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Vascular Endothelial Growth Factor Enhances Compensatory Lung Growth in Piglets

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Abstract

Background: Vascular endothelial growth factor has been found to accelerate compensatory lung growth after left pneumonectomy in mice. The aim of this study was to determine the natural history and the effects of vascular endothelial growth factor on compensatory lung growth in a large animal model.

Methods: To determine the natural history of compensatory lung growth, female Yorkshire piglets underwent a left pneumonectomy on days of life 10–11. Tissue harvest and volume measurement of the right lung were performed at baseline ($n = 5$) and on postoperative days 7 ($n = 5$), 14 ($n = 4$), and 21 ($n = 5$). For pharmacokinetic studies, vascular endothelial growth factor was infused via a central venous catheter, with plasma vascular endothelial growth factor levels measured at various time points. To test the effect of vascular endothelial growth factor on compensatory lung growth, 26 female Yorkshire piglets underwent a left pneumonectomy followed by daily infusion of vascular endothelial growth factor at 200 $\mu\text{g/kg}$ or isovolumetric 0.9% NaCl (saline control). Lungs were harvested on postoperative day 7 for volume measurement and morphometric analyses.

Results: Compared with baseline, right lung volume after left pneumonectomy increased by factors of 2.1 ± 0.6 , 3.3 ± 0.6 , and 3.6 ± 0.4 on postoperative days 7, 14, and 21, respectively. The half-life of VEGF ranged from 89 to 144 minutes. Lesser doses of vascular endothelial growth factor resulted in better tolerance, volume of distribution, and clearance. Compared with the control group, piglets treated with vascular endothelial growth factor had greater lung volume ($P <$

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0.0001), alveolar volume ($P=0.001$) septal surface area ($P=0.007$) and total alveolar count ($P=0.01$).

Conclusion: Vascular endothelial growth factor enhanced alveolar growth in neonatal piglets after unilateral pneumonectomy.

Introduction

Vascular endothelial growth factor (VEGF) is one of the major regulators of angiogenesis and plays a key role in promoting endothelial cell survival, proliferation, migration, and permeability.¹ These functions render VEGF a prominent factor in regulating organ growth and regeneration. VEGF is critically important in pulmonary development, because it both directs the sprouting capillary network² and facilitates the interaction between developing airways and newly formed blood vessels.³ Furthermore, treatment of human fetal lung explants with VEGF results in both increased epithelial cell proliferation and increased production of surfactant.⁴ Therefore, VEGF has potent mitogenic properties on both pulmonary endothelial and epithelial cells via an autocrine or paracrine fashion, making it a key growth factor in lung development.

Dysregulation in VEGF expression has been linked to several diseases of pulmonary development. One of these diseases is congenital diaphragmatic hernia (CDH), a neonatal condition associated with a high mortality rate and extraordinary costs of care.⁵ The displacement of abdominal organs into the thoracic cavity in children with CDH contributes to the development of bilateral pulmonary hypoplasia characterized by an arrest in airway branching and alveolar maturation.⁶ Rodents with nitrofen-induced CDH exhibit a deficiency in pulmonary expression of both VEGF and its receptors, especially in the parenchymal periphery.^{7,8} Impaired VEGF expression in the alveolar stage of lung development has been confirmed in lung samples from patients with CDH⁹ secondary to a dysregulation of the entire VEGF pathway, which starts with its upstream regulator hypoxia-induced factor (HIF) 2 α .¹⁰

In the model of compensatory lung growth (CLG) after unilateral pneumonectomy, VEGF signaling also proves to be critical for alveolar regeneration. Activation of VEGF receptor 2 (VEGFR2) is required for expansion of the bronchial alveolar stem cells and differentiation of the progenitor bronchial alveolar stem cells into alveolar epithelial and capillary endothelial cells.¹¹ Previous work from our group revealed the ability of systemically administered murine VEGF to accelerate CLG and enhance alveolarization in mice.^{12,13} To determine the efficacy of recombinant human VEGF in accelerating lung growth, we employed a piglet model of CLG because of its better homology and the proven interaction between human VEGF and porcine targets.¹⁴ The aims of this study were to establish the natural history of CLG in neonatal piglets and to determine the pharmacokinetics of VEGF and its effects on lung growth in a large-animal model.

