Lgl1 Is Suppressed in Oxygen Toxicity Animal Models of Bronchopulmonary Dysplasia and Normalizes During Recovery in Air

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ABSTRACT: Bronchopulmonary dysplasia (BPD), a major cause of morbidity in premature infants, is characterized by arrest of lung growth and inhibited alveologenesis. We had earlier cloned late-gestation lung 1 (LGL1), a glucocorticoid (GC)-induced, developmentally regulated gene in lung mesenchyme, and showed that reduced levels of late-gestation lung 1 protein (lgl1) inhibit lung branching. Maximal fetal expression of LGL1 is concordant with the onset of alveolar septation, suggesting an additional role for lgl1 in alveologenesis. At postnatal d 7, during the period of maximal septation in postnatal rat lung, lgl1 concentrates at the tips of budding secondary alveolar septa. We studied two models of impaired postnatal alveologenesis generated by exposure of newborn rats to 60% O₂ for 2 wk or 95% O₂ for 1 wk. A profound decrease of lgl1 expression with oxygen exposure was observed in both animal models. Animals exposed to 95% O₂ for 1 wk recovered in air over a 3-wk period, associated with normalization of lgl1 levels. Changes in lung levels of α -actin (a marker of myofibroblast differentiation associated with alveologenesis) and the mesenchymal marker vimentin were significant but less marked. Our findings support a role for lgl1 in postnatal lung development. We speculate that deficiency of lgl1 contributes to the arrested alveolar partitioning observed in BPD and that recovery is associated with normalization of lgl1 levels. (*Pediatr Res* 59: 389–395, 2006)

Chronic lung disease of prematurity (BPD) affects 30% of newborns with birth weights <1 kg (1). Recently, BPD has been associated with abnormal development of alveoli and conducting airways, secondary to premature birth, supplemental O₂ and/or mechanical ventilation (2,3). GCs have been used to treat or prevent BPD (4). While systemic administration of GC can improve lung mechanics in the short term, the early beneficial effects of postnatal GC are outweighed by an increased risk of short- and long-term adverse effects, including premature cessation of secondary septal formation, impaired growth, and neurodevelopmental delay (5,6). Therefore, the routine use of GC for prevention or treatment of BPD

is no longer recommended (4). An improved understanding of downstream targets of GC that regulate fetal lung maturation is an essential prerequisite to the now compelling need for the establishment of safe and effective therapies for BPD.

We cloned LGL1, a GC and developmentally regulated gene encoding a secreted glycoprotein (lgl1) that is, in the lung, expressed predominantly in mesenchyme (7,8). LGL1 was also detected in the fetal heart, intestine, kidney, liver, and spleen (7). We demonstrated antisense LGL1 inhibition of airway branching in rat lung explant culture (9), suggesting that lgl1 stimulates airway branching morphogenesis. Maximal fetal GC-responsive LGL1 expression, however, occurs at gestational d 20–21 in the rat, when branching of conducting airways is complete (7). At this time, mesenchymal lgl1 concentrates in the smooth muscle α -actin-positive myofibroblasts, located adjacent to the epithelium, that regulate the formation of new alveolar units (9). Moreover, beginning in late canalicular lung, secreted lgl1 is associated with distal lung epithelial cells *in vitro* (8). We therefore hypothesized that lgl1 would have a *distinct* functional role during alveologenesis and that reduced lgl1 levels at this time might be associated with the impaired alveolar growth observed in BPD. We therefore elected to investigate lgl1 expression in oxygen toxicity models of neonatal lung injury that demonstrate pathologic characteristics associated with BPD.

Exposure of neonatal rats to $\geq 95\%$ O₂ for 1 wk, a widely used model of neonatal lung injury, results in arrested lung growth without the dysplastic lesions observed in chronic human neonatal lung injury (10). Recovery of these animals in room air, following 95% O₂ exposure, is essentially complete by 4 wk (10,11). By contrast, animals exposed to 60% O₂ for 14 d, like infants with BPD, show a marked heterogeneity of response to injury in different lung regions (12). However,

Received June 30, 2005; accepted October 10, 2005.

