Published in final edited form as:

Angiogenesis. 2012 March ; 15(1): 23–32. doi:10.1007/s10456-011-9236-y.

Spatial Dependence of Alveolar Angiogenesis in Post-Pneumonectomy Lung Growth

Moritz A. Konerding¹, Barry C. Gibney², Jan Houdek¹, Kenji Chamoto², Maximilian Ackermann¹, Grace S. Lee², Miao Lin², Akira Tsuda³, and Steven J. Mentzer²

¹Institute of Functional and Clinical Anatomy, University Medical Center of Johannes Gutenberg-University, Mainz, Germany

²Laboratory of Adaptive and Regenerative Biology, Brigham & Women's Hospital, Harvard Medical School, Boston MA

³Molecular and Integrative Physiological Sciences, Harvard School of Public Health, Boston, MA

Abstract

Growth of the remaining lung after pneumonectomy has been observed in many mammalian species; nonetheless, the pattern and morphology of alveolar angiogenesis during compensatory growth is unknown. Here, we investigated alveolar angiogenesis in a murine model of post-pneumonectomy lung growth. As expected, the volume and weight of the remaining lung returned to near-baseline levels within 21 days of pneumonectomy. The percentage increase in lobar weight was greatest in the cardiac lobe ($p < .001$). Cell cycle flow cytometry demonstrated a peak of lung cell proliferation ($12.02 \pm 1.48\%$) 6 days after pneumonectomy. Spatial autocorrelation analysis of the cardiac lobe demonstrated clustering of similar vascular densities (positive autocorrelation) that consistently mapped to subpleural regions of the cardiac lobe. Immunohistochemical staining demonstrated increased cell density and enhanced expression of angiogenesis-related factors VEGFA, and GLUT1 in these subpleural regions. Corrosion casting and scanning electron microscopy 3–6 days after pneumonectomy demonstrated subpleural vessels with angiogenic sprouts. The monopodial sprouts appeared to be randomly oriented along the vessel axis with interbranch distances of 11.4 ± 4.8 μm in the regions of active angiogenesis. Also present within the regions of increased vascular density were frequent “holes” or “pillars” consistent with active intussusceptive angiogenesis. The mean pillar diameter was 4.2 ± 3.8 μm and the pillars were observed in all regions of active angiogenesis. These findings indicate that the process of alveolar construction involves discrete regions of regenerative growth, particularly in the subpleural regions of the cardiac lobe, characterized by both sprouting and intussusceptive angiogenesis.

Keywords

scanning electron microscopy; corrosion casting; neoalveolarization; intussusceptive angiogenesis; microCT

Introduction

Compensatory growth of the remaining lung after pneumonectomy has been described in many species including rats (1), mice (2), dogs (3), cats (4), rabbits (5) and ferrets (6). The growth of the regenerating lung, coincident with increases in protein (1), nucleic acids (7)

and cells (8), occurs within days of pneumonectomy. Recent studies have demonstrated that the growth of the remaining lung reflects not simply an increase in alveolar size, but an increase in the number of alveoli (9).

The rapid increase in the number of new alveoli is particularly intriguing given the structural constraints of neoalveolarization. As the primary gas exchange unit of the lung, the functional efficiency of the alveolus is determined by alveolar volume, surface accessibility and endothelial permeability (10, 11). Even small changes in alveolar geometry or blood flow can produce a significant change in oxygen flux (12, 13). Because discordant development of gas transport or blood perfusion can result in ineffective gas exchange, neoalveolarization must involve a highly integrated process of alveolar construction (14).

A central constituent of lung construction is the alveolar capillary network. Blood vessel number, density and spatial distribution contribute to the efficiency of gas exchange (15, 16). In development, classic studies by Burri and co-workers have outlined three phases of postnatal lung maturation: 1) rapid structural alveolation (mouse 4-14 days; humans birth to 1.5 years); 2) maturation of the secondary septa and major angiogenesis (mouse 14-21 days; humans 1.5-2 years); and 3) uniform growth and expansion (mouse 21-90 days; humans >2 years) (17-19). Although there is some evidence of later alveolation (16), the majority of angiogenesis is likely to occur within 21 days in mice.

In studies of post-pneumonectomy compensatory growth, studies in dogs have demonstrated an increase in endothelial cells proportionate to other lung constituents (20). The presumed increase in regenerating blood vessels appears to efficiently participate in gas exchange. Takeda and colleagues have demonstrated normal diffusion gradients and oxygen/carbon dioxide gas exchange after post-pneumonectomy lung regeneration (21). Related work in a variety of post-pneumonectomy animal models has implicated a diverse set of angiogenic and growth-related genes including epidermal growth factor (*Egr1*) (22), keratinocyte growth factor (*Fgf7*) (23), hepatocyte growth factor (*Hgf*) (24), hypoxia-inducible factor-1 α (*Hif1a*) (25), endothelial nitric oxide synthase (*Nos3*) (26), platelet-derived growth factor β (*Pdgfb*) (27), and vascular endothelial growth factor (*Vegfa*) (28). Although isolated endothelial cells produced a transcriptional signature compatible with angiogenesis (29), attempts to define transcriptional regulation using microarrays and bulk RNA have identified few genes clearly associated with capillary angiogenesis (30, 31).