Methods

Operative model of compensatory lung growth in neonatal piglets

Female Yorkshire piglets (Parsons Pig Farm, Hadley, MA) arrived at our animal facility on day of life (DOL) 3–6 for acclimation and preoperative nutritional optimization. Animals were housed in groups of 2 or 3 and bottle-fed grade A Ultra 24 Milk Replacer (Milk Products, Chilton, WI) four times a day. On DOL 10–11, piglets underwent a left pneumonectomy as described previously.¹⁵ Briefly, piglets were anesthetized with inhaled isoflurane (1%–4%) and intubated. Central venous catheters were placed via the external jugular (EJ) vein for VEGF infusion. A left thoracotomy through the fourth intercostal space and caudal retraction of the lung apex allowed for exposure of the pulmonary hilum. Dissection and division of hilar structures began at the most ventrocranial aspect and proceeded caudally through the superior pulmonary vein (PV), pulmonary artery, main-stem bronchus (or its main branches) and inferior PV. After division of the inferior pulmonary ligament, the lung was removed, and the bronchial stump was tested for air leaks with gentle, positive-pressure ventilation. After closure, piglets recovered from anesthesia in a humidified incubator and were monitored for signs and symptoms of hypoglycemia, hypoxia, and hypothermia. Analgesia and antibiotics were administered and feeding was resumed as soon as the piglets achieved full recovery. All procedures were carried out according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals and approved by the Institutional Animal Care and Use Committee (IACUC) at Boston Children's Hospital.

Determination of natural history of compensatory lung growth in neonatal piglets

Five, female, Yorkshire piglets were euthanized on DOL 10–11 and the lungs removed en bloc with the tracheobronchial tree for baseline measurement of total and right lung volumes. Neutral buffered 10% formalin (Sigma-Aldrich, St. Louis, MO) was infused through the trachea at 25–30 cm H₂O, and total lung volume (TLV) measurement was determined with a water displacement method.¹⁶ The left lung was removed after ligation of its main-stem bronchus, and the remaining right lung volume (RLV) was measured by water displacement. Lung volume was normalized by body weight to generate TLV/body weight (TLV/BW) and RLV/body weight (RLV/BW) ratios.

Nineteen piglets underwent left pneumonectomy as described previously. The 14 piglets who survived were euthanized on post-operative days (POD) 7 ($n = 5$), 14 ($n = 4$), and 21 ($n = 5$) for determination of RLV and RLV/BW, as described previously.

Tolerance of VEGF infusion and VEGF pharmacokinetics in neonatal piglets

Neonatal piglets' tolerance to VEGF infusion was tested at three different doses, 400 ($N = 1$), 200 ($n = 2$), and 100 ($n = 2$) $\mu\text{g/kg}$. These piglets did not undergo left pneumonectomy. Instead, female Yorkshire piglets on DOL 10 were anesthetized and intubated, followed by insertion of a right external jugular (EJ) central venous catheter for infusion of recombinant human VEGF₁₆₅ (Shire, Lexington, MA). The right femoral vein was accessed separately for blood draws during the infusion. A right carotid arterial catheter was also placed for blood pressure monitoring during the infusion. The volume of infusion was standardized at

3.3 mL/kg. The infusion was held at the discretion of the supervising veterinarian whenever persistent profound hypotension, defined as a mean arterial pressure (MAP) < 50 mm Hg, ensued. After the infusion, the EJ venous line was left in place while both the arterial and femoral venous lines were removed and the vessels ligated.

To determine the pharmacokinetics of VEGF in neonatal piglets, blood samples were collected before infusion and every 10 min during infusion via the femoral venous line. After infusion, blood collection occurred at 10, 20, and 40 min and at 1, 1.5, 2, 4, 8, 12, and 24 hours via the EJ line. Less than 0.5 mL of blood was removed with each collection for a total of 7–8 mL over a period of 24 hours; this volume and rate of removal was deemed a safe amount by the IACUC at our institution and well tolerated by all piglets. Blood was collected in ethylenediaminetetraacetic acid collection tubes, and plasma was obtained by centrifuging blood samples at 2000g and 4°C for 15 min. Levels of VEGF were determined with an anti-human VEGF, enzyme-linked immunosorbent assay (ELISA) (R&D Systems, Minneapolis, MN). The half-life, volume of distribution (V_d), and rate of clearance (Cl) for VEGF were calculated using Pheonix Version 6.4 (Certara, Princeton, NJ).

