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Mesenchymal-epithelial interactions in lung development and repair: are modeling and remodeling the same process?

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Demayo, F., P. Minoo, C. G. Plopper, L. Schuger, J. Shannon, and J. S. Torday. Mesenchymal-epithelial interactions in lung development and repair: are modeling and remodeling the same process? *Am J Physiol Lung Cell Mol Physiol* 283: L510–L517, 2002; 10.1152/ajplung.00144.2002.—We propose that lung morphogenesis and repair are characterized by complex cell-cell interactions of endodermal and mesodermal origin, leading to (or returning back to) an alveolar structure that can effectively exchange gases between the circulation and the alveolar space. We provide the developmental basis for cell/molecular control of lung development and disease, what is known about growth and transcription factors in normal and abnormal lung development, and how endodermal and mesodermal cell origins interact during lung development and disease. The global mechanisms that mediate mesenchymal-epithelial interactions and the plasticity of mesenchymal cells in normal lung development and remodeling provide a functional genomic model that may bring these concepts closer together. We present a synopsis followed by a vertical integration of the developmental and injury/repair mechanisms.

bronchopulmonary dyplasia; smooth muscle; terminal sac

THE PREMISE OF THIS SYMPOSIUM was that lung morphogenesis and repair are characterized by complex cell-cell interactions of endodermal and mesodermal origin, leading to (or returning back to) an alveolar structure that can effectively exchange gases between the circulation and the alveolar space. The presenters provided the developmental basis for cellular/molecular control of lung development and disease, what is known about growth and transcription factors in normal and abnormal lung development, and how endodermal and mesodermal cell origin interacts during lung development and disease. The global mechanisms that mediate mesenchymal-epithelial interactions and the plasticity of mesenchymal cells in normal lung development and remodeling provide a functional genomic model that may bring these concepts closer together. The following is a synopsis of each presentation, followed by an integration of the developmental and injury/repair mechanisms.

MOLECULAR MECHANISMS OF LUNG DEVELOPMENT[1]

In general, neonatal lung disease overlaps ongoing lung development. Thus bronchopulmonary dysplasia (BPD) may be viewed as the consequence of arrested normal development (alveologenesis) combined with

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[1]Presented by P. Minoo.

abnormal repair of immature, injured lungs. This is an important concept because mechanisms of repair recapitulate fundamental strategies of normal development. Thus the etiology of BPD may be related to interactions between untimely or spatially inappropriate signaling that arises from injury [e.g., inflammatory mediators, transforming growth factor (TGF)-β, etc.] with morphogenetic signaling and transcriptional pathways that control normal development and repair [e.g., fibroblast growth factor (FGF), Nkx2.1].

Lung development. The focus on lung developmental biology is relatively recent and, as in other organs, owes much to the analysis of *Drosophila* developmental genes. To be pertinent to BPD, the focus of the current discussion will be on the "terminal sac" phase of lung development.

Two forces drive lung development. Intrinsic forces consist of the integrated functional activities of a complex set of morphoregulatory molecules that fall into three classes: transcription factors [e.g., Nkx2.1, GATA, hepatocyte nuclear factor (HNF)-3]; signaling molecules [FGF, bone morphogenetic protein (BMP)-4, platelet-derived growth factor (PDGF), *Sonic hedgehog* (*Shh*), TGF-β]; and extracellular matrix proteins and their receptors (collagen, laminin, integrins, cadherins). Expression of each molecule occurs in a temporally, spatially, and cell type-specific manner. Also, the role of each molecule should be deemed not in terms of its isolated function in specific cells but, rather, in the context of epithelial-mesenchymal interactions, a process that is central to lung morphogenesis. The single most important extrinsic force in fetal lung development is lung fluid.

The developmental journey for the lung commences with the specification of a primordium, a group of endodermal cells set aside with explicit instructions to form the lung. Specification appears to involve the activity of the Gli family of mesenchymally localized transcription factors (28). Gli proteins presumably activate downstream genes whose products participate in cell-cell interactions to instruct the adjacent endoderm to form the lung primordium. Subsequently, the lung primordium forms the trachea and grows against the splanchnic mesenchyme. Both *Shh* and Nkx2.1 are required for tracheoesophageal septation (26, 31).

Further development of the lung requires branching morphogenesis, which is dependent on epithelial-mesenchymal interactions through the activity of a complex network of molecules, including peptide growth factors, transcriptional regulators, and members of the extracellular matrix proteins and their receptors. The most profound interruption in branching morphogenesis is observed in FGF-10(−/−) lungs, which lack structures distal to mainstem bronchi (38). Functional deletion of Nkx2.1 results in the absence of distal lung structures beyond the secondary or tertiary bronchi (26). BMP-4, a member of the TGF-β superfamily of signaling molecules, is reduced or absent in Nkx2.1(−/−) lungs. When BMP-4 signaling is interrupted through lung-specific transgenic means (43), distal lung development is inhibited. In *Shh(−/−)* lungs, a similar phenotype as in Nkx2.1(−/−) lungs is found, but there is evidence of limited distal cellular differentiation (31). Thus lung development can be viewed as a two-step process. Proximal lung morphogenesis is independent of Nkx2.1 and *Shh*, whereas distal lung morphogenesis is strictly dependent on Nkx2.1, which appears to occur through the BMP-4 signaling pathway. In the context of understanding BPD, precise knowledge of proximo-distal compartmentalization of the lung is critical, since the latter is the predominant mechanism by which the functional architecture of the distal lung and spatial organization of differentiated epithelial cell types (i.e., alveologenesis) are established.