In this report, we investigated the possibility that pneumonectomy does not trigger uniform, or global, alveolar capillary angiogenesis, but rather a pattern of angiogenesis that reflects discrete growth regions within the regenerating lung. To investigate this hypothesis, we employed microCT imaging, histology and immunohistochemistry as well as corrosion casting and scanning electron microscopy.

Methods

Mice

C57/B6 mice (Jackson Laboratory, Bar Harbor, Maine), 8 to 12 weeks old, were used in all experiments. The care of the animals was consistent with guidelines of the American Association for Accreditation of Laboratory Animal Care (Bethesda, Md) and approved by the Institutional Animal Care and Use Committee.

Pneumonectomy

After general anesthesia and intubation (32), the animal was maintained on a Flexivent rodent ventilator (SCIREQ, Montreal, QC Canada) at 200 bpm, 10 ml/kg, and PEEP of 2 cmH₂O with a pressure limited constant flow profile (32). The pneumonectomy was

performed through a 5th intercostal space left thoracotomy. With minimal manipulation of the lung, the hilum was ligated en bloc with a 5-0 surgical silk tie (Ethicon, Somerville, NJ). The entire left lung distal to the hilar ligature was sharply excised, the lung was removed and the thoracotomy closed with interrupted 5-0 silk sutures (Ethicon). Once spontaneous muscle activity returned, the animal was extubated and transferred to a warming cage. Sham thoracotomy involved an identical left thoracotomy incision and closure without surgical manipulation of the left lung.

Flow cytometry cell cycle analysis

The lung was processed in a modification of a procedure previously described (29). Briefly, the lung was minced into 1mm³ pieces and processed by enzymatic digestion: 1 mg/ml collagenase (Sigma, St. Louis, MO) and 2.5 U/ml dispase solution (Collaborative Biomedical Products, Bedford MA). The suspension was incubated at 37°C on a rotary shaker for 40 minutes. The lung was triturated using an 18g needle and filtered through a 100 µm mesh screen (BD Biosciences, San Jose, CA) washed and re-suspending at a concentration of 3×10^7 cells/ml. Using a technique similarly applied to lung endothelial cells (29), the whole lung cells were treated with red blood cell lysis buffer (BD Biosciences) and washed in 3% serum containing medium (29). The cells were pre-warmed to 37°C, stained with 1 mg/ml Hoechst 33342 (Molecular Probes, Carlsbad, CA) and incubated for 45 minutes at 37°C. The cells were washed and analyzed using dual excitation laser (BD FACSCanto II; 325nm and 488nm ex) flow cytometer. After defining ModFit software parameters (Verity, Topsham, ME) the viable lung cells were gated based on forward and side scatter parameters to exclude debris. Because of nuclear density interference and other staining nonlinearities (33), the G2/G1 ratio was typically modified using the ModFit autolinenarity algorithm. Autolinenarity G2/G1 ratios ranged from 1.94 to 1.99.

Lobar weights

With a beating heart, a caval venotomy and an absorptive pad facilitated drainage of intravascular blood volume. Blood-free wet weight was subsequently obtained. Lung dry weight was obtained after drying in an oven at 60°C until the weight was constant. The wet-to-dry ratio was calculated using the blood-free wet and dry lung weights.

MicroCT

Microtomography measurements were performed using a ProCon X-Ray CT-Alpha instrument (ProCon, Garbsen, Germany) equipped with a YXLON FXE 160.51 X-ray tube and a Hamamatsu flat-panel sensor detector (Hamamatsu Photonics, Hamamatsu, Japan). Images were obtained at 2048×2048 pixels (50 µm/pixel on the detector); some test measurements were also performed at a resolution of 1024×1024 pixels.

Spatial autocorrelation

Spatial autocorrelation measures were based on the vascular patterns obtained by microCT imaging of whole lung corrosion casts. The patterns largely reflected vessel densities in individual lobes. After pneumonectomy, the casts were imaged and spatial autocorrelation measures were applied to the 3D lung using Moran's I as previously described (34) (35) using a 64 bit version of Matlab 7.8 software (MathWorks, Natick, MA). Systematic image stacking and visualization was performed with Amira 5.2.2 (Visage Imaging, Richmond, Australia). The images were encoded using a 4 color indexing palette of pink (highest), green, white and black (lowest).

Immunohistochemical staining

As previously described (29), cryostat sections were warmed and blocked with 20% sheep serum, 20% goat serum, 0.1% azide in PBS. The slides were treated with anti-PCNA (Clone PC10, Dako, Hamburg, Germany) or anti-Ki-67 (Clone TEC-3, Dako), anti-VEGF (A-20, Santa Cruz, Heidelberg, Germany) or anti-GLUT1 (H-43, Santa Cruz) antibodies at 10-20 ug/ml. The slides were washed twice and treated with avidin-biotin-peroxidase complex (Vectastatin ABC-Kit, Vector Laboratories) or with the Envision® kit (Dako, Hamburg, Germany). After 15 minute incubation, the slides were washed twice, developed and counterstained with hematoxylin (Sigma).