Effects of VEGF on compensatory lung growth in neonatal piglets

Twenty-six female neonatal Yorkshire piglets underwent a left pneumonectomy and placement of an EJ central line on DOL 10–11 as described previously. Animals were randomized to either the VEGF ($n = 13$) or 0.9% NaCl (saline control) ($N = 13$) group. Piglets received 200 µg/kg VEGF or isovolumetric saline daily over a period of 60–90 minutes. The infusion volume was standardized to 3.3 mL/kg. Piglets were killed on POD 7, and the right lung, heart, liver, spleen, and left kidney were harvested. Lung specimens were infused with 10% formalin at 25–30 cm H₂O, and RLV was measured with the water displacement method.¹⁶ RLV and organ weight measurements were normalized for body weight. Infused lungs and organs were fixed in 10% formalin at 4°C for 48 hours before being placed in 70% ethanol for preservation.

Histologic analyses

For each lung specimen, a peripheral and central 5-µm section was obtained from the upper, middle, and lower lobes. Quantitative microscopy was performed at 100 × 100X magnification on lung sections stained with hematoxylin and eosin, following the principles of systemic uniform random sampling.^{17,18} Morphometric data, including parenchymal volume, alveolar volume, and septal surface area, were generated from a point and intersection counting technique using a 42-point test lattice. Alveolar density was measured with the transection counting technique of Weibel and Gomez,¹⁹ and the total alveolar count was determined from the product of alveolar density and parenchymal volume. Histologic analyses of cardiac tissues were performed by a blinded independent pathologist.

Statistical analyses

Comparisons of body weight, lung volume, and morphometric data between the control and VEGF groups were analyzed with the two-way Student's *t* test and expressed as the mean ± standard error. Lung volume measurements of the natural history of CLG experiment and VEGF pharmacokinetic data were expressed as the mean ± standard deviation. Data

analyses were performed with GraphPad Prism Version 7 (GraphPad Software, La Jolla, CA).

Results

Natural history of compensatory lung growth in neonatal piglets

Five of 19 piglets that underwent left pneumonectomy died in the perioperative period. Two died from postoperativepost-operative respiratory distress and three from intraoperative complications (traumatic intubation, intraoperative injury to the PV, and barotrauma). All technical complications occurred in the beginning of the study. The mean RLV at baseline (POD 0) was 92.1 ± 30.6 mL, which increased to 192.5 ± 55.5 , 303.7 ± 51.9 , and 332.7 ± 38.1 mL on POD 7, 14, and 21, respectively, after left pneumonectomy (Fig. 1A). These values represented 2.1 ± 0.6 -, 3 ± 0.6 -, and 3.6 ± 0.4 -fold increases from POD 0, respectively. When normalized for body weight, RLV/BW peaked on POD 7 at $6.34 \pm 0.94 \times 10^{-2}$ mL/g (Fig. 1B); this represented a 37% increase from RLV/BW on POD 0. RLV/BW on POD 0, 7, 14, and 21 accounted for 56%, 77%, 71%, and 66% of TLV/BW, respectively.

Piglet tolerance to VEGF infusion

Dose 400 μ g/kg—One piglet was studied at this dosage. The infusion was initiated at the rate of 6.67μ g/kg/min and produced an immediate decrease in MAP from 66 to 38 mm Hg (Fig. 2A); this hypotension was managed by withholding the infusion for 5 minutes and restarting at a rate of 3.33μ g/kg/min with improved MAP. The piglet eventually tolerated the infusion at 5μ g/kg/min; however, this piglet was slow to recover from anesthesia and exhibited pro-longed somnolence and lethargy for 6 hours after the conclusion of VEGF infusion.

Dose 200 μ g/kg—Two piglets were studied at this dosage. Both exhibited a decrease in MAP at the beginning of infusion. The first piglet in this group was initially intended to receive the dose of 400μ g/kg; however, this piglet tolerated a rate of only 1.67μ g/kg/min and completed the total dose of 200μ g/kg in 80 minutes. The second piglet tolerated the rate of 3.33μ g/kg/min and completed the infusion in 60 minutes (Fig. 2B, C).

Dose 100 μ g/kg—Two piglets were studied at this dosage. The infusion in both piglets was started at 1.67μ g/kg/min. Both piglets initially required a decrease in infusion rate to 0.83μ g/kg/min because of hypotension; however, both eventually tolerated the rate of 1.67μ g/kg/min and completed the infusion in 60 and 70 minutes (Fig. 2D and E, respectively).