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Supported by grants from the Canadian Institutes for Health Research to Feige Kaplan and Neil B. Swezey and by a Montreal Children's Hospital Research Institute Scholarship to Katia Nadeau.

DOI: 10.1203/01.pdr.0000198819.81785.f1

Abbreviations: BPD, bronchopulmonary dysplasia; GC, glucocorticoid; LGL1, late-gestation lung 1 gene and mRNA; lgl1, late-gestation lung protein 1

recovery of these animals is hard to assess in the short term. We therefore chose to evaluate the impact of hyperoxia on IgI1 in both animal models.

METHODS

In Vivo Interventions. Procedures involving animals were according to criteria established by the Canadian Council for Animal Care and approved by Animal Care Committees at Toronto Western Hospital, University Health Network, and the McGill University Health Centre. Timed pregnant Sprague-Dawley rats were obtained from Taconic (Germantown, NY). Experiments were conducted as paired exposures with one chamber receiving 60% (four litters) or 95% O₂ (four litters) and the other receiving air. Procedures for O₂ exposure were as described (11,13).

Immunohistochemistry. Perfused lung tissue sections (four animals per group) were inflation fixed and prepared for immunohistochemistry using the avidin-biotin-peroxidase technique as described (13). A rabbit polyclonal IgI1 antibody was raised against two synthetic IgI1 peptides (9) by Medicorp (Montreal, QC, Canada). The antibody identifies the expected protein band at 52 kD. Confirmation of the IgI1 target was previously demonstrated by preabsorption of the antibody with 5-fold excess of each peptide, which eliminated IgI1 staining (9). The primary antiserum was diluted 1:100 for anti-rat IgI1 antibody and 1:200 for mouse α -actin (Neomarkers) and anti-vimentin antibodies (Sigma Chemical Co.) and left overnight at room temperature for all except vimentin, which was kept at 4°C. Antibody specificity was verified by omitting the primary antibody. Biotinylated anti-mouse (Vector Laboratories) or anti-rabbit (Sigma Chemical Co.) IgG secondary antibodies (1:300) were added at room temperature for 2 h. After immunostaining, digital images were taken using a Spot camera (Diagnostic). Northern Eclipse image analysis software (Empix Imaging Inc.) was used for semiquantification of antibody staining according to supplier. For each section at magnification $\times 400$ ($n = 4$), two different thresholds were analyzed: a high threshold to obtain the value of the total surface area of lung tissue without air space and a lower threshold to obtain pixel counts of immunoreaction product. The relative amount of protein is expressed as the integrated immunostaining intensity over a given tissue area. For alveolar tips, eight tip regions were compared with 10 nearby nontip regions.

Isolation of Total Lung RNA. Lungs of entire litters were flash frozen in liquid nitrogen, homogenized in 4 M/L guanidinium thiocyanate, and extracted in phenol/chloroform extraction according to Chomczynski and Sacchi (14). RNA was resuspended in RNASecure (Ambion).

Northern blot analysis. Thirty micrograms of total lung RNA of rats exposed to 60% O₂ (four litters) or air (four litters) was hybridized to a 1.4-kb LGL1 probe generated by polymerase chain reaction (PCR) using the forward primer 5'TCAGAGTTCGCGCAGAACG3' and the reverse primer 5' CACCAAAAGCCACGGAGGCC. An 18S biotinylated probe was used to control for loading. Similar exposures of blots were used for densitometric analysis. All samples were within the linear range for densitometry. Hybridization signals were quantified using the Fuji Image Gauge V3 program.

