Overexpression of PDGF-A in the Lung Epithelium of Transgenic Mice Produces a Lethal Phenotype Associated with Hyperplasia of Mesenchymal Cells

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Transgenic mice expressing platelet-derived growth factor A chain (PDGF-A) in the distal lung epithelium from the surfactant protein C (SPC) promoter were generated to investigate the role of this growth factor in lung development. Expression of the SPC-PDGFA transgene resulted in an enlarged, nonfunctional lung and perinatal lethality caused by failure to initiate ventilation. Histologic analysis of embryonic day (E) 16.5 lungs revealed increased mesenchymal cells and acinar buds and decreased bronchioles and dilated airspaces in SPC-PDGFA transgenic mice. At E18.5, nontransgenic lungs exhibited lung morphology typical of the saccular stage of lung development, including dilated airspaces, thin respiratory epithelium and mesenchyme, and elastin fiber deposition in primary septa. In contrast, E18.5 transgenic lungs retained many features of the canalicular stage of lung development, including undilated airspaces, cuboidal respiratory epithelium, thickened mesenchyme, and lack of parenchymal elastin deposition. These results indicate that PDGF-A is a potent growth factor for mesenchymal cells in the developing lung and that the downregulation of PDGF-A expression that normally occurs in the lung during late gestation is required for transition from the canalicular to the saccular stage of lung development.

INTRODUCTION

Mouse lung development begins with the formation of the primitive lung buds around embryonic day (E) 9.5. The system of conducting airways develops during the pseudoglandular period of lung development (E9.5–E16) by branching of the primitive epithelial tubules under the influence of the underlying mesenchyme. Demarcation of distinct proximal and distal epithelium becomes apparent at the end of the pseudoglandular period. In the subsequent canalicular stage (E16.5–E17.5), the terminal acinar buds form and begin to dilate. Further dilation of the terminal buds and thinning of the epithelium and underlying mesenchyme occur in the saccular stage (E17.5–postnatal day 5), resulting in the development of the functional gas exchange units. In the final stage of lung development, which occurs postnatally, secondary septa grow to subdivide the sacculles and form the alveoli found in the adult lungs (Ten Have-Opbroek, 1991).

Reciprocal signaling between the pulmonary epithelium and mesenchyme is a critical component of developmental mechanisms in the embryonic lungs. Early evidence of this phenomenon was the induction of supernumerary budding of tracheal epithelium when placed in contact with more distal bronchial mesenchyme (Alescio and Cassini, 1962; Wessells, 1970). Subsequently, it has been recognized that soluble factors secreted by epithelial and mesenchymal cells are important mediators of the reciprocal communication that occurs between these cell types during lung development.

Platelet-derived growth factor (PDGF) is expressed in the developing lung and is likely to mediate interactions between the epithelium and mesenchyme. PDGF exists as dimers of A and B subunits that can form three isoforms: AA, AB, and BB (Hammacher et al., 1988; Heldin et al., 1986). PDGF receptors (PDGFR) also function as dimers of...
subunits designated α and β. PDGF-BB can interact with all three PDGFR dimers, whereas PDGF-AA can only bind to PDGFR-αα (Hart et al., 1988; Seifert et al., 1989). PDGF is a potent mitogen for mesenchymal cells, including lung fibroblasts and airway smooth muscle cells (Buch et al., 1994; Hirst et al., 1996; Osornio-Vargas et al., 1995). PDGF-A is widely expressed in lung epithelium during the pseudoglandular stage, but its expression declines until it is not detected in late gestation (Bostrom et al., 1996; Lindahl et al., 1997). PDGFR-α is expressed in mesenchymal cells underlying the lung epithelium (Lindahl et al., 1997; Orr-Urteger and Lonal, 1992). Studies in which PDGF-A expression is inhibited suggest an important role for this growth factor in the regulation of lung morphogenesis. Inhibition of PDGF-A or PDGF-B in embryonic lung explants using antisense oligonucleotides reduced lung size, but only inhibition of PDGF-A expression reduced branching morphogenesis (Souza et al., 1994, 1995). In PDGF-A knockout mice, no histologic abnormalities were reported before birth, but postnatally the mice developed emphysema caused by a defect in alveogenesis (Bostrom et al., 1996). To further explore PDGF function in the embryonic lung, we have generated transgenic mice in which expression of PDGF-A was targeted to the distal epithelium under control of the surfactant protein C (SPC) promoter. Here, we describe that PDGF-A overexpression results in increased lung mesenchyme and lung size, failure of airspace development, and perinatal death. These results demonstrate that PDGF-A is an important factor regulating the growth of the lung and that the restriction of PDGF-A expression that normally occurs in late gestation is important for the development of the airspaces.

