Development 124, 3943-3953 (1997)
Printed in Great Britain © The Company of Biologists Limited 1997
DEV4897

Alveogenesis failure in PDGF-A-deficient mice is coupled to lack of distal spreading of alveolar smooth muscle cell progenitors during lung development

Per Lindahl[1], Linda Karlsson[1], Mats Hellström[1], Samuel Gebre-Medhin[1], Karen Willetts[2,‡], John K. Heath[2] and Christer Betsholtz[1,*]

[1]Department of Medical Biochemistry and Microbiology, University of Göteborg, Medicinaregatan 9A, S-413 90 Göteborg, Sweden
[2]School of Biochemistry, University of Birmingham, Edgbaston, Birmingham B15 2TT, UK

*Author for correspondence (e-mail: Christer.Betsholtz@medkem.gu.se)
‡Present address: INSERM Unité 372, Campus de Luminy, Marseille 13276, Cedex 9, France

SUMMARY

PDGF-A$^{-/-}$ mice lack lung alveolar smooth muscle cells (SMC), exhibit reduced deposition of elastin fibres in the lung parenchyma, and develop lung emphysema due to complete failure of alveogenesis. We have mapped the expression of PDGF-A, PDGF receptor-α, tropoelastin, smooth muscle α-actin and desmin in developing lungs from wild type and PDGF-A$^{-/-}$ mice of pre- and postnatal ages in order to get insight into the mechanisms of PDGF-A-induced alveolar SMC formation and elastin deposition. PDGF-A was expressed by developing lung epithelium. Clusters of PDGF-Rα-positive (PDGF-Rα$^+$) mesenchymal cells occurred at the distal epithelial branches until embryonic day (E) 15.5. Between E16.5 and E17.5, PDGF-Rα$^+$ cells multiplied and spread to acquire positions as solitary cells in the terminal sac walls, where they remained until the onset of alveogenesis. In PDGF-A$^{-/-}$ lungs PDGF-Rα$^+$ cells failed to multiply and spread and instead remained in prospective bronchiolar walls. Three phases of tropoelastin expression were seen in the developing lung, each phase characterized by a distinct pattern of expression. The third phase, tropoelastin expression by developing alveolar SMC in conjunction with alveogenesis, was specifically and completely absent in PDGF-A$^{-/-}$ lungs. We propose that lung PDGF-Rα$^+$ cells are progenitors of the tropoelastin-positive alveolar SMC. We also propose that postnatal alveogenesis failure in PDGF-A$^{-/-}$ mice is due to a prenatal block in the distal spreading of PDGF-Rα$^+$ cells along the tubular lung epithelium during the canalicular stage of lung development.

Key words: mouse, lung, smooth mucle, alveogenesis, PDGF-A

INTRODUCTION

Platelet-derived growth factors are homo- or heterodimers of A- (PDGF-A) or B-chains (PDGF-B) that exert their action via binding to and dimerization of two types of receptor tyrosine kinases, α-receptors (PDGF-Rα) and β-receptors (PDGF-Rβ) (Heldin, 1992). PDGF was originally isolated from blood platelets as a growth factor for connective tissue and glial cells, but the two PDGF genes are now known to be expressed by a variety of cell types in developing, as well as in adult, vertebrates. The role of the PDGFs and their receptors during development has recently been studied using targeted gene inactivation in mice (Boström et al., 1996; Levéen et al., 1994; Lindahl et al., 1997; Soriano, 1994, 1997), using expressed dominant negative PDGF receptor mutants in *Xenopus* frogs (Ataliotis et al., 1995), and by application of neutralizing antibodies in *Lytechinus* sea urchins (Ramachandran et al., 1995) and in mice (Schatteman et al., 1996). Together these studies point to multiple roles of PDGFs and PDGF receptors at different stages of embryonic development, such as gastrulation (Ataliotis et al., 1995), cardiovascular development (Lindahl et al., 1997; Schatteman et al., 1996), and the development of kidney glomeruli (Levéen et al., 1994; Soriano, 1994). We recently showed that a proportion of mouse PDGF-A null mutants (PDGF-A$^{-/-}$) survive birth and develop generalized lung emphysema, involving the complete loss of alveolar smooth muscle cells (SMC) (Boström et al., 1996)*. Such cells, embedded in elastin fibres, normally build up the sphincter-like structures (alveolar ring muscles) around the openings of alveoli into alveolar ducts (Kapanchi and Gabbiani, 1997; Miller, 1921). The absence of alveolar SMC in PDGF-A$^{-/-}$ mice coincided with the loss of septal elastin deposits and failure of alveolar septal formation, suggesting a critical role of alveolar SMC in these processes. We also showed specific loss of scattered PDGF-Rα-positive cells in the lung parenchyma at embryonic day (E) 18.5 (plug scored E0.5; term=E19), and proposed that these cells were the progenitors of the alveolar

*In our previous publication (Boström et al., 1996) we used the term 'alveolar myofibroblasts' for the cells missing in PDGF-A-/- lung. This term has also been used for fibroblasts with contractile properties, which are abundant in the lung parenchyma (Kapanchi et al., 1974; Kapanchi and Gabbiani, 1997). However, there was no doubt that the cells specifically lost in PDGF-A-/- lungs were the smooth muscle cells normally situated at the entrance of the alveoli – the constituent cells of the alveolar ring muscles. In the present paper, we have therefore chosen to refer to them as 'alveolar smooth muscle cells'.

SMC (Boström et al., 1996). During embryonic development, lung epithelial tubular cells express PDGF-A, whereas mesenchymal cells express PDGF-Rα, indicating the existence of paracrine signalling between the epithelium and the mesenchyme (Boström et al., 1996; Orr-Urtreger and Lonai, 1992). The aim of the present study was to analyze how PDGF-A deficiency leads to failure of alveolar SMC formation during lung development. We hypothesized that alveolar SMC progenitors should express both PDGF-Rα and tropoelastin, and we have therefore studied in detail the expression patterns of these two genes, as well as the expression of smooth muscle α-actin and desmin, in developing wild-type and PDGF-A$^{-/-}$ lungs.

MATERIALS AND METHODS

Mice

PDGF-A$^{+/-}$ mice line #29 (Boström et al., 1996), bred as 129Ola/C57Bl6 hybrids, were crossed and embryos removed by Caesarean section at different embryonic ages. Tail or yolk sac tissue was used for genotyping by Southern blot analysis as described (Boström et al., 1996) or by a three-primer PCR. The forward primer 5′CCTTTGGCTCTAGGGTGGAATTTC and the two reverse primers 5′TGGATGTGGAATGTGTGCGAG and 5′ACACGAATGAACAGGGATGGG yielded 470 bp wild type and 368 bp mutant allele products in a 40-cycle reaction (96°C for 30 seconds, 55°C for 30 seconds, 65°C for 3 minutes).

In situ hybridization

We used a modification of a protocol described for non-radioactive in situ hybridization (Boström et al., 1996; Henrique et al., 1995). Briefly, embryos were fixed overnight in 4% buffered paraformaldehyde (PFA), infiltrated with 30% sucrose, 0.02% sodium azide, embedded in OCT compound, cryo-sectioned, and stored at –80°C. Prior to hybridization, sections were treated with 10 μg/μl of Proteinase K and refixed in PFA for 15 minutes. Prehybridization occurred in a solution containing 50-55% (55% were used for PDGF-B- and PDGF-Rα-probes, 50% for the others) deionized formamide, 10% dextran sulphate, 1 mg/ml yeast tRNA, 1× Denhardt's solution, 5 mM EDTA, 0.2 M NaCl, 0.013 M Tris-HCl, 5 mM NaH_2PO_4, 5 mM $NaHPO_4$, pH 7.5. Pre-heated probes (see below) were added at a concentration of 3-8 μg/ml hybridization solution, and the sections were incubated overnight. Post-hybridization washes were carried out in 1× SSC, 50-55% formamide and 0.1% Tween 20. The entire process from pre- to post-hybridization was performed at 65-72.5°C (72.5°C were used for PDGF-B- and PDGF-Rα-probes, 65°C for the others). Digoxygenin (DIG)-labelled RNA probes, and their detection on sections using an alkaline phosphatase-conjugated antibody, was done using DIG-labelled UTP, the 'DIG RNA labelling kit' and the 'DIG nucleic acid detection kit' (Boehringer Mannheim), according to the manufacturer's instructions.

DNA-probes

PDGF-B sense and antisense probes were generated from a 800 bp mouse cDNA containing the full length coding sequence cloned in pBS-SK (kindly provided by Dr P. Soriano). PDGF-Rβ probes were generated from a 461 bp *Sac*I fragment cloned in pGEM-2 (kindly provided by Dr J. Escobedo). PDGF-A probes were generated from a 900 bp *Eco*RI fragment cloned in pGEM-1 (kindly provided by Dr W. D. Richardson). Two PDGF-Rα fragments, a 917 bp *Eco*RI-*Eco*RV and a 719 bp *Eco*RV-*Eco*RI fragment, were cut out from a receptor extracellular domain-encoding 1.6 kbp *Eco*RI fragment (kindly provided by Dr W. D. Richardson), subcloned into pBS-SK, and used for probe generation. Probes against tropoelastin mRNA were generated from a 1.1 kbp *Eco*RI rat cDNA fragment cloned into pBS (kindly provided by Dr J. Foster) and a 644 bp *Bam*HI-*Kpn*I mouse cDNA fragment cloned into pBS-SK. The mouse cDNA were obtained by RT-PCR on postnatal lung tissue, using a forward primer 5′TTATCCCATCAAAGCACC and reverse primer 5′AACCCCACCAACACCAACTC, which yielded a 895 bp fragment. We show the use of antisense probes on 14 μm thick sections. As negative controls, the corresponding sense probes were used. No consistent hybridization signals were obtained with any of the sense probes (data not shown). Photography was done in a Nikon microphot-FXA microscope. Unstained sections in combination with Nomarski optics were used to allow for good sensitivity and resolution.