Quantitative real-time reverse transcriptase (RT)-PCR. Quantitative real-time RT-PCR was performed on the Mx4000 QPCR system from Stratagene (Stratagene, La Jolla, CA) using the Quantitect One-Step Probe RT-PCR Kit

(Qiagen). Gene-specific primers and FAM-labeled probes for rat cDNA sequences of LGL1, smooth muscle α -actin and vimentin were designed using Qiagen's online QuantiProbe Design Software (Table 1). Quantitect Gene Expression Assay for mouse 18S (Qiagen) was used to normalize for the input of RNA. The results were analyzed according to the standard curve method (15). One-step real-time RT-PCR reactions were performed in 25- μ L volume for 45 cycles, using 10 ng of total RNA for LGL1 and smooth muscle α -actin, 50 ng for vimentin, and 50 pg for 18S. PCR conditions are as directed by the manufacturer. Individual runs were performed in triplicates (four litters, all groups).

Statistical analysis. All data are presented as mean $\pm$ SEM. Statistical significance was determined by two-way analysis of variance. Pairwise group comparisons were assessed using the Student-Neuman-Keuls test.

RESULTS

Lgl1 localization in postnatal lung. In the rat, alveolar septation occurs in the first 2 wk of life and is $\sim 70\%$ complete by postnatal d 7 (16,17). Immunolocalization of IgI1 at postnatal d 7 and 14 (Fig. 1A–C) showed a diffuse distribution of IgI1 protein in the mesenchyme, in alveolar cells, and in the epithelia of larger conducting airways. Lgl1 levels increased until d 14 and then declined. At postnatal d 7, the IgI1 protein was concentrated at the tips of septating alveoli (Fig. 2A–C).

Lgl1 protein and mRNA levels are reduced in 60% O₂ toxicity models of BPD. Chronic lung injury in the neonatal rat exposed to 60% O₂ for 14 d is characterized by an overall inhibition of lung growth and DNA synthesis (13) and inhibition of myofibroblast differentiation (12) interspersed with distinct areas of parenchymal thickening with active DNA synthesis (13). We assessed IgI1 protein levels in this BPD model at postnatal d 7 and 14 by immunohistochemistry. A dramatic reduction in IgI1 protein was observed in the lungs of rats exposed to 60% O₂ when compared with air-exposed controls (Fig. 3A, B, E). Diminished IgI1 levels in 60% O₂ exposed animals remained pronounced at postnatal d 14 when the extent of lung injury most closely resembled the human BPD phenotype (Fig. 3C–E).

Northern analysis confirmed that effects of hyperoxia on IgI1 protein were accompanied by a profound reduction in LGL1 mRNA expression. LGL1 mRNA levels increased from postnatal d 7 to d 14 in animals exposed to air (Fig. 4A). LGL1 mRNA concentrations were decreased following 7, 10, and 14 d of exposure of animals to 60% O₂, when compared with

Table 1. Gene-specific primers for real-time RT-PCR

Gene	Accession no.	Primer	Probe
LGL1	NM_138518	Forward: CATCTACGCTGACACTTC Reverse: ATCACATCTGCATAGCCACCAA	ATCTGTAAGGCGGCTG
α -Actin	X06801	Forward: TGTCGCTCTGGACTTTGA Reverse: CAGGCAGTTCGTAGCTCT	TGCTTCTCTTCTTCCC
Vimentin	NM031140	Forward: CCTGAACCTGAGAGAACTA Reverse: TCTTTTGGAGTGGGTGTCAAC	CCTGGAGTCACTTCCT

Figure 1. Immunolocalization of rat lung IgI1 protein at postnatal d 7 (A) and 14 (B) ($n = 4$). Insets: Control without IgI1 primary antibody. Bar = 50 μ m. (C) Semiquantification of immunoreactive IgI1 protein. $*p \leq 0.001$ for O₂ vs air.

Figure 2. (A,B) Lgl1 protein at the tips of septating alveolar buds at postnatal d 7 ($n = 4$). Insets: Semiquantification of lgl1 protein in delimited regions ($n \geq 8$). Bar = 25 μm . (C) Semiquantification of immunoreactive lgl1 protein. * $p < 0.001$ for O_2 vs air.