MATERIALS AND METHODS

Generation of Transgenic Mice

The human PDGF-A cDNA (clone 13.1), was kindly provided by Christer Betsholtz (University Hospital, Uppsala, Sweden) (Rorsman et al., 1988). A 1.1-kb EcoRI fragment containing the PDGF-A cDNA was inserted between the 3.7-kb human SPC promoter (Glasser et al., 1991; Morris et al., 1998) and SV40 intron and polyadenylation signals. The 6.1-kb SPC-PDGFA construct was released by partial digestion with SacI and purified by agarose gel electrophoresis. A 3-ng/ml solution of the linear construct was microinjected into pronuclei of fertilized B6SJLF2 eggs (Brinster et al., 1985). Mice that developed from injected eggs were allowed to proceed to term or were obtained by Cesarean section on E16.5 or E18.5. The day the copulation plug was observed was considered E0.5. Embryos were genotyped by hybridization of DNA with a radiolabeled human SPC probe.

Tissue Preparation

Cesarean section was performed after euthanasia of foster females on gestational days 16.5 and 18.5. Twelve hours before collection of E16.5 embryos, each foster mother received an intraperitoneal injection of 40 mg/kg 5’-bromodeoxyuridine (Brdu) in PBS. E16.5 embryos were given a lethal injection of 2,2,2-tribromoethanol solution (Aldrich, Milwaukee, WI) and then fixed by immersion in 10% buffered formalin overnight at 4°C. The following day, samples were incubated in PBS two times for 30 min, and the lungs were removed. The samples were weighed, embedded in paraffin, and sectioned at 5 μm. E18.5 embryos were delivered and then placed on a 37°C warming plate. After 20 min of observation, live embryos were administered a lethal injection of tribromoethanol solution. Lungs of E18.5 embryos were fixed by intratracheal infusion of 0.1 ml 10% buffered formalin solution and then the body and inflated lungs were immersed in fixative overnight at 4°C.

In Situ Hybridization

Lung sections were hybridized with a 0.5-kb probe derived from SV40 sequences contained in the 3′ untranslated region of the SPC-PDGFA transgene message. The antisense and sense riboprobes were generated by in vitro transcription with T3 and T7 RNA polymerases, respectively (Promega, Madison, WI). Probes were labeled by incorporation of digoxigenin-11-UTP (Boehringer Mannheim, Indianapolis, IN). In situ hybridization was performed as described (Liu et al., 1998). In brief, sections were prehybridized with 50% formamide, 4× SSC at 37°C for 15 min, and then incubated with probe in hybridization solution (40% formamide, 10% dextran sulfate, 1× Denhardt’s solution, 4× SSC, 10 mM DTT, 1 μg/μl yeast tRNA, and 1 μg/μl sperm DNA) at 37°C for 4 h. After posthybridization washes, sections were treated with 10 μg/ml RNase in 2× SSC solution at 37°C for 30 min. Probe hybridization was detected by using anti-digoxigenin-alkaline-phosphatase Fab fragments (Boehringer Mannheim) followed by staining with nitro blue tetrazolium/
5-bromo-4-chloro-3-indolyl phosphate as described by the manufacturer (Boehringer Mannheim). No staining was observed when sections from transgenic or nontransgenic mice were hybridized with the sense riboprobe.