Deciphering key mechanisms of early lung development has been the major focus of investigation in the last decade. Because BPD occurs in lungs that are in the late (terminal sac) phase of development, a pertinent question is whether and how the information derived from such studies will be relevant to understanding, prevention, and/or treatment of BPD. Two lines of evidence demonstrate the relevance of such information. First, temporal expression of many key mediators of lung morphogenesis occurs throughout embryonic lung development, and some persist to adulthood, suggesting that they may indeed be critical for late lung development and alveologenesis. For example, Nkx2.1 expression, which occurs coincidently with the emergence of the lung primordium, persists in adult alveolar type II cells and is likely necessary for maintenance of differentiated cellular phenotypes, including surfactant protein synthesis. Similarly, HNF-3, GATA-6, and members of the signaling molecules, FGF and PDGF, are expressed throughout fetal and postnatal lung development.

Second, direct evidence for the involvement of specific genetic pathways in alveologenesis has recently emerged. These data demonstrate that a specific molecule may have distinctly different roles during early vs. late lung development. Thus the FGF pathway, whose abrogation leads to disruption of early lung morphogenesis, also participates in alveologenesis. All four FGF receptors (FGFR) are expressed in neonatal lung (47). Mice mutant in both FGFR-3 and FGFR-4 loci are normal at birth but die prematurely with symptoms of chronic pulmonary insufficiency and growth retardation. The lungs of FGFR-3/FGFR-4 knockout mice reveal a phenotype consistent with a complete block in alveologenesis, a phenotype resembling alveolar hypoplasia found in BPD (47). Another important clue is provided by the role of PDGF, PDGF signaling. PDGF ligands interact with various cell types through two types of receptor tyrosine kinases, PDGF-Rα and PDGF-Rβ (23). PDGF-A is expressed in the embryonic lung by epithelial cells; its receptors are found on the mesenchymal cells surrounding the branching epithelium. The latter configuration establishes a paracrine signaling pathway with important implications for epithelial-mesenchymal cross talk. Targeted disruption of PDGF-A disrupts alveolar septation and causes early neonatal death, consistent with the pathomorphological features of human BPD (7). These data clearly indicate that identification and characteriza-

tion of critical regulatory factors in early lung development provide pertinent information regarding neonatal lung disease. Furthermore, elucidation of mechanisms such as FGF or PDGF signaling will provide the rationale for innovative interventional strategies. The questions confronting us now are *1*) whether in neonates at risk for BPD, FGF and/or PDGF signaling is disrupted, and *2*) what mediator(s) of injury in fact disrupts the latter signaling pathways?

Inflammation. BPD is a highly complex, chronic lung disease of multifactorial etiology. We have proposed that the main pathway through which the effects of various insults, such as antenatal infection, surfactant insufficiency (volutrauma), or oxygen toxicity are translated into lung injury, is "inflammation." The close association between preterm labor and maternal infection is well established (15). Also, antenatal inflammation, as assessed by elevated tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-8, or IL-6, correlates with adverse neonatal outcome (53). In preterm neonates, we and others (18) have found that mediators of inflammation are present in the lungs as early as the first hour of life. Alveolar macrophages from preterm neonates at risk for BPD exhibit a robust "termlike" capacity to produce TNF-α and IL-1β (M. J. Blahnik, R. Ramanathan, C. R. Riley, C. A. Jones, and P. Minoo, unpublished observation). Notably, neonatal alveolar macrophages show reduced potential for expression of IL-10, an anti-inflammatory mediator that is key in controlling self-injury caused by dysregulated, smoldering inflammation (18). Although incapable of producing IL-10, neonatal macrophages retain normal capacity for responding to exogenous IL-10, raising the possibility for effective IL-10 recombinant peptide or gene therapy as a plausible and rational therapeutic strategy (21).

At the interface of inflammation and development. Ultimately, the etiology of BPD must be explained in the context of alveolar hypoplasia. If inflammation is the main culprit, what are the potential linkages connecting inflammation and morphogenesis? Recent data regarding the regulation of Nkx2.1 by various mediators are tantalizing. First, decreased expression of Nkx2.1 has been documented in the lungs of neonates who died with BPD (40). Second, TNF-α, which is abundantly expressed in the lungs of preterm neonates at risk for BPD, negatively regulates Nkx2.1 gene expression (30). These data provide examples of potential interactions between mediators of injury and those of normal morphogenetic pathways. Disruption of key factors such as Nkx2.1 potentially derails both ongoing morphogenesis and repair.

Another potential candidate that bridges the gap between inflammation and development is TGF-β. Members of the TGF-β family of peptide growth factors play vital roles from early embryogenesis to adulthood (13). Both morphogenetic and pathological roles of TGF-β have been well documented (11). At present, our knowledge of the possible role of TGF-β in pathogenesis of BPD is confined to associative implications; the lungs of preterm neonates at risk for BPD contain copious amounts of bioactive TGF-β. Importantly, a significant correlation exists between lung TGF-β levels immediately following premature birth and adverse pulmonary outcome (22). TGF-β inhibits epithelial cell proliferation/differentiation and lung morphogenesis in general (54). Smads, a family of transcriptional factors, are activated downstream of TGF-β and mediate its biological effects (54). TGF-β inhibits surfactant protein B (SP-B) gene expression (20). Inhibition of SP-B by TGF-β occurs through interactions between TGF-β-activated Smad3 and Nkx2.1, the latter being a key regulator of SP-B gene (C. Li, J. Xiao, K. Hormi, Z. Borok, and P. Minoo, unpublished observation). In this case, in contrast to its well-recognized role as an activator of gene transcription, Smad3 acts as a repressor of an Nkx2.1 target gene. Because Nkx2.1 in the neonatal lung controls development by regulating morphoregulatory target genes, the latter interactions may inevitably inhibit lung development, cellular differentiation, and production of pulmonary surfactant.