Figure 3. Lgl1 protein levels in animals exposed to 60% O_2 . Lgl1 protein levels in lungs of animals exposed to normal air for 7 d (A), 60% O_2 for 7 d (B), 14 d of normal air (C), 60% O_2 for 14 d (D) ($n = 4$). (E) Semiquantification of immunoreactive lgl1 protein. * $p \leq 0.001$ for O_2 vs air. Bar = 50 μm .

those of control litters exposed to room air. LGL1 mRNA levels in 60% O_2 -exposed animals ranged from 10% to 28% of that observed in air-exposed controls (Fig. 4A). The effects on LGL1 mRNA levels were validated by quantitative real-time RT-PCR analysis (Fig. 4B). All further mRNA analyses were carried out using quantitative real-time RT-PCR.

Smooth muscle α -actin protein and mRNA levels are reduced in 60% O_2 toxicity models of BPD. Lung injury in the neonatal rat exposed to 60% O_2 for 14 d is associated with inhibition of myofibroblast differentiation (13). We therefore sought to establish whether levels of smooth muscle α -actin (believed to modulate tissue remodeling in terminal lung development) would be affected by O_2 exposure. In air-exposed control animals, α -actin protein concentrated at the tips of budding septa at postnatal d 7 (Fig. 5A) and levels decreased with maturation and mesenchymal thinning (Fig. 5A and C). Animals exposed to 60% O_2 for 1 wk had significantly reduced α -actin levels when compared with air-exposed controls (Fig. 5A, B, E). Reduction in α -actin protein was still significant at 14 d when α -actin in air-exposed animals had diminished (Fig. 5C–E). The reduction in α -actin protein in 60% O_2 -exposed lungs was accompanied by a significant reduction in α -actin mRNA (Fig. 4C).

Vimentin protein and mRNA levels are reduced in 60% O_2 toxicity models of BPD. In the rat, interstitial fibroblasts undergo a period of rapid proliferation from postnatal d 4 to 13 (18). Following this period of rapid alveologenesis is a period of marked mesenchymal loss as alveolar walls become thinner. As a marker of mesenchymal cells, we assessed levels of vimentin in 60% O_2 -exposed animals. Vimentin levels were

Figure 4. LGL1, α -actin, and vimentin mRNA levels in rats exposed to 60% O_2 . Northern blot analysis for LGL1 (A). Real-time RT-PCR mRNA quantification of LGL1 (B), α -actin (C), and vimentin (D) ($n = 4$). * $p \leq 0.001$ for O_2 vs air. $\dagger p \leq 0.008$ for O_2 vs air.

significantly reduced in animals exposed to 60% O_2 for 1 wk (Fig. 6A, B, E). This difference was even more pronounced following 2 wk of exposure to 60% O_2 (Fig. 6C–E).

Changes in vimentin immunoreactivity following 1-wk exposure to 60% O_2 were accompanied by a significant reduction in vimentin mRNA (Fig. 4D). These differences were even more pronounced at postnatal d 10 and 14, in parallel to changes observed in vimentin protein expression. In air-exposed animals, vimentin mRNA levels increased from d 7 to d 10 and then

Figure 5. α -Actin protein levels in animals exposed to 60% O₂. α -Actin protein levels in lungs of animals exposed to normal air for 7 d (A), 60% O₂ for 7 d (B), normal air for 14 d (C), 60% O₂ for 14 d (D) ($n = 4$). (E) Semiquantification of immunoreactive Ig11 protein. Bar = 50 μ m. * $p \leq 0.001$ for O₂ vs air; † $p \leq 0.008$ for O₂ vs air.

Figure 6. Vimentin protein levels in animals exposed to 60% O₂. Vimentin protein levels in lungs of animals exposed to normal air for 7 d (A), 60% O₂ for 7 d (B), normal air for 14 d (C), 60% O₂ for 14 d (D) ($n = 4$). (E) Semiquantification of immunoreactive Ig11 protein. Bar = 50 μ m. * $p \leq 0.001$ for O₂ vs air.

declined, consistent with the expected rapid proliferation of lung mesenchyme followed by a period of mesenchymal loss.