Pharmacokinetics of VEGF in neonatal piglets

Figure 2, F depicts the plasma levels of VEGF in the 5 piglets during infusion and at various time points for 24 hours after infusion. Because this assay is known to cross-react with porcine VEGF,²⁰ the pre-infusion values of 0–18 pg/mL most likely represent the endogenous plasma levels of VEGF in piglets. The half-lives of VEGF₁₆₅ at the doses of 400, 200, and 100μ g/kg were 89.3, 107.9 ± 5.5 , and 125.3 ± 19.2 minutes, respectively (Table 1). The volume of distribution (V_d) was 126.2 mL/kg at 400μ g/kg and increased to 147.1 ± 63.9 at 200μ g/kg and 381.8 ± 69.9 mL/kg at 100μ g/kg. Similarly, the CI was 1.92

mL/min/kg at 400 µg/kg and increased to 2.46 ± 0.90 at 200 µg/kg and 6.10 ± 1.75 mL/min/kg at 100 µg/kg.

Effects of VEGF on compensatory lung growth in neonatal piglets

Of the 13 piglets who underwent left pneumonectomy in the control group, 3 died on either POD 1 or 2 from respiratory distress. Of the 13 piglets who underwent left pneumonectomy in the VEGF group, 1 died on POD 2 from respiratory distress. Two piglets in the VEGF group were excluded from the study after developing severe diarrhea in the postoperative period, presumably from viral gastroenteritis, because they were housed together. No intraoperative complications were encountered during this phase of the study. In the end, 10 piglets from each group were available for analyses.

Each infusion was initiated at the rate of 1.67 µg/kg/min and increased gradually to the established maximal rate of 3.33 µg/kg/min. Every piglet in the VEGF group developed somnolence and decreased appetite during and immediately after infusion. The rate of infusion was decreased if the piglet developed shivering or emesis. All piglets required 60–90 minutes to complete the daily infusion of 200 µg/kg.

Piglets in the VEGF and control groups had similar weights at the time of operation (2789 ± 49 vs. 2765 ± 61 g, respectively). At the time of euthanasia, piglets in the VEGF group had similar body weights compared with the control group, (3264 ± 60 vs 3489 ± 154 g, $P=0.2$) (Fig. 3A). Piglets in the VEGF group had a greater RLV/BW on POD 7 compared with the control group ($6.35 \pm 0.16 \times 10^{-2}$ vs $5.46 \pm 0.08 \times 10^{-2}$ mL/g, $P<0.0001$) (Fig. 3B). Compared with the control group, the VEGF group also had greater liver/body weight (3.14 ± 0.12 vs $2.76 \pm 0.08 \times 10^{-2}$, $P=0.02$) and spleen/body weight (3.91 ± 0.24 vs $2.97 \pm 0.15 \times 10^{-3}$, $P=0.004$) ratios (Fig. 3C, D). There was also a trend toward greater heart/body weight ratio in the VEGF group (7.55 ± 0.25 vs $6.98 \pm 0.17 \times 10^{-3}$, $P=0.07$) (Fig. 3E) and lesser kidney/body weight ratio in the VEGF group (3.62 ± 0.22 vs $4.09 \pm 0.09 \times 10^{-3}$, $P=0.07$) (Fig. 3F). Histologic examination of cardiac tissues revealed increased size and number of endothelial cells within the pericardial fat in the VEGF group (data not shown); no other microscopic abnormalities explaining the differences in organ weights across groups were observed.

Morphometric analyses

Compared with the control group, piglets in the VEGF group had greater parenchymal volume (5.12 ± 0.15 vs $4.26 \pm 0.09 \times 10^{-2}$ mL/g, $P=0.0001$), greater alveolar volume (3.25 ± 0.14 vs $2.64 \pm 0.07 \times 10^{-2}$ mL/g, $P=0.001$), greater septal surface area (10.67 ± 0.41 vs 9.09 ± 0.32 cm²/g, $P=0.007$), and greater alveolar count (6.02 ± 0.25 vs $4.69 \pm 0.40 \times 10^8$, $P=0.01$) (Fig. 4).

Discussion

Because of the limited number of large-animal studies that employ the model of left pneumonectomy in the postnatal period, the lack of data on the feasibility and natural history of CLG in neonatal piglets presented the first major challenge for this study. Previous experience in our laboratory utilizing the neonatal piglet model had revealed that

postoperative survival depended largely on preoperative nutritional optimization, operative technique, and postoperative monitoring and support.¹⁵ In piglets, the early post-natal period is marked by rapid redistribution of pulmonary blood flow,²¹ maturation in mechanical association between vascular tone and alveolar pressure,²² functional development of alveolar macrophages,²³ and improvement in pulmonary compliance and elastic recoil.^{24,25} When compared with other species, however, porcine lungs exhibit a remarkable degree of maturity at birth as demonstrated by well-developed pulmonary septa, activated type II pneumocytes, and an organized capillary network.²⁶ These features may have contributed to survivability despite substantial derangements in hemodynamic status after pneumonectomy in piglets.^{27,28}