The most direct evidence for a functional linkage between inflammatory mediators and developmental pathways comes from transgenic mouse studies. Alveolar hypoplasia can be experimentally induced in transgenic mice with lung-specific ectopic and/or overexpression of TNF-α (27) and IL-6 (12). Interestingly, regulated ectopic expression of IL-11 before the saccular phase of fetal lung development results in neonatal alveolar hypoplasia, whereas expression subsequent to alveolar formation has no effect (34). This finding provides a potential mechanistic explanation for phenotypic differences between "new" vs. "old" BPD. Further studies are needed to elucidate the identity of the developmental pathways that may be the target of the latter cytokines. Finally, inflammation activates the ubiquitous transcription factor nuclear factor (NF)-κB, which mediates many of its biological effects. Muraoka et al. (29) observed that experimentally induced perturbations in NF-κB gene expression disrupted epithelial-mesenchymal interactions, repressed BMP-4 and FGF-10 expression, and resulted in abnormal lung morphogenesis. We have shown that the BMP-4 promoter is negatively regulated by TNF-α and NF-κB in lung epithelial cells (C. Li, N. L. Zhu, R. C. Tan, P. L. Ballard, R. Derynck, and P. Minoo, unpublished observations). Thus NF-κB can modulate both inflammatory and morphoregulatory genes, thereby establishing a tight operational and functional linkage between inflammation and development.

EPITHELIAL-MESENCHYMAL INTERACTIONS IN LUNG[2]

The importance of epithelial-mesenchymal interactions in normal lung development was first demonstrated 70 years ago by Dr. Dorothy Rudnick, who showed that removal of mesenchyme from chick lungs grafted onto chorioallantoic membranes in ovo resulted in arrested lung development. Subsequent studies in mammals extended these observations to mammals and demonstrated the inductive potency of lung mesenchyme (LgM).

[2] Presented by J. Shannon.

One example of this was the experiments of Alescio and Cassini, who showed that grafting a piece of distal LgM onto trachea denuded of its own mesenchyme resulted in the induction of a supernumerary bud that subsequently branched in a lunglike pattern. These studies, however, did not examine whether the tracheal epithelium (TrE) had been induced to express markers of lung differentiation. When we grafted distal LgM onto TrE we also observed that the induced epithelium would invade the grafted tissue and branch within it. We further observed that the TrE exhibited all of the ultrastructural characteristics of alveolar type II cells, including numerous osmiophilic lamellar bodies. Expression of surfactant protein C (SP-C), a specific marker of the distal lung, was also induced in the grafted TrE and was apparent by 24 h postgrafting. We next analyzed the epithelial phenotype of reciprocal recombinants of embyronic lung epithelium (LgE) and tracheal mesenchyme (TrM). In this case, the TrM reprogrammed the distal LgE to assume a tracheal phenotype, including the induction of ciliated and mucous cells; all distal lung epithelial markers were suppressed. From these experiments, we concluded that mesenchyme specifies both epithelial morphogenesis and differentiation and that the entire respiratory epithelium, from the larynx to the distal tips, exhibits a significant plasticity in its eventual phenotype that is dependent on the inductive cues it receives from the mesenchyme.

The inductive factors responsible for specification of distal lung epithelial phenotype are diffusible and active over a short distance. To determine whether the reprogramming of TrE to a lung phenotype required direct epithelial-mesenchymal contact, we constructed recombinations of LgE, LgM, TrE, and TrM in which a 40-μm-thick meshtype Teflon filter with a nominal pore size of 0.45 μm was interposed between the two tissues. Under the influence of LgM, both LgE and TrE grew and branched within a Matrigel substratum. Under the influence of TrM, both LgE and TrE grew but did not branch, instead forming an epithelial cyst. TrE-cultured transfilter to LgM was induced to express SP-C mRNA in the cells in the most distal aspects of the epithelium. The inductive influence of LgM, however, showed spatial constraints because TrE that was positioned to the side of the LgM did not respond. We have demonstrated the spatial influence of LgM in experiments in which LgE and LgM were both enrobed in Matrigel but separated by a distance of ~200 μm. In these cultures, the LgE was drawn to the LgM by chemoattraction and would then invade and branch within the LgM. Studies in other laboratories have shown that FGF-10 is a potent chemoattractant found in LgM. Increasing the distance between the two tissues to >300 μm abolished the chemoattractive effect.

Control of lung epithelial growth and differentiation are multifactorial. We designed a culture system in which embyronic LgE will traverse the full developmental pathway from undifferentiated columnar epithelial cells to fully differentiated alveolar type II cells in the absence of LgM. We further refined this system to demonstrate the TrE could be reprogrammed to an alveolar type II cell phenotype in the absence of mesenchyme and that members of the FGF family, notably FGF-7 and FGF-1, were critical to this process. Somewhat surprisingly, FGF-10 was not able to induce reprogramming of TrE.

BMP-4 is a secreted protein found in the distal LgE. Disruption of BMP-4 signaling in the distal lung disrupts lung morphogenesis and correct spatial epithelial differentiation. Because BMP-4 has been shown to be induced by FGF-10, it has been hypothesized that BMP-4 is involved in specification of distal epithelial phenotype. We first tested this hypothesis by examining the effects of the different FGF family members found in the lung (FGF-1, -2, -7, -9, -10, and -18) on the expression of both BMP-4 and SP-C in cultured TrE. We found that although all of the FGFs induced BMP-4, only FGF-1, -2, -7, and -9 induced SP-C. Second, we antagonized the effects of BMP-4 in tracheal epithelial cultures with the protein Noggin, which acts by binding directly to BMP-4. We observed that a concentration of Noggin sufficient to block the effects of exogenously added BMP-4 had no effect on tracheal epithelial growth or the induction of SP-C. Third, direct addition of BMP-4 to cultures of TrE did not induce SP-C expression and actually inhibited rudiment growth and SP-C expression. From these three lines of investigation, we conclude that the role of BMP-4 in lung development is not to specify distal lung phenotype.