Ig11 protein levels are reduced in rats exposed to 95% O₂ but normalize following recovery in air. Newborn rats exposed to 95% O₂ for 1 wk exhibit a complete arrest of lung growth (10). No regions of residual growth, such as those observed following exposure to 60% O₂, are seen. When these animals are restored to room air, catch-up growth to normal lung structure and function is essentially complete by 4 wk (10,11). Similar to our findings in the 60% O₂-exposed rat model, lung levels of Ig11 in animals exposed to 95% O₂ for 1 wk were dramatically reduced throughout the lung when compared with air-exposed controls (Figs. 7A and B and 8A). Following a 1-wk recovery period in room air, pups that had been exposed to 95% O₂ showed increased Ig11 immunoreactivity throughout the lung (Figs. 7C and D and 8A). Interestingly, a low but detectable enrichment of Ig11 was observed at the tips of many budding septa at this time (Fig. 7D). After a recovery period of 2 wk (Figs. 7E and F and 8A), Ig11 protein levels continued to increase and appeared to be diffusely distributed throughout the lung. Following a 3-wk recovery period in air, Ig11 levels in 95% O₂-exposed animals were generally indistinguishable from levels in air-exposed controls (Figs. 7G and H and 8A).

Quantitative real-time RT-PCR analysis confirmed that effects on Ig11 protein following exposure to 95% O₂ were accompanied

by a similar change in LGL1 mRNA expression (Fig. 9A). LGL1 mRNA was decreased by 60% following a 1-wk exposure to 95% O₂, when compared with control litters exposed to room air. LGL1 mRNA levels remained significantly below normal after a 1-wk recovery period in air. Following a 2-wk recovery period in air differences detected in mRNA levels of animals exposed to 95% O₂ were no longer significantly different from those of air-exposed controls.

α -Actin and vimentin are reduced in 95% O₂ toxicity models of BPD. We also assessed changes in levels of α -actin and vimentin in animals recovering from exposure to 95% O₂ (Figs. 8B, 9B, 10, and 11). An apparent reduction in alveolar α -actin expressing myofibroblasts was observed in air-exposed animals with maturation and mesenchymal thinning (compare parts C, E, and F in Figure 10). Animals exposed to 95% O₂ for 1 wk displayed a significant reduction in α -actin levels when compared with air-exposed controls (Figs. 8B and Fig. 10A and B). Following a 1-wk recovery period in air, α -actin immunoreactivity remained significantly below normal but was more clearly defined at the tips of septating alveoli (Fig. 10D). There were no significant differences in α -actin immunoreactivity following a 2- to 3-wk recovery period in air (Figs. 8B and Fig. 10E-H).

Similarly, a significant decrease in α -actin mRNA was detected by quantitative real-time RT-PCR following exposure to 95% O₂ for 1 wk (Fig. 9B). Following a 2-wk recovery

Figure 7. Lgl1 protein levels in animals exposed to 95% O₂ for one week and allowed to recover in air. Lgl1 protein levels in lungs of animals exposed to normal air, 7 d (A) 95% O₂, 7 d (B) normal air, 14 d (C) 95% O₂, 7 d; normal air, 7 d (D), at this time, Lgl1 can be clearly seen in septating tips, see arrows) normal air, 21 d (E) 95% O₂, 7; normal air, 14 d (F) normal air, 28 d (G) 95% O₂, 7 d; normal air, 21 d (H), (*n* = 4). Bar = 50 μ m.

period in air, differences in α -actin mRNA expression between 95% O₂-exposed animals and air-exposed controls were no longer significant.

