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Control Mechanisms of Lung Alveolar Development and Their Disorders in Bronchopulmonary Dysplasia

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ABSTRACT

Bronchopulmonary dysplasia (BPD) is a chronic lung disease that occurs in very premature infants and is characterized by impaired alveologenesis. This ultimate phase of lung development is mostly postnatal and allows growth of gas-exchange surface area to meet the needs of the organism. Alveologenesis is a highly integrated process that implies cooperative interactions between interstitial, epithelial, and vascular compartments of the lung. Understanding of its underlying mechanisms has considerably progressed recently with identification of structural, signaling, or remodeling molecules that are crucial in the process. Thus, the pivotal role of elastin deposition in lung walls has been demonstrated, and many key control-molecules have been identified, including various transcription factors, growth factors such as platelet-derived growth factor, fibroblast growth factors, and vascular endothelial growth factor, matrix-remodeling enzymes, and retinoids. BPD-associated changes in lung expression/content have been evidenced for most of these molecules, especially for signaling pathways, through both clinical investigations in premature infants and the use of animal models, including the premature baboon or lamb, neonatal exposure to hyperoxia in rodents, and maternal-fetal infection. These findings open therapeutic perspectives to correct imbalanced signaling. Unraveling the intimate molecular mechanisms of alveolar building appears as a prerequisite to define new strategies for the prevention and care of BPD. **(*Pediatr Res* 57: 38R–46R, 2005)**

Abbreviations

BPD, bronchopulmonary dysplasia
ECM, extracellular matrix
EMAP II, endothelial-monocyte activating polypeptide II
FGF, fibroblast growth factor
GC, glucocorticoids
MMP, matrix metalloproteinase
PDGF, platelet-derived growth factor
RA, retinoic acid
TGF, transforming growth factor
VEGF, vascular endothelial growth factor
TIMP, tissue inhibitor of metalloproteinases

BPD was initially defined as a disorder occurring in infants ventilated for neonatal respiratory distress; the described features included mucosal metaplasia of airways, emphysema, and widespread interstitial fibrosis (1). Over the years, probably as a consequence of both progress in therapeutic strategies and survival in greater proportion of highly premature infants, BPD has been characterized by a reduced frequency of airway injury, but an increase in alveolar growth disorders (2,3). This led to a "new" definition of BPD in which impairment of alveolar formation is the prominent feature, leading to long-term global reduction in alveolar number and gas-exchange surface area (4,5). BPD is now considered as resulting from the impact of injury, including oxygen toxicity, barotrauma/volutrauma, and infection, on a very immature lung, which leads in turn to arrest of normal maturation (6), with possible variable susceptibility due to some gene polymorphisms. Prenatal injury consecutive to glucocorticoid exposure or chorioamnionitis may also be involved (7). Unlike injury to the adult lung that is essentially growth arrested, BPD indeed occurs in a growing lung with uncompleted morphogenesis. The formation of definitive alveoli by secondary septation of primitive saccules is effectively an essentially postnatal event: if the alveolar phase of human lung development extends from about 36 wk gestation to 18 mo postnatally, the majority of alveologenesis (various synonym terms have been used to designate the process, including alveogenesis, alveologenesis, alveolization, and alveolarization, of which alveologenesis and alveolization are the most correct etymologically) occurs within 5–6 mo of term birth (8). Infants susceptible to develop BPD are therefore born in the early saccular phase, or even in the canalicular phase of lung development for the most premature of them (8). The pathophysiological mechanisms leading to BPD have been appraised at a variety of levels including cell proliferation (9), inflammation and fibrotic process (10), oxidative stress (11), infection (12), or microvascular development (13). The purpose of the present review is to focus on

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recent knowledge about the control mechanisms of alveolar septa formation and their impairments in BPD and its animal models. Much information has effectively been gained from *in vivo* models that reproduce abnormalities encountered in BPD. The closest model to the human condition is probably the prematurely delivered, ventilated baboon (14). In rodents that present totally postnatal alveologenesis (between d 4 and 20), neonatal exposure to hyperoxia that inhibits septal formation and affects alveologenesis (15, 16) has been widely used for more than 20 years as a model to study associated cell and molecular alterations. Experimental chorioamnionitis has also been developed (17), although used to a lesser extent.