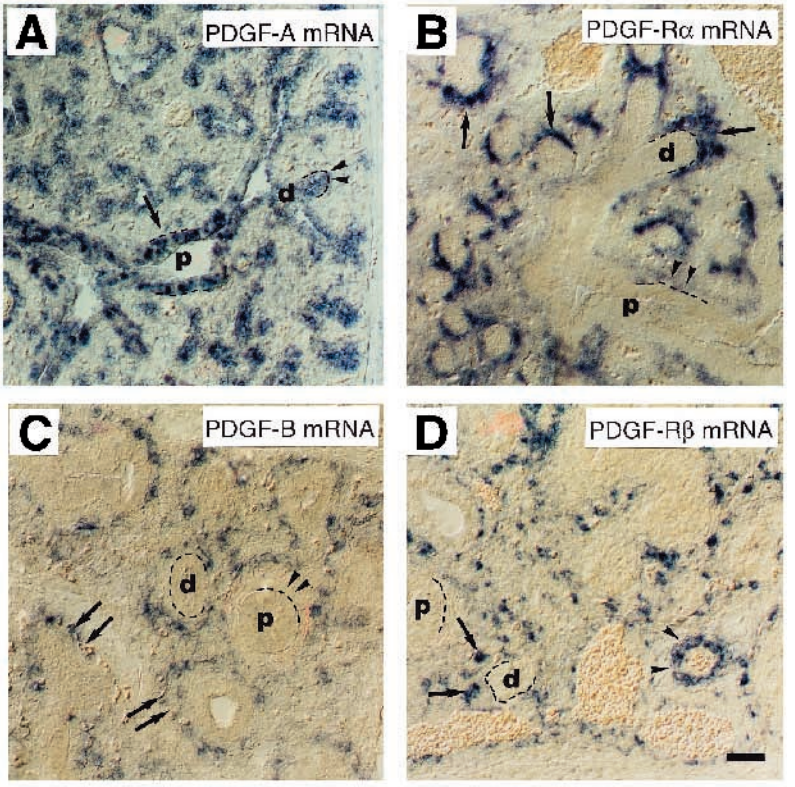


Fig. 1. PDGF and PDGF receptor expression patterns in the developing lung. Non-radioactive in situ hybridization (positive reaction indicated by blue stain) of sections from E14.5 wild-type lungs. Examples of proximal (p) and distal (d) tubules are shown, where the basal epithelial surface is indicated by a dotted line. (A) PDGF-A is expressed by epithelial cells of both proximal and distal tubules. Non-labelled cells could be found (arrows) intermingled with strongly labelled cells in the proximal tubules, while epithelial cells of distal tubules (acinar tubules) were uniformly strongly labelled (arrowheads). (B) PDGF-Rα is strongly expressed by 1-2 layers of clustered mesenchymal cells (arrows) lining the distal epithelial tubules (d) or epithelial buds. Proximal epithelial tubules are lined by cells showing weak PDGF-Rα expression (arrowheads). (C) PDGF-B is expressed by mesenchymal cells located at some distance from the epithelial cells (arrowheads). Erythrocytes (arrows) are often surrounded by PDGF-B-positive cells, consistent with an endothelial expression of PDGF-B. (D) PDGF-Rβ is expressed by mesenchymal cells clustered in arterial walls (arrowheads), or scattered in blood capillary-containing regions of the mesenchyme (arrows). Bar, 80 μm.

Immunohistochemistry

Immunohistochemistry was performed using antibodies directed against smooth muscle α-actin (clone 1A4; Dako) and desmin (clone D33; Dako), according to protocols supplied by the manufacturer.

RESULTS

PDGF-A and PDGF-Rα expression patterns in the developing lung

PDGF-A mRNA is expressed by many types of epithelium during embryonic development (Orr-Urtreger and Lonai, 1992). In the E14.5-16.5 developing lung, we found that PDGF-A mRNA was expressed in the epithelial tubules of both proximal and distal location (Fig. 1A). The patterns were slightly different at the two locations; in proximal (developing bronchial) epithelium, there was a mixture of strongly labelled cells with weakly or non-labelled cells. At distal locations (the acinar tubules), the epithelial labelling for PDGF-A was uniformly strong.

PDGF-Rα mRNA is expressed in lung mesenchyme from the onset of lung development (Orr-Urtreger and Lonai, 1992, and data not shown). At E14.5-15.5, strong expression of PDGF-Rα was seen in the 1-2 layers of mesenchymal cells surrounding the distal epithelial branches and terminal buds (Fig. 1B) (strongly PDGF-Rα-positive cells are hereafter referred to as PDGF-Rα$^+$ cells). Weaker but significant PDGF-Rα expression was also seen in the mesenchyme surrounding the proximal epithelium of prospective bronchi and bronchioles.

Since alveolar SMC are specifically absent in postnatal PDGF-A$^{-/-}$ mice (Boström et al., 1996), and since PDGF-Rα is the only known receptor for PDGF-A (Heldin, 1992), we assumed that the alveolar SMC progenitors should carry PDGF-Rα. We suspected that the PDGF-Rα$^+$ cells at the terminal buds could be such progenitors and therefore compared the PDGF-Rα expression patterns in PDGF-A$^{-/-}$ and PDGF-A$^{+/+}$ lungs. At E14.5-15.5, these patterns were indistinguishable (Fig. 2A,B), but at E16.5 and later, they differed significantly (Fig. 2C-L). In E16.5 PDGF-A$^{+/+}$ lungs, PDGF-Rα$^+$ cells were more numerous and appeared to be more spread compared to the PDGF-A$^{-/-}$ lung, in which their distribution was essentially unchanged from E15.5 (Fig. 2C,D). At