Air-exposed control animals exhibited a reduction in immunoreactive vimentin associated with maturation and mesenchymal thinning [Figs. 8C and 11 (compare A and C with E and G)]. Vimentin levels were reduced in animals exposed to 95% O₂ when compared with air-exposed controls (Fig. 11A–F). A gradual increase in vimentin to normal levels

was observed during the 3-wk recovery period in air. At age 4 wk, vimentin levels in animals exposed to air or 95% O₂ were indistinguishable. Changes in vimentin immunoreactivity following exposure to 95% O₂ for 1 wk were accompanied by a significant reduction in vimentin mRNA as assessed by quantitative real-time RT-PCR (Fig. 9C), which persisted for 2 wk during recovery in air.

DISCUSSION

The first 2 wk of postnatal life represent the period of maximal lung septation in the rat. Our detection of high levels of lgl1 protein during this period, and particularly its localization to growing epithelia, is consistent with the hypothesis that lgl1 has a distinct functional role in alveologenesis. Our observation that lgl1 concentrates at the distal tips of septating buds at postnatal d 7 suggests a role for lgl1 in septal growth and alveolar proliferation. Concordant with these observations, we have recently found that neonatal LGL1 mice, in which transgenic LGL1 expression is driven in the lung by a mesenchyme-specific versican promoter, display precocious alveolar septation and mesenchymal thinning (19).

The hyperoxic rat models demonstrate the major feature of modern BPD, namely, arrested/delayed alveolar growth, and therefore can be used to study the role of factors that may regulate this process. We found a profound suppression of lgl1 expression following both a 2-wk exposure to 60% O₂ and a 1-wk exposure to 95% O₂, indicating that impaired alveologenesis is associated with deficiency of lgl1. Both O₂ exposure protocols are associated with a reduction in lung cell DNA synthesis (11,13). The impact of hyperoxia on alveolar formation is concentration dependent. As previously assessed by measurement of mean linear intercept for animals used in this study, alveolar formation was arrested by a 1-wk exposure to 95% O₂ (11). In the 60% O₂ exposure model, the mean linear intercept, though not statistically significantly different from that observed in air-exposed animals, showed a significant increase in variance, consistent with the observed heterogeneous effect on alveolar formation (20).

Suppression of lgl1 protein and LGL1 mRNA levels in animals exposed to 95% O₂ for 1 wk was followed by a gradual increase to normal levels during a 3-wk recovery period in air, consistent with a requirement for lgl1 for normal postnatal lung growth, alveologenesis, and repair. Normalization of lgl1 levels was concordant with the reported rapid

Figure 8. Semiquantification of immunoreactive lgl1 protein (A), α -actin (B), and vimentin (C) in animals exposed to 95% O₂ for 1 wk and allowed to recover in air. **p* $\leq$ 0.001 for O₂ vs air.

Figure 9. mRNA quantification of LGL1 (A), α -actin (B), and vimentin (C) by real-time RT-PCR in animals exposed to 95% O₂ for 1 wk and allowed to recover in air. * $p \leq 0.001$ for O₂ vs air.

catch up in alveolar formation during recovery, as assessed by measurement of mean linear intercept (11).

α -Actin protein and mRNA levels were also affected in both animal models of O₂ toxicity. Changes in the levels of α -actin in recovering lung of 95% O₂-exposed animals are of interest. Perinatal rat lung myofibroblasts localize to alveolar septa (21). Mitchell *et al.* (22) demonstrated an abundance of individual cells staining for smooth muscle α -actin in the saccular interstitium during secondary septal formation, suggesting that α -actin expressing myofibroblasts may be an important component in tissue remodeling during lung development. Moreover, conditions for lung remodeling after injury may also depend on reactivation of α -actin expression. We suggest that Ig11, like α -actin, may be required for tissue remodeling and alveolar formation during postnatal lung development and following lung injury.

Both immunoreactive vimentin and vimentin mRNA levels were reduced in animals exposed to hyperoxia, with a gradual recovery to normal after recovery in air. The findings of diminished vimentin in the face of delayed thinning of the mesenchyme suggest that O₂ toxicity may result in changes in the protein composition of mesenchymal tissue.