Scanning electron microscopy

After systemic heparinization with 2000 U/kg heparin IP, the mice were thoracotomized in deep anesthesia. The pulmonary artery was cannulated through the right ventricle with an olive-tipped cannula and perfused with 10 ml of 37°C saline followed by 10 ml of a buffered 2.5% glutaraldehyde solution (Sigma) at pH 7.40. After casting of the microcirculation with 10 ml of the polyurethane-based casting resin PU4ii (vasQtec, Zurich, Switzerland) and caustic digestion, the microvascular corrosion casts were imaged after coating with gold in an argon atmosphere with a Philips ESEM XL30 scanning electron microscope. Stereopair images were obtained by using tilt angles of 6°. The quality of the filling of the corrosion casts was also evaluated by comparisons with the vascular densities in semithin light microscopic sections stained with methylene blue.

Statistical analysis

Flow cytometry statistical analysis was based on measurements in at least three different mice. The unpaired Student's t-test for samples of unequal variances was used to calculate statistical significance. The data was expressed as mean $\pm$ 1 SD. The significance level for the sample distribution was defined as $P < .05$.

Results

Time course of compensatory lung growth

As expected, the remaining lung after murine pneumonectomy increased its weight and volume sufficiently to approximate the preoperative baseline (Figure 1A). Immunohistochemistry of the cell cycle proteins Ki-67 and PCNA demonstrated a marked proliferative response of cells within the alveolar septa (Figure 1B and 1C). To provide a more quantitative assessment of cell proliferation, the post-pneumonectomy lungs were digested and the lung cells were analyzed by flow cytometry. Cell cycle analysis demonstrated an initial indication of cell proliferation on Day 3 (S-phase $3.90 \pm 1.08\%$; G2-phase $0.40 \pm 0.71\%$) followed by a peak of cells in G2+S phase ($12.02 \pm 1.48\%$) on Day 6 (Figure 2). By 14 days after pneumonectomy, the total number of cells in G2+S phase ($3.34 \pm 3.01\%$) approached the baseline of $1.19 \pm 0.81\%$ ($N=4-5$ mice per time point). Based on the cell cycle activity, the subsequent morphology experiments focused on the time period between days 3 and 6 after pneumonectomy.

Spatial dependence of growth

To investigate the spatial dependence of lung growth, we measured the relative growth of the 4 lobes (upper, middle, lower and cardiac) of the post-pneumonectomy right lung. The percentage increase in lobar weight was greatest in the cardiac lobe ($p < .001$) (Figure 3). To assess the spatial dependence of blood vessels within the lobes, we performed vascular casting and microCT imaging (Figure 4A). Reconstructions of the post-pneumonectomy lung demonstrated significant heterogeneity in vascular density. Moran's I, an

autocorrelation statistic that reflects the degree of spatial dependency, was mapped for the post-pneumonectomy lung (Figure 4B). Spatial autocorrelation demonstrated clustering of similar vascular densities (positive autocorrelation) in the subpleural regions of the cardiac lobe (Figure 4C-F).

Subpleural histology

The subpleural regions of the cardiac lobe were studied in detail by standard histology and immunohistochemical staining. Histology analysis demonstrated increased cell density in discontinuous regions of the peripheral cardiac lobe. In most cases, the dominant focus of cellularity was within 300 μ m of the pleural surface. Immunohistochemical staining demonstrated increased expression of angiogenesis-associated factors VEGF and GLUT1 (Figure 5). Maximal expression of these factors was observed in the subpleural regions with variable extension into the subjacent lung parenchyma (Figure 5).

Mechanism of angiogenesis

To investigate the possibility of active angiogenesis within these subpleural regions, the cardiac lobe was studied by vascular corrosion casting and scanning electron microscopy. The pleural vessels, forming a polygonal plexus in the visceral pleura, demonstrated significant variability, but regions with increased vessel caliber and vascular density were observed (Figure 6A, ellipse). The subjacent regions demonstrated increased vascular density consistent with active angiogenesis (Figure 6B). To investigate the mechanism of the increased vascular density, corrosion casting and SEM performed 3-6 days after pneumonectomy demonstrated subpleural vessels with angiogenic sprouts (Figure 7A,B; circles). The monopodial sprouts appeared to be randomly oriented along the vessel axis. Sampling in subpleural regions with the highest vascular density demonstrated interbranch distances of 11.4 ± 4.8 μ m. Also present within these regions were frequent “holes” or “pillars” suggesting active intussusceptive angiogenesis (Figure 7B,C; arrows); the mean intussusceptive pillar diameters was 4.2 ± 3.8 μ m. By day 9 after pneumonectomy, a double layer capillary morphology was frequently observed in these subpleural regions (Figure 7D, arrow).