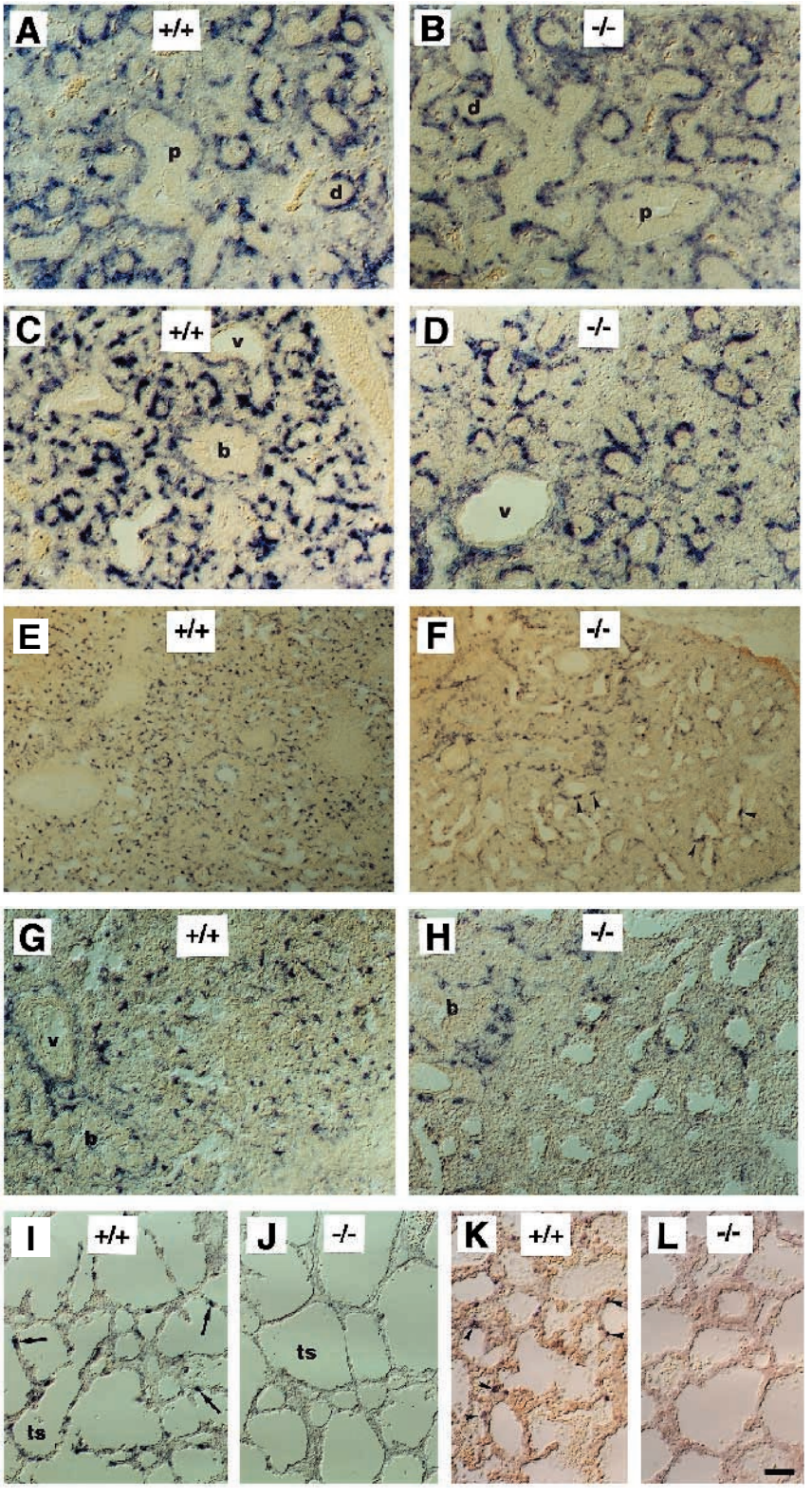
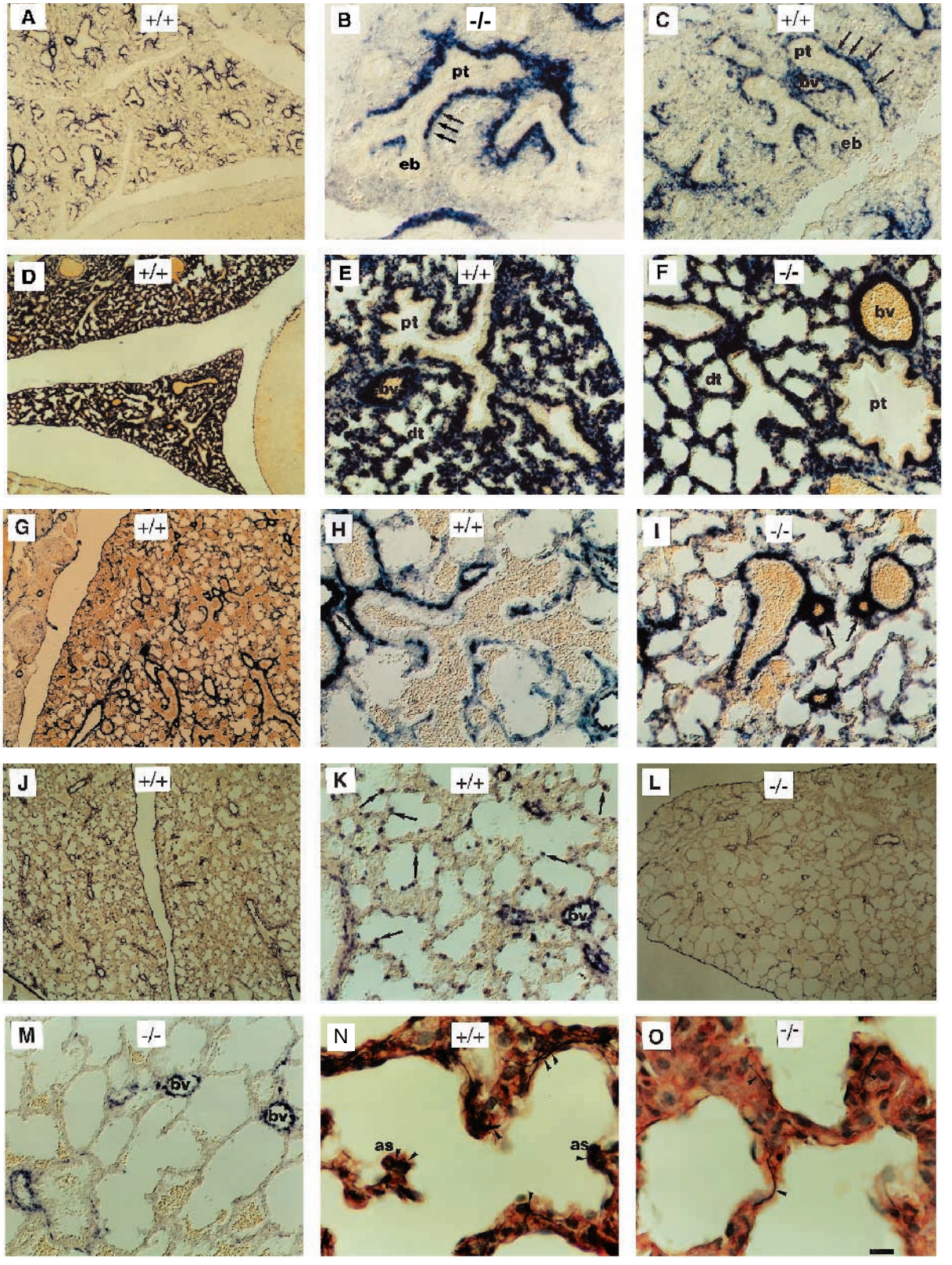


Fig. 2. PDGF-Rα expression patterns in developing wild-type and PDGF-A$^{-/-}$ lungs. PDGF-Rα expression was compared at E15.5 (A,B), E16.5 (C,D), E17.5 (E-H), P1 (I,J) and P7 (K,L). p, proximal tubule; d, distal tubule, b, bronchiole; v, blood vessel; ts, terminal sac. Arrowheads in F point at clustered PDGF-Rα$^+$ cells at proximal tubules. Arrows in I and arrowheads in K point at scattered PDGF-Rα$^+$ cells situated in the walls of alveolar sacs. Note that scattered PDGF-Rα$^+$ cells are fewer in E16.5-17.5 PDGF-A$^{-/-}$ lungs and virtually absent in postnatal lungs compared with wild-type (+/+) littermate controls. The presence of erythrocytes in the alveolar sacs of postnatal lungs is due to aspiration of blood following decapitation of the animal. Bar, 80 μm (160 μm for E and F, which show an overview of the E17.5 lung).

E17.5 PDGF-Rα^{+} cells appeared completely scattered in the PDGF-A$^{+/+}$ lung; at this time most of them occurred as solitary cells lining the lumen of the prospective terminal sacs (Fig. 2E-H). Weak staining for PDGF-Rα was also seen in vascular walls and in cells surrounding the epithelium at more proximal sites; in prospective bronchial and bronchiolar walls. In E17.5 PDGF-A$^{-/-}$ lungs on the other hand, the appearance of numerous scattered PDGF-Rα^{+} cells was not seen, and in regions where thin-walled tubules of the prospective terminal sacs dominated, hardly any PDGF-Rα^{+} cells could be found



(Fig. 2F,H). Instead, the few PDGF-Rα$^+$ cells seen in E17.5 lungs were situated in small clusters at more proximal epithelial tubules (Fig. 2F,H). At E18.5 and postnatal day (P)1, the difference between PDGF-Rα expression patterns in PDGF-A$^{+/+}$ and PDGF-A$^{-/-}$ lungs was pronounced in that no, or very few, PDGF-Rα$^+$ cells were seen in the walls of terminal sacs in PDGF-A$^{-/-}$ lungs, whereas such cells were numerous in PDGF-A$^{+/+}$ lungs (Fig. 2I,J). At P4-P7 the density of scattered PDGF-Rα$^+$ cells declined in the PDGF-A$^{+/+}$ lung, suggesting down-regulation of PDGF-Rα in differentiated cells (Fig. 2K). PDGF-A$^{-/-}$ lungs lacked PDGF-Rα$^+$ cells also at P4-7 (Fig. 2L). The weak PDGF-Rα expression was similar in PDGF-A$^{+/+}$ and PDGF-A$^{-/-}$ lung tissue, and occurred typically in developing bronchial and vascular walls. This expression remained in the early postnatal lungs but declined before P7.

Tropoelastin expression and elastin deposition in developing normal and PDGF-A-deficient lungs

Elastin deposition in the alveolar septum is believed to be accomplished by interstitial myofibroblast-like cells (Collett and Des Biens, 1974; Fukuda et al., 1983; Noguchi and Samaha, 1991; Vaccaro and Brody, 1978), but the exact identity of the elastin-producing cell(s) has remained unclear, considering the presence of three types of contractile mesenchymal cells in the alveolar septum: the alveolar ring SMC, alveolar myofibroblasts and microvascular pericytes (Kapanchi and Gabbiani, 1997). We therefore studied the tropoelastin mRNA expression pattern in PDGF-A$^{+/+}$ and PDGF-A$^{-/-}$ embryonic and postnatal lungs. At E15.5, expression of tropoelastin mRNA was confined to mesenchymal cells lining the proximal tubules, apparently leaving the buds free from deposited elastin (Fig. 3A-C). At E18.5, when branching of the lung is terminated (Hilfer, 1996), essentially all of the lung mesenchyme was strongly tropoelastin-positive (Fig. 3D-F). Tropoelastin expression was also abundant in the developing vasculature, and was seen both in vascular endothelium and in the developing vascular wall (Fig. 3E,F,K,M). Postnatally, the lung tropoelastin expression became progressively restricted to a subset of interstitial cells. Already on P1 the general mesenchymal expression of tropoelastin mRNA was significantly down-regulated (Fig. 3G-I), and later, at P7-P14, expression was limited to a scattered population of mesenchymal cells having the expected location of alveolar SMC progenitors (Fig. 3J,K), namely at the edge of growing alveolar septa, and to developing vascular and bronchial wall cells. In PDGF-A$^{-/-}$ lungs, the tropoelastin expression pattern at prenatal ages was similar to that of PDGF-A$^{+/+}$ lungs (Fig. 3C,F,I). Postnatally, however, there was complete and selective loss of the scattered mesenchymal tropoelastin-positive cells in the PDGF-A$^{-/-}$ lungs (Fig. 3L,M), whereas the vascular and bronchial wall expression was indistinguishable in PDGF-A$^{+/+}$ and PDGF-A$^{-/-}$ lungs (Fig. 3J,L).