Complexity in studying alveologenesis arises from the fact that the process is coordinated by multiple interactions through paracrine mechanisms between the fibroblastic, epithelial, and microvascular lung components, and with extracellular matrix (ECM). Defects in one of these components have repercussions on the whole alveolar development. The basis for intervention to prevent or reverse impaired alveologenesis depends on clarification of these complex interrelationships that are operative during normal lung development.

ELASTOGENESIS IS ESSENTIAL TO ALVEOLAR SEPTATION

To provide gas-exchange efficiently in the postnatal organism, the lung undergoes dramatic tissue growth and remodeling. The formation of new interalveolar walls known as alveolar septation is a necessary step to increase blood-gas interface and meet the respiratory requirements of the growing organism. Among the variety of factors that participate in the control of budding of secondary septa, elastin deposition in the thickness of primary septa appears to have a spatially instructive role inasmuch as the specific sites of elastic fiber formation correspond precisely to the location of future buds. New septa then extend that are composed of a double capillary layer, and elastin localizes at their tip (8). Later in development, microvascular maturation takes place with fusion of the double capillary layer into a single medial layer facing both alveolar lumens of the septum (8), and alveolar walls become thinner by apoptosis (18). Elastin is elaborated by cross-linking of a soluble precursor, tropoelastin, under the action of lysyl oxidase. Septal tropoelastin is produced by interstitial cells that express smooth-muscle actin, and are designated myofibroblasts.

The essential role of elastin for distal lung development was evidenced by different approaches. Although the lungs of mice devoid of elastin gene developed until the saccular stage, they displayed fewer, dilated distal air sacs with attenuated tissue septa, a condition reminiscent of emphysema (19). However, evaluating the consequences for secondary septation was not possible using this model, inasmuch as elastin null mice did not survive beyond the 4th postnatal day. The requirement for elastin deposition was indirectly evidenced by invalidation of platelet-derived growth factor A (PDGFA) gene. In this model, a profound deficiency in alveolar myofibroblasts and associated bundles of elastin fibers resulted in absence of secondary septa and definitive alveoli (20,21). Importantly, the loss of myofibroblast staining and elastin was limited to the lung parenchyma, whereas vascular and bronchial smooth muscle cells with accompanying elastin deposition were clearly observed, which emphasizes the specificity of septal elastogenesis blockade (20,21). It seems that this was due to a failure of myofibroblasts or their precursors to migrate from more proximal to the peripheral sites of the lung where alveolar elastin deposits should occur. These observations evidence a crucial role of PDGFA, which is produced by epithelial cells, as a chemoattractant for fibroblasts before the onset of septation (21). It has been suggested that this migration to sites of septal budding is not a random phenomenon, but conversely, that a morphogen gradient would provide instruction for the precise and specific localization of septa (22). Retinoic acid (see "Alveolar Formation Is Antagonistically Influenced by RA and GC") and sonic hedgehog have been proposed as candidate molecules for establishing such a gradient that would in turn lead to precise regulation of PDGFA production to provide appropriate guidance to myofibroblasts (22). These interrelationships are depicted in Figure 1, which tentatively summarizes the various cell–cell and cell–matrix interactions involved in the control of alveolization process.

Elastin was found to be increased in ventilated infants who died of BPD (23). Elastin deposition and expression were also enhanced in a ventilated preterm lamb model (24). These findings, however, may relate to the fibrotic repair process prominent in "old" BPD. Some confusion may effectively arise from the fact that myofibroblasts are essential as a unique source of connective tissue material in the normal process of septa formation but are also involved in the fibrotic process that often occurs in the reparative phase of lung injury. Furthermore, increased elastin turnover (25) and paucity of elastic fibers in alveolar walls because of destruction consecutive to imbalance between protease and antiprotease activities (26) have also been reported in BPD. In rat pups exposed to hyperoxia during alveologenesis, disruption of elastin fibers was also found (27), but this went along with decreased tropoelastin expression (28). Whether changes in septal elastin deposition occur in early stages of BPD is unknown and calls for further investigation.