Proteoglycans (PGs) are multifunctional macromolecules found in the extracellular matrix as well as on cell surfaces. We have investigated the role of PGs in lung development by treating cultured lung explants with sodium chlorate, which prevents sulfation of the glycosaminoglycan side chains that are attached to PG core proteins. We found that sulfated PGs are required for growth and branching morphogenesis in intact lung tips as well as in recombinants consisting of LgM plus LgE or LgM plus TrE. Quantitative real time PCR showed that expression of SP-C was significantly diminished in the intact tips and LgM plus LgE recombinants and essentially abolished in LgM plus TrE recombinants. We next analyzed the effects of specifically inhibiting chondroitin sulfate PGs by treating cultured tissues with chondroitinase ABC lyase (EC 4.2.2.4). We found that chondroitinase treatment inhibited growth and branching morphogenesis in a manner identical to that seen in chlorate-treated tissues. When we examined SP-C expression, however, we found that chondroitinase treatment did not affect the induction or maintenance of SP-C mRNA. These data suggest that chondroitin sulfate PGs play a critical role in lung growth and patterning but seem not to be involved in specification of lung epithelial differentiation.

ROLE OF DEVELOPMENTALLY IMPORTANT TRANSCRIPTION AND GROWTH FACTORS IN ADULT LUNG PHYSIOLOGY AND PATHOBIOLOGY[3]

The revolution in molecular biology that occurred over the last two decades saw new and exciting approaches in defining factors that regulate lung development and differentiation. In vitro and in vivo analyses have identified

[3] Presented by F. DeMayo.

the transcription and growth factors that regulate lung development from the early stages of branching morphogenesis through alveolarization (47). Many of these factors have also been found to regulate the function of the adult lung. Of the transcription factors found to regulate lung function are such factors as Nkx2.1 (6), HNF-3β (1, 6, 33), and CCAAT enhancer binding protein-α (C/EBP-α) (8). Of the growth factors known to be critical for the regulation of lung development, the FGF signaling pathway has been shown to be important for regulating adult pulmonary physiology.

Transcription factor analysis of the promoters for the regulation of cell specific gene expression in the lung have identified several transcription factors as being important for the regulation of lung gene expression. The promoter for the Clara cell secretory protein (CCSP) has been used as a model to identify the elements regulating the expression of pulmonary epithelial genes. CCSP is expressed preferentially in the nonciliated secretory cells (Clara cells of the airways reviewed in Ref. 9). Promoter analysis of CCSP gene expression has identified Nkx2.1 (33), HNF-3β (9), and C/EBP-α (8) as being important in regulating the expression of CCSP. Ablation of these genes results in neonatal lethality. Ablation of Nkx2.1 (26) and C/EBP-α results in pulmonary defects. Ablation of HNF-3β results in embryo lethality at a stage before lung development (1). However, analysis of the physiological and pathophysiological regulation of CCSP expression demonstrates these genes are important for the regulation of CCSP.

CCSP is involved in protecting the lung from hyperoxic, bacterial, and viral insults (14, 16, 17, 25). Analysis of the regulation of CCSP has demonstrated roles for cytokines such as IFN-γ and hyperoxia (9). There are two candidate regions in the CCSP promoter that may mediate IFN-γ stimulation. The promoter contains a γ-activation sequence that may mediate IFN-γ activation of CCSP. Mediation of IFN-γ stimulation may also be mediated by HNF-3β (24).

CCSP is important for the protection of the lung from hyperoxic injury (25). Hyperoxia represses the expression of CCSP. Transcriptional analysis of CCSP expression reveals that the repression of CCSP by hyperoxia may occur by several mechanisms. First, activator protein-1 binding may repress CCSP expression. Second, hyperoxia may alter the binding of Nkx2.1 to the CCPS promoter. Finally, there may be altered binding of C/EBP-α to the CCSP promoter. This analysis demonstrates that developmentally important transcription factors are pivotal in regulating lung physiology. The same can also be said for developmental growth factors.

FGF signaling is important for pulmonary development. Ablation of FGF-10 (3) as well as its receptor FGFR-2 (32) signaling disrupts lung branching morphogenesis. The development of gene switch technology has allowed the investigation of the disruption of FGF signaling in the adult mouse in vivo (10, 44, 47). Altered expression of FGF-3 in the adult mouse lung causes two phenotypes, depending on the level of induction of FGF-3. Low levels of FGF-3 induction cause increased macrophage invasion of the lung, presumably due to increased activity of the alveolar type II cells. High levels of FGF-3 expression cause a significant increase in alveolar type II cells. Therefore, this developmentally important signaling pathway regulates the influx of inflammatory cells into the lung as well as the differentiation of alveolar type II cells. This discussion demonstrates that understanding the function of processes important for lung development will also lead to the identification of factors important in lung pathobiology and physiology.

ROLE OF THE MYOFIBROBLAST IN LUNG BIOLOGY AND PATHOLOGY[4]

Bronchial myogenesis begins in the trachea (around *day 10* of gestation in the mouse) and extends in a proximal to distal fashion along the developing bronchial tree. Smooth muscle (SM) differentiation is preceded by a change in peribronchial cell shape from round to elongated that also follows a proximal-distal path. We found that this change in shape is crucial for bronchial myogenesis. Regardless of the organ of origin, embryonic mesenchymal cells remain undifferentiated in culture if forced to stay round and become SM cells if allowed to elongate, even if the cells come from an organ that would not normally develop significant SM, such as the kidney (52). In the embryonic lung, the process of cell elongation is promoted, at least in part, by the developing bronchial basement membrane (35, 37, 51). New epithelial-mesenchymal contacts produced during branching morphogenesis stimulate the synthesis of laminin-α_1 chain by both cell types (37). Laminin-1, the main basement membrane constituent, is then produced and polymerizes at the epithelial-mesenchymal interface. Apposed mesenchymal cells use this polymer to spread and elongate, a process that triggers SM differentiation (35, 50). Concomitantly, mesenchymal cell elongation induces laminin-2 synthesis and deposition, which further stimulates myogenesis (35).