The deposition of elastin fibres was studied using van Gieson-elastin staining of tissue sections. Before P4, i.e. before the onset of alveogenesis, no difference could be discerned between PDGF-A$^{+/+}$ and PDGF-A$^{-/-}$ lungs (data not shown). For example, in the wall of the prealveolar sacs at P4, elastic fibres were seen of the same abundance and thickness in both genotypes. After onset of alveogenesis a dramatic difference was noticed, however; the elastin deposition associated with alveolar septum formation did not occur in PDGF-A$^{-/-}$ lungs (Fig. 3N,O).

Fig. 3. Tropoelastin expression in developing lungs. Non-radioactive in situ hybridization of wild-type (+/+; A,C,D,E,G,H,J,K,N) and PDGF-A$^{-/-}$ (B,F,I,L,M,O) sections. (A-C) In E15.5 (B) and E16.5 (A,C) lungs, mesenchymal cells adjacent to the epithelial tubules produce tropoelastin mRNA. Expression is intense in mesenchymal cells (arrows) lining the proximal epithelial tubules (pt), while the mesenchyme surrounding the epithelial buds (eb) is devoid of tropoelastin expression. Tropoelastin mRNA is also produced by endothelial and SMC progenitors in blood vessels (bv). In E15.5-16.5 lungs, tropoelastin expression does not seem to be affected by PDGF-A deficiency. (D-F) At E18.5 tropoelastin mRNA is produced by all mesenchymal cells in the lung, at proximal (pt) as well as distal (dt) sites, and in blood vessel (bv) endothelial and SMC cells. PDGF-A deficiency did not affect this second phase of tropoelastin expression. (G-I) Postnatally, at P1, the general expression of tropoelastin mRNA is significantly reduced, and most mesenchymal cells are weakly stained. The high level of expression is maintained in blood vessels (arrows). (J-M) At P7, a population of scattered mesenchymal cells, typically located at the tips of ingrowing alveolar septa (arrows), express tropoelastin. The scattered tropoelastin-positive cells are completely absent in PDGF-A$^{-/-}$ lungs (L,M). The presence of tropoelastin expressing blood vessels (bv) in PDGF-A$^{+/+}$ and PDGF-A$^{-/-}$ lungs provides an internal control of hybridization efficiency. (N,O) Elastin fibers (arrowheads), visualised by van Gieson-elastin staining, are deposited in bundles in the alveolar septa (arrows) of P20 PDGF-A$^{+/+}$ lungs. Consistent with the total absence of scattered tropoelastin producing cells in the alveolar sac walls of PDGF-A$^{-/-}$ lungs, elastin fibers are dramatically reduced in number in the P20, PDGF-A$^{-/-}$ lungs. The presence of erythrocytes in the alveolar sacs of postnatal lungs is due to aspiration of blood following decapitation of the animal. Bar, 320 μm for A,D,G,J,L; 80 μm for C,E,F,H,I,K,M; 60 μm for B and 25 μm for N and O.

PDGF-Rα and tropoelastin expression do not overlap in the alveolar SMC lineage

The lack of distal spreading of PDGF-Rα$^+$ cells seen prenatally correlates with the later occurring absence of tropoelastin-producing cells in growing alveolar septa as well as with the subsequent lack of mature alveolar SMC. We assumed that these cells represent the same lineage, in which PDGF-Rα expression preceeds that of tropoelastin. In P4 PDGF-A$^{+/+}$ lungs (at the onset of alveogenesis), both PDGF-Rα and tropoelastin were expressed in mesenchymal cells occurring at roughly the same abundance (Fig. 4). However, whereas the tropoelastin-positive cells were preferentially associated with epithelial folds typical of forming alveolar septa (Fig. 4A,B), this localization was not apparent for the PDGF-Rα$^+$ cells, which were scattered, but not preferentially located to forming alveolar septa (Fig. 4C,D). Although a few PDGF-Rα$^+$ cells appear to localize to forming alveolar septa (arrow in Fig. 4C), the divergent expression patterns are consistent with down-regulation of PDGF-Rα expression immediately prior to, or following, the onset of tropoelastin expression in alveolar SMC progenitors. Lack of overlapping expression of PDGF-Rα and tropoelastin was seen also at P7 when alveogenesis had proceeded further, by which time fewer PDGF-Rα$^+$ cells, but a larger number of tropoelastin-positive cells, were seen compared to P4, and where preferential location of the tropoelastin-positive, but not the PDGF-Rα$^+$ cells, occurred in developing alveolar septa (compare Figs 2K and 3K). Tropoelastin and strong PDGF-Rα expression was also not overlap-

ping at the pseudoglandular stage (compare Figs 2A and 3C); at this time PDGF-Rα expression occurred in distal mesenchyme and tropoelastin expression in proximal mesenchyme.

Smooth muscle α-actin expression in the developing mouse lung

Prenatally, smooth muscle α-actin was expressed in prospective bronchial, bronchiolar and vascular walls (Fig. 5A-D) but not in the PDGF-Rα$^+$ mesenchyme at the terminal buds. This pattern was indistinguishable in PDGF-A$^{+/+}$ and PDGF-A$^{-/-}$ lungs. Postnatally, a similar location of smooth muscle α-actin-positive cells was seen; positive cells occurred in bronchi and large blood vessels (not capillaries), but did not show a scattered distribution similar to that of PDGF-Rα$^+$ or tropoelastin-positive cells (Fig. 5E-H and data not shown). We have shown previously that smooth muscle α-actin is expressed in alveolar SMC once alveogenesis is completed, from approximately 3 weeks of postnatal age (Boström et al., 1996). However, at this age, PDGF-Rα or tropoelastin were no longer expressed in these cells (data not shown). It appears that the expression of smooth muscle α-actin occurs relatively late in the ontogeny of alveolar SMC, i.e. in alveolar ring muscles only once the process of alveogenesis is completed, but is already seen in bronchial or vascular wall SMC prenatally.

Although smooth muscle α-actin did not label specifically the alveolar SMC cell lineage, abnormal vascular expression of this protein was revealed in the P14 PDGF-A$^{-/-}$ lung. Here, the expression was strongly up-regulated in arterioles (Fig. 5H). We have shown previously that 3-week-old PDGF-A$^{-/-}$ pups have right-sided cardiac myocardium hypertrophy (Boström et al., 1996). It is possible that both these phenotypes reflect the same secondary changes in the lung, rather than being primarily caused by PDGF-A deficiency.

Desmin expression in the developing mouse lung

Desmin has been suggested as a marker for alveolar ring muscles and alveolar myofibroblasts (Kapanchi and Gabbiani, 1997). We found that desmin was expressed in a large proportion of mesenchymal cells in the developing pre- and postnatal lung. This expression pattern did not specifically overlap with that of PDGF-Rα or tropoelastin after P2. No apparent differences were noticed between the desmin expression patterns in PDGF-A$^{+/+}$ and PDGF-A$^{-/-}$ lungs at prenatal or postnatal ages (Fig. 5F-O). We conclude that desmin is not a specific marker for alveolar SMC progenitors, but shows an expression pattern and distribution consistent with alveolar myofibroblasts, or possibly microvascular pericytes. Whereas both alveolar SMC and myofibroblasts have been suggested to be desmin positive by others, lung pericytes were suggested to be negative (Kapanchi and Gabbiani, 1997). In this study, desmin was also found in bronchial epithelial cells, specifically located to the ciliae (Fig. 5N).

PDGF-B and -Rβ expression patterns in the developing lung

As shown above, the expression patterns of PDGF-A and -Rα mRNA in the developing lung suggest that a PDGF-A-containing PDGF dimer released by lung epithelial cells promotes spreading of PDGF-Rα-positive progenitor cells. Since PDGF-B can bind to and activate PDGF-Rα and since PDGF-B can form heterodimers with PDGF-A, it was of interest to see if PDGF-B was expressed in the developing lung. By in situ hybridization, PDGF-B was shown to be expressed in a subset of cells in the lung mesenchyme (Fig. 1C). At E14.5 these cells locate to a region outside the clusters of PDGF-Rα-positive mesenchymal cells, and appear at sites where extensive blood vessel formation takes place (Ten Have-Opbroek, 1981). We have recently shown that PDGF-B is expressed by capillary and small arteric endothelial cells in many tissues of the mouse embryo, including the lung, as well as in fetal liver megakaryocytes (Lindahl et al., 1997). The absence of any overlap between PDGF-A and -B expression in the developing lung suggests that the lung epithelium produces PDGF-AA dimers and the lung capillary endothelium produces PDGF-BB dimers, and that no PDGF-AB dimers are formed. It is clear from the PDGF-A null phenotype that the PDGF-Rα-positive alveolar ring SMC require PDGF-AA. Whether they also require endothelium-derived PDGF-BB is at present not clear, but studies of the spreading of PDGF-Rα-positive lung mesenchymal cells in E17.5-18.5 PDGF-B knockout embryos should answer this question.

