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Comparative Analysis of the Mechanical Signals in Lung Development and Compensatory Growth

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Abstract

This review compares the manner in which physical stress imposed on the parenchyma, vasculature and thorax, and the thoraco-pulmonary interactions, drive both developmental and compensatory lung growth. Re-initiation of anatomical lung growth in the mature lung is possible when the loss of functioning lung units renders the existing physiologic-structural reserves insufficient for maintaining adequate function and physical stress on the remaining units exceeds a critical threshold. The appropriate spatial and temporal mechanical interrelationships, and the availability of intra-thoracic space, are crucial to growth initiation, follow-on remodeling and physiological outcome. While the endogenous potential for compensatory lung growth is retained and may be pharmacologically augmented, supra-optimal mechanical stimulation, unbalanced structural growth, or inadequate remodeling, may limit functional gain. Finding ways to optimize the signal-response relationships and resolve structure-function discrepancies are major challenges that must be overcome before the innate compensatory ability could be fully realized. Partial pneumonectomy reproducibly removes a known fraction of functioning lung units and remains the most robust model for examining the adaptive mechanisms, structure-function consequences, and plasticity of the remaining functioning lung units capable of regeneration. Fundamental mechanical stimulus-response relationships established in the pneumonectomy model directly inform the exploration of effective approaches to maximize compensatory growth and function in chronic destructive lung diseases, transplantation and bioengineered lungs.

Keywords

Mechanotransduction; mechanical stress; lung development; pneumonectomy; lung regeneration

Introduction

Compensatory lung growth (CLG) refers to the innate ability for accelerated cell proliferation and/or hypertrophy, addition of matrix and fibers and the formation of new anatomical structures such as airways and capillaries that occur in response to the loss of functional lung units due to disease or surgical removal. Regardless of the inciting cause, compensation depends on adaptation of the remaining functioning units capable of responding. Signals from the loss of lung units stimulate coordinated activation of metabolic pathways, balanced generation of all normal tissue components and appropriate remodeling leading to augmented structure and function of the remaining lung units. The potential for

CLG is retained in young and adult mammals and from small to large species. Mechanical signals play a pivotal role in both developmental and compensatory lung growth, and the latter recapitulates many of the active pathways during development, although there are also important selective differences. To fully appreciate the signals, potential and challenges of CLG it is imperative to understand the signals and limits of developmental lung growth. This review summarizes the current knowledge in this field with emphasis on a comparison of mechanosensitive relationships among the parenchyma, microvasculature and thorax during development and in a post-pneumectomy (PNX) model of re-initiated lung growth and compensation.

Mechanical Signals in Lung Development

Living cells and organs exemplify the concept of tensegrity (Wang, et al., 2001) – constant physical tension is required to maintain their three-dimensional structure and function. Physical tension within cells are transmitted via the cytoskeleton; the balance of physical forces governs fundamental processes such as cell size, shape, permeability, ion transport, migration, proliferation, gene activation and signal transduction. Lung architecture also exemplifies tensegrity – the balance of physical tension, stress and strain fundamentally determine lung structure and function. Unlike most solid organs, the elastin-rich lung tissue and capillaries are confined within a closed thorax while undergoing large tonic and cyclic mechanical stress and deformation with each breath and each cardiac contraction. Mechanical signals generated by the two disparate cardio-respiratory pumps and their interactions within the mechanical constraints of the thorax transduce and modulate every aspect of lung development, growth, remodeling, and function (Hsia, 2004b, Hsia, et al., 2015).

Mechanical forces and parenchyma development

Physical tension on the lung is maintained by a continuum network of elastic fibers that courses from the trachea to the most distal gas exchange units and the pleura (Weibel, 2009). At functional residual capacity, inward elastic recoil of the lung balances the outward recoil of the rib cage. During inspiration, physical stress on the lung and rib cage increases due to the negative pressure generated by contractions of the diaphragm and other respiratory muscles that expands lung volume. Respiratory muscle relaxation during expiration allows elastic recoil of the lung to expel air. In the developing fetus a steadily enlarging bony thorax and cyclic respiratory movements generate pressure gradients between the tracheal lumen and the surrounding parenchyma that provide the signals for lung growth and branching morphogenesis (Figure 1). Constrained by thoracic geometry and modulated by diffusion-reaction interactions among myriad growth modulators or morphogens (Turing, 1990), the iterative asymmetric bifurcations of conducting airways continue until birth, while airways in the respiratory region continue to bifurcate after birth, ultimately creating 14–16 generations of conducting airways and another 6 generations of respiratory bronchioles and alveolar ducts, terminating in some 400 million alveolar sacs in the adult human lung (Ochs, et al., 2004). Increasing physical stress in this system, e.g., by fetal tracheal occlusion, accelerates airway formation and branching (Grushka, et al., 2010, Unbekandt, et al., 2008) while reducing stress, e.g., by restricting respiratory movements, has the opposite effect

(Harding and Hooper, 1996, Harding, et al., 1993). Stratified airway bifurcation follows a fractal pattern (Mandelbrot, 1983, Weibel, 1991), filling all available spaces and delivering the inspired air bolus in series to successive airway generations while alveolization maximizes total air-tissue interface area to facilitate diffusive conductance for gas transfer. Airway luminal diameters are dynamically regulated by smooth muscles, actin and other contractile elements (Oldmixon, et al., 2001) present throughout the airway generations to ensure sufficient ventilatory delivery to the gas exchange surfaces without excessively increasing anatomical dead space or respiratory muscle work.

Mesenchymal smooth muscle cells (SMCs) surrounding the bronchovasculture generate mechanical stress and direct epithelial branching morphogenesis. In the fluid-filled embryonic lung, SMCs are present in the walls of proximal but not distal airways (Tollet, et al., 2001); this localization pattern is essential for fetal branching morphogenesis and alveoli formation (Kim and Vu, 2006, Sparrow and Lamb, 2003). Rhythmic airway SMC contractions produce peristaltic waves propelling lung fluid from proximal to distal airways (Schittny, et al., 2000). Coupled with a continuous production of lung fluid, the positive airway pressure distends distal epithelial buds where SMCs are absent, spurring branching and alveolization. Increasing fluid pressure leads to hyperplastic lungs (Blewett, et al., 1996) while decreasing fluid pressure leads to hypoplastic lungs (Harding, et al., 1993). Cyclic mechanical strain feeds back to regulate airway SMC proliferation and migration through matrix metalloproteinase release and activation (Hasaneen, et al., 2005).