In more recent studies, we tried to understand why a change in cell shape could induce SM differentiation. Because spreading/elongation might be sensed by the cytoskeleton as mechanical tension and because SM develops at sites that sustain mechanical tension, we determined whether stretch may induce an undifferentiated mesenchymal cell to differentiate into a SM cell. By stretching round embryonic mesenchymal cells, we found that stretching was sufficient to initiate SM myogenesis, but only if it caused cell elongation (35). A similar effect was seen in embryonic lung explants by controlling the intrabronchial hydrostatic pressure. Both cell stretching and spreading induced SM myogenesis by a mechanism involving serum response factor (SRF) and its dominant negative isoform SRFΔ5, produced by alternative splicing of exon 5 (2). We found that undifferentiated mesenchymal cells synthesize SRF and SRFΔ5, but during bronchial myogenesis, or on spreading/stretching, SRFΔ5 synthesis is sup-

[4] Presented by L. Schuger.

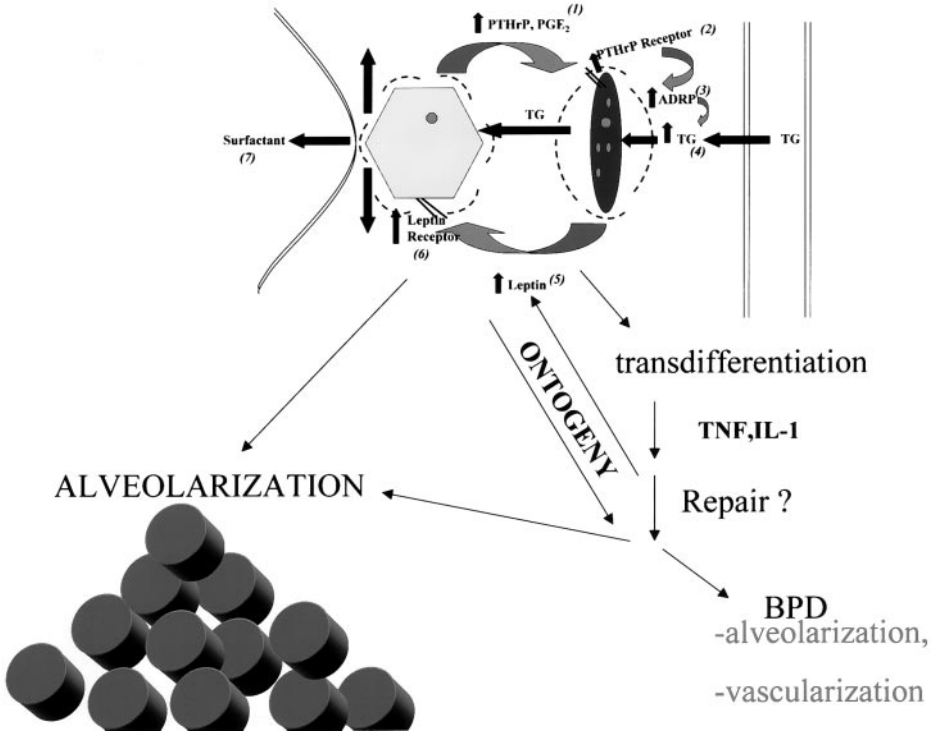

Fig. 1. Mild fluid distension of the embryonic lung upregulates parathyroid hormone-related protein (PTHrP) and prostaglandin E_2 (PGE_2) (*1*) and its mesodermal receptor (*2*), stimulating fibroblast expression of adipocyte differentiation-related protein (ADrP) (*3*), uptake of triglyceride (TG) by fibroblasts (*4*), and expression of leptin (*5*), which characterize the lipofibroblast phenotype. Leptin then acts retrograde to bind to its epithelial receptor (*6*), stimulating surfactant phospholipid synthesis (*7*). Overdistension, hyperoxia, or inflammatory cytokines [interleukin (IL)-1, tumor necrosis factor (TNF)-α] can interrupt this paracrine mechanism, causing the transdifferentiation of lipofibroblasts to myofibroblasts, the signature cell type for fibrosis. Mechanistically, lipofibroblasts promote epithelial type II cell growth and differentiation, whereas myofibroblasts do not (43), potentially explaining the hypoplasia of bronchopulmonary dysplasia (BPD).

pressed in favor of increased SRF production. Furthermore, human hypoplastic lungs related to conditions that hinder cell stretching continue to synthesize SRFΔ5 and show a marked decrease in bronchial and interstitial SM cells and tropoelastin (50).

In more recent studies, we searched for genes that were differentially expressed during SM differentiation. One of these genes was *rhoA*, a member of the GTPase family involved in the control of the cytoskeleton (5). RhoA levels were high in round, undifferentiated mesenchymal cells in vivo and in vitro and drastically decreased on SM differentiation (4). Functional studies using agonists and antagonists of RhoA activation and dominant positive and negative plasmid constructs demonstrated that high RhoA activity was required to maintain the round, undifferentiated mesenchymal cell phenotype. This was, in part, achieved by restricting the localization of SRF and SRFΔ5 mostly to the cytoplasm. Upon spreading on laminin-2, but not on other main components of the extracellular matrix, the activity and level of RhoA rapidly decreased, accompanied by disappearance of SRFΔ5 and translocation of SRF to the nucleus. All this was prevented by overexpression of dominant positive RhoA (4). Our studies, therefore, suggest that a change in SRF alternative splicing, together with its enrichment in the nucleus, is an important stimulus of SM myogenesis.