PDGF-Rβ was expressed in yet another subset of mesenchymal cells, these being either located in the developing lung arteric

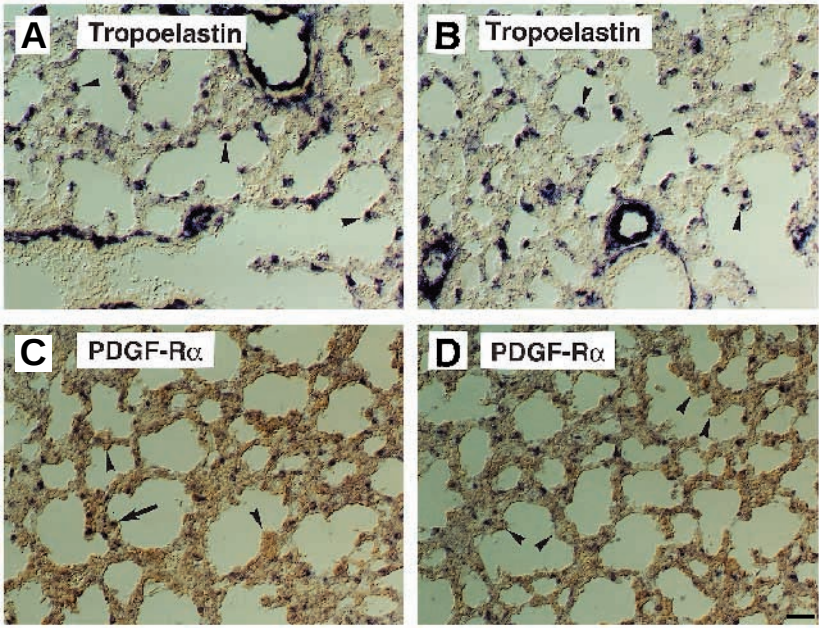


Fig. 4. Tropoelastin and PDGF-Rα expression at P4.5. Two representative areas of lung tissue are shown for each hybridization. Close comparison of the expression patterns reveal that most tropoelastin-positive cells (arrowheads indicate examples in A and B) are located to growing alveolar septa, whereas most PDGF-Rα$^+$ cells did not have an apparent localization to alveolar septa (arrowheads in C and D). A few PDGF-Rα$^+$ cells were seen in association with structures that may be alveolar septa, as indicated by the arrow in C. Bar, 80 μm.

walls, or intermingled with the PDGF-B-positive cells and having a scattered location (Fig. 1D). This pattern is distinctly different from that of the PDGF-Rα expression, since the PDGF-Rβ-positive cells are already scattered in the lung mesenchyme at E14.5, i.e. during the pseudoglandular stage, at which PDGF-Rα expression occurs in clusters of mesenchymal cells at the epithelial buds (Fig. 1B). We have recently shown that developing microvascular pericytes in other locations express PDGF-Rβ and spread along sprouting capillary endothelium in a PDGF-B-dependent manner (Lindahl et al., 1997). Indeed, the PDGF-Rβ-positive cells in the lung also require PDGF-B for spreading (Lindahl et al., 1997, and data not shown). It is therefore likely that the scattered lung PDGF-Rβ-positive cells represent developing microvascular pericytes. In summary, the two genes encoding the constituent chains of PDGF ligands and the two PDGF receptor genes are all expressed in the developing mouse lung, probably in different cell types, as schematically illustrated in Fig. 6.

DISCUSSION

Epithelial-mesenchymal interactions in lung development

Four stages of lung development can be

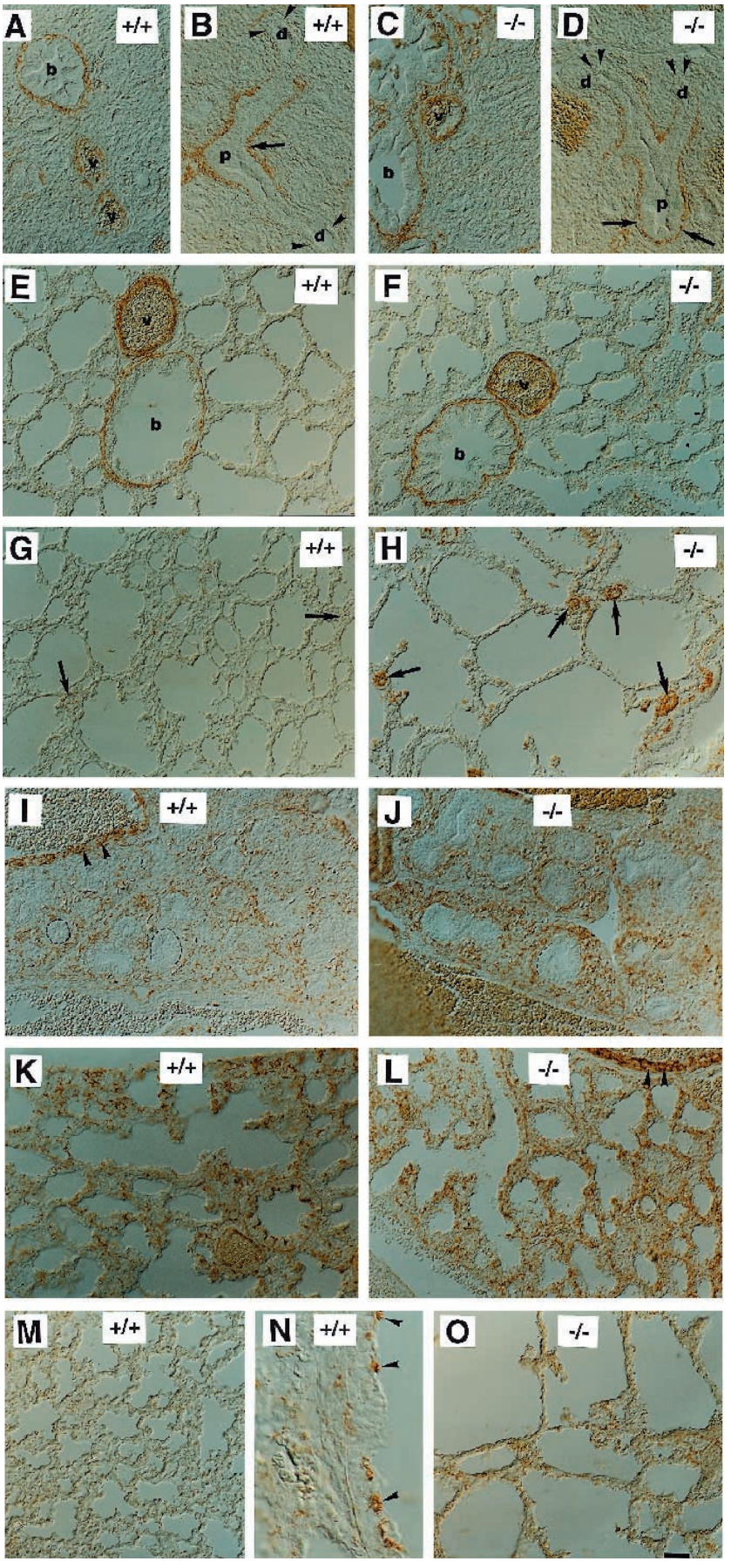


Fig. 5. Smooth muscle α-actin and desmin expression in developing lungs. Smooth muscle α-actin immunohistochemistry is shown for E15.5 (A-D), P1 (E,F) and P14 (G,H) lungs. Expression in seen in bronchial (b) and blood vessel (v) walls and in the mesenchyme surrounding proximal tubular epithelium (p) (arrows in B and D) but not distal epithelium (d) (arrowheads in B and D). Thus smooth muscle α-actin expression does not occur at detectable levels in the PDGF-Rα+ mesenchyme, which is situated surrounding the distal epithelium and the epithelial buds (see Figs 1 and 2). No difference was noticed between the patterns of expression of smooth muscle α-actin in wild-type (+/+) or PDGF-A$^{-/-}$lungs. At P14, smooth muscle α-actin was up-regulated in arterioles of the emphysematous PDGF-A$^{-/-}$ lung (compare G with H; arterioles indicated by arrows). Desmin immunohistochemistry is shown for E 15.5 (I,J), P1 (K,L) and P14 (M-O). At E15.5, desmin expression occurs in mesenchymal cells at some distance from the epithelial tubules (examples of basal epithelial surfaces are indicated by dotted lines), thus coinciding with the region where extensive blood capillary formation takes place and where PDGF-B and PDGF-Rβ expression occurs. Thus desmin expression does not seem to occur in the PDGF-Rα^{+} cells (see Figs 1 and 2). The tropoelastin-positive cells situated in developing alvoelar septa were also desmin negative, or expressed very low levels of desmin (M). No difference was apparent between the desmin expression patterns in wild-type or PDGF-A$^{-/-}$ lungs at prenatal or early postnatal ages. Desmin is also expressed in the ciliae of bronchial epithelial cells (N, arrowheads). Bar, 80 μm (40 μm for N).

discerned, based on histological appereance (Ten Have-Opbroek, 1981, 1991). During the pseudoglandular stage (E9.5-E16.6 in the mouse) the primitive lung bud forms the basic pattern of bronchi through dichotomous branching (Hilfer, 1996). This branching appears to be induced by the mesenchyme surrounding the distal ends of the epithelial tubules. During the canalicular stage (E16.6-E17.4 in the mouse), the terminal respiratory portion of the lungs is established through continued centrifugal branching. Moreover, the epithelial cells lining the prospective terminal sacs develop a flattened appereance, and close contacts are formed between blood vessels and the developing respiratory units. The terminal sac stage (E17.4-P5 in the mouse) is characterized by expansion of the terminal tubules into sacs with a flat epithelium composed of differentiated pneumocytes. The last stage, alveogenesis, is initiated postnatally (starting approximately at P5 in the mouse) by the formation of alveolar septa.