Discussion

In this report, we have contributed several spatial and temporal observations relevant to the process of murine post-pneumonectomy lung growth. First, cell cycle analysis demonstrated enhanced lung cell proliferation peaking 6 days after pneumonectomy. Second, lung growth after pneumonectomy was not uniform, but demonstrated spatial dependencies with the greatest growth in the cardiac lobe and in discontinuous subpleural regions. Third, the subpleural regions demonstrated enhanced expression of angiogenesis and growth-related factors (VEGF and GLUT1). Finally, corrosion casting and SEM of subpleural regions 3-6 days after pneumonectomy demonstrated morphologic evidence of both sprouting and intussusceptive angiogenesis. Together, these findings in post-pneumonectomy mice suggest that the pattern of blood vessel growth has distinct spatial dependencies and the process involves both sprouting and intussusceptive angiogenesis.

In our studies, spatial dependency refers to the co-variation of angiogenesis and anatomic location. Stimulated by earlier work demonstrating nonuniform growth (36, 37), our initial measurements of lobar weight and autocorrelations of microCT data were exploratory analyses intended to identify the spatial patterns of lung growth. The identification of spatial “hot spots” did not imply new vessel growth or angiogenesis per se, but rather defined the scale and distribution of vascular heterogeneity within the lung. Using this pattern as a

guide, diagnostic features of sprouting and intussusceptive angiogenesis were subsequently confirmed using corrosion casting, and scanning electron microscopy.

Although it is tempting to apply rigorous statistical tests to our data, spatial analytic tools currently have several practical limitations. First, the linkage of a biologic event such as angiogenesis and anatomic location violates standard statistical assumptions of feature independence. Because most statistical tools do not compensate for spatial dependency, and lead to unstable parameter estimates, our measure of spatial autocorrelation was presented as topographic information rather than as a statistical comparison. Second, our spatial analysis of angiogenesis was contrasted with presumed uniformity—an obvious oversimplification of such complex space as the post-pneumonectomy lung. We recognize that our observations of discontinuous subpleural angiogenesis likely reflects anatomic and physiologic explanations that are currently unrecognized.

Investigations of lung growth have been complicated by the unique structural features of the lung; namely, repeating gas exchange units within an elastic skeleton. In contrast to other regenerative organs, an increase in lung volume may simply reflect alveolar distension and not tissue growth. Because of these limitations, structural assessments of the lung have relied upon histologic sections and designed-based sampling techniques (Fehrenbach et al., 2008; Ochs et al., 2004; Weibel, 1963). Morphometric measurements, such as mean linear intercept (MLI), do not depend upon alveolar geometry or geometric similarity, but only upon the random orientation of the sampling (38). An advantage of this approach is that measurements of volume and intercept can be linked, at least indirectly, to the surface area of the lung participating in gas exchange. A disadvantage is that MLI is a summary descriptor without spatial information relevant to the process of lung growth.

Pulmonary angiogenesis is a growth process that has been difficult to identify; in part, because of the high density of blood vessels in normal conditions and the difficulty in identifying angiogenesis in 2-dimensional histologic sections. Morphologic evidence of angiogenesis in the adult lung is rare; largely restricted to unusual animal models (39-41) and human disease (42). Our finding that growth areas were discontinuous, and within a relatively narrow temporal window, further complicated the unequivocal demonstration of angiogenic forms. Classic developmental studies of the lung have suggested that pulmonary vessel growth would likely involve intussusceptive angiogenesis (43, 44). Intussusception is a form of angiogenesis involving the division of a single vessel into two lumens. The intussusceptive “pillar”, a tissue bridge that leads to septation of the vessel lumen, appears as a “hole” in vascular corrosion casts (45). Although early work in lung development characterized pillars as 1-2.5µm in diameter (44), our findings suggest a wider range of pillar diameters—likely reflecting a dynamic process in adult mice. Practically, intussusceptive pillars can only be visualized by SEM. To facilitate our search for evidence of pulmonary angiogenesis, we focused the casting and SEM studies on the time of maximal proliferation (3-6 days after pneumonectomy) and in the subpleural regions of the lung. Consistent with our histologic predictions, we were able to demonstrate striking examples of intussusceptive angiogenesis. Similarly, these regions of intussusceptive angiogenesis uniformly demonstrated spatially coincident sprouting angiogenesis—a finding consistent with a common stimulus for both forms of angiogenesis.

Also reflecting the spatial dependency of lung growth, the cardiac lobe demonstrated a significant increase in weight relative to the other lobes of the right lung. Among mammals, the mouse has unusual pulmonary anatomy. The mouse has 4 lobes in the right lung: the standard right upper lobe, right middle lobe and right lower lobe as well as a lobe that is located in the lower mediastinum. Various referred to as the cardiac, infracardiac, postcaval, median and inferior lobe, this lobe is uniquely located in the inferior midline of

the ventral mediastinum. Our findings of differential growth in the cardiac lobe is consistent with other rodent studies that have demonstrated relatively increased volume (8, 46-48), weight (49) and cell proliferative activity (8, 50, 51) in the post-pneumonectomy cardiac lobe. Although the unique location and shape of the cardiac lobe are potentially relevant factors, the reasons for differential growth of the cardiac lobe remain unclear.

Consistent with our findings, a number of investigators have noted evidence of increased cellular proliferation in subpleural (8, 52-55) and peribronchial (8) areas of the post-pneumonectomy lung. Nattie et al have argued that the constancy of alveolar size in the subpleural regions after pneumonectomy, despite a demonstrable increase in lung volume, indicates that proliferation of the subpleural alveoli has occurred (56). The expression of VEGFA and GLUT1 in the subpleural regions is also consistent with the findings of angiogenic gene transcription in isolated lung cells (29, 57, 58) and suggests intercellular signaling networks within discrete subpleural regions.

