ALVEOLAR FORMATION AND STRUCTURE

The lung provides an interface for gas exchange between the air and blood to supply oxygen and remove carbon dioxide. Before birth, the lung develops from the foregut endoderm through the interaction between epithelial cells and mesenchymal cells. Lung epithelium undergoes multiplication of branches through embryonic [embryonic day 9 (E9)–E12 in mice], pseudoglandular (E12–E15 in mice), canalicular (E15–E17 in mice), and saccular (E17–birth in mice) stages, as demonstrated in Fig. 1.

After birth, maturation of the lung continues, entering the alveolar stage. The alveolarization period starts from the late embryonic period to the neonatal period; during this period, pulmonary surfactant is secreted, primarily by nonciliated bronchiolar epithelial cells (Clara cells) and alveolar type II epithelial cells. Alveolar septation is developmentally regulated, occurring in mice during the last month of gestation and the first 8 years of life. In the mature lung, the alveolus, alveolar sac, alveolar duct, and alveolar pore form the basic alveolar structure. The interalveolar walls are supplied with capillaries, which are supported by elastic fibers and basement membranes. Their surface is covered by squamous alveolar type I epithelial cells. The round secretory alveolar type II epithelial cells are scattered around alveolar type I epithelial cells, usually in the corner of the alveoli. Alveolar type II epithelial cells contain highly distinctive granules known as lamellar bodies rich in phospholipids. When phospholipid secretory products (known as pulmonary surfactant) are released from alveolar type II epithelial cells, they spread over to form a membrane that covers the surface of the interalveolar walls. Pulmonary surfactant reduces the surface tension force at the air-water interface and facilitates expansion of alveoli. In addition to alveolar type I and II epithelial cells, alveolar macrophages with engulfed particles are also present in free alveolar air spaces and interalveolar walls.

PULMONARY SURFACANT

Pulmonary surfactant is composed of structurally heterogeneous lipoproteins and is synthesized in alveolar type II epithelial cells as a vesicular structure called tubular myelin, which forms the lamellar body. After secretion from cells, pulmonary surfactant forms an insoluble film at the air-liquid interface of the alveolar surface. Around 90–95% of the pulmonary surfactant compositions are lipids. The majority of surfactant lipids are phospholipids (~80%), principally dipalmitoylphosphatidylcholine and phosphatidylglycerol. Working together with surfactant proteins, phospholipids reduce alveolar surface tension to promote lung expansion on inspiration and to prevent lung collapse on expiration.

There are ~10% neutral lipids in pulmonary surfactant. Until recently, the functional roles of neutral lipids have been poorly understood in the lung. Cholesteryl ester and triglycerides are major components in neutral lipids. Lysosomal acid lipase (LAL) hydrolyzes cholesteryl ester and triglycerides in the lysosomes of cells to generate free cholesterol and free fatty acids (10, 11). Using a genetic ablation approach, researchers can reveal, via blockade of cholesteryl ester and triglyceride metabolism in LAL knockout mice (lal−/−), severe disruption of the alveolar structure, including pulmonary emphysema and remodeling (20). Associated with these phenotypes, a high level of neutrophil influx is observed in the lungs. The numbers of bronchoalveolar macrophages appear foamy and are gradu-
ally increased. Affymetrix GeneChip array analyses show increased mRNA levels of proinflammatory cytokines (including IL-1β, IL-6, and TNF-α) and matrix metalloproteinases (including MMP-8, MMP-9, and MMP-12). In addition, Clara cell hypertrophy and hyperplasia are developed in conducting airways. The severity of pathophysiological phenotypes in the lal−/− mouse lungs is age dependent. It is known that many neutral lipid metabolites serve as ligands for nuclear receptors that are potent transcription factors controlling gene expression of cytokines/chemokines, proteinases, and structural proteins, which are essential for the maintenance of normal alveolar functions in various physiological conditions and host defenses. Identification of these ligands and nuclear receptors will be the major task in the pulmonary field to understand lung biology and host defenses.