**Antibodies and Immunohistochemistry**

Goat serum against human PDGF-A (R&D Systems) was used at a dilution of 1:100. Mouse monoclonal antibody against α-smooth muscle actin (Dako, Carpinteria, CA) and mouse monoclonal antibody against desmin (Dako) were used at a dilution of 1:400. Rabbit serum against surfactant protein B (SPB) (Dr. Jo Rae Wright, Duke University, Durham, NC) was used at a dilution of 1:1000. Rabbit serum against rat Clara cell secretory protein (CCSP) (Dr. Gurmukh Singh, VA Medical Center, Pittsburgh, PA) was used at a dilution of 1:5000. Rat monoclonal anti-bromodeoxyuridine (BrdU) antibody (Accurate, Westbury, NY) was used at a dilution of 1:50. Immunohistochemistry for CCSP and SPB was performed by using an immunoperoxidase technique as previously described (Liu et al., 1997). For immunohistochemistry with mouse monoclonal antibodies against desmin and α-smooth muscle actin, a technique was used to inhibit background caused by endogenous mouse IgG. Before inactivation of endogenous peroxidase by incubation in 3% hydrogen peroxide in methanol, sections were incubated in biotinylated goat anti-mouse IgG (Jackson Immunoresearch, West Grove, PA) at 1:4000 at room temperature for 30 min. After washing, sections were incubated with inactivated horseradish peroxidase-conjugated streptavidin at 1:2000 (500 ng/ml) at room temperature for 30 min. Subsequent steps were performed as described (Liu et al., 1997). For human PDGF-A immunohistochemistry, sections were treated with 0.05% trypsin at room temperature for 20 min prior to incubation with the primary antibody. Bound antibody was detected with peroxidase-conjugated donkey anti-goat IgG followed by diaminobenzidine and hydrogen peroxide. BrdU immunohistochemistry was performed as described (Brass et al., 1999).

**Morphometric Analysis**

Samples for morphometric analysis were derived from three litters collected on the same day in which the lungs of all wild-type
embryos were of similar developmental stage. Lung sections (5 μm) were stained with hematoxylin and eosin. To analyze bronchiole numbers and airspace changes in the transgenic lungs, five to seven vertical sections per embryo at 40- to 50-μm intervals from the three heaviest E16.5 transgenic and nontransgenic left lungs were studied. Images were captured using a 4× objective and digitized for analysis. The total number of bronchioles on each section was counted and the area of each section was measured by using NIH Image software. To analyze the proportion of the sections occupied by airways and airspaces, black-and-white images were then used to measure section areas with or without the “Include Interior Holes” option. The difference between the two measurements is the area occupied by the lumens of airways and acinar tubules. To analyze epithelial and mesenchymal compartments as well as the density of terminal buds in the peripheral lung, four lung sections per mouse were photographed from five transgenic and five nontransgenic littermates in areas lacking large blood vessels and airways. For nontransgenic lungs, areas without dilated airspaces in terminal buds were selected so they could be compared to transgenic lungs in which pulmonary acini were undilated. Acini were outlined on the photographs with a black marker, and acinar buds were counted. The photographs were then scanned and analyzed by using NIH Image. The area of the circled acini was calculated, blood vessels and conducting bronchioles were erased manually, and the remaining area was calculated as the mesenchymal area. To estimate the size of terminal respiratory units in transgenic and nontransgenic mice, the method of Emery and Mithal was used (Emery and Mithal, 1960). Photographs were taken with a 10× objective from three transgenic and three nontransgenic mice. For each terminal bronchiole, a perpendicular line was drawn from the center of the respiratory tube to the nearest edge of the lung. The number of acinar buds crossed by this line was counted and the length of the line was measured. Nine to ten such measurements were made from each mouse.

**Data Analysis**

Data are presented as group means ± SEM. Group means were compared using unpaired t tests except for BrdU labeling data, for which a paired t test was used. In the latter case, transgenic mice were paired with nontransgenic litter mates that were immediately adjacent in utero to compensate for developmental stage differences within and among litters.

**RESULTS**

**Generation of SPC-PDGFA Transgenic Embryos**

The SPC-PDGFA construct shown in Fig. 1 was microinjected into fertilized eggs to generate transgenic mice in which PDGF-A expression was targeted to lung epithelium. In initial experiments, microinjection of the SPC-PDGFA construct failed to generate any mice carrying the transgene at weaning (Table 1). This result suggested lethality at an earlier stage. Thereafter, fetuses developing from microinjected eggs were obtained by Cesarean section at E16.5 and E18.5, representing the canalicular and saccular stages of mouse lung development, respectively. From these experiments, 11 of 47 E16.5 fetuses and 4 of 26 E18.5 fetuses were identified as carrying the SPC-PDGFA transgene (Table 1).
SPC-PDGFα Mice Have Increased Lung Size and Die Perinatally