The subpleural region, also referred to as the “subpleural mantle” (59), has unique structural features. In normal rodents, the subpleural zone has distinctive characteristics including a reduced volume fraction of septal tissue and a reduced air space surface density (59). These observations that have led to caution in interpreting subpleural morphometric data. Although a reasonable morphometric consideration, these unique anatomic features may also facilitate rapid post-pneumonectomy growth. For example, the pleura and subpleural zones are associated with both systemic and pulmonary vessels (60)—a pre-existing vascular supply presumed to be necessary for alveolar construction. Similarly, the mesothelium, a recently described reservoir for stem cells (61), demonstrates an early proliferative response after pneumonectomy (50) and an unanticipated capacity for adaptive growth (41). In future studies, we suspect that the spatial “hot spots” defined here will provide valuable insights into the process of lung regeneration.

Acknowledgments

This work was supported by NIH grants HL75426, HL94567 and HL007734 as well as the Uehara Memorial Foundation and the JSPS Postdoctoral Fellowships for Research Abroad.

Abbreviations

2D	2-dimensional
3D	3-dimensional
CL	cardiac lobe
IP	intraperitoneal
LWI	lung weight index
MLI	mean linear intercept
PBS	phosphate buffered saline
RLL	right lower lobe
RML	right middle lobe
RUL	right upper lobe
SD	standard deviation
SEM	scanning electron microscopy
TBW	total body weight

References

1. Addis T. Compensatory hypertrophy of the lung after unilateral pneumectomy. *J. Exp. Med.* 1928; 47:51–56. [PubMed: 19869400]
2. Tatar-Kiss S, Bardocz S, Kertai P. Changes in L-ornithine decarboxylase activity in regenerating lung lobes. *FEBS Lett.* 1984; 175:131–134. [PubMed: 6479330]
3. Heuer GJ, Dunn GR. Experimental pneumectomy. *Bull Johns Hopkins Hosp.* 1920; 31:31–42.
4. Bremer JL. The fate of the remaining lung tissue after lobectomy or pneumonectomy. *J. Thorac. Surg.* 1936; 6:336–343.
5. Sery Z, Keprt E, Obrucnik M. Morphometric analysis of late adaptation of residual lung following pneumonectomy in young and adult rabbits. *J. Thorac. Cardiovasc. Surg.* 1969; 57:549–557. [PubMed: 5774634]
6. McBride JT. Lung-volumes after an increase in lung distension in pneumonectomized ferrets. *J. Appl. Physiol.* 1989; 67:1418–1421. [PubMed: 2793743]
7. Romanova LK, Leikina EM, Antipova KK. Nucleic acid synthesis and mitotic activity during development of compensatory hypertrophy of lung in rats. *Bulletin of Experimental Biology and Medicine, USSR.* 1967; 63:303–306.
8. Voswinckel R, Motejl V, Fehrenbach A, Wegmann M, Mehling T, Fehrenbach H, Seeger W. Characterisation of post-pneumonectomy lung growth in adult mice. *Eur. Respir. J.* 2004; 24:524–532. [PubMed: 15459128]
9. Fehrenbach H, Voswinckel R, Michl V, Mehling T, Fehrenbach A, Seeger W, Nyengaard JR. Neoalveolarisation contributes to compensatory lung growth following pneumonectomy in mice. *Eur. Respir. J.* 2008; 31:515–522. [PubMed: 18032439]
10. Kumar H, Tawhai MH, Hoffman EA, Lin CL. The effects of geometry on airflow in the acinar region of the human lung. *J. Biomech.* 2009; 42:1635–1642. [PubMed: 19482288]
11. Sznitman J, Heimsch T, Wildhaber JH, Tsuda A, Rosgen T. Respiratory Flow Phenomena and Gravitational Deposition in a Three-Dimensional Space-Filling Model of the Pulmonary Acinar Tree. *Journal of Biomechanical Engineering-Transactions of the Asme.* 2009; 131:1–16.
12. Chang HK, Cheng RT, Farhi LE. Model study of gas-diffusion in alveolar sacs. *Respir. Physiol.* 1973; 18:386–397. [PubMed: 4746962]
13. Tippe A, Tsuda A. Recirculating flow in an expanding alveolar model: experimental evidence of flow-induced mixing of aerosols in the pulmonary acinus. *J. Aerosol Sci.* 2000; 8:979–986.
14. Thompson, DW. *On Growth and Form.* Cambridge University Press; New York: 1945. The forms of tissues, or cell-aggregates.; p. 465-565.
15. Ciurea D, Gil J. Morphometry of capillaries in three zones of rabbit lungs fixed by vascular perfusion. *Anat. Rec.* 1996; 244:182–192. [PubMed: 8808393]
16. Schittny JC, Mund SI, Stampanoni M. Evidence and structural mechanism for late lung alveolarization. *Am. J. Physiol.-Lung Cell. Mol. Physiol.* 2008; 294:L246–L254. [PubMed: 18032698]
17. Burri, PH. *Handbook of Physiology. The Respiratory System.* Am. Physiol Soc.; Bethesda, MD: 1985. Development and growth of the human lung.; p. 1-46.
18. Burri PH. Structural aspects of postnatal lung development - Alveolar formation and growth. *Biol. Neonate.* 2006; 89:313–322. [PubMed: 16770071]
19. Zeltner TB, Caduff JH, Gehr P, Pfenninger J, Burri PH. The postnatal-development and growth of the human-lung. 1. morphometry. *Respir. Physiol.* 1987; 67:247–267. [PubMed: 3575905]
20. Yan X, Bellotto DJ, Foster DJ, Johnson RL, Hagler HK, Estrera AS, Hsia CCW. Retinoic acid induces nonuniform alveolar septal growth after right pneumonectomy. *J. Appl. Physiol.* 2004; 96:1080–1089. [PubMed: 14617528]
21. Takeda SI, Hsia CCW, Wagner E, Ramanathan M, Estrera AS, Weibel ER. Compensatory alveolar growth normalizes gas-exchange function in immature dogs after pneumonectomy. *J. Appl. Physiol.* 1999; 86:1301–1310. [PubMed: 10194216]
22. Landesberg LJ, Ramalingam R, Lee K, Rosengart TK, Crystal RG. Upregulation of transcription factors in lung in the early phase of postpneumonectomy lung growth. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 2001; 281:L1138–1149. [PubMed: 11597905]