In addition to lipids, pulmonary surfactant contains ~5–10% surfactant proteins, including surfactant protein A, B, C, and D (SP-A, SP-B, SP-C, and SP-D). Genetic ablations of genes encoding for these molecules have been performed in mice. Although SP-A, SP-C, and SP-D gene knockout mice are viable, they display some phenotypes similar to lal−/− knockout mice, including alveolar emphysema and inflammation-related remodeling (13–15, 17, 40). These observations suggest some common pathophysiological mechanisms between LAL and surfactant proteins. On the other hand, SP-B gene knockout mice result in lethal respiratory atelectasis after birth (6). Deficiency of SP-B did not alter embryonic lung development in this mouse model. Similar to the observation made in mice, an inherited mutation of SP-B has been identified in humans and results in lethal respiratory distress syndrome in newborn infants (29). SP-B is a 79-amino acid amphipathic peptide, produced by the proteolytic cleavage of SP-B proprotein (proSP-B) in Clara cells and alveolar type II epithelial cells. The SP-B peptide is stored in lamellar bodies and secreted with phospholipids into the airway lumen. It facilitates the stability and rapid spreading of surfactant phospholipids during respiratory cycles and is essential for maturation of alveolarization and postnatal respiratory adaptation in newborns (41).

Although SP-B deficiency has no effect on lung development, expression of the SP-B gene starts at the onset of lung formation and is developmentally controlled in a highly tissue-specific manner. The tissue- and cell-type specificities are primarily controlled by cis-acting DNA elements and trans-acting transcription factors. In a transgenic mouse model line carrying the human SP-B 1.5-kb 5′-flanking regulatory region and the lacZ reporter gene, expression of the human SP-B 1.5-kb lacZ reporter gene recapitulates the endogenous SP-B gene expression, which starts at mouse embryonic day 9 and is restricted to epithelial cells throughout lung development (Fig. 2). In lung explant cultures, the human SP-B 1.5-kb lacZ reporter gene is highly expressed in newly formed epithelial tubules during the respiratory branching process, as demonstrated in Fig. 2 (48). There are two cis-acting DNA elements highly conserved in both the human and murine SP-B 5′-flanking regulatory sequence that are essential for SP-B transcriptional activation (3, 5, 46). Deletion of the enhancer region, which binds to thyroid transcription factor 1, retinoic acid receptor (RAR), signal transducers and activators of transcription 3 (Stat3), and nuclear receptor coactivators (SRC-1, ACTR, TIF2, and CBP/p300) in the human SP-B 1.5-kb lacZ gene, abolishes lacZ reporter gene expression in Clara cells and significantly reduces its expression in alveolar type II epithelial cells in transgenic mice (48).

NUCLEAR RECEPTORS

Gene transcriptional control is an important mechanism for alveolar maturation and maintenance. Transcription factors and
coactivators regulate gene expression of structural proteins, growth factors, cytokines, chemokines, and proteinases in lung development and in adult lungs when responding to various physiological changes. For many years, hormones have been used to treat lung-associated inflammation and diseases (such as chronic obstructive pulmonary disease and asthma). Given the fact that the lung is a highly lipogenic organ, hormone activated-nuclear receptors (a unique group of transcription factors) are especially important for the formation and the maintenance of the alveolar structure. Hormonal ligands for various nuclear receptors come from lipid metabolism. Disruption of normal lipid metabolism can cause severe pulmonary inflammation and alveolar abnormality (20).

All nuclear receptors contain similar functional domains. A central DNA-binding domain is composed of two highly conserved zinc fingers and specifically binds to various hormone response elements on the target genes. A ligand-binding domain is responsible for recognition of hormones to ensure selective and specific physiological responses. An NH₂-terminal transactivation domain (AF1) and a COOH-terminal ligand transactivation domain (AF2) are essential for transcriptional activation of nuclear receptors. Nuclear receptors can be monomeric, homodimeric, heterodimeric with retinoid X receptor (RXR), or both. When hormone ligands bind, nuclear receptors bind to hormone response elements and recruit nuclear receptor coactivators, including SRC-1, ACTR, TIF2, CBP/p300, p/CIP and P/CAF (5a, 16a, 16b, 36a), through the AF2 domain. These coactivators possess histone acetyltransferase (HAT) domains with intrinsic histone acetylation activity (2a, 5a, 5b, 29a, 34a). Coactivators with HATs tend to interact with each other to form a large transcription activation complex. Recruitment of multiple HATs leads to chromatin remodeling, transcription factor modification, and target gene activation (18).