FGF signaling is also critical for alveologenesis. FGF receptors (FGFR) 1–4 are expressed in the developing lung with specific spatial and temporal profiles. Alveolar formation co-

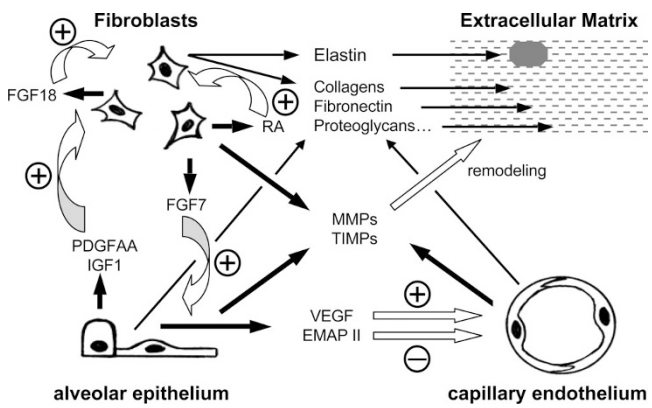

Figure 1. Principal cell–cell and cell–matrix interrelationships at work during alveologenesis that are targets of disturbances in BPD.

incides with increased expression of FGFR3 and 4 (29). Mice devoid of both FGFR3 and 4 manifest a failure of secondary septation not observed in either single mutant (30). The underlying mechanisms have not yet been cleared up. FGF18, which is produced by fibroblasts (Fig. 1), may represent one of the implicated ligands acting in an autocrine manner, because, on the one hand, its expression is markedly increased concomitantly with alveolar septation, and, on the other hand, FGF18 enhanced myofibroblast growth and expression of tropoelastin and lysyl oxidase (31). Moreover, elastogenesis also involves the synthesis of microfibril proteins such as fibrillins and fibulins that act as scaffold for elastin assembly and are essential to the process (32,33). Their pulmonary expression is up-regulated during alveologenesis (34), and FGF18 stimulated expression of fibulins 1 and 5 in myofibroblasts (31). Nevertheless, elastin deposition in primary septa occurred in the FGFR3/4 null mice and even failed to cease with aging (30). Therefore, other FGF-driven mechanisms must occur that i) condition septal surge and ii) stop elastogenesis. FGF2 might constitute an attractive candidate for the latter process, based on its negative effect on tropoelastin production in neonatal lung fibroblasts *in vitro* (35). Because no lung abnormality was reported in FGF2 null mice, the involvement of other mediators is, however, likely.

Little is known about the status of peptide factors that control alveolar septation and/or elastogenesis in BPD and its models. Delayed expression of PDGFA (36) and reduced expression of FGFR4 (37) were observed in the lung of rat neonates exposed to hyperoxia, but these molecules have thus far not been explored in infants with BPD. Although FGF2 was found to be elevated in tracheal aspirates of preterm neonates who died or developed BPD and to correlate with apoptosis (38), it is not known whether this participated to the outcome of BPD. By contrast, a decrease of FGF2 followed by an increase under recovery in air were observed in neonatal rats exposed to 95% oxygen, and, interestingly, intraperitoneal injection of soluble inactive FGFR1, which is a receptor of FGF2, arrested compensatory lung growth and secondary septation in recovering animals (39). These findings suggest a role of FGF2 in repair process.

OTHER ECM COMPONENTS, ECM REMODELING, AND INTERACTIONS WITH MEDIATORS

Alveolar septation also implies the deposition of other ECM components, including collagens and proteoglycans (Fig. 1), as well as activity of enzymes that elaborate the carbohydrate components of the latter. Their gene expression is up-regulated in the lung postnatally (34). Moreover, the relative lung contents of collagen and fibronectin markedly increase coincidentally with alveolar septation (40,41). Collagen architecture was markedly distorted in infants with BPD, with thickened, tortuous, and disorganized fibers (40). Fibronectin mRNA and protein were increased and decreased in early acute and chronic phases of BPD, respectively (42), but the specimens in this study were characteristic of "old" BPD.