Lung injury in animals exposed to 60% O₂ for 14 d is typified by an overall reduction in lung growth, with patchy areas of DNA synthesis and parenchymal thickening characterized by an increase in type II epithelial cells. Enrichment of type II cells in regions of hypercellularity may reflect altered expression of growth agonists or antagonists essential to the transition of type II to type I cells associated with normal alveologenesis. Limited information is available regarding mesenchymal factors that stimulate type II to type I transition. We have shown that (1) maximal LGL1 expression in late gestation at the onset of alveolar septation is in α -actin expressing myofibroblasts, which are believed to stimulate alveologenesis (9); (2) in late gestation and throughout the postnatal period, Ig11 protein is associated with lung epithelium (8); (3) neonatal LGL1 transgenic mice exhibit precocious alveolar septation and mesenchymal thinning (19); and, in preliminary studies, (4) the LGL1 5' proximal promoter includes binding sites for both GC and retinoic acid receptors, important modulators of late gestation and postnatal lung development. It is thus tempting to speculate that Ig11, regulated by GC and/or retinoic acid and secreted by fibroblasts, may be one factor involved in the transition of epithelial type

II to type I cells and that deficient Ig11 may contribute to the epithelial hypercellularity and disrupted alveolarization observed in O₂ toxicity models of BPD. Suitable experiments using *in vitro* and *in vivo* animal models will be necessary to evaluate this hypothesis. Moreover, whether the reduction in Ig11 observed in these animal models is a direct consequence of increased O₂ levels on fibroblasts, secondary to effects of hyperoxia on other lung cell types, or the result of other neurohumoral effects known to aggravate hyperoxia-induced

Figure 10. α -Actin protein levels in animals exposed to 95% O₂ for one week and allowed to recover in air. α -Actin protein levels in lungs of animals exposed to normal air, 7 d (A) 95% O₂, 7 d (B) normal air, 14 d (C) 95% O₂, 7 d; normal air, 7 d (D, at this time α -actin can be clearly seen in septating tips [C,D, arrows]) normal air, 21 d (E) 95% O₂, 7 d; normal air, 14 d (F) normal air, 28 d (G) 95% O₂, 7 d; normal air, 21 d (H), ($n = 4$). Bar = 50 μ m.

Figure 11. Vimentin protein levels in animals exposed to 95% O₂ for one week and allowed to recover in air. Vimentin protein levels in lungs of animals exposed to normal air, 7 d (A) 95% O₂, 7 d (B) normal air, 14 d (C) 95% O₂, 7 d; normal air, 7 d (D) normal air, 21 d (E) 95% O₂, 7 d; normal air, 14 d (F) normal air, 28 d (G) 95% O₂, 7 d; normal air, 21 d (H), (*n* = 4). Bar = 50 μ m.

lung injury [e.g. elevated bombesin-like peptides (23), circulating GC levels (24)] remains to be determined.

Many key mediators of lung morphogenesis are expressed throughout fetal development and into postnatal life, suggesting that they may be critical for both airway branching (early) and alveologenesis (late). Examples include fibroblast growth factors (25), transforming growth factor β (26,27), BMP4 (28), and platelet-derived growth factor α (29,30). Our observations suggest that LGL1 may be a new member of this group of multifunctional developmental regulators of lung organogenesis (29).

The routine use of GC for prevention or treatment of BPD is no longer recommended due to the potential long-term adverse effects on lung growth and neurodevelopment (2–4). There is therefore a compelling need to identify more specific downstream targets of GC that may provide the beneficial effects of GC on lung mechanics in the context of BPD without incurring the attendant GC toxicity. Our present findings support a role for Lgl1 in alveologenesis and suggest that deficiency of Lgl1 may contribute to the arrested alveolar partitioning observed in BPD. As such, Lgl1 may have relevance to the development of future strategies for prophylaxis and treatment of BPD.

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