The most well-characterized nuclear receptor family members in alveolarization are the RARs. RARs include three isotypes, designated α, β, and γ. RARs and RXRs have been previously detected in respiratory epithelial cells by immuno-histochemical staining (27, 28, 44). RARα and RARβ double-null mutant mice die in utero and have severely hypoplastic lungs, suggesting that they are required for lung development (26). Retinoic acids (RAs) are the ligands for RARs. RAs are vitamin A derivatives and lipophilic hormones that can be readily diffusible through cell membranes. Early information regarding physiological functions and clinical applications of RA is derived from vitamin A-deficient animals and prenatal and postnatal infants (42, 43). Clinical studies in premature infants show a correlation between low serum levels of vitamin A and chronic lung disease after respiratory distress syndrome. Vitamin A supplementation from the early postnatal period reduces the morbidity associated with bronchopulmonary dysplasia (31–33). Importantly, treatment of animals with all-trans-RA increases the number of alveoli and reverses elastase-induced pulmonary emphysema in vivo (21, 22). RAs stimulate SP-B expression in respiratory epithelial cells (12, 27, 44). Delivery of all-trans RA with the use of pulmonary surface-active material to alveoli causes elevation of cellular retinol binding protein-1 mRNA in a lung-specific manner (Massaro DJ, Massaro GD, and Clerch LB, unpublished observations).

To elucidate the functional role of RARs in pulmonary alveolarization, both transgenic and knockout mouse systems have been used by several laboratories. In doxycycline-controlling double-transgenic mouse systems, the normal RA/RAR signaling pathway is interfered with overexpressing a dominant-negative RARe (dnRARα) in respiratory epithelial cells under the control of the human SP-C promoter or the rCCSP promoter. Overexpression of dnRARα in neonatal lungs from day 1 to day 21 (a critical period for alveolar maturation) leads to substantial alveolar abnormality with the increased air space, larger but fewer alveoli, and diminished alveolar surface area (as demonstrated in Fig. 3). In these animals, numbers of alveolar epithelial cells are significantly reduced. Expression of the SP-B gene is inhibited in alveolar type II epithelial cells (49). This finding supports a concept that the RA/RAR axis plays a critical role in neonatal alveoli maturation. Because dnRARα blocks RAR elements on target genes for all three forms of RARs (α, β, γ) in an indiscriminate fashion, it cannot address diverse and differential roles played by individual RAR isoforms in alveolarization.

To address this issue, several laboratories use genetic knockout mice to assess functional roles of each of the RA isoforms in the lung. In 14-day-old RARα−/− mice, the volume of individual alveoli, the number of alveoli, and alveolar surface area remain unchanged compared with wild-type mice. However, at age 50 days, the volume of individual alveoli is larger and the number of alveoli and the alveolar surface area are smaller in RARα−/− than in wild-type mice (23). RARβ−/− mice exhibit premature septation and form alveoli twice as fast as wild-type mice during the period of septation but at the same rate as wild-type mice thereafter. RARβ agonist treatment of

Fig. 3. Disruption of the alveolar structure in the neonatal lungs of dominant-negative retinoic acid receptor-α (dnRARα) double-transgenic mice. WT, wild-type mice; CCSP/dnRAR, doxycycline-controlled CCSP/dnRAR double-transgenic mice; SP-C/dnRAR, doxycycline-controlled SP-C/dnRAR transgenic mice. [From Yang et al. (49).]
newborn animals impairs septation (24). Therefore, RARβ is an endogenous inhibitor of alveolar formation during but not after the perinatal period. RARγ gene deletion results in a decrease in alveolar number and whole lung elastic tissue and an increase in cord length of alveoli at postnatal day 28. The additional deletion of RXRα allele results in a decrease in alveolar surface area and alveolar number and an increase in cord length of alveoli (25).

**ALVEOLAR INJURY DURING HYPEROXIA**

Patients with acute and chronic cardiopulmonary disorders are often given supplemental oxygen to enhance the alveolar and arterial oxygen levels. The prolonged administration of inspired oxygen at a high concentration leads to various forms of alveolar tissue damage, including acute lung injury and adult respiratory distress syndrome (7, 34). In animal models, exposure to a lethal dose of oxygen (e.g., 95%-100%) leads to acute lung inflammation and destruction of alveolar parenchyma.