Branching morphogenesis is mediated by a host of transcriptional factors including thyroid transcription factor-1 (TTF-1), Forkhead orthologs (FOX), GATA, SOX, and ETS family members (Maeda, et al., 2007), sonic hedgehog (SHH), fibroblast growth factors (FGFs), bone morphogenetic protein-4 (Bmp4), platelet-derived growth factor- α (PDGF- α), retinoic acid, and Wnt signaling pathways (Kim and Vu, 2006) (McGowan and McCoy, 2014) (Perl and Gale, 2009) (Kadzic, et al., 2014, Shu, et al., 2005), among others. FGF and SHH signaling promotes airway SMC differentiation in proximal airways (Jesudason, et al., 2006, Yi, et al., 2009). Bmp4 signaling favors endodermal cell differentiation into distal respiratory epithelium, while lack of Bmp4 favors differentiation into proximal bronchiolar epithelium (Weaver, et al., 1999). Within the alveolar septal interstitium, contractile myofibroblasts containing alpha-smooth muscle actin are the counterpart of proximal airway SMCs and likely share the same origin as SMCs (Kim and Vu, 2006). Myofibroblasts produced tropoelastin, a precursor of elastin (Chailley-Heu, et al., 2005), which is essential for secondary alveolar septation. A new interalveolar septal bud arises as a double capillary layer with elastin localized at the tip. Tension on elastin fibers causes lengthening of the new septa (Figure 2) (Oldmixon, et al., 1988) (Weibel, 2009, Wood, et al., 1998). Myofibroblasts exhibit asymmetric localization of phosphorylated epidermal growth factor receptor (phospho-EGFR), which directs cell migration. Disruption of the asymmetric phospho-EGFR localization leads to unstable cell polarity, aberrant myofibroblast localization and impaired alveolar septation (Li, et al., 2012). Dysregulation of airway SMCs and alveolar myofibroblasts is implicated in epithelial mesenchymal transition leading to myofibroblast accumulation, upregulation of profibrotic mediators (Cabrera-Benitez, et al., 2012, Hinz, 2012, Zhang, et al., 2015), and excessive connective tissue deposition, thereby contributing to the evolution of diverse diseases such as congenital diaphragmatic hernia (CDH)

(Jesudason, et al., 2006), asthma (Gabbrielli, et al., 1994), and pulmonary fibrosis (Gharaee-Kermani, et al., 2009).

Mechanical forces and microvascular development

Pulmonary blood vessels receive the entire right ventricular cardiac output and distribute erythrocytes in parallel channels throughout an extensive alveolar microvascular network such that nearly all of the ferrous heme binding sites on the hemoglobin of mixed venous blood are completely oxygenated during a median alveolar capillary transit time of 1.2 s at rest (Hogg, et al., 1994) and 0.42–0.46 s at exercise (Warren, et al., 1991). Maintenance of a low pulmonary microvascular pressure is enabled by the air-filled acinar architecture that allows alveolar septal capillaries to distend with increasing perfusion and alveolar corner vessels to dilate with lung expansion with little impediment, thereby ensuring high microvascular capacitance. In addition to the structural design, a low microvascular pressure is actively maintained via balanced vasomotor regulatory mediators such as endothelin (Roberts, et al., 1998), endothelial nitric oxide (NO) (Kawai, et al., 1995), prostaglandins (Bonanno, et al., 2016), and even surfactant, which causes smooth muscle relaxation in a prostanoid-mediated, epithelium-dependent manner without involving NO synthase (Koetzler, et al., 2006).

Independent of ventilatory mechanical stress, the microvascular network experiences physical stress including tonic and cyclic distension and flow-related shear stress that provide mechanical signals for adaptation (Hasaneen, et al., 2007). Vasculogenesis refers to the differentiation of precursor cells (angioblasts) into endothelial cells and the *de novo* formation of a primitive vascular network in the embryonic lung whereas angiogenesis refers to the growth of new capillaries from pre-existing vessels (Risau, 1997) by sprouting and/or intussusception (Burri, 2006). It has been stated that vasculogenesis is induced by physical forces, predominantly fluid shear stress (Chatterjee, et al., 2008), whereas angiogenesis is induced by hypoxia (Heil, et al., 2006). In fact, the distinction is not clear-cut, because the two signals – physical stress and hypoxia – are intrinsically intertwined. Shear stress activates NADPH and generates reactive oxygen species (ROS) (Browning, et al., 2012, Chatterjee, et al., 2008) and may create oxygen tension gradients within and among cells that alter regional hypoxia-activated responses. Flow-related stress is detected by pulmonary vascular endothelial cells via a caveolae and membrane protein complex initiating depolarization via potassium-ATP ion channel closure followed by activation of NADPH oxidase (NOX2) and NO synthase (eNOS), and signaling through MAP kinases to generate ROS and NO (Chatterjee, et al., 2008). Both ROS and NO promote vasodilation, endothelial cell proliferation, angiogenesis and vascular remodeling as compensation for a perceived need for tissue oxygen delivery (Chatterjee, et al., 2008, Tian, et al., 2011). Chronically increased pulmonary blood flow induced by shunting in an ovine model is associated with the upregulation of multiple proangiogenic genes and a neonatal “angiogenesis burst” between 1 and 4 weeks of age (Tian, et al., 2011).