Another gene differentially expressed during SM myogenesis was *p311*. P311 is an 8-kDa protein with several PEST-like motifs found in neurons and muscle (42, 43). P311 transfection into two fibroblast cell lines, NIH/3T3 and C3H/10T1/2, induced phenotypic changes consistent with myofibroblast transformation, including upregulation of SM-α actin and SM22, induction of FGF-2, vascular endothelial growth factor, and PDGF and PDGF receptors, stimulation of integrins α_3 and α_5, and increased proliferation and migration rate. The P311-induced changes differed, however, from the well-characterized fibrogenic myofibroblast in that P311 inhibited TGF-β1, TGF receptor II, and TGF-β1-activating matrix metalloproteinase (MMP)-2 and MMP-9, with the resultant decrease in collagen 1 and 3 expression. Supporting a role for P311 in vivo, immunohistochemical examination of human wounds showed P311 only in myofibroblasts and their activated precursors (D. Pan, X. Zhe, S. Jakkaraju, G. A. Taylor, and L. Schuger, unpublished observations). These studies are the first to implicate P311 in myofibroblast transformation, to demonstrate that transformation can occur independently of TGF-β1, and to suggest that myofibroblasts, long considered the main source of fibrosis, may also have the potential to prevent it. Our current goal is to better understand the role of laminins, SRF, and P311 in lung myogenesis and lung diseases characterized by abnormal SM-like cells.

A FUNCTIONAL GENOMIC APPROACH TO UNDERSTANDING HOW OVERDISTENSION MAY CAUSE BPD[5]

Each of the presenters has addressed a key aspect of lung development, alluding to the use of this basic knowledge in understanding lung pathology. The laboratory in which I conduct research has taken a functional genomic approach to understanding the role of paracrine factors in cell cross talk as a means of identifying up- and downstream signaling pathways in-

[5] Presented by J. S. Torday.

volved in lung development and pathophysiology. The stretch-sensitive parathyroid hormone-related protein (PTHrP) gene (45) is an excellent candidate for such a study because it is necessary for normal lung development (36), is expressed during the terminal sac phase of lung development (36), and has been linked to neonatal lung disease (39). We are interested in paracrine factors like PTHrP that are mediated by ligands of endodermal origin with cognate receptors on mesoderm (or visa versa) as a way of getting at functionally relevant up- and downstream signaling elements.

Alveolar distension is an important mechanism of lung morphogenesis and dysmorphogenesis alike (Fig. 1). The paracrine paradigm in Fig. 1 incorporates many of the molecular regulatory motiffs enumerated by the previous presenters. Dr. Minoo highlighted the importance of *Shh* in normal lung development. *Shh* determines the expression of PTHrP in type II epithelial cells. Dr. DeMayo underscored the importance of C/EBP-α, which regulates adipocyte differentiation-related protein and leptin expression in fibroblasts. As earlier indicated, when PTHrP signaling is interrupted, the mesodermal lipofibroblasts default to the myofibroblasts, which Dr. Schuger has elucidated for us. Dr. Shannon has elegantly shown us how epithelial-mesenchymal interactions determine lung morphogenesis. Thus we have forged a link between normal and abnormal lung development through common pathways of homeostasis and repair.