Stat3 was originally identified as the acute phase response factor (1, 50). Stat3 is mainly activated by proinflammatory IL-6 family of cytokines (IL-6, IL-11, ciliary neurotrophic factor, oncostatin M, and leukemia inhibitory factor) that share the common gp130 receptor subunit (19, 36). Stat3 becomes activated by phosphorylation in response to extracellular signaling molecules (8, 35). Phosphorylation causes dimerization and translocation of Stat3 into the nucleus to activate downstream target genes. Similar to the dnRAR study, overexpression of a dominant-negative Stat3 mutation at the phosphorylation site in respiratory epithelial cells in doxycycline-controlled double-transgenic mice causes alveolar destruction, and animal death is increased during hyperoxia (47). Stat3C is an artificial form that mimics the phosphorylated Stat3 action with substitution of two cystine residues within the COOH-terminal loop of the SH2 domain of Stat3 (3). Overexpression of Stat3C in respiratory epithelial cells in a doxycycline-controlled double-transgenic mouse system protects lung inflammation and injury caused by hyperoxia. In this mouse line, more than 50% of transgenic mice survive to 95% oxygen exposure at day 7, compared with 0% survival of wild-type mice. Immunohistochemical study indicates that overexpression of Stat3C protects acute capillary leakage of red blood cells and neutrophil infiltration into the alveolar region (Fig. 4).

The extracellular matrix is the major component in the endothelial-epithelial interstitial structure along the alveolar wall. MMPs, including collagenase, gelatinase, elastase, stromelysin, and matrilysin, are zinc-dependent matrix-degrading proteinases that share structural and functional similarities. They degrade almost every component of the extracellular matrix and are required for normal lung development and remodeling after lung injury. Exuberant or aberrant expression of MMPs can cause tissue damage and has been associated with many lung diseases, including asthma, chronic obstructive pulmonary disease, cancer, and adult respiratory distress syndrome (30). Under hyperoxic conditions, neutrophil-associated MMP-9 influx to the lung is dramatically increased in wild-type animals but not in Stat3C-overexpressing double-transgenic mice. Zymographic analysis of bronchoalveolar lavage fluids shows a much higher MMP-9 enzymatic activity in oxygen-treated wild-type mice than in Stat3C-overexpressing transgenic mice (Lian X, Yang L, Xu H, Clarke A, Li T, Du H, and Yan C, unpublished observations). MMP-12 is another important proteinase that degrades elastin and type IV collagen (2, 30). After oxygen exposure, MMP-12 expression is significantly induced in the interstitial region along the alveolar wall in wild-type animals but not in Stat3C-overexpressing double-transgenic mice (Lian et al., unpublished observations). These studies support a concept that activation of the Stat3 pathway delays MMPs influx or de novo synthesis in the alveolar region to prevent alveolar wall damage and capillary leakage.

Other mechanisms may also account for Stat3 protection against oxygen-induced alveolar injury. Stat3C promotes synthesis of SP-B, which maintains the surfactant integrity. Stat3C is a potent activator for SP-B gene expression (45). Intratracheal treatment with exogenous SP-B improves survival and decreases lung injury during hyperoxia in mice (16). DNA fragmentation has also been reported in the lungs under hyperoxic condition (38). Because Stat3C is a growth-promoting molecule and prevents apoptosis, overexpression of Stat3C in respiratory epithelial cells may prevent or delay hyperoxia-induced apoptosis. Importantly, the IL-6 family of cytokines also protects against oxygen-induced lung injury (37, 39). Therefore, the IL-6 family of cytokines and the Stat3 signaling axis, which is generally regarded as a proinflammatory pathway, exhibits an anti-inflammatory function in hyperoxia-induced lung inflammation and injury.

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Fig. 4. Protection of hyperoxia-induced alveolar hemorrhage by overexpressing signal transducers and activators of transcription 3 (Stat3C). Arrows indicate clusters of red blood cells. Stat3C, doxycycline-treated Stat3C-overexpressing transgenic mice.
REFERENCES