ECM remodeling is an important parameter of harmonious pulmonary development. MMP2, also designated gelatinase A, appears to play particularly important role in the postnatal lung. In the rat, MMP2 activity was maximal in the first 11 d, and the major part of the enzyme was present in the active form (43). In addition to defects in branching morphogenesis, MMP2 null mice exhibited abnormal saccular development, and similar features were found to be associated with low expression of MT1-MMP, an activator of MMP2, in mice lacking EGF receptor (44). Moreover, the dynamic induction of MMP2 seen in neonatal lungs during the first days of life was significantly impacted by hyperoxia (45). Consistent with the assumption of a major role of MMP2 in septation process, lowered MMP2 activity appears as a characteristic feature of infants who develop BPD, during the initial, acute phase of the disease (46–48). Elevated levels of the other gelatinase, MMP9, and high MMP9/TIMP1 ratio rather appear to be associated with the later, regenerative and chronic phases of the disease, and with fibrosis (48,49). A similar pattern was found in the extremely premature ventilated baboon (50).

Lastly, ECM has not only structural properties, but functions as a dynamic modulator through the selective sequestration and subsequent release of growth factors and cytokines. A striking example of the importance of this function for alveologenesis is given by disturbance of alveolar septation despite normal lung cell differentiation, including myofibroblasts, in mice deficient in fibrillin-1 (51). This appears likely to result from enhanced proportion of active TGF-β through greater local activation (51). Imbalanced production of this cytokine effectively appears as a major mechanism in the pathogenesis of BPD. Increased levels of TGF-β have been detected in airway secretions of preterm infants with BPD (52). In animal models developed to enhance endogenous production of TGF-β (53), or to induce TGF-β overexpression (54,55), pulmonary morphologic changes consistent with those seen in human BPD have been observed, including enlarged alveolar sacs, poor secondary septation, thick and hypercellular septa, and decreased platelet endothelial cell adhesion molecule (PECAM) expression indicative of abnormal capillary development. The adverse effects of TGF-β on septation appear paradoxical inasmuch as TGF-β is known to up-regulate elastin production and gene expression in alveolar fibroblasts (56,57). Possibly, myofibroblast proliferative effects and defects in angiogenesis may be determinant (see "Vascular Growth Is Required for Normal Alveolar Development").

DEFECTS IN ALVEOLAR DEVELOPMENT CAN RESULT FROM ALTERED RESPIRATORY EPITHELIAL CELL GROWTH AND DIFFERENTIATION

Alveologenesis is characterized by an extensive proliferation of alveolar type II (ATII) cells. Sufficient ATII cell number is important because they serve as stem cells for alveolar type I (ATI) cells that line most of the alveolar surface and form air-blood barriers, and because they ensure adequate surfactant production around birth. Conditional deletion of the winged helix transcription factor Foxa2 (or HNF3β) has evidenced its requirement for ATII cell differentiation (58). Moreover, when Foxa2 was deleted in late gestation, extensive airspace enlargement and altered septation were displayed (59). Full ATII cell

differentiation therefore appears to be required for alveolar septation, illustrating the concept that epithelial signals are essential to the interstitial events of alveologenesis. The zinc-finger transcription factor GATA6 is also necessary to fetal lung maturation, including differentiation of both ATI and ATII cells (60), but, paradoxically, maintenance of elevated transcription of GATA6 in mice during the postnatal period impaired alveolar septation (61). Control of Foxa2 and GATA6 expression levels appears essential for regulating the expression of genes involved in lung development both through up and down transcriptional regulations. Last, it is worth mentioning that in mice null for T1α, an ATI cell surface marker, abnormal distal lung cell proliferation, and narrower and irregular air spaces were observed at birth (62). However, the underlying mechanism is unknown.