Epithelial-mesenchymal reciprocal signalling is known to occur during the development of many parenchymal organs. For example, the role of the distal lung mesenchyme for inducing branch formation is well established (Alescio and Cassini, 1962; Goldin et al., 1984; Goldin and Wessels, 1979; Hilfer, 1996; Wessels, 1970). How this signalling is governed at the molecular level is not fully clear, but a number of molecules have been implicated. Transgenic overexpression, or misexpression, of bone morphogenetic protein-4 (Bellusci et al., 1996), sonic hedgehog (Bellusci et al., 1997), transforming growth factor (TGF)-α (Korfhagen et al., 1994), TGF-β1 (Zhou et al., 1996) or fibroblast growth factor (FGF)-7 (Simonet et al., 1995) lead to defects in lung development. Inhibition of FGF-receptor signalling by expression of dominant negative FGF-R2 inhibits branching beyond two main bronchi (Peters et al., 1994). Involvement of transforming growth factor-β members in lung development is also suggested from the knockout of TGF-β3 (Kaartinen et al., 1995) and from in vitro studies of the effect of TGF-β1 on lung epithelial branching (Serra et al., 1994). Epidermal growth factor receptor (EGFR) signalling is implicated in lung development since EGFR null mice display abnormal lung development (Miettinen et al., 1995).

The lung defect seen in PDGF-A$^{-/-}$ mice combined with the normal developmental expression of PDGF-A and its receptor, suggest that paracrine PDGF-A – PDGF-Rα receptor signalling constitutes a critical part of the epithelial-mesenchymal signalling in the developing lung, and that epithelial-mesenchymal interaction in the lung is also important for functions other than branch initiation. Specifically, the present work suggests that when the final bronchial branches are induced, epithelial cell signalling involving PDGF-AA is essential for a subset of mesenchymal cells to multiply and move to a new, more distal location, at which they can complete the last stage of lung morphogenesis: alveogenesis.

PDGF-A dependent multiplication and spreading of PDGF-Rα+ cells in the developing lung

PDGF-A is expressed in the tubular epithelium during the pseudoglandular stage. At this stage strong PDGF-Rα expression occurs in mesenchymal cells surrounding the end buds and the distal parts of the same tubules. During the canalicular stage an important change occurs in the distribution of the PDGF-Rα^{+} cells as they spread along the surface of the prospective terminal sacs. The scattered PDGF-Rα^{+} cells remain in this position until the onset of alveogenesis, at which time the PDGF-Rα mRNA is down-regulated. The details of the expression patterns in PDGF-A$^{+/+}$ compared to PDGF-A$^{-/-}$ animals suggests that the specific absence of the scattered PDGF-Rα^{+} cells in PDGF-A$^{-/-}$ lungs is due to a block in proliferation and distal spreading of these cells. PDGF-A$^{-/-}$ lungs contain far fewer PDGF-Rα^{+} cells at E17.5, but in addition, the remaining cells occur mainly at a proximal location, lining prospective bronchioles, and little if any scattering of PDGF-Rα^{+} cells is seen. These observations are consistent with a model where PDGF-Rα^{+} cells lining the acinar tubules relocate from this site to the epithelial surface of the prospective terminal saccules in response to PDGF-AA (Fig. 7). In the absence of PDGF-AA, spreading does not occur, and conse-

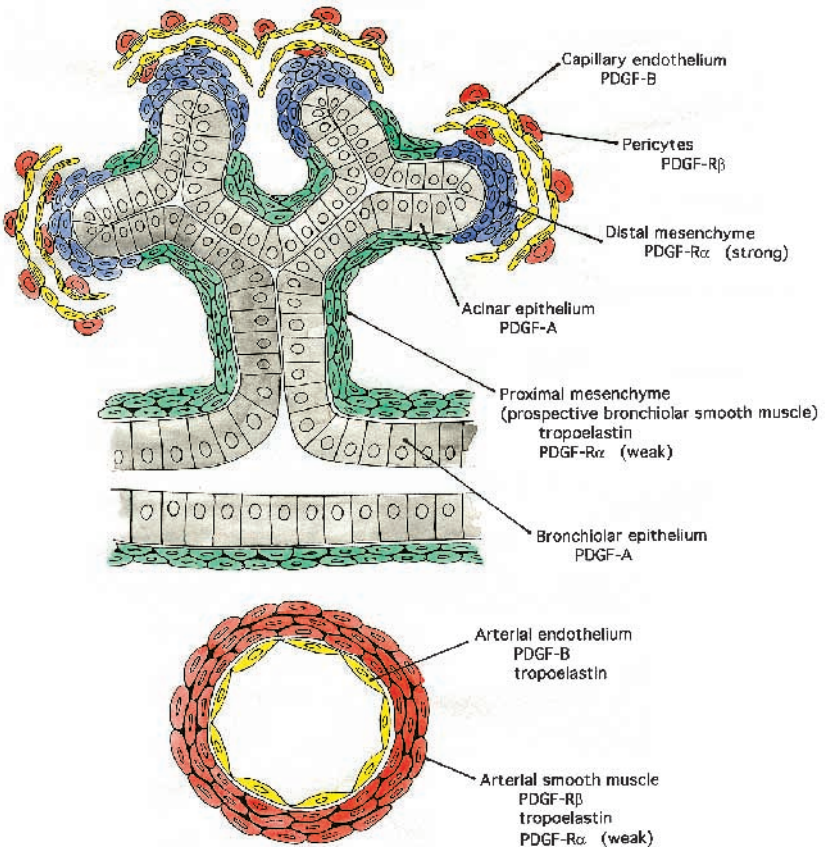


Fig. 6. PDGF, PDGF receptor and tropoelastin expression in the developing lung. Schematical illustration of expression sites at the pseudoglandular stage of lung development in proximal and distal epithelial tubules and associated mesenchyme and in lung arteries and arterioles. The following cell types are shown in different colours: vascular endothelium (yellow), lung epithelium (grey), vascular wall SMC progenitors (red), bronchiolar SMC progenitors (green) and alveolar SMC progenitors (blue).

quently the PDGF-Rα$^+$ cells remain at proximal (bronchiolar) sites along the epithelial branches. The failure of spreading of PDGF-Rα$^+$ cells may therefore reflect the lack of PDGF-AA-induced cell proliferation and migration. The proposed model would also be compatible with alternative, or additional, functions of PDGF-A such as regulation of cell survival and differentiation. However, we found no histological evidence of increased apoptosis in PDGF-A$^{-/-}$ lungs, and undetectable apoptosis by TUNEL labelling in wild-type or PDGF-A$^{-/-}$ lungs (data not shown). This appears consistent with the previously reported very low apoptosis frequency in the late mouse embryonic lung (Bellusci et al., 1997, 1996).

The PDGF-Rα$^+$ cells are probably alveolar SMC progenitors

Because of the lack of overlapping markers, we have failed to produce direct evidence that the PDGF-Rα$^+$ cells that spread distally at the canalicular stage of lung development are indeed the progenitors of the tropoelastin-positive cells actively involved in the process of alveogenesis from P4, and the later occurring smooth muscle α-actin-positive alveolar SMC. Our assumption that these cells represent the same cell lineage, and consequently that PDGF-Rα is down-regulated at the onset of tropoelastin expression and alveogenesis, is, however, strongly supported by several pieces of indirect evidence. The absence of scattered PDGF-Rα$^+$ cells in PDGF-A$^{-/-}$ lungs from E17.5 correlates with the absence of scattered tropoelastin-expressing cells from P4, as well as with the specific and complete absence of differentiated alveolar SMC at 3 weeks. By transmission electron microscopy, no other cells but the alveolar SMC were found to be missing in the PDGF-A$^{-/-}$ lung (Boström et al., 1996). These studies did not address the abundance of alveolar myofibroblasts and pericytes, but the desmin stainings shown here suggest that the alveolar myofibroblast compartment is unaffected. Pericytes are also probably not affected since pericyte loss leads to vascular leakage, microaneurysm formation and hemorrhage, as occurs in PDGF-B$^{-/-}$ mice (Lindahl et al., 1997) but not in PDGF-A$^{-/-}$ mice. The spatial distribution and abundance of PDGF-Rα$^+$ cells in PDGF-A$^{+/+}$ lungs are in agreement with a role in alveogenesis. It is also reasonable to predict that a cell type (or its progenitor) that is specifically lost in PDGF-A-deficient mice should carry the receptor for PDGF-A at a stage in development when PDGF-A is expressed and also be located close to the source of ligand, as is the case for the PDGF-Rα$^+$ mesenchymal cells discussed.