The external appearance of transgenic mice was similar to that of their normal littermates at E16.5 and E18.5. The SPC-PDGFα transgene did not affect the viability of embryos at E16.5. The external appearance and lung morphology of one litter delivered at E16.5 appeared to be of a more advanced developmental stage (ca. E17.5). Fetuses in this litter were considered separately in some of the analyses as noted below. The lungs and heart were removed en bloc from E16.5 fetuses. Some lungs were observed to be abnormally large. These lungs were photographed with lungs from nearby littermates (Figs. 2A and 2B). Embryos with these large lungs were later identified by DNA typing as transgenic embryos. After the lungs and heart were weighted en bloc, lung lobes were dissected for histologic analysis and the left lobe was weighed. The average weight of lungs and heart of 9 transgenic embryos from these 4 litters was 1.8 times that of the 27 wild-type littermates (58.9 ± 8.9 mg vs. 32.5 ± 1.8 mg, P = 0.02). The weight of the left lobe of transgenic mice was 2.5 times that of the normal left lobe (17.0 ± 3.1 mg vs. 6.8 ± 0.4 mg, P = 0.01) (Fig. 3).

Nontransgenic mice delivered by Cesarean section on E18.5 initiated ventilation normally and turned pink within a few minutes. In contrast, transgenic embryos (as revealed by subsequent genotyping analysis) gasped a few times and then died. Postmortem inspection revealed that the lungs of transgenic mice did not collapse after dissection of thoracic cage. Injecting any amount of fixative into the lungs of transgenic mice was difficult, whereas the lungs of wild-type littermates were easily inflated with 0.1 ml of fixative. The lungs from transgenic embryos were larger than those from wild-type embryos even after inflation of the latter with fixative (Figs. 2C and 2D). Indentations from the ribs and vertebral column indicated that the transgenic lungs grew to press against the wall of the thoracic cage. These findings showed that the SPC-PDGFα transgene resulted in increased prenatal growth of the lung that interfered with lung function. One transgenic embryo and one of its adjacent littermates died in utero. Histologic examination of the lungs from the one dead transgenic embryo did not reveal any abnormal pathology, which suggested that the transgene was not expressed in the lung and that the mouse died of unrelated causes.

Expression of the SPC-PDGFα Transgene

Expression of the PDGF-A transgene was analyzed by in situ hybridization of lung sections with a probe derived from SV40 sequences in the 3′ untranslated region of the transgene. As expected, no hybridization signal was detected in nontransgenic lung (Fig. 4A). Transgene expression as evidenced by hybridization of the SV40 probe was detected in epithelial cells in transgenic embryos on both E16.5 (Fig. 4B) and E18.5 (not shown). Transgene mRNA was detected only in epithelial cells of terminal lung buds but not of bronchioles. The hybridization signal for the transgene was consistent with the known expression pattern driven by the human SPC promoter in transgenic mice (Glasser et al., 1991; Wert et al., 1993). Expression of the transgene polypeptide was demonstrated by performing immunohistochemistry using antibodies against human PDGF-A. Staining was observed in SPC-PDGFα transgenic lung at E16.5 (Fig. 4D) and E18.5 (not shown), but not in nontransgenic lung (Fig. 4C). Staining for PDGF-A in transgenic lungs was observed in epithelial cells of terminal lung buds, which was consistent with the location of mRNA expression found by in situ hybridization.