Compensatory lung growth is a physiologic process that depends on the age and species studied.²⁹ Two of the most important factors that affect this process are the mechanical stress imposed by an empty hemithorax and hypoxia.³⁰ In swine, CLG post-lobectomy is rapid and accompanied by marked expression of epidermal growth factor receptor.³¹ Results of this study revealed that CLG postpneumonectomy was also a rapid process, where maximal regeneration of volume was seen between POD 7 and 14. It is also noteworthy that, unlike rodents in which CLG completely returned to preoperative lung volumes,¹² porcine CLG was incomplete, and only 80% of baseline TLV/BW was achieved on POD 7. Despite the lack of pulmonary functional experiments in this study, it is likely that further remodeling of lung tissue and improvement in pulmonary mechanics continued even beyond the period of volume expansion.

VEGF has been studied previously in large animals and clinical trials as a therapy for ischemic heart disease via direct coronary infusion.^{32,33} In these studies, VEGF delivery was consistently accompanied by hypotension and required the concomitant administration of an inhibitor of inducible nitric oxide synthase (iNOS). In another study using newborn Yorkshire piglets, VEGF was reported to exert a strong vasodilatory effect on the pulmonary vasculature via a NO-dependent mechanism.³⁴ Our study further confirmed the hypotensive side effect of VEGF infusion. However, it should be noted that by decreasing the initial rate of infusion and by gradually titrating the rate on reinfusion based on clinical symptoms, for example, shivering and somnolence, all piglets completed the daily infusion without the need for an iNOS inhibitor or any other type of pharmacologic support. Furthermore, NO production might be a desirable side effect should VEGF be developed into a therapy for CDH, a disease in which management of pulmonary hypertension is a challenge in itself and inhaled NO is used frequently as a supportive treatment.³⁵ In children with severe CDH who are maintained on extracorporeal membrane oxygenation, systemic vasodilation can usually be managed with the support of the circuit. Alternatively, VEGF could also be co-administered with an iNOS inhibitor should managing systemic hypotension become too challenging.

The half-life of VEGF in this study ranged from 89 to 144 minutes, substantially greater than that reported in the literature.^{36,37} This observation could be due to the piglets' immature metabolic function and suboptimal uptake of VEGF into tissues. In fact, pharmacokinetic data revealed that lesser doses of VEGF resulted in greater V_d and Cl, both of which implied better tissue uptake. For this reason, and given the piglet's poor tolerance

of the VEGF infusion at the dose of 400 µg/kg, 200 µg/kg was chosen as the testing dose for the treatment arm of the study.

Daily treatment with VEGF at 200 µg/kg enhanced CLG in neonatal piglets as demonstrated by both increased lung volume and improved morphometric data, including total alveolar count. Unlike what has been seen in the rodent model, results from this study revealed an increase in organ weight of the liver, spleen, and heart. These changes may be explained by an improvement in blood flow, a result of the vasodilatory effect of VEGF. It should be stressed that except for evidence of endothelial hyperplasia, there were no microscopic abnormalities on histologic analyses of cardiac tissues in either of the experimental groups.

The degree of pulmonary hypoplasia has been used extensively as a prognostic factor for survival in children with CDH.³⁸ Innovative therapies that target this aspect of the disease have been developed over the years.³⁹ Perhaps the most studied of such therapies is fetal tracheal occlusion, which utilizes mechanical stretching and alveolar distention as a stimulus for lung expansion.⁴⁰ Unfortunately, its clinical efficacy remains unproven, and preterm labor associated with fetal interventions poses a major barrier to its clinical applicability.⁴¹ For this reason, our goal remains the development of a new, medical therapy that accelerates post-natal lung growth and circumvents the need for fetal intervention. Because of its proliferative effects on postnatal lung growth in a large-animal model, VEGF may hold promise as a noninvasive adjunct therapy for treatment of the most severe cases of CDH.