Among growth factors acting on alveolar epithelial cells, FGF7 (or keratinocyte growth factor), whoch is released by lung fibroblasts (Fig. 1) has early been recognized as a potent proliferation stimulus for adult ATII (63). This mediator has also a potent stimulatory effect on proliferation and maturation of developing ATII cells (64). In tracheal aspirates from premature neonates within 5 d after birth, FGF7 was found to be significantly higher in survivors without BPD than in those with BPD; a concentration higher than 110 pg/mL had a positive predictive value of 95% for absence of BPD (65). FGF7 has also been shown to prevent lung epithelial injury induced by different forms of aggressions, including oxidative stress (66–68), mechanical ventilation (69), and infection (70), which emphasizes its key regulatory role for the alveolar epithelial compartment. Consistently, transgenesis of a soluble FGF receptor that bound FGF7 rendered mice more susceptible to hyperoxia (71). However, FGF7 failed to protect against hyperoxic inhibition of postnatal alveolar formation and early pulmonary fibrosis in newborn rats (67). Because altered cyclin and Cdk expression consistent with G1 or G2 arrest has been reported in epithelial cells in the premature baboon model of BPD (72), FGF7 might reveal useful to enhance ATII proliferation. Interestingly, FGF7 induced new alveolar formation after pneumonectomy in adult lungs (73). Taken together, these investigations not only suggest that FGF7 determination may help evaluating the risk for BPD in preemies but that supplying exogenous FGF7 may protect the alveolar epithelium from BPD-associated injuries.

Another aspect of epithelial-interstitial cell interactions in alveologenesis and BPD is the involvement of IGFs. Leprechaunism, a disease caused by a defect of IGF-I receptor (IGF-IR), is associated with reduced lung surface area and larger, less numerous alveoli (74). Consistently, it has recently been reported that alveolar development correlates with IGF-I level: comparison between normal and dexamethasone- or retinoic-acid-treated neonatal rats indicated that the stronger the IGF-I and -II expression, the better the alveolar development (75). Moreover, IGF-I produced by epithelial cells (Fig. 1) stimulated *in vitro* migration and proliferation of lung fibroblasts (76). However, hyperoxia enhanced IGF-I and -II expression in neonatal rat lung *in vivo* (77) and in explant cultures (78). Simultaneously, IGF-IR were increased in fibroblasts (78). IGF-I in epithelium and IGF-IR in myofibroblasts were also intensely increased in the lung of patients with BPD (79). The significance of these findings is therefore not fully clear. Presumably, IGF-I is a positive enhancer of alveolar development, and its increase in BPD is associated with repair process rather than with pathogenesis of the disease.

VASCULAR GROWTH IS REQUIRED FOR NORMAL ALVEOLAR DEVELOPMENT

VEGF signaling plays a major role for microvascular lung development (13). VEGF is released principally by respiratory epithelial cells and enhances migration, proliferation, and differentiation of adjacent endothelial cells *via* paracrine signaling to receptors Flt-1 and Flk-1 (Fig. 1). The requirement of normal angiogenesis for alveologenesis has been demonstrated by the use in the developing rat of angiogenesis inhibitors, including VEGF receptor inhibitor of neutralizing antibody (80,81,81a). These inhibitors not only impaired pulmonary vascular growth, but also reduced septation and final alveolar number. Nitric oxide (NO) is a downstream regulator of VEGF, and, interestingly, NO synthase was considerably reduced after treatment with VEGF receptor inhibitor, whereas inhaled NO corrected alveolar disorders in this model (82). The heparan-sulfate-binding isoform $VEGF_{188}$, which strongly increases its expression shortly before birth (83,84), appears especially important inasmuch as mice only expressing the freely diffusible $VEGF_{120}$ isoform presented at birth reduced peripheral airspaces and microvasculature, with fewer air-blood barriers (85) The essential role of VEGF signaling for the maintenance of alveolar structure was also evidenced by occurrence of emphysema in adult rats treated with VEGF receptor inhibitor (86), and in adult mice with lung-targeted VEGF inactivation (87). However, VEGF overexpression in neonatal mouse lung increased mortality and caused pulmonary hemorrhage, hemosiderosis, alveolar remodeling, and inflammation (88), which indicates that, although being necessary for postnatal lung development, VEGF expression must be strictly controlled.