23. Kaza AK, Kron IL, Leuwerke SM, Tribble CG, Laubach VE. Keratinocyte growth factor enhances post-pneumonectomy lung growth by alveolar proliferation. *Circulation*. 2002; 106:1120–1124. [PubMed: 12354720]
24. Sakamaki Y, Matsumoto K, Mizuno S, Miyoshi S, Matsuda H, Nakamura T. Hepatocyte growth factor stimulates proliferation of respiratory epithelial cells during postpneumonectomy compensatory lung growth in mice. *Am. J. Respir. Cell Mol. Biol*. 2002; 26:525–533. [PubMed: 11970903]
25. Zhang Q, Bellotto DJ, Ravikumar P, Moe OW, Hogg RT, Hogg DC, Estrera AS, Johnson RL Jr, Hsia CC. Postpneumonectomy lung expansion elicits hypoxia-inducible factor-1 α signaling. *Am. J. Physiol. Lung Cell. Mol. Physiol*. 2007; 293:L497–504. [PubMed: 17513452]
26. Leuwerke SM, Kaza AK, Tribble CG, Kron IL, Laubach VE. Inhibition of compensatory lung growth in endothelial nitric oxide synthase-deficient mice. *Am. J. Physiol. Lung Cell. Mol. Physiol*. 2002; 282:L1272–1278. [PubMed: 12003783]
27. Yuan SZ, Hannam V, Belcastro R, Cartel N, Cabacungan J, Wang JX, Diambomba Y, Johnstone L, Post M, Tanswell AK. A role for platelet-derived growth factor-BB in rat postpneumonectomy compensatory lung growth. *Pediatr. Res*. 2002; 52:25–33. [PubMed: 12084843]
28. Jancelewicz T, Grethel EJ, Chapin CJ, Clifton MS, Nobuhara KK. Vascular Endothelial Growth Factor Isoform and Receptor Expression During Compensatory Lung Growth. *J. Surg. Res*. 2008; 160:107–113. [PubMed: 19535097]
29. Lin M, Chamoto K, Gibney B, Lee GS, Collings-Simpson D, Houdek J, Konerding MA, Tsuda A, Mentzer SJ. Angiogenesis gene expression in murine endothelial cells during post-pneumonectomy lung growth. *Resp. Res*. 2011 In press.
30. Paxson JA, Parkin CD, Iyer LK, Mazan MR, Ingenito EP, Hoffman AM. Global gene expression patterns in the post-pneumonectomy lung of adult mice. *Respir Res*. 2009; 10:1–15. [PubMed: 19134192]
31. Wolff JC, Wilhelm J, Fink L, Seeger W, Voswinckel R. Comparative gene expression profiling of post-natal and post-pneumonectomy lung growth. *Eur. Respir. J*. 2010; 35:655–666. [PubMed: 19717484]
32. Gibney B, Lee GS, Houdek J, Lin M, Chamoto K, Konerding MA, Tsuda A, Mentzer SJ. Dynamic determination of oxygenation and lung compliance in murine pneumonectomy. *Exp. Lung Res*. 2011; 37:301–309. [PubMed: 21574875]
33. Wersto RP, Chrest FJ, Leary JF, Morris C, Stetler-Stevenson M, Gabrielson E. Doublet discrimination in DNA cell-cycle analysis. *Cytometry*. 2001; 46:296–306. [PubMed: 11746105]
34. Marwan N, Kurths J, Thomsen JS, Felsenberg D, Saporin P. Three-dimensional quantification of structures in trabecular bone using measures of complexity. *Physical Review E*. 2009; 79
35. Wald MJ, Vasilic B, Saha PK, Wehrli FW. Spatial autocorrelation and mean intercept length analysis of trabecular bone anisotropy applied to in vivo magnetic resonance imaging. *Med. Phys*. 2007; 34:1110–1120. [PubMed: 17441256]
36. Yilmaz C, Ravikumar P, Dane DM, Bellotto DJ, Johnson RL, Hsia CCW. Noninvasive quantification of heterogeneous lung growth following extensive lung resection by high-resolution computed tomography. *J. Appl. Physiol*. 2009; 107:1569–1578. [PubMed: 19729592]
37. Ravikumar P, Yilmaz C, Dane DM, Johnson RL, Estrera AS, Hsia CCW. Developmental signals do not further accentuate nonuniform postpneumonectomy compensatory lung growth. *J. Appl. Physiol*. 2007; 102:1170–1177. [PubMed: 17138837]
38. Campbell H, Tomkeieff SI. Calculation of the internal surface of a lung. *Nature*. 1952; 170:117–117. [PubMed: 14957041]
39. Schraufnagel DE, Malik R, Goel V, Ohara N, Chang SW. Lung capillary changes in hepatic cirrhosis in rats. *Am. J. Physiol.-Lung Cell. Mol. Physiol*. 1997; 272:L139–L147.
40. Schraufnagel DE. Monocrotaline-induced angiogenesis. Differences in the bronchial and pulmonary vasculature. *Am. J. Pathol*. 1990; 137:1083–1090. [PubMed: 1700616]
41. Wagner EM, Petrache I, Schofield B, Mitzner W. Pulmonary ischemia induces lung remodeling and angiogenesis. *J. Appl. Physiol*. 2006; 100:587–593. [PubMed: 16210444]

