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MWSPR Plenary Session I

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GALECTIN-1 EXPRESSION IS INCREASED IN THE SECONDARY ALVEOLAR TIPS OF MOUSE LUNG.

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Bronchopulmonary dysplasia, a common complication of prematurity, is characterized by inadequate alveolarization. The process of alveolarization, by which the lung forms mature gas-exchanging units, is not well understood. In mice, alveolarizaion occurs during postnatal days four through twelve, when the formation of secondary septa creates thin walled alveoli. The purpose of this study was to investigate the genes involved in this process. RNA was isolated from dissected tips of secondary septa and whole lung tissue of day six postnatal mouse lung. The tips of secondary septa were obtained via laser capture microscopy of frozen sections. Total RNA was isolated from the tip and whole lung samples and amplified in the same manner. Affymetrix gene profiling was then performed, using mouse U74Av2 GeneChips. The signal for galectin-1 mRNA was six fold higher in the secondary septal tips than in whole lung tissue ($p<0.05$). Galectins, or S-type lectins, are beta-galactoside-binding proteins involved in the regulation of cell proliferation, differentiation and apoptosis. Galectin-1 is a homodimer of two 14 kDa subunits. It is the most abundant galectin in the lung. To confirm the relative abundance of galectin-1 in secondary septal tips versus whole lung, immunostaining of sections of day six postnatal and adult mouse lung tissue was performed. Staining for galectin-1 was concentrated in the tips of the secondary septa in the day six postnatal tissue. Furthermore, staining was dramatically increased in the day six mouse lung tissue when compared to staining levels in the adult lung sections. Immunoblot analysis of lung homogenates obtained at different stages of lung development demonstrated that a peak of galectin-1 expression occurs at postnatal days six and twelve, corresponding to the time of alveolarization. The increased expression of galectin-1 at the site and time of ongoing alveolarization suggests that it may play a role in this important aspect of lung development. Supported by NIH grants HL-62861 and HL-07638.

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SU1498 INHIBITS ALVEOLARIZATION IN NEWBORN MICE.

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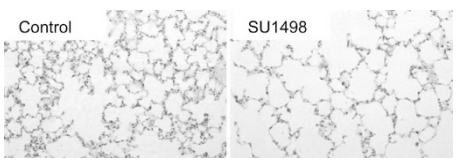
BACKGROUND: Bronchopulmonary dysplasia (BPD) in premature infants is characterized by inhibited alveolarization and vasculogenesis. Inhibitors of angiogenesis induce emphysema in newborn rats resulting in a phenotype similar to BPD in premature infants. Our goal was to generate a mouse model of inhibited alveolarization that could be employed to explore the mechanisms resulting in, and interventions for BPD. SU1498 is a commercially available compound that inhibits vascular endothelial growth factor receptors. **METHODS:** Three day old C3H/HeNHsd mice were injected with a single dose of SU1498 (30mg/kg, SC). Lungs of control (sham-injected) and treated mice were inflation fixed on postnatal day 21. Tissue sections were mounted and morphometric analysis was performed to determine the volume density (VD) of air space, tissue, large blood vessels, conducting airways, and alveolar surface area. Lungs were also harvested for electron microscopic analysis of alveolar structures. **RESULTS:** The VD of airspace ($63.7\pm1.9\%$ vs. $53.2\pm1.2\%$) and conducting airways ($3.0\pm0.9\%$ vs. $0.9\pm0.4\%$) were significantly greater in treated versus control mice ($n=8$, $P<0.05$). The VD of large blood vessels was not different between the two groups. The alveolar tissue VD ($31.0\pm1.5\%$ vs. $43.9\pm1.3\%$) and the alveolar surface area (318.6 ± 31.6 cm^2 vs. 238.1 ± 22.8cm^2) were significantly less in treated versus control mice ($n=8$, $P<0.05$). Electron microscopy demonstrated a decrease in alveolar wall thickness in the lungs of treated mice. Treatment also resulted in fewer but enlarged capillaries compared to controls.