Lung Histology in SPC-PDGFα Transgenic Mice

Histologic analysis of E16.5 and E18.5 lungs revealed distinct morphological changes in SPC-PDGFα transgenic mice compared with their wild-type littermates (Fig. 5). Transgenic mice (Fig. 5B) appeared to have reduced numbers of bronchioles compared with nontransgenic mice (Fig. 5A). This observation was confirmed by morphometric analysis at E16.5, which revealed that transgenic mice had only 18% of the number of bronchioles per unit area compared with nontransgenic mice (Fig. 6A). In addition, the percentage of the section area occupied by the lumens of bronchioles and pulmonary acini was significantly reduced in SPC-PDGFα transgenic mice (Fig. 6B). Transgenic lung sections had an increased ratio of mesenchymal area to acinar area, a result consistent with the action of PDGF on mesenchymal cells (Fig. 6C). Transgenic lung sections had slightly reduced numbers of acinar buds per unit area (Fig. 6D), but because transgenic lungs were over twice the size of nontransgenic lungs, the total number of acinar buds was larger in transgenic mice. An estimate of the size of the terminal respiratory units in transgenic and nontransgenic mice was made by measuring distances from the ends of terminal bronchioles to the nearest edge of the section and also counting the number of acinar buds within this distance (acinar bud index). Results of this analysis showed that both these indicators were increased in SPC-PDGFα transgenic lungs (Fig. 6E), suggesting an increased branching of the respiratory tubules. The organization of the epithelium in E16.5 SPC-PDGFα lung appeared normal (Fig. 5B, inset). In the litter with the developmental appearance of E17.5, lungs from nontransgenic mice were in the early saccular stage (not shown). The transgenic embryos in this litter did not show any signs of progression to the saccular stage (not shown).

Wild-type E18.5 fetuses exhibited normal histology for the saccular stage of lung development, characterized by dilated airspaces, flattened epithelium, and a thin mesenchymal cell layer between the airspaces (Figs. 5C and 5E). SPC-PDGFα transgenic lungs lacked the normal saccular appearance and retained many characteristics of the canalicular stage (Figs. 5D, 5F–5G). Transgenic lungs were hypercellular and contained a thickened mesenchyme surrounding the airspaces. The appearance of terminal tubules...
varied, with some being dilated and others undilated. The dilated tubules were clustered (Fig. 5D, arrow) and possessed a characteristic morphology that differed from the normal sacculles in nontransgenic mice. The proportion of dilated tubules varied among different lobes from the same mouse and among different mice. In transgenic lungs, undilated terminal tubules were lined by cuboidal epithelial cells protruding into the airspaces with relatively clear cytoplasm (Fig. 5G), whereas dilated terminal tubules were lined with squamous and cuboidal epithelial cells (Fig. 5F).

Some sloughing of cuboidal epithelium was observed in these dilated tubules. The most extensively dilated terminal tubules were lined only with squamous epithelial cells. The clustered distribution of dilated terminal buds suggests an obstruction of the bronchioles to which these tubules drain.

**Effect of PDGF-A on Cell Proliferation**

Because of the increased size of SPC-PDGFA lungs and the known mitogenic properties of PDGF, cell proliferation was examined by BrdU incorporation in lungs from E16.5 transgenic fetuses and their wild-type littermates. Pregnant dams carrying SPC-PDGFA and nontransgenic embryos were exposed to BrdU, and embryos were collected 12 h later at E16.5. BrdU incorporation was analyzed by immunohistochemistry, and labeled cells in the lung were counted by light microscopy. A small but significant increase in proliferative rate was observed in the transgenic lungs (difference in BrdU-labeled cells between transgenic and nontransgenic lungs = 2.2 ± 0.96%, P = 0.03) (Fig. 7).

**Epithelial Differentiation in SPC-PDGFA Transgenic Lungs**

To investigate whether epithelial differentiation was altered in SPC-PDGFA transgenic lung, Clara cell secretory protein (CCSP) and surfactant protein B (SPB) were detected by immunohistochemistry (Fig. 8). CCSP is synthesized in Clara cells, nonciliated epithelial cells of the airways. SPB is expressed in the distal epithelium and bronchiolar epithelial cells located in the airspaces and terminal bronchioles. CCSP and SPB expression were not detected in E16.5 transgenic and nontransgenic lungs (not shown). However, in the litter with the developmental appearance of E17.5, CCSP was detected in nontransgenic embryos but not in transgenic mice (Figs. 8A and 8B). At E18.5, CCSP immunostaining appeared weaker in transgenic lungs than nontransgenic lungs (Figs. 8C and 8D). These results indicate that there is a delay in the differentiation of the airway epithelium in SPC-PDGFA mice. Nontransgenic lungs at E18.5 displayed the normal pattern of SPB staining in type II alveolar epithelial cells of the sacculles (Figs. 8E and 8G). Lungs from SPC-PDGFA mice of the same age exhibited more SPB-positive cells in lining the airspaces, and prominent SPB-positive secretions were observed in the airways and airspaces of transgenic mice (Figs. 8F, 8H, and 8I).