Hypoxia, defined as a reduction in oxygen partial pressure, is fundamentally a molecular physical gradient and a primordial stimulus for the development of gas exchange organs across the animal kingdom including the mammalian placenta (Zamudio, 2003) and the

postnatal lung (Burri and Weibel, 1971, Hsia, et al., 2005, Ravikumar, et al., 2015). Chronic hypoxia exposure or residence at high altitude has been reported to increase total pulmonary vessel length, volume, endothelial surface area and number of endothelial cells in vivo (Howell, et al., 2003). Cellular hypoxia impairs the mechanical function of contractile elements (Chen, et al., 2013, John, 2010, Moon, et al., 2010), which alters regional force distribution and deformation within and among cells. Hypoxia stimulates mitochondrial ROS generation, which stabilizes hypoxia-inducible transcriptional factor-1 α (HIF-1 α) (Chandel, et al., 2000, Guzy and Schumacker, 2006). HIF-1 α signals to an array of homeostatic pathways including pro-angiogenic genes such as paracrine erythropoietin (Epo) and vascular endothelial growth factor (VEGF) that mediate microvascular formation and growth (Marti, 2005), and NO synthase (Jung, et al., 2000, Zhang, et al., 2011) and endothelin-1 (Li, et al., 2014) that regulate vasomotor tone and perfusion distribution, as an extensive self-regulated network preventing cell injury and death under conditions of compromised tissue oxygen delivery. The HIF-1 α network is activated by both hypoxia (Marti and Risau, 1998) and mechanical stress (Chang, et al., 2003, Chun, et al., 2002, Kim, et al., 2002), underscoring the interrelationship between these two fundamental adaptive stimuli and their convergence upon ROS as a common initiating factor for lung development, growth, remodeling and repair (Figure 3).

Mechanical interactions regulate growth vs. remodeling

Growth refers to the addition of new cells, matrix, fibers, and structural elements, while *remodeling* refers to the modification and redistribution of existing structural elements to minimize physical and metabolic stress and optimize organ function. Growth of new tissue is normally accompanied and/or followed by rearrangement of cell/tissue elements and architectural modification at micro- and macro-scopic scales and different organizational levels. Whether growth or remodeling predominates is governed by the stage of somatic maturation and the rate of thoracic enlargement; the latter rate determines the magnitude of mechanical pre-stress on the developing lung.

After birth, the outward elastic recoil of the thorax progressive increases, thought to be due to rib cage ossification, increasing intercostal muscle tone and respiratory muscle generation of larger negative intrathoracic pressures (Blackburn, 2013). As somatic growth and thoracic expansion occur most rapidly during the first few years after birth, presumably pre-stress of lung parenchyma is also high during this period, coinciding with vigorous formation of intra-acinar airways and alveoli while the existing branches remodel by lengthening and dilatation. Simultaneously the extra-acinar conducting bronchovascular branches lengthen and dilate in response to the increased ventilation and cardiac output to reduce air and blood flow resistance as well as the mechanical stress on distal lung units. A later pubertal growth spurt also affects the long bones first, preceding the time of peak growth velocity of the thorax by 6 mo to 1 year (DeGroot, et al., 1988, Simon, et al., 1972, Tanner, et al., 1966a, Tanner, et al., 1966b). Thoracic shape becomes elongated and the residual volume (as % of vital capacity) increases; these changes reflect reduced thoracic compliance and increased outward recoil of the chest wall at a low lung volume that are not balanced by an increase in expiratory pressures generated by respiratory muscles (DeGroot, et al., 1988).

Remodeling of alveolar septa during maturation is evident from the preferential shift of connective tissue elements to the “thick side” of the tissue-capillary barrier to provide physical support while minimizing gas diffusion resistance across the “thin side”. Gradual septal thinning by apoptosis (Bruce, et al., 1999) and lengthening transform the typical “double capillary” septal morphology of an immature lung into the “single capillary” morphology of a mature lung (Burri, 2006), which expands total capillary blood volume and surface area. As the rate of somatic maturation slows with age, thoracic expansion and lung pre-stress also decrease, associated with cessation of alveolization while lung maturation continues with predominantly air space enlargement and modification of the existing scaffold. Upon reaching adulthood with epiphyseal union of the bony ribcage in large animals and humans, the thorax reaches its final fixed dimension, which along with its relative rigidity dictates the final size of the lung. Thereafter, parenchymal pre-stress stabilizes or declines to a minimal level and active growth/remodeling ceases. In contrast to large mammals, the epiphyses do not fully close in rodents (Dawson, 1934, Hsia, 2004a); somatic growth and thoracic expansion slowly continue throughout life. Without a stable final thoracic size and with a less rigid rib cage, thoraco-pulmonary pre-stress remains elevated. Rodent lungs are less stratified and without respiratory bronchioles (Bal and Ghoshal, 1988), suggesting reduced ability to regulate mechanical stress and ventilatory distribution. These inter-species differences in developmental pattern contribute to differential susceptibility to mechano-sensitive signals.

Mechanical Signals in Compensatory Lung Growth

Compensatory lung growth (CLG) refers to the innate ability to accelerate or re-initiate active lung growth in response to a loss of functioning lung units, resulting in augmented structure and function of the remaining lung units to compensate for the initial loss. Experimental pneumonectomy (PNX) is the most robust and extensively characterized model of CLG mimicking the consequences of anatomical loss of lung units. Mechanical stress has emerged as the major stimulus for the *de novo* re-initiation of growth in the mature lung (Hsia, 2004b, Wirtz and Dobbs, 2000) while exogenous growth factors (Ravikumar, et al., 2011, Yan, et al., 2005) and ambient hypoxia (Burri and Weibel, 1971, Hsia, et al., 2005) modify the activated growth pathways. As lung units are lost due to surgery or disease, re-direction of air and blood flow to the remaining lung units cause air space expansion, tissue stretch, capillary distention and flow shear, which provide mechanical stimuli for adaptation. Where these stimuli are below a growth-initiating threshold, adaptation occurs via recruitment of the remaining physiological reserves and remodeling of the remaining structure (Carlin, et al., 1991, Herazo, et al., 1992, Hsia, 2004b, Hsia, et al., 1992). Alveolar microvascular reserves may be recruited via unfolding of septa, opening and distention of capillaries, and more uniform distribution of erythrocytes within and among capillaries (Figure 4). Remodeling of the remaining structure via apoptosis (Sakurai, et al., 2007) attenuates alveolar wall thickness and the diffusion barrier. Recruitment and remodeling are passive adjustments that increase lung diffusing capacity with minimal metabolic energy expenditure. In contrast, active tissue-capillary growth is metabolically costly, and only invoked when the stimuli exceed a critical threshold and the reserves and remodeling are insufficient for maintaining adequate gas exchange.