42. Schraufnagel DE, Sekosan M, McGee T, Thakkar MB. Human alveolar capillaries undergo angiogenesis in pulmonary veno-occlusive disease. *Eur. Respir. J.* 1996; 9:346–350. [PubMed: 8777975]
43. Caduff JH, Fischer LC, Burri PH. Scanning electron microscope study of the developing microvasculature in the postnatal rat lung. *Anat. Rec.* 1986; 216:154–164. [PubMed: 3777448]
44. Burri PH, Tarek MR. A novel mechanism of capillary growth in the rat pulmonary microcirculation. *Anat. Rec.* 1990; 228:35–45. [PubMed: 2240600]
45. Lee GS, Filipovic N, Lin M, Gibney BC, Simpson DC, Konerding MA, Tsuda A, Mentzer SJ. Intravascular pillars and pruning in the extraembryonic vessels of chick embryos. *Dev. Dyn.* 2011; 240:1335–1343. [PubMed: 21448976]
46. Fernandez LG, Le Cras TD, Ruiz M, Glover DK, Kron IL, Laubach VE. Differential vascular growth in postpneumonectomy compensatory lung growth. *J. Thorac. Cardiovasc. Surg.* 2007; 133:309–316. [PubMed: 17258553]
47. Rannels DE, Stockstill B, Mercer RR, Crapo JD. Cellular-changes in the lungs of adrenalectomized rats following left pneumonectomy. *Am. J. Respir. Cell Mol. Biol.* 1991; 5:351–362. [PubMed: 1910820]
48. Sekhon HS, Thurlbeck WM. A comparative-study of postpneumonectomy compensatory lung response in growing male and female rats. *J. Appl. Physiol.* 1992; 73:446–451. [PubMed: 1399964]
49. Rannels DE, White DM, Watkins CA. Rapidity of compensatory lung growth following pneumonectomy in adult rats. *J. Appl. Physiol.* 1979; 46:326–333. [PubMed: 422449]
50. Cagle PT, Langston C, Goodman JC, Thurlbeck WM. Autoradiographic assessment of the sequence of cellular proliferation in postpneumonectomy lung growth. *Am. J. Respir. Cell Mol. Biol.* 1990; 3:153–158. [PubMed: 2378749]
51. Thet LA, Law DJ. Changes in cell number and lung morphology during early postpneumonectomy lung growth. *J. Appl. Physiol.* 1984; 56:975–978. [PubMed: 6725076]
52. Fisher JM, Simnett JD. Morphogenetic and proliferative changes in regenerating lung of rat. *Anat. Rec.* 1973; 176:389–395. [PubMed: 4723402]
53. Brody JS, Burki R, Kaplan N. Deoxyribonucleic-acid synthesis in lung-cells during compensatory lung growth after pneumonectomy. *Am. Rev. Respir. Dis.* 1978; 117:307–316. [PubMed: 637412]
54. Holmes C, Thurlbeck WM. Normal lung growth and response after pneumonectomy in rats at various ages. *Am. Rev. Respir. Dis.* 1979; 120:1125–1136. [PubMed: 507530]
55. Foster DJ, Yan X, Bellotto DJ, Moe OW, Hagler HK, Estrera AS, Hsia CCW. Expression of epidermal growth factor and surfactant proteins during postnatal and compensatory lung growth. *Am. J. Physiol.-Lung Cell. Mol. Physiol.* 2002; 283:L981–L990. [PubMed: 12376351]
56. Nattie EE, Wiley CW, Bartlett D. Adaptive growth of lung following pneumonectomy in rats. *J. Appl. Physiol.* 1974; 37:491–495. [PubMed: 4411866]
57. Chamoto K, Gibney BC, Lee GS, Lin M, Simpson DC, Voswinckel R, Konerding MA, Tsuda A, Mentzer SJ. CD34+ progenitor to endothelial cell transition in post-pneumonectomy angiogenesis. *Am. J. Resp. Cell Mol. Biol.* 2011 In revision.
58. Chamoto K, Gibney BC, Lee GS, Lin M, Konerding MA, Tsuda A, Mentzer SJ. Alveolar macrophage dynamics in post-pneumonectomy lung growth. *J. Immunol.* 2011 In revision.
59. Zeltner TB, Bertacchini M, Messerli A, Burri PH. Morphometric estimation of regional differences in the rat lung. *Exp. Lung Res.* 1990; 16:145–158. [PubMed: 2328712]
60. Marchand P, Gilroy JC, Wilson VH. An anatomical study of the bronchial vascular system and its variations in disease. *Thorax.* 1950; 5:207–221. [PubMed: 14776713]
61. Herrick SE, Mutsaers SE. Mesothelial progenitor cells and their potential in tissue engineering. *Int. J. Biochem. Cell Biol.* 2004; 36:621–642. [PubMed: 15010328]