REFERENCES

1. **Ang SL, Wierda A, Wong D, Stevens KA, Cascio S, Rossant J, and Zaret KS.** The formation and maintenance of the definitive endoderm lineage in the mouse: involvement of HNF3/forkhead proteins. *Development* 119: 1301–1315, 1993.
2. **Belaguli NS, Zhou W, Trinh TH, Majesky MW, and Schwartz RJ.** Dominant negative murine serum response factor: alternative splicing within the activation domain inhibits transactivation of serum response factor binding targets. *Mol Cell Biol* 19: 4582–4591, 1999.
3. **Bellusci S, Grindley J, Emoto H, Itoh N, and Hogan BL.** Fibroblast growth factor 10 (FGF10) and branching morphogenesis in the embryonic mouse lung. *Development* 124: 4867–4878, 1997.
4. **Beqaj S, Jakkaraju S, Mattingly RR, Pan D, and Schuger L.** High RhoA activity maintains the undifferentiated mesenchymal cell phenotype, whereas RhoA downregulation by laminin-2 induces smooth muscle myogenesis. *J Cell Biol* 156: 893–903, 2002.
5. **Bishop AL and Hall A.** Rho GTPases and their effector proteins. *Biochem J* 348: 241–255, 2000.
6. **Bohinski RJ, Di Lauro R, and Whitsett JA.** The lung-specific surfactant protein B gene promoter is a target for thyroid transcription factor 1 and hepatocyte nuclear factor 3, indicating common factors for organ-specific gene expression along the foregut axis. *Mol Cell Biol* 14: 5671–5681, 1994.
7. **Bostrom H, Willetts K, Penky M, Leyeen P, Lindhal P, Hedstrand H, Penka M, Hellstrom M, Gebre-Medhin S, Schalling M, Nilsson M, Kurland S, Tornell J, Heath JK, and Betsholtz C.** PDGF-A signaling is a critical event in lung alveolar myofibroblast development and alveogenesis. *Cell* 85: 863–873, 1996.
8. **Cassel TN, Nordlund-Moller L, Andersson O, Gustafsson JA, and Nord M.** C/EBPα and C/EBPδ activate the Clara cell secretory protein gene through interaction with two adjacent C/EBP-binding sites. *Am J Respir Cell Mol Biol* 22: 469–480, 2000.
9. **Chang A, Ramsay P, Zhao B, Park M, Magdaleno S, Reardon MJ, Welty S, and DeMayo FJ.** Physiological regulation of uteroglobin/CCSP expression. *Ann NY Acad Sci* 923: 181–192, 2000.
10. **Clark JC, Tichelaar JW, Wert SE, Itoh N, Perl AK, Stahlman MT, and Whitsett JA.** FGF-10 disrupts lung morphogenesis and causes pulmonary adenomas in vivo. *Am J Physiol Lung Cell Mol Physiol* 280: L705–L715, 2001.
11. **Coker RK and Laurent GJ.** Pulmonary fibrosis: cytokines in the balance. *Eur Respir J* 11: 1218–1221, 1998.
12. **DiCosmo BF, Geba GP, Picarella D, Elias JA, Rankin JA, Stripp BR, Whitsett JA, and Flavell RA.** Airway epithelial cell expression of interleukin-6 in transgenic mice. Uncoupling of airway inflammation and bronchial hyperreactivity. *J Clin Invest* 94: 2028–2035, 1994.
13. **Dunker N and Krieglstein K.** Targeted mutations of transforming growth factor-beta genes reveal important roles in mouse development and adult homeostasis. *Eur J Biochem* 267: 6982–6988, 2000.
14. **Graham RM, Friedman M, and Hoyle GW.** Sensory nerves promote ozone-induced lung inflammation in mice. *Am J Respir Crit Care Med* 164: 307–313, 2001.
15. **Greci LS, Gilson GJ, Nevils B, Izquierdo LA, Qualls CR, and Curet LB.** Is amniotic fluid analysis the key to preterm labor? A model using interleukin-6 for predicting rapid delivery. *Am J Obstet Gynecol* 179: 172–178, 1998.
16. **Harrod KS, Mounday AD, Stripp BR, and Whitsett JA.** Clara cell secretory protein decreases lung inflammation after acute virus infection. *Am J Physiol Lung Cell Mol Physiol* 275: L924–L930, 1998.
17. **Hayashida S, Harrod KS, and Whitsett JA.** Regulation and function of CCSP during pulmonary *Pseudomonas aeruginosa* infection in vivo. *Am J Physiol Lung Cell Mol Physiol* 279: L452–L459, 2000.
18. **Jones C, Cayabyab R, Kwong K, Stotts C, Wong B, Hamdan H, Minoo P, and deLemos R.** Undetectable IL-10 and persistent IL-8 expression early in hyaline membrane disease: a possible developmental basis for the predisposition to chronic lung inflammation in preterm newborns. *Pediatr Res* 39: 966–975, 1996.
19. **Kimura S, Hara Y, Pineau T, Fernandez-Salguero P, Fox CH, Ward JM, and Gonzalez FJ.** The T/ebp null mouse: thyroid-specific enhancer-binding protein is essential for the organogenesis of the thyroid, lung, ventral forebrain, and pituitary. *Genes Dev* 10: 60–69, 1996.
20. **Kumar AS, Gonzales LW, and Ballard PL.** Transforming growth factor-β(1) regulation of surfactant protein B gene expression is mediated by protein kinase-dependent intracellular translocation of thyroid transcription factor-1 and hepatocyte nuclear factor 3. *Biochim Biophys Acta* 1492: 45–55, 2000.
21. **Kwong KY, Jones CA, Cayabyab RG, Lecart C, Khuu N, Rhandhawa I, Hanley JM, Ramanathan R, deLemos RA, and Minoo P.** The effects of IL-10 on proinflammatory cytokine expression (IL-1β and IL-8) in hyaline membrane disease (HMD). *Clin Immunol Immunopathol* 88: 105–113, 1998.
22. **Lecart C, Cayabyab R, Buckley S, Morrison J, Kwong K, Warburton D, Jones C, and Minoo P.** Bioactive transforming growth factor-β in the lungs of extremely low birthweight (ELBW) neonates predicts the need for oxygen requirement. *Biol Neonate* 77: 217–223, 2000.
23. **Lindahl P, Bostrom H, Karlsson L, Hellstrom M, Kalen M, and Betsholtz C.** Role of platelet-derived growth factors in angiogenesis and alveogenesis. *Curr Top Pathol* 93: 27–33, 1999.
24. **Magdaleno SM, Wang G, Jackson KJ, Ray MK, Welty S, Costa RH, and DeMayo FJ.** Interferon-γ regulation of Clara cell gene expression: in vivo and in vitro. *Am J Physiol Lung Cell Mol Physiol* 272: L1142–L1151, 1997.
25. **Mango GW, Johnston CJ, Reynolds SD, Finkelstein JN, Plopper CG, and Stripp BR.** Clara cell secretory protein deficiency increases oxidant stress response in conducting airways. *Am J Physiol Lung Cell Mol Physiol* 275: L348–L356, 1998.