Role of PDGF-A in lung elastogenesis

Elastin is the major extracellular matrix constituent of the lung, making up approximately 25% of the dry weight of the adult lung. The tropoelastin mRNA expression patterns in developing PDGF-A$^{+/+}$ and PDGF-A$^{-/-}$ lungs reveal three phases of lung elastogenesis, brought about in part by different cell types, and showing different dependence on PDGF-A/Rα signalling. At the pseudoglandular stage, elastin is expressed in mesenchyme apposed to the proximal lung epithelium, whereas the distal mesenchyme, which is PDGF-Rα positive, lacks tropoelastin expression at this stage (Fig. 3 A-C). Considering the polarised expression of tropoelastin, and the different properties of proximal and distal mesenchyme for inducing branch formation (Heine et al., 1990; Hilfer et al., 1985), elastin fibers deposited at proximal sites may be important for prevention of ectopic branching of the lung epithelium. Conversely, the lack of elastin deposition at the buds during the pseudoglandular stage may permit further branching. This first phase of lung elastogenesis is unaffected by the absence of PDGF-A. During the terminal sac stage, at which time epithelial branching has ceased, tropoelastin is strongly expressed by a majority of lung mesenchymal cells both at proximal and distal sites, apparently including smooth muscle progenitors as well as lung fibroblasts. This expression likely leads to deposition of elastic fibres in the walls of the terminal sac s, which provides sufficient elasticity in these structures to allow for efficient breathing during the first postnatal days. This second phase of lung elastogenesis is not detectably influenced by the absence of PDGF-A. The third phase involves deposition of elastin in alveolar septa by prospective alveolar SMC, a process intimately coupled to alveolar septation. This phase shows an obligatory requirement for PDGF-A. The hypothesis has been put forward that elastin deposition by alveolar cells provides a critical driving force in the morphogenesis of alveolar septa, based on morphological studies of lung development (Burri and Weibel, 1977; Emery, 1970; Noguchi et al., 1989). Our data support this hypothesis by correlating the loss of alveolar septal elastin deposition with

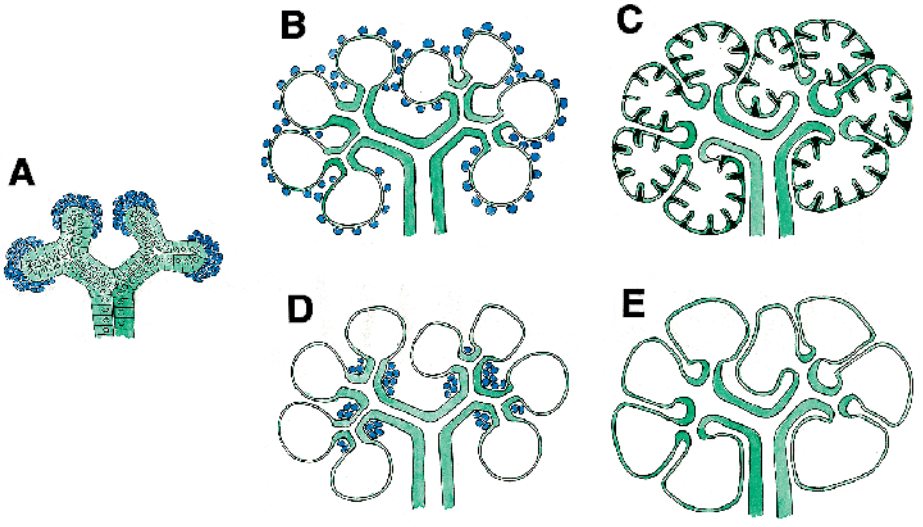


Fig. 7. Model of alveolar SMC development. Alveolar SMC progenitors (blue) originate at the pseudoglandular stage as clustered PDGFRα$^+$ mesenchymal cells located at the epithelial buds (A). In conjunction with the canalicular stage, these cells spread to acquire positions surrounding the prospective terminal sacs (B). During the terminal sac to alveolar stages, the cells down-regulate PDGFRα expression and up-regulate tropoelastin expression (black) (C). In PDGF-A$^{-/-}$ lungs the distal spreading of PDGFRα$^+$ progenitors does not occur (D), and consequently, alveolar septal elastin deposition (and alveogenesis) fails (E).

loss of septum formation in PDGF-A$^{-/-}$ mice. Irrespective of whether or not elastin deposition is a morphogenetic force in alveogenesis, the importance of elastin to alveolar septum integrity is supported by studies of the pathogenesis of human lung emphysema. In this condition(s) septal elastin destruction appears to be critical, brought about either by genetic anti-elastase deficiency, or by increased release of inflammatory elastases (Janoff, 1988; Muley et al., 1994; Snider, 1984). Thus although implying a common critical denominator, alveolar septal elastin, the pathogenesis of emphysema in human beings and in PDGF-A-deficient mice appears to be different.

Arteriolar reaction and cor pulmonale in PDGF-A$^{-/-}$ mice

Up-regulation of smooth muscle α-actin in blood vessel wall cells is often found in conjunction with hypertension, and is seen in hypertensive aorta (Clements et al., 1997), in arterioles in pulmonary hypertension (Jones et al., 1997) and in mesangial cells during the development of hypertensive renal damage (Kimura et al., 1996). Thus both the up-regulated expression of smooth muscle α-actin seen in the arterioles of the emphysematous lungs of 2-week-old PDGF-A$^{-/-}$ pups (this study), and the cor pulmonale seen at 3 weeks (Boström et al., 1996) are indicative of pulmonary hypertension. In human patients with emphysema, muscularization of pulmonary arterioles sometimes occurs, which leads to pulmonary hypertension and cor pulmonale (Marshall and Marshall, 1997). It is believed that the cause of these changes is persistent hypoxia, which leads to prolonged pulmonary vasoconstriction and secondary vascular changes (Marshall and Marshall, 1997; Riley et al., 1997). It can be assumed that the advanced and generalized emphysema of the PDGF-A$^{-/-}$ mice leads to hypoxia. Angiotensin II is known to up-regulate smooth muscle α-actin (Andrawis et al., 1996) and stimulate vascular smooth muscle cell hypertropy (Morrell et al., 1996; Patton et al., 1995), and is therefore a possible mediator of the vascular reaction seen in the emphysematous PDGF-A$^{-/-}$ lung.

In summary, the lung arteriolar phenotype and the cor pulmonale of PDGF-A$^{-/-}$ mice from 2 weeks of postnatal age may both be secondary to hypoxia occurring as a result of severe lung emphysema.

Generic features of PDGF-A and PDGF-B function

By phenotypical analysis of PDGF knockout mice, we have found generic features of PDGF-A and PDGF-B function. Three types of smooth muscle cells/myofibroblasts show an obligatory requirement for different PDGF isoforms during development; alveolar SMC depend on PDGF-A (Boström et al., 1996); this study) and microvascular pericytes and kidney glomerular mesangial cells depend on PDGF-B (Levéen et al., 1994; Lindahl et al., 1997; Soriano, 1994). In all three cases, locally produced PDGF appears to trigger PDGF receptor-positive progenitor cells to multiply and spread from a proximal to a distal location. A similar function may also be performed by PDGF-A during Xenopus gastrulation (Ataliotis et al., 1995), at which time mesodermal cells move along the blastocoel roof on a posterior-to-anterior route. Taken together, the available data thus suggest that PDGFs provide selective signals during embryonic development, by acting on the PDGF receptor-carrying progenitor cells and allowing for their proliferation and spreading on PDGF-producing endothelial or epithelial sheet/tubes. It is possible that this selective process involves proliferation and migration only, processes known to be regulated by PDGF in vitro (Betsholtz and Raines, 1997), but it may involve other functions as well, such as cell survival or differentiation.

We would like to thank Sara Beckman for technical assistance, and Drs J. Escobedo, J. Foster, W.D. Richardson, and P. Soriano for DNA probes. This study was supported by grants from the Swedish Cancer Foundation, the Swedish Medical Research Council, the Ingabritt and Arne Lundberg Research Foundation, the Cancer Research Campaign UK and the Wellcome Trust.

REFERENCES

Alescio, T., and Cassini, A. (1962). Induction in vitro of tracheal buds by pulmonary mesenchyme grafted on tracheal epithelium. *J. Exp. Zool.* **150**, 83-94.

Andrawis, N. S., Wang, E., and Abernethy, D. R. (1996). Endothelin-1 induces an increase in total protein synthesis and expression of the smooth muscle alpha-actin gene in vascular smooth muscle cells. *Life Sci*. **59**, 523-528.

Ataliotis, P., Symes, K., Chou, M. M., Ho, L., and Mercola, M. (1995). PDGF signalling is required for gastrulation in *Xenopus laevis*. *Development* **121**, 3099-3110.

Bellusci, S., Furuta, Y., Rush, M. G., Henderson, R., Winnier, G., and Hogan, B. L. M. (1997). Involvement of Sonic hedgehog (Shh) in mouse embryonic lung growth and morphogenesis. *Development* **124**, 53-63.

Bellusci, S., Henderson, R., Winnier, G., Oikawa, T., and Hogan, B. L. M. (1996). Evidence from normal expression and targeted misexpression that bone morphogenetic protein-4 (Bmp-4) plays a role in mouse embryonic lung morphogenesis. *Development* **122**, 1693-1702.

Betsholtz, C., and Raines, E. (1997). Platelet-derived growth factor: A key regulator of connective tissue cells in embryogenesis and pathogenesis. *Kidney Int.* **51**, 1361-1369.

Boström, H., Willetts, K., Pekny, M., Levéen, P., Lindahl, P., Hedstrand, H., Pekna, M., Hellström, M., Gebre-Medhin, S., Schalling, M., Nilsson, M., Kurland, S., Törnell, J., Heath, J. K., and Betsholtz, C. (1996). PDGF-A signaling is a critical event in lung alveolar myofibroblast development and alveogenesis. *Cell* **85**, 863-873.

Burri, P. H., and Weibel, E. R. (1977). Ultrastructure and morphometry of the developing lung. In *Development of the Lung, Part 1: Structural Development* (ed. W. A. Hodson), pp. 215-268. New York: Marcel Dekker.

Clements, M. L., Banes, A. J. and Faber, J. E. (1997). Effect of mechanical loading on vascular alpha 1D- and alpha 1B-adrenergic receptor expression. *Hypertension* **29**, 1156-1164.

Collett, A. J. and Des Biens, G. (1974). Fine structure of the myogenesis and elastogenesis in the developing rat lung. *Anat. Rec.* **179**, 343-360.

Emery, J. L. (1970). The postnatal development of the human lung and its implication for lung pathology. *Respiration* **27**, 41-50.

Fukuda, Y., Ferrans, V. J. and Crystal, R. G. (1983). The development of alveolar septa in fetal sheep lung. An ultrastructural and immunohistochemical study. *Am. J. Anat.* **167**, 405-439.