Alveolar-capillary regenerative ability is retained

The occurrence of CLG has been reported in multiple mammalian species – rodent, rabbit, ferret, dog (Burri and Sehovic, 1979, Cagle and Thurlbeck, 1988, Davies, et al., 1982, Hsia, 2004a, Hsia, et al., 2004, Kirchner and McBride, 1990, Langston, et al., 1977, McBride, 1985, Sekhon and Thurlbeck, 1992, Thurlbeck and Langston, 1989), children (Ciric, et al., 2003, Ijsselstijn, et al., 1997, Laros and Westermann, 1987, Marven, et al., 1998, McBride, et al., 1980) and adult humans (Butler, et al., 2012) – resulting in higher than expected function of remaining lung units (Birath, et al., 1965, Burrows, et al., 1960, Herazo, et al., 1992, Hijazi, et al., 1998). In young animals and in children after unilateral pneumonectomy (PNX) or CDH repair, vigorous “*catch-up*” CLG nearly normalizes long-term lung function (Ciric, et al., 2003, Ijsselstijn, et al., 1997, Laros and Westermann, 1987, Marven, et al., 1998, McBride, et al., 1980, Takeda, et al., 1999a), although bronchovascular flow resistance remains elevated (Takeda, et al., 1999a, Takeda, et al., 1999b, Takeda, et al., 1997, Takeda, et al., 1994). In adult humans, post-PNX CLG has been indirectly inferred by imaging techniques (Butler, et al., 2012). Direct assessment of human CLG is hindered by the invasive measures required for assessment and a prolonged time course of adaptation necessary to remodel a highly stratified scaffold. In diffuse lung injury, the responses of normal lung units cannot be clearly separated from those of diseased units while ongoing injury and/or inflammation may suppress innate compensatory responses. Regardless of how lung units are destroyed, compensation depends on adaptation of the remaining units capable of responding.

While definitive human CLG is difficult to validate because the remaining lung is usually abnormal, robust data from large animals, i.e., the canine model, strongly support the retained potential for re-initiation of alveolar-capillary growth. The canine PNX model has several unique features compared to the more widely studied rodent model: **a)** Patterns of rib cage, lung and somatic maturation more closely mimic that in human development. The thorax in large animals reaches fixed dimensions following epiphyseal closure upon reaching adulthood and constrains mechanical thoraco-pulmonary interactions in a manner similar to that in humans. **b)** Acinar airway system is stratified and more similar to humans. In addition to alveolar ducts, several generations of respiratory bronchioles are present and play a role in the regulation of ventilation-perfusion and mechanical stress distributions, which in turn provide stimuli for adaptation. **c)** Fractional lung loss by surgical removal of selected lobes is controllable and reproducible, and the compensatory responses in the remaining lobes are readily quantifiable. **d)** Time course of adaptation is similar to that in humans. **e)** Functional outcome is readily quantified in anesthetized and conscious states and from rest to exercise. **f)** Plasticity of CLG may be examined by selectively perturbing the individual putative stimuli. **g)** Post-PNX responses have been extensively characterized at physiological, structural, cellular and molecular levels, allowing cohesive interpretation of the mechanisms of growth initiation, regulation and limitation. This model is well suited for integrated structure-function assessment of preclinical interventions to amplify endogenous CLG. Cumulative insights obtained from observations in the PNX model are summarized below.

Balanced addition of alveolar septal constituents in CLG

Multiple cell lineages and nearly all major homeostatic mediators and molecular pathways are activated in the post-PNX remaining lung (Gilbert and Rannels, 1998, Hogan, et al., 2014, Kho, et al., 2013, Paxson, et al., 2009, Wolff, et al., 2010, Yuan, et al., 2002, Zhang, et al., 2007, Zhang, et al., 2006). There is a disproportionally large early post-PNX increase in the volume of septal interstitium (Hsia, et al., 2016b), which is associated with increased alveolar endothelial permeability (Waller, et al., 1996) and/or an influx of migratory cells (Doerschuk and Sekhon, 1990). Alveolar type-2 (AT2) cells are the first progenitors to proliferate and hypertrophy (Hsia and Johnson, 2006, Thet and Law, 1984). Other stem or progenitor cell types have not shown significant or reliable response (Nolen-Walston, et al., 2008, Paxson, et al., 2013, Voswinckel, et al., 2003). It is highly unlikely for a single or a few progenitor cell types alone to account for the intensity, rapidity and widespread spatial distribution of post-PNX proliferative response. It is more probable that differentiated resident cells also undergo mechanically induced division and/or hypertrophy, i.e., the spectrum of “progenitor cells” may be broader and more prevalent than traditionally held. Over the course of several weeks, cell proliferation and matrix/fiber deposition increase alveolar septal extravascular tissue volume by more than two-fold, accompanied by gradual attenuation of the volume of interstitium. All constituents of the septum increase in volume; alveolar-capillary surfaces areas also increase while acinar airways increase in branches as well as diameter and length (Hsia, et al., 2000) with long-term normalization of distal lung morphology. Accelerated angiogenesis is evident from the upregulation of various pro-angiogenic molecules (Foster, et al., 2004, Foster, et al., 2002, Hogan, et al., 2014, Zhang, et al., 2007, Zhang, et al., 2006, Zhang, et al., 2008) and the appearance of more numerous double capillary segments (Ravikumar, et al., 2014); the latter are morphological markers of new capillary formation arising from existing capillaries by the process of intussusception (Djonov, et al., 2003). Subsequent remodeling and lengthening of alveolar septa separate the double capillaries into single capillaries and attenuate septal thickness to increase total capillary blood volume and surface area and reduce diffusive resistance in a process reminiscent of that during development, (Hsia and Ravikumar, 2015).