**Mesenchymal Differentiation in SPC-PDGFA Transgenic Lungs**

Elastin fibers were detected in mesenchyme surrounding airways and blood vessels but were absent in distal tubules at E16.5 in both transgenic and nontransgenic lungs (not shown). A network of fine elastin fibers occurred in the thin mesenchyme of primary septa in nontransgenic lungs at E18.5 (Fig. 8J). In the lungs of transgenic mice of this age, normal elastin staining was observed around blood vessels and airways, but the normal pattern of elastin fibers was not present around the airspaces (Fig. 8K). No difference in staining for α-smooth muscle actin (myofibroblast/smooth muscle cell marker), desmin (smooth muscle cell marker), or Von Willebrand’s factor (endothelial cell marker) was observed between transgenic and nontransgenic lungs.

**DISCUSSION**

In this study, overexpression of PDGF-A during lung development resulted in increased lung growth, failure of the airspaces to develop, and perinatal death caused by the inability to initiate ventilation. This dramatic phenotype indicates that PDGF-A is a potent growth factor in lung development, and the results are in accordance with other studies in which expression of PDGF-A was inhibited in the developing lung. Treatment of lung organ cultures with antisense PDGF-A oligonucleotides inhibited cellular proliferation and resulted in smaller explants with reduced branching and terminal buds (Souza et al., 1995). Our results examining PDGF-A in the whole animal are consistent with the concept that PDGF-A stimulates prenatal lung growth and branching in terminal respiratory tubules. Targeted inactivation of the PDGF-A gene in mice resulted in death by 2–3 weeks postnatally from alveogenesis failure (Bostrom et al., 1996). Lungs from PDGF-A knockout mice appeared normal before birth, suggesting that other mitogens may compensate for the lack of PDGF-A prenatally in these animals. Alternatively, the small fetus size in PDGF-A knockout mice may mask an inhibition of lung growth.

The appositional expression of PDGF-A and its cognate receptor during development suggests a paracrine stimulation of PDGF-A on the adjacent mesenchymal cells. In wild-type mice, PDGF-A is expressed throughout the pulmonary epithelium on E14.5, but its expression declines so that it cannot be detected by E18.5 (Bostrom et al., 1996; Lindahl et al., 1997). The PDGF-α receptor is widely expressed in mesenchymal cells underlying the epithelium on E14.5 and subsequently becomes restricted to a small number of cells in the saccular mesenchyme. These cells are required for postnatal development of alveoli, because secondary septal growth is impaired in PDGF-A knockout mice that lack these cells (Lindahl et al., 1997). In SPC-PDGFA transgenic mice, widespread epithelial PDGF-A expression is maintained in the latter stages of lung development and results in a
lung at E18.5 that retains many features of the canalicular stage, including undilated tubules with cuboidal epithelium and a thick mesenchymal cell layer lacking elastin fibers between the tubules. These results suggest that downregulation of PDGF-A expression is an important event in the transition from the canalicular to saccular stage and that widespread PDGF-A expression in the distal epithelium is incompatible with the dilation of the airspaces and thinning of the underlying mesenchyme that normally occur during this time.

Interference with sacculation in SPC-PDGFA mice may be a direct effect of mesenchymal cell proliferation producing thickened septa that prevent the formation of air sacs. In addition, the altered physical environment in SPC-PDGFA lungs may interfere with formation of the airspaces. Distention of the lung, as occurs during normal fetal breathing movements or after tracheal ligation, stimulates dilation of the airspaces and thinning of the distal epithelium and mesenchyme (Carmel et al., 1965; De Paepe et al., 1998; Hooper et al., 1993). The abnormal growth of SPC-PDGFA lungs is likely to interfere with lung distention and may inhibit fetal breathing movements so that an essential signal generated by distention of the lung tissue is lacking.