Goldin, G. V., Hindman, H. M. and Wessells, N. K. (1984). The role of cell proliferation and cellular shape change in branching morphogenesis of the embryonic mouse lung: analysis using aphidicolin and cytochalasins. *J. Exp. Zool.* **232**, 287-296.

Goldin, G. V. and Wessels, N. K. (1979). Mammalian lung development: the possible role of cell proliferation in the formation of supernumerary trachea buds and in branching morphogenesis. *J. Exp. Zool.* **208**, 337-346.

Heine, U. I., Munoz, E. F., Flanders, K. C., Roberts, A. B. and Sporn, M. B. (1990). Colocalization of TGF-beta 1 and collagen I and III and glycosaminoglycans during lung branching morphogenesis. *Development* **109**, 29-36.

Heldin, C.-H. (1992). Structural and functional studies of platelet-derived growth factor. *EMBO J.* **11**, 4251-4259.

Henrique, D., Adam, J., Myat, A., Chitnis, A., Lewis, J. and Ish-Horowicz, D. (1995). Expression of a Delta homologue in prospective neurons in the chick. *Nature* **375**, 787-790.

Hilfer, S. R. (1996). Morphogenesis of the lung: Control of embryonic and fetal branching. *Annu. Rev. Physiol.* **59**, 93-113.

Hilfer, S. R., Rayner, R. M. and Brown, J. W. (1985). Mesenchymal control of branching pattern in the fetal mouse lung. *Tissue Cell* **17**, 523-538.

Janoff, A. (1988). Emphysema: Proteinase-antiproteinase imbalance. In *Inflammation: Basic Principles and Clinical Correlates* (ed. J. Gallin et al.), pp. 803-825. New York: Raven Press.

Jones, P. L., Cowan, K. N. and Rabinovitch, M. (1997). Tenascin-C, proliferation and subendothelial fibronectin in progressive pulmonary vascular disease. *Am. J. Pathol.* **150**, 1349-1360.

Kaartinen, V., Voncken, J. W., Shuler, C., Warburton, D., Bu, D., Heisterkamp, N. and Groffen, J. (1995). Abnormal lung development and cleft palate in mice lacking TGF-beta3 indicates defects of epithelial-mesenchymal interaction. *Nature Genetics* **11**, 415-421.

Kapanchi, Y., Assimacopoulos, A., Irle, C., Zwahlen, A. and Gabbiani, G. (1974). Contractile interstitial cells in pulmonary alveolar septa: A possible regulator of ventilation/perfusion ratio? *J. Cell Biol.* **60,** 375-392.

Kapanchi, Y. and Gabbiani, G. (1997). Contractile cells in pulmonary alveolar tissue. In T*he Lung. Scientific Foundations*, 2nd edition (ed. R. G. Crystal, J. B. West, E. R. Weibel and P. J. Barnes), pp. 697-707. Philadelphia: Lippincott-Raven Publishers.

Kimura, K., Suzuki, N., Ohba, S., Nagai, R., Hiroi, J., Mise, N., Tojo, A., Nagaoka, A., Hirata, Y., Goto, A., Yazaki, Y. and Omata, M. (1996). Hypertensive glomerular damage as revealed by the expression of alpha-smooth muscle actin and non-muscle myosin. *Kidney Int. Suppl.* **55**, S169-172.

Korfhagen, T. R., Swants, R. J., Wert, S. E., McCarty, J. M., Kerlakian, C. B., Glasser, S. W. and Whitsett, J. A. (1994). Respiratory epithelial cell expression of human transforming growth factor-alpha induces lung fibrosis in transgenic mice. *J. Clin. Invest.* **93**, 1691-1699.

Levéen, P., Pekny, M., Gebre-Medhin, S., Swolin, B., Larsson, E. and Betsholtz, C. (1994). Mice deficient for PDGF B show renal, cardiovascular and hematological abnormalities. *Genes Dev.* **8**, 1875-1887.

Lindahl, P., Johansson, B., Levéen, P. and Betsholtz, C. (1997). Pericyte loss and microaneurysm formation in PDGF-B-deficient mice. *Science* **277**, 242-245.

Marshall, B. E. and Marshall, C. (1997). Pulmonary hypertension. In *The Lung. Scientific Foundations*, 2nd edition (ed. R. G. Crystal, J. B. West, E. R. Weibel and P. J. Barnes), pp. 1581-1588. Philadelphia: Lippincott-Raven.

Miettinen, P. J., Berger, J. E., Meneses, J., Phung, Y., Pedersen, R. A., Werb, Z. and Derynck, R. (1995). Epithelial immaturity and multiorgan failure in mice lacking epidermal growth factor receptor. *Nature* **376**, 337-341.

Miller, W. S. (1921). The musculature of the finer divisions of the bronchial tree and its relation to certain pathological conditions. *Am. Rev. Tuberc.* **5**, 689-698.

Morrell, N. W., Grieshaber, S. S., Danilov, S. M., Majack, R. A. and Stenmark, K. R. (1996). Developmental regulation of angiotensin converting enzyme and angiotensin type1 receptor in the rat pulmonary circulation. *Am. J. Respir. Cell. Mol. Biol.* **14**, 526-537.

Muley, T., Wiebel, M., Schultz, V. and Ebert, W. (1994). Elastinolytic activity of alveolar macrophages in smoking-associated pulmonary emphysema. *Clin. Invest.* **72**, 269-276.

Noguchi, A., Reddy, R., Kursar, J. D., Parks, W. C. and Mecham, R. P. (1989). Smooth muscle isoactin and elastin in fetal bovine lung. *Exp. Lung Res.* **4**, 537-552.

Noguchi, A. and Samaha, H. (1991). Developmental changes in tropoelastin gene expression in the rat lung studied by in situ hybridization. *Am. J. Respir. Cell. Mol. Biol.* **5**, 571-578.

Orr-Urtreger, A. and Lonai, P. (1992). Platelet-derived growth factor-A and its receptor are expressed in separate but adjacent cell layers of the mouse embryo. *Development* **115**, 1045-1058.

Patton, W. F., Erdjument-Bromage, H., Marks, A. R., Pempst, P. and Taubman, M. B. (1995). Components of the protein synthesis and folding machinery are induced in vascular smooth muscle cells by hypertrophic and hyperplastic agents. Identification by comparative protein phenotyping and microsequencing. *J. Biol. Chem.* **270**, 21404-21410.

Peters, K., Werner, S., Liao, X., Wert, S. E., Whitsett, J. and Williams, L. T. (1994). Targeted expression of a dominant negative FGF receptor blocks branching morphogenesis and epithelial differentiation of the mouse lung. *EMBO J.* **13**, 3296-3301.

Ramachandran, R. K., Govindarajan, V., Seid, C. A., Patil, S. and Tomlinson, C. R. (1995). Role for platelet-derived growth factor-like and epidermal growth factor-like signaling pathways in gastrulation and spiculogenesis in the Lytechinus sea urchin embryo. *Dev. Dynam.* **204**, 77-88.

Riley, D., Thakker-Varia, S., Poiani, G. J. and Tozzi, C. A. (1997). Vascular remodelling. In *The Lung. Scientific Foundations*, 2nd edition (ed. R. G. Crystal, J. B. Weste, E. R. Weibel and P. J. Barnes), pp. 1589-1597. Philadelphia: Lippincott-Raven.

Schatteman, G. C., Loushin, C., Li, T. and Hart, C. E. (1996). PDGF-A is required for normal murine cardiovascular development. *Dev. Biol.* **176**, 133-142.

Serra, R., Pelton, R. W. and Moses, H. L. (1994). TGF-beta1 inhibits branching morphogenesis and N-myc expression in lung bud organ cultures. *Development* **120**, 2153-2161.

Simonet, W. S., Derose, M. L., Bucay, N., Nguyen, H. Q., Wert, S. E., Zhou, L., Ulich, T. R., Thomason, A., Danilenko, D. M. and Whitsett, J. A. (1995). Pulmonary malformations in transgenic mice expressing human keratinocyte growth factor in the lung. *Proc. Nat. Acad. Sci. USA* **92**, 12461-12465.

Snider, G. L. (1984). Two decades of research in the pathogenesis of emphysema. *Schweiz Med. Wochenschr.* **114**, 898-906.

Soriano, P. (1994). Abnormal kidney development and hematological disorders in PDGF beta-receptor mutant mice. *Genes Dev.* **8**, 1888-1896.

Soriano, P. (1997). The PDGF α receptor is required for neural crest cell development and for normal patterning of the somites. *Development* **124**, 2691-2700.

Ten Have-Opbroek, A. A. W. (1981). The development of the lung in mammals: an analysis of concepts and findings. *Am. J. Anat.* **162**, 201-219.

Ten Have-Opbroek, A. A. W. (1991). Lung development in the mouse embryo. *Exp. Lung Res.* **17**, 111-130.

Vaccaro, C. and Brody, J. S. (1978). Ultrastructure of developing alveoli. I. The role of the interstitial fibroblast. *Anat. Rec.* **192**, 457-480.

Wessels, N. K. (1970). Mammalian lung development: interactions in formation and morphogenesis of tracheal buds. *J. Exp- Zool.* **175**, 455-466.

Zhou, L., Dey, C. R., Wert, S. E. and Whitsett, J. A. (1996). Arrested lung morphogenesis in transgenic mice bearing an SP-C-TGF-beta1 chimeric gene. *Dev. Biol.* **175**, 227-238.

(Accepted 6 August 1997)