Threshold, optimal range, and upper limit of CLG

After the loss of ~42% of lung units by left PNx, expansion of the remaining units, microvascular recruitment, and attenuation of blood-gas diffusion barrier are sufficient to maintain near-normal maximal O₂ uptake and requiring little new tissue-capillary growth (Carlin, et al., 1991, Hsia, et al., 1993a, Hsia and Johnson, 2006, Hsia, et al., 1991, Hsia, et al., 1990). Only the remaining infra-cardiac lobe that undergoes the largest anisotropic expansion across the midline shows significant gains in alveolar septal tissue volume and surface areas (Hsia and Johnson, 2006). Following the removal of 58% of lung units by right PNx, supra-threshold mechanical stress stimulates tissue-capillary growth in all remaining lobes; functional compensation per unit of initial fractional remaining lung is greater following 58% than 42% resection (Hsia, et al., 1994a, Hsia, et al., 1993b, Hsia, et al., 1994b). Following loss of nearly 70% of lung units by bilateral partial lung resection, heterogeneous mechanical stress on the remaining lobes is accentuated (Yilmaz, et al., 2009, Yilmaz, et al., 2011); compensatory alveolar-capillary growth remains vigorous in all remaining lobes but with diminishing gains as its magnitude either reaches a plateau or

begins to decline (Hsia, et al., 2008, Ravikumar, et al., 2014). This pattern indicates the existence of a threshold, an optimal range, and an upper limit of CLG.

Growth and remodeling phases of CLG

Early post-PNX alveolar septal growth is accompanied by progressive remodeling throughout the remaining lung that attenuates the non-uniform increase in mechanical stress and strain. Analysis of regional lung air and tissue volumes and deformation using high resolution computed tomography obtained at two inflation pressures before and after extensive lung resection show markedly non-uniform regional mechanical properties (Figure 5A) and anatomical growth within and among the remaining lobes, with an initial proliferative phase marked by increasing air and parenchymal tissue-blood volumes, a progressive remodeling phase marked by tissue relaxation and an increase in specific lobar compliance, and followed by delayed improvement in whole lung compliance and diffusing capacity more than 3–4 months later (Hsia, et al., 2008, Yilmaz, et al., 2009, Yilmaz, et al., 2011). Long-term lobar tissue growth correlates inversely with principal lung strain, suggesting that CLG and remodeling relieves tissue strain (Figure 5B). Both strain and growth are greater in the periphery than the center of each lobe (Yilmaz, et al., 2011); the peripheral location has fewer and less rigid support structures, exhibits greater air space enlargement, and experiences higher mechanical strain at a given lung volume. Concurrent and sequential growth and remodeling are crucial for ensuring coordinated adjustment among different components at molecular, cellular, tissue and architectural levels to minimize scaffold distortion and maximize functional gain.

Additive stimuli for CLG

Compared to the rapid vigorous post-PNX CLG in young animals that completely normalizes long-term gas exchange and exercise capacities (Takeda, et al., 1999a), adult canine CLG requires more intense stimuli for initiation and a longer adaptive time course (Butler, et al., 2012) whereby early proliferation is followed by acinar remodeling over several months (Yilmaz, et al., 2011) to partially normalize total alveolar-capillary surface areas and diffusing capacity (Ravikumar, et al., 2014). The maturity-related differences suggest that developmental and post-PNX signals exert additive effects. Various reports of accentuated post-PNX lung growth by the addition of hypoxia (Sekhon, et al., 1993) and growth factors (Kaza, et al., 2002, Kaza, et al., 2000, Yan, et al., 2004) also support the susceptibility of the remaining lung to additive signals. Furthermore, with increasing lung resection up to 70%, an upper limit of post-PNX alveolar tissue re-growth is reached while new double capillary formation increases steadily without reaching an upper limit (Ravikumar, et al., 2014), suggesting the existence of independent stimuli for tissue vs. capillary growth. These stimuli have been separately examined in the canine model, summarized below.

Stimuli Related to Lung expansion

To temporally isolate in vivo signals resulting from lung expansion (tissue deformation) from those resulting from increased perfusion to the remaining lobes (capillary distention/shear), the right lung of adult canine was removed and replaced with custom-shaped space-occupying inflatable silicone prosthesis that was kept either inflated (INF) or deflated (DEF)

(Hsia, et al., 2003, Hsia, et al., 2001, Wu, et al., 2000, Zhang, et al., 2007) or initially inflated followed by delayed acute deflation after full recovery from surgery (Figure 6–7) (Dane, et al., 2013, Ravikumar, et al., 2013). Results were compared to pre-PNX control (the removed right lung in the same animal), matched post-PNX animals with continually inflated (INF) prosthesis, and unoperated normal animals. Post-PNX perfusion per unit of lung increased similarly from pre-PNX baseline in both INF and DEF groups. Post-PNX lung expansion following deflation of prosthesis upregulated the HIF-1 α -Epo-VEGF signaling cascade in a coordinated fashion while the corresponding expression in the INF group was unchanged (Figure 6). In the INF group, post-PNX lung volumes and diffusing capacity at a given pulmonary blood flow remained 25–40% below pre-PNX baseline. In contrast, in the DEF group both alveolar septal growth and function increased up to 157% post-deflation. While post-PNX lung expansion in the DEF group was associated with greater increases in all components of extravascular alveolar tissue volume, the corresponding increases in capillary blood volume and double capillary formation are similar to that in the INF control group, suggesting an effect of lung expansion on parenchyma growth but not capillary growth (Figure 7). On average, expansion related responses could explain about one-half of the total post-PNX structure-function compensation.

Stimuli Related to Increased Perfusion

To create differential perfusion changes among post-PNX remaining lobes, adult dogs underwent thoracotomy with either banding of the left caudal lobar pulmonary artery (PAB, to its average normal *ex vivo* circumference) or SHAM banding, followed 3 weeks later by right PNX (Dane, et al., 2016). Before PNX, PAB reduced basal perfusion to the banded left caudal lobe and redirected perfusion equally to the lobes of the right lung. After right PNX, the average expected increase in lobar perfusion was greater to the unbanded remaining lobes than to the banded lobe (Figure 8A). Compared to SHAM banding, post-PNX expansion and gain of extravascular alveolar tissue components were impaired in all remaining lobes of PAB animals; impairment was more severe in the banded lobe (Figure 8B). In contrast, the expected post-PNX increase in double capillary formation was exaggerated in all lobes, especially the unbanded left cranial lobe (Figure 8C) (Dane, et al., 2016). Thus, restricting the post-PNX perfusion increase to one lobe impairs alveolar expansion and tissue growth but selectively stimulates intussusceptive capillary formation, indicating distinct actions of perfusion-related signals from expansion-related signals on CLG. Overall perfusion-related signals could explain about one-half of the total post-PNX structure-function compensation.