A major limitation of this study was the lack of pulmonary function tests to corroborate volumetric and morphometric data. Because of the requirement for repeated sedation and intubation, which could cause barometric injury to the remaining right lung, these tests were avoided to ensure accurate lung volume measurement. Another limitation involved the use of left pneumonectomy as a model for CDH. Operative removal of the left lung was chosen because there was no other validated model for pulmonary hypoplasia in neonatal piglets. In addition, the systemic administration of VEGF can raise concern over the theoretic risk of carcinogenesis; however, a safety study on patients receiving VEGF gene therapy for the treatment of ischemic limb disease revealed that there was no difference in the incidence of cancer or retinopathy at 10-year follow-up.⁴² Furthermore, ongoing work by our group is exploring the efficacy of topical VEGF as an alternative for systemic administration in the model of CLG. These results, if proven, may lead to a more targeted approach that can potentially decrease the side effects and risks associated with systemic delivery.

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References

1. Nieves BJ, D'Amore PA, Bryan BA. The function of vascular endothelial growth factor. *Biofactors*. 2009;35:332–337. [PubMed: 19415738]

2. Acarregui MJ, Penisten ST, Goss KL, Ramirez K, Snyder JM. Vascular endothelial growth factor gene expression in human fetal lung *in vitro*. *Am J Respir Cell Mol Biol*. 1999;20:14–23. [PubMed: 9870913]
3. Healy AM, Morgenthau L, Zhu X, Farber HW, Cardoso WV. VEGF is deposited in the subepithelial matrix at the and leading edge of branching airways stimulates neovascularization in the murine embryonic lung. *Dev Dyn*. 2000;219:341–352. [PubMed: 11066091]
4. Brown KR, England KM, Goss KL, Snyder JM, Acarregui MJ. VEGF induces airway epithelial cell proliferation in human fetal lung *in vitro*. *Am J Physiol Lung Cell Mol Physiol*. 2001;281:L1001–L1010. [PubMed: 11557604]
5. Raval MV, Wang X, Reynolds M, Fischer AC. Costs of congenital diaphragmatic hernia repair in the United States—Extracorporeal membrane oxygenation foots the bill. *J Pediatr Surg*. 2011;46:617–624. [PubMed: 21496527]
6. Chandrasekharan PK, Rawat M, Madappa R, Rothstein DH, Lakshminrusimha S. Congenital diaphragmatic hernia—A review. *Matern Heal Neonatol Perinatol*. 2017;3:6.
7. Chang R, Andreoli S, Ng YS, Truong T, Smith SR, Wilson J, et al. VEGF expression is downregulated in nitrofen-induced congenital diaphragmatic hernia. *J Pediatr Surg*. 2004;39:825–828. [PubMed: 15185205]
8. Sbragia L, Nassr AC, Goncalves FL, Schmidt AF, Zuliani CC, Garcia PV, et al. VEGF receptor expression decreases during lung development in congenital diaphragmatic hernia induced by nitrofen. *Braz J Med Biol Res*. 2014;47:171–178. [PubMed: 24519134]
9. van der Horst IWJM, Rajatapiti P, van der Voorn P, van Nederveen FH, Tibboel D, Rottier R, et al. Expression of hypoxia-inducible factors, regulators, and target genes in congenital diaphragmatic hernia patients. *Pediatr Dev Pathol*. 2011;14:384–390. [PubMed: 21671771]
10. Woik N, Kroll J. Regulation of lung development and regeneration by the vascular system. *Cell Mol Life Sci*. 2015;72:2709–2718. [PubMed: 25894695]
11. Ding B-S, Nolan DJ, Guo P, Babazadeh AO, Cao Z, Rosenwaks Z, et al. Endothelial-derived angiocrine signals induce and sustain regenerative lung alveolarization. *Cell*. 2011;147:539–553. [PubMed: 22036563]
12. Sakurai MK, Lee S, Arsenault DA, Nose V, Wilson JM, Heymach JV, et al. Vascular endothelial growth factor accelerates compensatory lung growth after unilateral pneumonectomy. *Am J Physiol Lung Cell Mol Physiol*. 2007;292:L742–L747. [PubMed: 17122356]
13. Dao DT, Vuong JT, Anez-Bustillos L, Pan A, Mitchell PD, Fell GL, et al. Intranasal delivery of VEGF enhances compensatory lung growth in mice. *PLoS One*. 2018;13.
14. Kere M, Siriboon C, Liao JW, Lo NW, Chiang HI, Fan YK, et al. Vascular endothelial growth factor A improves quality of matured porcine oocytes and developing parthenotes. *Domest Anim Endocrinol*. 2014;49:60–69. [PubMed: 25061966]
15. Dao DT, Anez-Bustillos L, O’Loughlin AA, Pan A, Nedder AP, Bolgen D, et al. Technique and perioperative management of left pneumonectomy in neonatal piglets. *J Surg Res*. 2017;212:146–152. [PubMed: 28550900]
16. Scherle W. A simple method for volumetry of organs in quantitative stereology. *Mikroskopie*. 1970;26:57–60. [PubMed: 5530651]
17. Ochs M, Mühlfeld C. Quantitative microscopy of the lung: A problem-based approach. Part 1: basic principles of lung stereology. *Am J Physiol Lung Cell Mol Physiol*. 2013;305:L15–L22. [PubMed: 23624789]
18. Mühlfeld C, Ochs M. Quantitative microscopy of the lung: a problem-based approach: Part 2. Stereological parameters and study designs in various diseases of the respiratory tract. *Am J Physiol Lung Cell Mol Physiol*. 2013;305:L205–L221. [PubMed: 23709622]
19. Weibel ER, Gomez DM. A principle for counting tissue structures on random sections. *J Appl Physiol*. 1962;17:343–348. [PubMed: 14005589]
20. Hellerud BC, Nielsen EW, Thorgersen EB, Lindstad JK, Pharo A, Tønnessen TI, et al. Dissecting the effects of lipopolysaccharides from nonlipopolysaccharide molecules in experimental porcine meningococcal sepsis. *Crit Care Med*. 2010;38:1467–1474. [PubMed: 20400898]