SPC-PDGFA transgenic lungs had dramatically increased mass on E16.5 and were clearly enlarged relative to non-transgenic lungs on E18.5. This increase in mass could...
FIG. 5. Lung histology. Histologic sections from SPC-PDGFA and wild-type lungs were stained with hematoxylin and eosin. (A) E16.5 nontransgenic lung. (B) E16.5 transgenic lung. (C) E18.5 nontransgenic lung. (D) E18.5 transgenic lung. Arrow indicates a cluster of dilated respiratory tubules. (E) Higher magnification of the nontransgenic lung in (C). (F, G) Higher magnification of dilated (F) and undilated (G) tubules from transgenic lung in (D). Bar in (C), 100 µm for (A–D); bar in inset of (A), 20 µm for insets in (A) and (B); bar in E, 20 µm for (E–G).
result from an increase in cell proliferation and/or a decrease in apoptosis. Previous studies have shown that the rate of apoptosis in the prenatal mouse lung is very low (Bellusci et al., 1996), so that the large difference in size we observed is likely caused by increased proliferation in transgenic lungs. Lungs from SPC-PDGFA mice exhibited a slightly higher percentage of BrdU-labeled cells at E16.5 compared to wild-type littermates. Because transgenic lungs were 2.5-fold larger than wild-type lungs at this time, it is likely that larger differences in proliferation rates occurred earlier in development. Proliferation in SPC-PDGFA lungs during late gestation may be inhibited once the lung grows to exert pressure on the thoracic cavity because fetal breathing movements, which stimulate proliferation in the lung, would be inhibited.

PDGF-A overexpression greatly increased fetal lung mesenchyme, as judged by the increase in fractional area of the mesenchyme and the overall increase in the size of the lungs. This finding is consistent with the known proliferative effect of PDGF on mesenchymal cells. As in wild-type lungs, cells in the mesenchymal layer surrounding epithelial tubules did not express α-smooth muscle actin or desmin. This result indicates that PDGF-A expression did not induce differentiation toward myofibroblast or smooth muscle lineages in these cells. In contrast, overexpression of TGF-β1 in the lung resulted in an increased mesenchymal cell compartment with alterations in the differentiation of these cells (Zhou et al., 1996). A clear morphological difference in the mesenchyme of SPC-PDGFA mice at E18.5 was the lack of elastin fibers surrounding the epithelial tubules, which contrasted with the normal density of elastin fibers observed around larger airways and blood vessels. Impaired elastin deposition in mice overexpressing PDGF-A may appear to be incongruent with the phenotype of PDGF-A knockout mice, in which elastin deposition during alveogenesis is also impaired (Bostrom et al., 1996). These differences can be reconciled by considering the pattern of tropoelastin expression, which proceeds in distinct phases in the developing lung. Prenatally, tropoelastin is expressed in mesenchymal cells underlying proximal tubules at E15.5 and is widely expressed throughout lung mesenchyme at E18.5. Postnatally, tropoelastin is expressed in bronchial and vascular walls and in growing septal tips during alveogenesis (Lindahl et al., 1997). PDGF-A overexpression affects prenatal elastin deposition in the lung parenchyma, whereas lack of PDGF-A affects postnatal elastin deposition during secondary septal formation.

Overexpression of PDGF-A had an inhibitory effect on}

![FIG. 6](image)

**FIG. 6.** Morphometric measurements from E16.5 lung sections. (A) Bronchiole counts. (B) Percent of section area occupied by airways and airspaces. (C) Ratio of mesenchymal to acinar area in lung parenchyma. (D) Acinar bud counts in lung parenchyma. (E) The size of terminal respiratory units was estimated by measuring the distance from the end of a terminal bronchiole to the edge of the section and counting the number of acinar buds crossed by this line (acinar bud index). These two indices are plotted with each symbol representing measurements from a single terminal respiratory unit. Average measurements ± SEM were: distance from edge: transgenic, 47.8 ± 2.9 μm; nontransgenic, 14.3 ± 1.3 μm, P < 0.0001; acinar bud index: transgenic, 6.0 ± 0.4; nontransgenic, 2.0 ± 1.3; P < 0.0001.