Impairments of growth, structure, and function of the developing pulmonary vessels in BPD and models have been extensively reviewed recently (13). Recent quantitative analysis has showed that infants with BPD present fewer air-blood barriers, less capillary loading, and more distant capillaries from the air surface than controls (89). Over time, however, primary septal walls adapt by thinning and increasing the number of air-blood barriers, thereby taking on the function of secondary septa (89). It is clear that vascular disorders result at least partly from altered signaling of angiogenic factors, their receptors, and NO synthase, as evidenced in human infants (90,91), in prematurely delivered baboons (92), in lambs exposed to intra-amniotic endotoxin (93) and in hyperoxia-exposed rat neonates (37). It is worth emphasizing that VEGF signaling was primarily affected, whereas angiopoietin–1, another angiogenic growth factor, and its receptor Tie2 were unchanged in the baboon model (92). Decreased Tie2 was observed in human infants with BPD, however (90). Similarly, hyperoxic lung injury in newborn rats reduced expression of VEGF, VEGF receptors, and HIF2α, a transcription factor involved in the

control of VEGF expression (94). It also reduced the $VEGF_{188}$ isoform in newborn rabbits (83). Last, providing inhaled NO to premature infants slightly decreased the incidence of BPD and death (95), presumably through VEGF production by epithelial cells and subsequent protection of lung vascular development.

On the other hand, EMAP II, an anti-angiogenic protein distributed to regions of epithelial-mesenchymal interactions (Fig. 1), may play a functional role during alveolar development as a putative negative regulator of vessel formation (96). Importantly, EMAP II is maintained at low expression level throughout postnatal life and in the adult, with the exception of a surge that correlates with microvascular maturation (96), which suggests that vascular growth must be down-regulated when fusion of the double capillary network into a single one occurs. Recently, it was reported that EMAP II abundance is elevated in the lung tissue of infants with BPD as well as in the premature baboon model (97), suggesting that this protein may contribute to the interruption of vascular development seen in BPD.

ALVEOLAR FORMATION IS ANTAGONISTICALLY INFLUENCED BY RA AND GC

Before septation, the lung contains a relatively large supply of vitamin A under the form of retinyl esters. These precursors are stored in lipid interstitial cells, a subset of fibroblasts concentrated at sites of alveolus formation, which convert them into RA (Fig. 1) (98). The RA-synthesizing enzymes aldehyde dehydrogenase 1 (Aldh-1) and retinaldehyde dehydrogenase 2 (Raldh-2) are up-regulated during the period of maximal alveolar-wall cell proliferation (99). RA enhances tropoelastin gene expression (100), and using inhibitors of Aldh-1, Raldh-2, and retinyl ester hydrolases, it has been demonstrated that endogenous retinoids increase the steady state level of tropoelastin transcripts in rat lung fibroblasts and fetal lung explants (101). Retinoid involvement in the control of septation was evidenced by several experimental approaches. In neonatal rat pups, RA enhanced ongoing alveologenesis by increasing the total number of alveoli (102), whereas vitamin A deficiency led to delayed alveolar development (103). Furthermore, simultaneous deletion of two RA receptors, RARγ and RXRα (104), or overexpression of dominant negative RARα (105) reduced alveolar number, whereas RARβ knockout mice exhibited higher alveolar number (106). These findings support the concept that endocrine RA and its receptors RARs/RXRs play a complex and critical role in alveolization during the neonatal period of the lung, including both stimulatory and inhibitory influences. In addition to elastin, RA or retinol have been shown to stimulate the expression of PDGFA/PDGFRα (107,108) and FGF18 (31). Finally, RA abrogated key features of emphysema in an elastase-generated model of the disease (109) that highlights the regenerative properties of RA and suggests that developmental and repair processes share common regulatory mechanisms.