Figure 1.

Post-pneumonectomy lung growth. A) After baseline pre- pneumonectomy (day=0), left pneumonectomy was performed. The remaining right lung increased volume (solid line) and dry weight (dashed line)($p<.015$). Volume measurements were made by displacement (Scherle) method; weight measurements were made as blood-free weights. Both volume and weight were normalized to total body weight (TBW) to produce lung volume and weight indices. The increase in volume and weight were significant ($p<.04$; $N=8-12$ mice per data point; error bars reflect 1 SD). Representative images of Ki-67 (B) and PCNA (C) immunostaining of the lung 6 days after pneumonectomy. Positive nuclear staining (brown) was prominent on day 6 after pneumonectomy (bar = 50 μ m).

Figure 2.

Cell cycle profiling of post-pneumonectomy lung cells obtained by enzymatic digestion and analyzed using flow cytometry (ModFit, Verity Software House). Automated analysis of the ModFit model components, processed by a Marquardt nonlinear least-squares analysis, excluded aggregates (green) and identified S phase and G2 phase lung cells at five time points (Day 0, 3, 6, 14 and 21). A) The combined analysis of the percentage of cells in S+G2 phase of the cell cycle at various time points after pneumonectomy (mean $\pm$ 1 SD; 4-5 mice per time point). B) Cell cycle analysis on day 3 after pneumonectomy. Prominent S phase population (cross-hatched; blue arrow) is shown. C) Cell cycle analysis on day 6 after pneumonectomy. Prominent G2 phase population (red arrow) is shown.

Figure 3.

Change in right lung lobar weight 21 days after left pneumonectomy. The weight of the right upper lobe (RUL), right middle lobe (RML), right lower lobe (RLL) and cardiac lobe (CL) were measured 21 days after left pneumonectomy and normalized to body weight to provide a lung weight index (LWI). The change in weight of the post-pneumonectomy lobe was compared to normal (gray) and sham thoracotomy (crosshatched) controls. The increase in weight was significant ($p < .05$; each data point $N=8$; error bars reflect 1 SD).

Figure 4. Representative spatial autocorrelation of the post-pneumonectomy lung 6 days after surgery. A) Vascular casting was followed by microCT imaging and 3D image reconstruction. B) Demonstration of the axial image sampling for spatial analysis. C-F) Moran's I spatial autocorrelation of four regions of the cardiac lobe, sampled at an equal spatial interval, are shown. Regions of significant positive autocorrelation are color encoded in a 4 color index: pink>green>white>black.

Figure 5. Immunohistochemistry of angiogenesis-associated growth factors in the cardiac lobe 6 days after pneumonectomy. Increased cell density in subpleural regions was readily recognized in lower resolution analysis of anti-VEGFA (A), and anti-GLUT1 (C) staining. B, D) Higher resolution imaging of the subpleural regions demonstrates active expression of the two angiogenesis-related factors. Bar = 200 μ m. Note the increased staining intensity in pleural mantle.

Figure 6.

Representative corrosion cast of the post-pneumonectomy lung on day 6 after left pneumonectomy. A) Variable caliber pleural vessels (ellipse) associated with increased vascular density was noted in subpleural regions of the lung (square). B) Higher resolution imaging demonstrated increased vascular density (arrow).

Figure 7.

Mechanism of post-pneumonectomy angiogenesis. Regions of active angiogenesis post-pneumonectomy (days 3-6) were demonstrated by corrosion casting of the pulmonary vasculature and imaging by SEM. A-C) Numerous angiogenic sprouts (e.g. circles) were noted at 10-15 μm interbranch distances. Spatially coincident with the sprouts were holes in the casts (e.g. arrows) consistent with intussusceptive pillars. D) Casts of the alveoli on day 9 after pneumonectomy demonstrated a double layer capillary morphology (arrow).