The above series of studies support the conclusion that post-PNX CLG is initiated by supra-threshold mechanical signals generated by separate increases in volume and perfusion to the remaining lobes. Taken together, these mechanical signals could fully account for the observed compensatory response.

Comparison of Mechanical Signal and Response in the Two Types of Lung Growth

The parallel features of developmental and compensatory lung growth are now apparent; both are driven by the balance of mechanical forces acting on the lung parenchyma-vasculature and on the thorax, and constrained by the physical size, shape and availability of space within the thorax. In both types of growth, generation of new tissue and structure is followed by remodeling leading to enhanced gas exchange and mechanic function of the remaining lung. The developing thorax enlarges gradually, imposing more symmetric and sustained mechanical stress on the lung; cardiac output also increases gradually, imposing symmetric and sustained pulmonary microvascular stress. Consequently, growth and remodeling are symmetric and sustained with mild to moderate spatial heterogeneity throughout fetal and postnatal life; proliferation, remodeling and the increase in diffusing capacity proceed in concert, gradually reducing thoraco-pulmonary mechanical stress; the intensity of lung growth slows with the rate of rib cage growth during maturation and ceases completely upon reaching adulthood.

In CLG following PNX (or surgical repairs of CDH and severe kyphoscoliosis), the increase in negative intra-thoracic pressure, the availability of more space, and the redistribution of ventilation-perfusion occur rapidly, imposing large, heterogeneous and asymmetric mechanical stress on lung tissue and microvasculature. The resulting CLG is also markedly heterogeneous and asymmetric, with an initial proliferative burst followed by slower remodeling and tissue relaxation that gradually relieve mechanical stress. Some lobes respond more vigorously than others (Ravikumar, et al., 2014, Yilmaz, et al., 2009, Yilmaz, et al., 2011). As in development, lung units in the periphery experience greater post-resection mechanical stress/strain and exhibit more vigorous growth activity. Adaptive activity persists as long as there are regions that experience supra-threshold mechanical signals; thus, functional improvement is delayed and continues at a slow rate for many months. Spatial and temporal heterogeneities reduce the overall magnitude of CLG compared to a homogeneous response; on the other hand, the presence of heterogeneity also means that adaptive pathways remain activated at least in some regions, thereby prolonging the “*window of susceptibility*” during which the imposition of additive stimuli and/or pharmacological interventions may modify the endogenous response.

Lineage tracing has shown that FGF10 expressing progenitor cells give rise to bronchovascular smooth muscle cells and alveolar lipofibroblasts (El Agha, et al., 2014). Platelet-derived growth factor receptor- α (PDGFR- α)-expressing lipofibroblasts mediate epithelial-mesenchymal crosstalk, a critical process shared between development and post-PNX CLG (Chen, et al., 2012, McGowan and McCoy, 2014, Paxson, et al., 2013). Serial post-PNX transcriptomic analysis of the remaining lung in mice has shown prominent early expression of genes related to proliferation (Ccnb1, Bub1, and Cdk1), extracellular matrix (Colla1, Eln, and Tnc), and proteases (Serpinb2 and MMP9) in a pattern consistent with late stage postnatal lung development rather than embryonic or early alveolization stages (Kho, et al., 2013). The post-PNX course resembles initial de-differentiation (3–7 days) followed by re-development (28–56 days), suggesting reversion of differentiated remaining lung cells

to a more primitive developmental stage with a higher proliferative potential. These findings are fully consistent with mechanical activation of a broad palette of resident lung cells beyond the “classical” alveolar stem cells, the formation of morphologically immature new capillaries, and the multi-phase adaptive response (vigorous non-sustained proliferation followed by slower remodeling leading to delayed functional improvement).

Translational Implications and Challenges for Lung Regeneration

Pharmacological augmentation of CLG

The incomplete restoration of structure-function by endogenous CLG in adult mammalian lung indicates retention of plasticity but also a need for ways to augment the innate response. While various factors such as hypoxia (Sekhon, et al., 1993), hormones (Rannels, et al., 1991), NO (Leuwerke, et al., 2002) and various growth regulators (Kaza, et al., 2001, Kaza, et al., 2002, Kaza, et al., 2000) have been reported to modify endogenous CLGR, none has been shown to successfully augment gas exchange function above that of the endogenous response. This pattern is clearly illustrated by post-PNX supplementation of *all-trans* retinoic acid (RA) in adult canines. When mechanical stimuli are *below* a growth-initiating threshold (following 42% lung resection), RA supplementation has little effect (Yan, et al., 2005). When mechanical stimuli exceed a growth-initiating threshold (following 58% lung resection), RA supplementation further increases the gain in extravascular alveolar tissue volume (~30%) and intussusceptive double capillary formation (100%) compared to post-PNX vehicle-treated controls. However, RA exerts nonuniform effects on alveolar septal cells, with stimulation of AT2 cell growth lagging behind that of other cell types. RA also distorts acinar architecture, leading to thicker alveolar septa and capillary basement membranes, smaller air spaces and lower lung compliance, while exercise performance and the gain in lung diffusing capacity are similar to that in vehicle-treated controls (Dane, et al., 2004, Ravikumar, et al., 2011, Yan, et al., 2004). These results uncover a critical *structure-function discrepancy* in pharmacological augmentation of CLG, i.e., augmentation of structural growth fails to augment function. Surmounting this discrepancy poses a major challenge to translational progress in regenerative medicine that will be critical to moving the field forward and fully realizing the potential of innate plasticity of the lung.

Cell-based therapy to augment CLG

Manipulation of key developmental pathways in murine and human pluripotent stem cells has led to the generation of lung endoderm precursor cells (Longmire, et al., 2012) as well as differentiated airway and alveolar epithelial cells (Ghaedi, et al., 2013, Huang, et al., 2014, Wong, et al., 2012) that hold considerable promise for cell-based therapy to facilitate tissue repair and augment CLG. We can reasonably expect that this emergent field of research will need to surmount similar obstacles of structure-function discrepancy as that seen in pharmacologically augmented CLG.