Blood retinol concentration has early been recognized to be lower in prematurely born than in full-term infants (110), and in those who develop BPD than in those who do not (111,112). In trials of vitamin A supplementation, reduced need for supplemental oxygen and mechanical ventilation was observed (113), but whether the incidence of BPD was reduced remains controversial with either no change (114) or slight decrease (115). As regards direct administration of RA, only experimental approaches in the hyperoxic rat model have been performed. RA treatment of rats exposed to hyperoxia from postnatal d 3 increased collagen in airspace walls and mean alveolar area, but neither improved septal formation and microvessel count, nor decreased airspace size on d 14 or 18 (116,117). By contrast, on d 45, lungs were no longer different from those in controls, indicating complete recovery, whereas deficient alveologenesis remained obvious in hyperoxia-exposed rats that were not RA treated (118).

Although GC hormones are widely used in preemies to accelerate maturation and surfactant synthesis and to prevent inflammatory process, they appear to exert deleterious effects on alveologenesis. The postnatal formation of alveoli is largely prevented by GC treatment, which accelerates alveolar wall thinning, fusion of the two capillary layers, and inhibits outgrowth of new septa leading to early termination of the septation process as shown in monkeys (119), rats (120,121) or lambs (122,123). Importantly, RA treatment antagonized GC effects and partially rescued failed septation induced by a GC hormone in mice and rats (102,124). The underlying mechanisms of these antagonistic effects are not completely understood. GC stimulate developing-lung elastogenesis (125), which may appear paradoxical in view of their effects on septation. However, this stimulation is unlikely to relate to alveolar-wall elastin inasmuch as dexamethasone was reported to prevent alveolar elastin deposition (126). GC-induced inhibition of septation in mice was shown to be associated with a block in angiogenesis due to down-regulation of VEGFR2, and this down-regulation was prevented by RA treatment (127). Recent observations indicated that RA enhanced and dexamethasone decreased IGF-I (75) and midkine (128) in rat lung. In addition, midkine expression was suppressed in neonatal mice exposed to hyperoxia (129). Taken together with the above-reported probable importance of IGF-I in alveologenesis, and with the influence of midkine on pulmonary vascular gene expression (130), these findings indicate possible links between GC and RA accounting for their antagonism.

Balance between RA and GC occurs in the course of normal lung development, and disturbances in this equilibrium may lead to abnormal alveolization (7). However, survival was enhanced in oxygen-exposed newborn rats by simultaneous treatment with RA and dexamethasone (116), which suggests possible complementary effects also. RA may compensate the accelerating effects of GC on septal maturation while keeping the benefit of GC treatment for lung epithelial maturation and for lowering inflammation. Consistent with this assumption, it was observed that blood vitamin A level was higher in premature ventilated infants whom respiratory function positively responded to GC as compared with those who displayed no respiratory improvement (131).

CONCLUSION AND PERSPECTIVES

The spotlight focusing on impaired septation as a prominent feature of BPD prompts the neonatologist to question about

Table 1. *Synopsis of the characteristics, functions, and alterations in BPD or models, of the principal identified control factors of pulmonary alveologenesis*