21. Glenny RW, Bernard SL, Lucht DL, Neradilek B, Polissar NL. The spatial-temporal redistribution of pulmonary blood flow with postnatal growth. *J Appl Physiol.* 2006;102:1281–1288. [PubMed: 17095640]
22. Mansell AL, McAteer AL, Pipkin AC. Maturation of interdependence between extra-alveolar arteries and lung parenchyma in piglets. *Circ Res.* 1992;71:701–710. [PubMed: 1499112]
23. Dickie R, Tasat DR, Alanis EF, Delfosse V, Tsuda A. Age-dependent changes in porcine alveolar macrophage function during the postnatal period of alveolarization. *Dev Comp Immunol.* 2009;33:145–151. [PubMed: 18775449]
24. Standaert TA, Wilham BE, Mayock DE, Watchko JF, Gibson RL, Woodrum DE. Respiratory mechanics of the piglet during the first month of life. *Pediatr Pulmonol.* 1991;11:294–301. [PubMed: 1758753]
25. Mansell AL, Collins MH, Johnson E, Gil J. Postnatal growth of lung parenchyma in the piglet: Morphometry correlated with mechanics. *Anat Rec.* 1995;241:99–104. [PubMed: 7879927]
26. Winkler GC, Cheville NF. The neonatal porcine lung: Ultrastructural morphology and postnatal development of the terminal airways and alveolar region. *Anat Rec.* 1984;210:303–313. [PubMed: 6507895]
27. Friedli B, Kent G, Kidd BS. The effect of increased pulmonary blood flow on the pulmonary vascular bed in pigs. *Pediatr Res.* 1975;9:547–553. [PubMed: 1161342]
28. Lan CC, Chang CY, Peng CK, Wu CP, Huang KL, Lee SC, et al. Effect of body positions on hemodynamics and gas exchange in anesthetized pigs shortly after pneumonectomy. *Shock.* 2010;34:4 82–4 87.
29. Paisley D, Bevan L, Choy KJ, Gross C. The pneumonectomy model of compensatory lung growth: Insights into lung regeneration. *Pharmacol Ther.* 2014;142:196–205. [PubMed: 2433263]
30. Hsia CCW. Comparative analysis of the mechanical signals in lung development and compensatory growth. *Cell Tissue Res.* 2017;367:687–705. [PubMed: 28084523]
31. Kaza AK, Cope JT, Fiser SM, Long SM, Kern JA, Tribble CG, et al. Contrasting natures of lung growth after transplantation and lobectomy. *J Thorac Cardiovasc Surg.* 2002;123:288–294. [PubMed: 11828288]
32. Henry TD, Annex BH, McKendall GR, Azrin MA, Lopez JJ, Giordano FJ, et al. The VIVA trial: Vascular endothelial growth factor in ischemia for vascular angiogenesis. *Circulation.* 2003;107:1359–1365. [PubMed: 12642354]
33. Sato K, Wu T, Laham RJ, Johnson RB, Douglas P, Li J, et al. Efficacy of intracoronary or intravenous VEGF165 in a pig model of chronic myocardial ischemia. *J Am Coll Cardiol.* 2001;37:616–623. [PubMed: 11216988]
34. Janvier A, Nadeau S, Baribeau J, Perreault T. Role of vascular endothelial growth factor receptor 1 and vascular endothelial growth factor receptor 2 in the vasodilator response to vascular endothelial growth factor in the neonatal piglet lung. *Crit Care Med.* 2005;33:860–866. [PubMed: 15818117]
35. McHoney M. Congenital diaphragmatic hernia, management in the newborn. *Pediatr Surg Int.* 2015;31:1005–1013. [PubMed: 26403792]
36. Kleinheinz J, Jung S, Wermker K, Fischer C, Joos U. Release kinetics of VEGF165 from a collagen matrix and structural matrix changes in a circulation model. *Head Face Med.* 2010;6(1): 17. [PubMed: 20642842]
37. Folkman J. Role of angiogenesis in tumor growth and metastasis. *Semin Oncol.* 2002;29(6Q) a50nc02906q0015.
38. Oluyomi-Obi T, Kuret V, Puligandla P, Lodha A, Lee-Robertson H, Lee K, et al. Antenatal predictors of outcome in prenatally diagnosed congenital diaphragmatic hernia (CDH). *J Pediatr Surg.* 2017;52:881–888. [PubMed: 28095996]
39. Eastwood MP, Russo FM, Toelen J, Deprest J. Medical interventions to reverse pulmonary hypoplasia in the animal model of congenital diaphragmatic hernia: A systematic review. *Pediatr Pulmonol.* 2015;50:820–838. [PubMed: 25994108]
40. DiFiore JW, Fauza DO, Slavin R, Peters CA, Fackler JC, Wilson JM. Experimental fetal tracheal ligation reverses the structural and physiological effects of pulmonary hypoplasia in congenital diaphragmatic hernia. *J Pediatr Surg.* 1994;29:248–256 discussion 256–7. [PubMed: 8176601]