Upper limit and structure-function discrepancy in CLG

Several factors contribute to the upper limit of endogenous CLG and the discrepancy between the ability to exogenously augment structural growth and the inability to translate

structural augmentation into functional enhancement above that achieved by the innate response:

- a. *Nonuniform growth stimulation and inadequate remodeling causing architectural distortion* In emphysematous rats, exogenous RA stimulates alveolar septation; however, the increase in alveolar number is offset by a smaller mean alveolar size without a concurrent increase in alveolar surface area, thus preventing a meaningful increase in lung diffusing capacity (Massaro and Massaro, 1997, Tepper, et al., 2000). In post-PNX canine lungs, RA-enhanced double capillaries do not fully remodel into single capillaries that are more favorable for gas exchange (Ravikumar, et al., 2011, Yan, et al., 2004) while a relative lack of RA stimulation of AT2 cell growth could adversely affect surfactant homeostasis and secondarily impair alveolar mechanics. Another example of growth-promoting intervention is tracheal occlusion in the fetal lamb model of CDH; tracheal occlusion accelerates mesenchyme growth but not AT2 cell proliferation, leading to alveolar distortion, surfactant deficit (Benachi, et al., 1998) and inefficient gas exchange for supporting respiration (Probyn, et al., 2000). Structural distortion in CLG is a direct consequence of heterogeneous mechanical signals; such distortion cannot be predicted from cellular-molecular studies but must be defined under physiological or pathophysiological conditions in the intact animal. Strategies to optimize the distribution of mechanical stress could enhance acinar remodeling, minimize distortion and enhance outcome.
- b. *Imbalance of oxidative stress vs. anti-oxidation* As a primordial signal transducing virtually all biological processes, mechanical stresses increase tissue permeability by perturbing cellular junctions, activate ion transport and receptors by distorting the membrane, upregulate gene transcription, protein synthesis and metabolism by increasing cytoskeletal tension that transmits directly to the nuclei and organelles, and intensify mitochondrial ROS production along the electron transport chain. Cell signaling by ROS originated in ancestral anaerobes to mediate fundamental cytoprotective pathways against ambient oxygen toxicity (Hsia, et al., 2016a). In eukaryotes, ROS are produced by, and in turn regulate, metabolic events including remodeling and growth. Mechanically stimulated ROS production is normally neutralized by endogenous antioxidants. Excessive mechanical stress or dysregulated growth can cause oxidation-antioxidation imbalance that in turn limit proliferation, remodeling, and function.

Under heterogeneous mechanical stress during post-PNX adaptation, some remaining lung units may experience supra-optimal physical and oxidative stress that threaten tissue integrity, mandating the deposition of connective tissue for physical support and the recruitment of cytoprotective mechanisms to prevent or mitigate oxidative damage, thus shifting metabolic energy away from growth stimulation towards defense against injury. This scenario is consistent with the observed upper limit of endogenous CLG as the severity of lung resection increases (Hsia and Johnson, 2006, Ravikumar, et al., 2014). Thus, the regulatory balance of ROS signaling could shift from predominantly remodeling/growth to cytoprotection. Adding pharmacological growth stimulants could further exaggerate metabolic oxidative stress in excess of endogenous antioxidant capacity and curtail

compensation. It is worth noting that all of the major pro-proliferative and pro-angiogenic mediators implicated in CLG, e.g., hepatocyte growth factor, HIF-Epo-VEGF, NO, among others, also exert potent protection against tissue injury. Strategies to restore and maintain redox balance during mechanically stimulated lung growth may protect metabolically active tissue, permit full expression of adaption, and enhance outcome.

Summary and Perspectives

In keeping with the tensegrity model where constant balance of tension modulates structure and function at all levels of cellular and tissue organization, separate mechanical stresses imposed on the parenchyma and vasculature and the thoraco-pulmonary mechanical interactions drive both developmental and compensatory lung growth. Alveolar growth is invoked only when a) existing physiologic-structural reserves prove insufficient for maintenance of function, b) parenchymal and/or microvascular stress exceed a critical threshold, and c) physical space is available to accommodate the enlarged alveolar-capillary volume and surfaces without crowding. Appropriate spatial and temporal mechanical interrelationships and feedback are crucial for growth initiation, follow-on remodeling and physiological outcome. The re-initiated growth pathways generally follow those during postnatal maturation, although the relative recruitability of specific pathways and mediators may differ. Ultimately the maximum lung dimension is constrained by the available thoracic space set by the rib cage size and diaphragm station. While innate potential for CLG in the mature lung is retained and may be pharmacologically augmented, supra-optimal mechanical stimulation, unbalanced structural growth, or inadequate remodeling may limit functional gain. Finding ways to optimize the signal-response relationships and resolve the structure-function discrepancy represent a major challenge in this field.

Partial lung resection or pneumonectomy remains a most robust model for examining structure-function consequences of lung destruction regardless of inciting etiology, evaluating the mechanisms, potential and limitations of CLG, and developing effective growth-promoting interventions. The mechanical stimulus-response paradigm observed post-PNX directly informs the adaptive mechanisms of remaining lung units in a variety of clinico-pathological conditions including CDH, kyphoscoliosis, major lung resection in the treatment of cancer, infection or trauma, diffuse parenchymal disease, and lung transplantation as well as tissue engineering. In ex vivo transplanted or bioengineered lungs, optimizing the distribution of force transmission will be essential for maintaining barrier integrity and facilitating cell repopulation, differentiation and matrix remodeling. In pulmonary emphysema, the elastic fiber scaffold is disrupted and alveolar capillaries obliterated; mechanical signals may decline below the threshold for initiating CLG while depletion of progenitor cells and/or accentuation of growth inhibition pathways may impair the ability to respond to the diminished mechanical signals. Under these conditions, attempts to pharmacologically induce CLG have been uniformly unsuccessful. Strategies to amplify mechanotransduction pathways in emphysema remain daunting but should be explored. In interstitial lung disease, inflammation and exaggerated oxidative stress preferentially direct the adaptive response towards anti-inflammation and cytoprotection instead of growth or remodeling. As the disease evolves, patchy areas of consolidation intermingling with areas of air space enlargement indicate aberrant force transmission that can result from and in turn

transduce unfavorable epithelial mesenchymal transition and myofibroblast accumulation and exacerbate fibrosis. Anti-inflammatory, cytoprotective, and anti-fibrotic therapy that promote more uniform mechanotransduction signal-response may permit the manifestation of beneficial remodeling and/or regenerative growth of the remaining functioning lung units. Owing to the complexity and the prolonged time course of CLG evolution in large mammals, interventions designed to optimize CLG and its outcome will need to be applied in a sustained fashion and targeted to the appropriate phase(s) of the natural response (Figure 9). The evolution of any enhancement of structure-function compensation is also likely to be gradual.