![FIG. 7](image)

**FIG. 7.** BrdU incorporation in E16.5 lungs. Pregnant dams carrying embryos from microinjected eggs were administered BrdU at E16.0, and embryos were collected 12 h later at E16.5. Embryos from litter 4 had the developmental appearance of E17.5. The percentage of BrdU labeled cells in transgenic and nontransgenic littermate lungs was 2.2% ± 0.96%, P = 0.03.
FIG. 8. CCSP, SPB, and elastin staining. (A–D) Immunoperoxidase staining for CCSP protein. (A) Nontransgenic lung from the litter with developmental appearance of E17.5. (B) Lung from transgenic littermate of mouse in (A). (C) E18.5 nontransgenic lung. (D) E18.5 transgenic lung. (E–I) Immunoperoxidase staining for SPB protein. (E) E18.5 nontransgenic lung. (F) E18.5 transgenic lung. (G) Higher magnification of the nontransgenic lung in (E). (H) Higher magnification of distal tubules in the transgenic lung in (F). (I) Higher magnification of bronchiole in the transgenic lung in (F) showing SPB-positive secretions in the lumen. (J) Elastin staining in E18.5 nontransgenic lung. Arrows indicate elastin fibers in the septal walls of the airspaces. (K) Elastin staining in E18.5 transgenic lung.
some aspects of epithelial cell differentiation. Epithelial tubules in E18.5 SPC-PDGFA lungs were lined with cuboidal epithelia rather than the thin type-1 cells in wild-type saccules. SPB expression was readily detected in the distal epithelium of transgenic mice starting at E18.5 as in wild-type lungs. In contrast, the proximal epithelium appeared normal structurally, but the expression of CCSP in the airways was somewhat retarded in the transgenic mice. Since PDGF-α receptor expression in the developing lung is restricted to mesenchymal cells (Lindahl et al., 1997), the effects of PDGF-A on epithelial cells would appear to be secondary to the mesenchymal cell growth and/or the resultant physical changes in the lung. Effects on epithelial cell growth and differentiation caused by perturbation of a mesenchymal cell growth factor underscore the intimate interactions between epithelial and mesenchymal cells during lung development.

In SPC-PDGFA transgenic lungs, the number of bronchi- oles decreased but the numbers of acinar buds increased, suggesting an increase in distal branching in the transgenic mice. In PDGF-A knockout mice, prenatal lung structure appeared normal, and deficiency of PDGF-A did not affect the proximal-distal differentiation of epithelial cells. In contrast, other growth factors have been reported to regulate the proximal-distal differentiation of lung epithelial cells. Increased proximal cells and concurrently reduced distal epithelial cells have been reported in animal models with disruption of bone morphogenetic protein 4 signaling or in fetal lung culture to which retinoid acid was added (Cardoso et al., 1995; Weaver et al., 1999). An increase in the proportion of distal tubules and epithelial cells, as occurs in SPC-PDGFA mice, has not been previously reported.

Manipulation of the expression of PDGF-A and PDGF-B subunits has demonstrated that these two molecules have distinct functions in vivo. In PDGF-A knockout mice, alveolar smooth muscle cell precursors expressing PDGF-α receptors failed to develop, thereby interfering with alveogenesis. In PDGF-B knockout mice, pericytes expressing PDGF-β receptors failed to develop, resulting in abnormal capillary structure and hemorrhage (Leveen et al., 1994). In these cases, the differences in phenotype between PDGF-A and PDGF-B deficiency can be attributed to the expression patterns of the growth factor ligands and receptors during development. We previously generated transgenic mice overexpressing PDGF-B from the SPC promoter. These mice survived to adulthood and developed lung disease characterized primarily by emphysematous lesions but also by focal areas of fibrosis (Hoyle et al., 1999). Neonatal SPC-PDGFβ mice exhibited increased airspace size and thickened septa, but no abnormalities were observed before birth. Since PDGF-B subunits can bind to both PDGF α and β receptors but PDGF-A subunits can only bind to PDGF α receptors, SPC-PDGFα mice would be expected to exhibit a phenotype similar to or milder than SPC-PDGFβ mice. The observed effects of PDGF-A overexpression, i.e., increased lung growth and perinatal lethality, did not occur in SPC-PDGFβ mice. Our observations are therefore somewhat unexpected based on the known activities of PDGF isoforms in vitro. The results may be explained by differences in intracellular signaling mediated by PDGF α and β receptors as has been suggested (Rosenkrantz and Kazlauskas, 1999) or by modulation of PDGF activity in vivo by binding proteins such as α2-macroglobulin, which is known to inhibit the activity of PDGF-B (Bonner and Brody, 1995; Bonner et al., 1989). Further studies will be required to elucidate the specific mechanisms by which overexpression of the PDGF-A chain appears to promote a more severe lung phenotype than that of PDGF-B chain.

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REFERENCES

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