26. **Minoo P, Su G, Drum H, Bringas P, and Kimura S.** Defects in tracheoesophageal and lung morphogenesis in Nkx2.1 (−/−) mouse embryos. *Dev Biol* 209: 60–71, 1999.
27. **Miyazaki Y, Araki K, Vesin C, Garcia I, Kapanci Y, Whitsett JA, Piguet PF, and Vassalli P.** Expression of a tumor necrosis factor-α transgene in murine lung causes lymphocytic and fibrosing alveolitis. A mouse model of progressive pulmonary fibrosis. *J Clin Invest* 96: 250–259, 1999.
28. **Motoyama J, Liu J, Mo R, Ding Q, Post M, and Hui CC.** Essential function of Gli2 and Gli3 in the formation of lung, trachea and esophagus. *Nat Genet* 20: 54–57, 1998.
29. **Muraoka RS, Bushdid PB, Brantley DM, Yull FE, and Kerr LD.** Mesenchymal expression of nuclear factor-κB inhibits epithelial growth and branching in the embryonic chick lung. *Dev Biol* 225: 322–338, 2000.
30. **Ohmori M, Harii N, Endo T, and Onaya T.** Tumor necrosis factor-α regulation of thyroid transcription factor-1 and Pax-8 in rat thyroid FRTL-5 cells. *Endocrinology* 140: 4651–4658, 1999.
31. **Pepicelli CV, Lewis ML, and McMahon P.** Sonic hedgehog regulates branching morphogenesis in the mammalian lung. *Curr Biol* 8: 1083–1086, 1998.
32. **Peters K, Werner S, Liao X, Wert S, Whitsett J, and Williams L.** Targeted expression of a dominant negative FGF receptor blocks branching morphogenesis and epithelial differentiation of the mouse lung. *EMBO J* 13: 3296–3301, 1994.
33. **Ray MK, Chen CY, Schwartz RJ, and DeMayo FJ.** Transcriptional regulation of a mouse Clara cell-specific protein (mCC10) gene by the NKx transcription factor family members thyroid transciption factor 1 and cardiac muscle-specific homeobox protein (CSX). *Mol Cell Biol* 16: 2056–2064, 1996.
34. **Ray P, Tang W, Wang P, Homer R, Kuhn C, Flavell RA, and Elias JA.** Regulated overexpression of interleukin-11 in the lung. Used to dissociate development-dependent and -independent phenotypes. *J Clin Invest* 100: 2501–2511, 1997.
35. **Relan NK, Yang Y, Beqaj S, Miner JH, and Schuger L.** Cell elongation induces laminin α2 chain expression in mouse embryonic mesenchymal cells. Role in visceral myogenesis. *J Cell Biol* 147: 1341–1350, 1999.
36. **Rubin LP and Torday JS.** Parathyroid hormone-related protein (PTHrP) biology in fetal lung development. In: *Endocrinology of the Lung,* edited by Mendelson CR. Totowa, NJ: Humana, 2000, p. 269–297.
37. **Schuger L, Skubitz AP, Zhang J, Sorokin L, and He L.** Laminin α_1 chain synthesis in the mouse developing lung: requirement for epithelial-mesenchymal contact and possible role in bronchial smooth muscle development. *J Cell Biol* 139: 553–562, 1997.
38. **Sekine K, Ohuchi H, Fujiwara M, Yamasaki M, Yoshizawa T, Sato T, Yagishita N, Matsui D, Koga Y, Itoh N, and Kato S.** Fgf10 is essential for limb and lung formation. *Nat Genet* 21: 138–141, 1999.
39. **Speziale MV, Mannino FL, Hastings RH, and Deftos LJ.** Parathyroid hormone-related protein in tracheal aspirates of newborn infants. *Pediatr Res* 43: 660–665, 1998.
40. **Stahlman MT, Gray ME, and Whitsett JA.** Expression of thyroid transcription factor-1 (TTF-1) in fetal and neonatal human lung. *J Histochem Cytochem* 44: 673–678, 1996.
41. **Stripp BR, Sawaya PL, Luse DS, Wikenheiser KA, Wert SE, Huffman JA, Lattier DL, Singh G, Katyal SL, and Whitsett JA.** Cis-acting elements that confer lung epithelial cell expression of the CC10 gene. *J Biol Chem* 267: 14703–14712, 1992.
42. **Studler JM, Glowinski J, and Levi-Strauss M.** An abundant mRNA of the embryonic brain persists at a high level in cerebellum, hippocampus and olfactory bulb during adulthood. *Eur J Neurosci* 5: 614–623, 1993.
43. **Taylor GA, Hudson E, Resau JH, and Vande Woude GF.** Regulation of P311 expression by Met-hepatocyte growth factor/scatter factor and the ubiquitin/proteasome system. *J Biol Chem* 275: 4215–4219, 2000.
44. **Tichelaar JW, Lu W, and Whitsett JA.** Conditional expression of fibroblast growth factor-7 in the developing and mature lung. *J Biol Chem* 275: 11858–11864, 2000.
45. **Torday JS, Tsai SW, Sun H, and Rubin LP.** Paracrine mediators of mechanotransduction in lung development. Cellular deformation: mechanics and mechanisms of physiologic response. *Am J Med Sci* 316: 205–208, 1998.
46. **Weaver M, Yingling JM, Dunn NR, Bellusci S, and Hogan BLM.** Bmp signaling regulates proximal-distal differentiation of endoderm in mouse lung development. *Development* 126: 4005–4015, 1999.
47. **Weinstein M, Xu X, Ohyama K, and Deng CX.** FGFR-3 and FGFR-4 function cooperatively to direct alveogenesis in the murine lung. *Development* 125: 3615–3623, 1998.
48. **Wenstrom KD, Andrews WW, Hauth JC, Goldenberg RL, DuBard MB, and Cliver SP.** Elevated second-trimester amniotic fluid interleukin-6 levels predict preterm delivery. *Am J Obstet Gynecol* 178: 546–550, 1998.
49. **Whitsett J.** A lungful of transcription factors. *Nat Genet* 20: 7–8, 1998.
50. **Yang Y, Beqaj S, Kemp P, Ariel I, and Schuger L.** Stretch-induced alternative splicing of serum response factor promotes bronchial myogenesis and is defective in lung hypoplasia. *J Clin Invest* 106: 1321–1330, 2000.
51. **Yang Y, Palmer KC, Relan N, Diglio C, and Schuger L.** Role of laminin polymerization at the epithelial mesenchymal interface in bronchial myogenesis. *Development* 125: 2621–2629, 1998.
52. **Yang Y, Relan NK, Przywara DA, and Schuger L.** Embryonic mesenchymal cells share the potential for smooth muscle differentiation: myogenesis is controlled by the cell's shape. *Development* 126: 3027–3033, 1999.
53. **Yoon BH, Romero R, Jun JK, Park KH, Park JD, Ghezzi F, and Kim BI.** Amniotic fluid cytokines interleukin-6, tumor necrosis factor-α, interleukin-1β, and interleukin-8 and the risk for the development of bronchopulmonary dysplasia. *Am J Obstet Gynecol* 177: 825–830, 1997.
54. **Zhou L, Dey CR, Wert SE, and Whitsett JA.** Arrested lung morphogenesis in transgenic mice bearing an SP-C-TGF-β1 chimeric gene. *Dev Biol* 175: 227–238, 1996.