41. Harrison MR, Keller RL, Hawgood SB, Kitterman JA, Sandberg PL, Farmer DL, et al. A randomized trial of fetal endoscopic tracheal occlusion for severe fetal congenital diaphragmatic hernia. *N Engl J Med.* 2003;349:1916–1924. [PubMed: 14614166]
42. Muona K, Mäkinen K, Hedman M, Manninen H, Ylä-Herttuala S. 10-year safety follow-up in patients with local VEGF gene transfer to ischemic lower limb. *Gene Ther.* 2012;19:392–395. [PubMed: 21776026]

Fig. 1. Natural history of compensatory lung growth in neonatal piglets. Right lung volume increased most rapidly between postoperative days (POD) 0 and 14 (A). Right lung volume/body weight (RLV/BW) ratio peaked on POD 7 and represented a 37% increase from POD 0 (B). Results are expressed as the mean $\pm$ standard deviation.

Fig. 2.

Tolerance of VEGF infusion and plasma VEGF levels during and after infusion. All piglets displayed a decrease in MAP with VEGF infusion. One piglet received VEGF infusion at the dose of 400 µg/kg (A), 2 at the dose of 200 µg/kg (B, C), and 2 at the dose of 100 µg/kg (D, E). Plasma levels of VEGF were measured every 10 min during infusion and at various time points for 24 h after infusion (F).

Fig. 3. Effects of VEGF on compensatory lung growth and organ weight in neonatal piglets. Piglets in the control and VEGF groups had similar body weight at the time of operation and when they were killed (A). VEGF-treated piglets had greater RLV/BW on POD 7 (B). VEGF-treated piglets also had greater liver (C), spleen (D), and heart (E) weight, but lesser kidney weight (F) at the time they were killed. Results are expressed as box and whisker plots.

Fig. 4. Morphometric analyses of lung tissues. VEGF-treated piglets had greater parenchymal volume (A), greater alveolar volume (B), greater septal surface area (C), and greater total alveolar count (D) compared with the control group. Representative micrographs at 100 × magnification of the control (E) and VEGF-treated (F) lungs are shown. Results are expressed as box and whisker plots.

Table 1

Pharmacokinetic data of VEGF in neonatal piglets.

Dose	Area under the curve (ng/mL*min)	Half-life (min)	Volume of distribution (mL/kg)	Clearance (mL/min/kg)
400 µg/kg	2.08 × 10 ⁵	89.3	126.2	1.92
200 µg/kg ⁻¹	1.28 × 10 ⁵	113.4	83.1	1.56
200 µg/kg ⁻²	2.96 × 10 ⁴	102.4	211.0	3.36
100 µg/kg ⁻¹	1.27 × 10 ⁴	144.5	451.7	7.85
100 µg/kg ⁻²	2.31 × 10 ⁴	106.0	311.8	4.35