Family	Factors	Main source or distribution	Main target	Functions during alveologenesis	Alterations in BPD and models	References
Growth/differentiation factors and receptors	EMAP II	Interstitial cells	Endothelial cells	Negative regulator of vascular formation	Increased in BPD	96, 97
	FGF2	Interstitial cells	Interstitial cells endothelial cells (FGFR1)	Decreased tropoelastin production *in vitro* but not demonstrated *in vivo*	Elevated in tracheal aspirates of preterm neonates who developed BPD; decreased in hyperoxia	35, 38, 39
	FGF7	Interstitial cells	Epithelium (FGFR2)	Stimulates proliferation and maturation	Decreased in tracheal aspirates of preterm neonates who developed BPD	63, 64, 65
	FGF18	Interstitial cells	Interstitial cells (FGFR3?)	Stimulates fibroblasts proliferation and myofibroblast differentiation; enhances elastogenesis	Unknown	31
	IGF1	Epithelium, macrophages	Interstitial cells (IGF1R)	Stimulates proliferation and migration; various other effects	IGF and IGF1R are increased in BPD and hyperoxia	74–79
	Midkine	Epithelium (mostly), interstitial cells	Various	Vascular development, epithelial maturation	Suppressed in hyperoxia	128–130
	PDGFA	Epithelium, macrophages	Interstitial cells (PDGFRα)	Chemotactic attractant for fibroblasts; lack of migration of fibroblasts and blockade of septal elastogenesis in null mice	Expression delayed in hyperoxia	20, 21, 36
	TGFβ1	Epithelium interstitial cells	Epithelium interstitial cells (TGFβR)	Enhances matrix deposition (elastin, etc.); overexpression inhibits alveolar development	Increased levels of TGFβ detected in airway secretion of preterm infants with BPD; induced by hyperoxia	51–57
	VEGF	Epithelium (mostly type II cells)	Endothelial cells (VEGFR1/R2)	Enhances migration, proliferation and differentiation of endothelial cells; required for pulmonary angiogenesis and septation	Decreased in BPD and hyperoxia	80, 81, 81a, 83, 84, 88, 90–92, 94
	VEGFR1/R2 (Flt-1/Flk-1)	Endothelial cell surface		Postnatal inhibition results in abnormal vascular development	Decreased in BPD and hyperoxia	90, 91, 94
	FGFR3/FGFR4	Interstitial cell surface		Simultaneous deletion, abrogates alveologenesis	FGFR4 is decreased in hyperoxia	29, 30, 37
Transcription factors	FOXA2 (HNF3β)	Distal epithelium		Lung maturation, cell differentiation	Unknown	58, 59
	GATA6	Epithelium		Lung maturation, cell differentiation	Unknown	60, 61
	RARα	Interstitial cells, epithelium		Ligand for retinoic acid; overexpression of dominant negative RARα reduces alveolar number	Unknown (exogenous RA compensates effects of hyperoxic exposure)	105
	RARβ	Interstitial cells, epithelium		Ligand for retinoic acid; deletion increases alveolar number		106
	RARγ/RXRα	Interstitial cells, epithelium		Ligands for retinoic acid; simultaneous deletion reduces alveolar number		104, 116–118
Matrix components and remodeling enzymes	Collagens	All cell types, ubiquitously distributed		Tissue architecture	Types I and III are increased in BPD	34, 40, 43, 122
	Elastin	Interstitial and smooth muscle cells, tip of septa		Tissue architecture, provides tissue elasticity, deposition in saccular walls determines secondary septation	Increased in "old" BPD; paucity of septal elastin in BPD; decreased in hyperoxia	8, 19, 23–28, 34
	Fibronectin	Ubiquitously distributed, principally in basement membranes		Tissue architecture, cell matrix interactions, cell adhesion	Dependent on "old" BPD phases	34, 41, 42
	MMP2/MMP9 (gelatinases A/B)	All cell types	Collagens	Matrix remodeling; abnormal saccular development in MMP2 null mice	Weak MMP2, high MMP9 activity in infants who developed BPD; increased in hyperoxia	43–49

disorders in the underlying molecular mechanisms. Although numerous elements in the process of alveolization have probably still to be determined, key control factors have already been identified during the last 10 years that indeed exhibit expression disturbances in BPD and/or models. Table 1 recapitulates the principal factors involved. Genes that regulate alveolar morphogenesis must be differentially expressed during periods of active and inactive alveolar formation. Global analyses of lung transcriptome or proteome that are increasingly developing in this field should therefore offer new prospects in the identification of candidate genes and help reaching a more comprehensive view of the process. Restoring balanced levels through supply of insufficient factors and inhibition of excessive factors appears as a promising clinical approach for prevention or treatment of the disease. However, it should be stressed that alveolization, like other developmental processes, undoubtedly depends on complex interactions that are normally tightly controlled and balanced in time and space. The antagonistic effects of glucocorticoids and retinoids and the possible harmful consequences of incautious use of glucocorticoids are especially illustrative in this respect. Future attempts to intervene in these processes must take into account not only the potential benefit of supplementing the immature organism with exogenous factors but also the serious potential for unanticipated adverse effects on both the lung and other organ systems.

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