
<tr>
<td>Alveolar tips volume per lung, ml</td>
<td>0.010 ± 0.0022</td>
<td>0.007 ± 0.0014</td>
</tr>
<tr>
<td>PDGFR-α(+) alveolar tips Vv</td>
<td>0.033 ± 0.0055</td>
<td>0.003 ± 0.0003*</td>
</tr>
<tr>
<td>PDGFR-α(+) alveolar tips volume per lung, ml</td>
<td>0.009 ± 0.0019</td>
<td>0.001 ± 0.0001†</td>
</tr>
<tr>
<td>PDGFR-α(+) alveolar tips volume/ alveolar tips volume per lung, ml</td>
<td>0.819 ± 0.0390</td>
<td>0.238 ± 0.0672*</td>
</tr>
</tbody>
</table>

Vv, volume density. Values are means ± SE; n = 5 for Room Air and n = 6 for Hyperoxia condition; different from Room Air group, *P < 0.001, †P < 0.01, unpaired t-test.
the tips of secondary alveolar crests, a condition that would favor reduced alveolarization.

Previously, we demonstrated that, compared with MSCs from infants not developing BPD, MSCs from infants developing BPD show higher phospho-glycogen synthase kinase-3β, β-catenin, and α-actin content (35), indicative of advanced myofibroblastic differentiation. We now show that MSCs from infants developing BPD express lower levels of PDGFR-α. One possible common factor in the development of this phenotype from a less differentiated mesenchymal progenitor cell may be exposure to TGF-β. Overexpression of TGF-β in newborn rodent lungs induces changes consistent with BPD, including hypoalveolarization (17, 39). We have previously shown that TGF-β1 induces neonatal lung MSC myofibroblastic differentiation (35). Finally, TGF-β has been shown to downregulate PDGFR expression in other lung mesenchymal cells including lung fibroblasts (27). In the present study, we found that low-dose TGF-β1 reduced PDGFR-α expression only in MSCs from infants who develop BPD. TGF-β1 did not affect the expression of the cell proliferation marker Ki67, suggesting that the reduced expression of PDGFR-α in MSCs from infants who developed BPD is not due to antimitogenic effects of TGF-β.

There are some limitations to our study. First, examining the components of tracheal aspirates may not provide an accurate picture of processes in the distal lung leading to the development of BPD. However, studies have shown neonatal tracheal aspirate fluid to have equal validity to bronchoalveolar lavage in the estimation of disaturated phosphatidylcholine (12) and IL-8 levels (11), and therefore these aspirates may reflect a practical alternative for obtaining lung fluid specimens. Second, it is possible that the lung MSCs isolated from infants who develop BPD represent a different population of cells that are recruited to the airway lumen, therefore explaining differences in PDGFR expression. Third, hyperoxic exposure of neonatal mice does not replicate the circumstances leading to human BPD. However, the lungs of neonatal mice and premature infants share similar developmental features. At birth, rodents exhibit a saccullar stage of lung development and alveolarization proceeds on the first 2 postnatal weeks (1). In the human lung, sacculles appear by 23 wk gestation (13, 22). The common reductions in PDGFR-α expression observed in MSCs from premature infants and lung tissue from hyperoxic newborn mice provide further evidence that the neonatal hyperoxia model constitutes a reasonably sound model of human BPD. We conclude that neonatal lung MSCs from infants who develop BPD show reduced PDGFR-α expression, leading to impaired cell migration. PDGFR-α expression is also reduced in the alveoli of infants with BPD in situ. Given the requirement of PDGFR for septation and alveolarization, these data strongly suggest that defective PDGFR-α signaling is a primary feature of human BPD. Further understanding of MSC biology may provide further insight into the pathogenesis of BPD and lead to new therapeutic interventions.

GRANTS

This work was supported by NIH grants R01 HL79339 and K23 HL109149.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

REFERENCES


AUTHOR CONTRIBUTIONS


GRANTS

This work was supported by NIH grants R01 HL79339 and K23 HL109149.
28. Madurga A, Mizikova I, Ruiz-Camp J, Morty RE.