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Figure 1.

Physical forces acting on the thorax and lung. The outward recoil of the thorax and inward recoil of the lung create mechanical stress that provide signals for lung growth and remodeling, which in turn relieve stress. Adapted from (Hsia, 2004b)

Figure 2.

Hypothesized mechanism of alveolar septation under mechanical stress. **Panel a:** Three configurations illustrate how elastin fibers (shown as a thick cable) within an alveolar wall under tension from elastic recoil and surface forces create a tent-like surface with an angle Φ . As force balance (and Φ) change (left to right), the elastin cable may separate from the plane of existing alveolar walls forming a new alveolar wall with elastin fibers at the tip (T). Confluence of 3 adjacent alveoli forms a junction (J) where alveolar surface tension and septal retractive forces balance and the elastin cable is absent. Red arrows represent forces acting on the alveolar walls. **Panel b:** Two micrographs illustrate the 3D relationship of alveoli, septa and ducts with the network of alveolar entrance rings forming the wall of an alveolar duct. The septal tips (T) form an entrance ring to an alveolar sac and part of the incomplete “wall” of an alveolar duct shown in scanning electron micrograph (left). Elastic

fibers (stained black) support the free tips of the alveolar septa (right). Adapted from (Oldmixon, et al., 1989, Weibel, 2009, Wood, et al., 1998).

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Figure 3.

An example of convergent signal transduction for lung growth and remodeling. Both mechanical stress and hypoxia generate reactive oxygen species (ROS) and signal through shared pathways, for example, hypoxia-inducible factors (HIF) and its targets erythropoietin (Epo), VEGF, and NO synthase, leading to complex overlapping tissue responses.

Figure 4.

Anatomical alveolar microvascular recruitment. **Panel a:** Three images of subpleural alveolar capillaries obtained by in vivo videomicroscopy show a progressive increase in the number of erythrocyte-containing capillary segments as pulmonary blood flow or pressure increases (left to right). Adapted from (Okada, et al., 1992). **Panel b:** Two electron micrographs from a lung fixed under a constant airway pressure illustrate anatomical heterogeneity of capillary hematocrit and erythrocyte distribution within and among patent alveolar capillaries.

Figure 5.

Parenchymal deformation (linear strain) was measured in adult canine lung from high resolution computed tomography images obtained at two inflation volumes (15 and 30 cm H₂O transpulmonary pressure) pre- and post-bilateral resection of about 70% of lung tissue (removing 35% from each side). **Panel a:** Heterogeneous distribution of regional parenchymal strain. Strain magnitudes are indicated by the length and color of individual vectors. Parenchymal strain is larger at the periphery and increases post-resection in the remaining lobes. **Panel b:** At 15 months post-70% lung resection, the gain of extravascular

tissue volume in the remaining lobes (expressed as Post/Pre-resection ratio) correlates inversely with the change in maximum principal lobar strain, suggesting that lung growth relieves tissue strain. Adapted from (Yilmaz, et al., 2011).

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Figure 6.

Lung expansion upregulates a major cytoprotective-growth factor cascade. Adult canines underwent right pneumonectomy (PNX) where the right lung was replaced by custom-shaped inflated (INF) space-occupying silicone prosthesis (P). In some animals, the prosthesis was deflated (DEF) 3 weeks later to allow lateral expansion of the remaining lung (**panel a**). Delayed deflation of prosthesis resulted in upregulated expression of HIF-1 α (**panel b**) and its targets erythropoietin receptor (EpoR, **panel c**) and VEGF (**panel d**) in a coordinated fashion compared to the pre-PNX baseline. The expression was unchanged in

control post-PNX animals with continuously INF prosthesis. Normal HIF-1 α expression in unoperated control animals is also shown in the middle panel. Mean $\pm$ SD. Adapted from (Zhang, et al., 2007).

Figure 7.

Lung volume, extravascular alveolar tissue volume, capillary blood volume and double capillary profiles (**panels a to d**, respectively) in the remaining left lung of adult canines 8 to 12 months after right PNx where the right lung was replaced by an inflated (INF) silicone prosthesis, which was acutely deflated (DEF) in some animals 4 months after surgery.

Results were compared to post-PNX animals with continuously inflated (INF) prosthesis and unoperated (NORMAL) control animals. Mean±SD. $P < 0.05$ by ANOVA: * vs. NORMAL, † vs. INF. Based on data from (Ravikumar, et al., 2013).

Figure 8.

Redistribution of pulmonary perfusion by selective lobar pulmonary artery (PA) banding creates differential changes in alveolar tissue-capillary response following subsequent PNX in adult canines. **Panel A:** Fold change (post/pre-PNX ratio) in lobar PA blood flow following banding of left caudal lobe PA (PAB) compared to SHAM PA banding. Means $\pm$ SD. Pulmonary perfusion before and after right PNX was measured by a microsphere technique while standing at rest. **Panels B and C:** Perturbation of lobar perfusion produces opposite effects on post-PNX extravascular alveolar septal tissue volume (mL.kg⁻¹, **panel B**) and the formation of double-capillary profiles (percent of total [single + double] capillary profiles, **panel C**) in post-PNX remaining lobes of the left lung in PAB and Sham-banding groups compared with the corresponding lobes of unoperated normal adult animals. Examples of single- and double-capillary morphology are also shown in **Panel C**. Mean $\pm$ SD. $P < 0.05$; * PAB vs. Sham. § vs. unoperated normal lobe. From (Dane, et al., 2016).

Compensatory Lung Growth

Interventions

Figure 9.

Challenges and interventional strategies in the optimization of compensatory lung growth.