CONCLUSION: A single of dose of the VEGFR inhibitor, SU1498, to newborn mice results in inhibition of alveolar development at 21 days. This phenotype provides a model for the investigation of mechanisms resulting in inhibited alveolarization. Such investigation may lead to strategies for the prevention or treatment of BPD in prematurely born infants.

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EXPRESSION OF EPIDERMAL GROWTH FACTOR-LIKE DOMAIN 7 IN NEONATAL RAT LUNGS DURING NORMOXIA AND HYPEROXIA.

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Purpose of Study: Preterm babies treated with ventilator support and supplemental oxygen frequently develop chronic lung disease (CLD) that has significant mortality and morbidity. Oxygen toxicity plays an important role in CLD etiology. Several lines of evidence have suggested that impairment of pulmonary angiogenesis is implicated in alveolization and the development of CLD. Epidermal growth factor-like domain 7 (EGFL-7) is a recently identified protein secreted from vascular endothelial cells and it regulates vascular tubulogenesis (*Nature* 2004;428:754). Aim of this study was to measure EGFL-7 expression in the neonatal lung during normoxia and hyperoxia. **Methods:** Rat pups at 4 days of age were randomly assigned to normoxic and hyperoxic groups. The rats in the normoxic and hyperoxic groups were treated with room air and 95% O_2 for 3, 6, and 10 days, respectively. The lung tissues were collected for total RNA isolation. EGFL-7 mRNA expression was measured by quantitative real-time reverse-transcription polymerase chain reaction (Q-RT-PCR). Separately, human umbilical vein endothelial cells (HUVEC) were cultured in 37°C, 5% CO_2 incubator, and were exposed to normoxia (room air) or hyperoxia (95% O_2). **Results** EGFL-7 mRNA in normoxic neonatal rat lung was consistently expressed from 7 days to 2 months of age ($n = 3$) at each time. EGFL-7 mRNA expression in the hyperoxic group was significantly decreased after oxygen exposure for 3, 6 and 10 days; it decreased 2.1 fold at day 3 ($n = 3$); 4.1 fold at day 6 ($n = 3$); and 3.1 fold at day 10 ($n = 3$) compared to time-matched normoxic group results, respectively. EGFL-7 mRNA expression in the hyperoxic group returned to nearly normal levels 2 weeks ($n = 3$) after discontinuing oxygen exposure, and it remained at normal levels during the 2 month recovery period ($n = 2$–3). In cultured HUVEC, EGFL-7 mRNA expression also decreased 2.6 fold after 95% O_2 exposure for 48 hours. **Conclusions:** Oxygen exposure is associated with the decrease of EGFL-7 mRNA expression in the neonatal rat lung and the expression level returns to normal after oxygen treatment. These findings imply that reduced levels of EGFL-7 at a critical lung development stage may be a contributing factor in the impairment of pulmonary angiogenesis and alveolization after hyperoxic lung injury.

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CHARACTERIZATION OF GENETIC VARIATION IN INTRON 4 OF THE SURFACTANT PROTEIN B GENE.

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Expression of the surfactant protein B gene is required for function of the pulmonary surfactant. The 9.5 kb surfactant protein B gene includes 10 translated and exons and 1 untranslated exon. Genetic variants in intron 4 characterized by insertions or deletions of 11 distinct motifs have been associated with respiratory distress in some populations of infants, adult respiratory distress syndrome, and risk of squamous cell carcinoma of the lung. Due to polymerase enzyme stutter, characterization of allelic variation by direct sequencing has been difficult. To examine genetic variation in intron 4 in a cohort of Missouri infants ($n=240$), we identified a polymerase enzyme with high fidelity for intron 4 amplification and analyzed product length by agarose gel electrophoretic mobility. In 480 alleles, we found 14.4% (69/480) variant alleles, 9.4% with insertions and 5.0% with deletions. Allelic diversity was significantly greater among African-Americans ($n=204$, 19.1% insertion alleles, 2.9% deletion alleles) than Caucasians ($n=244$, 2.0% insertion alleles, 7.4% deletion alleles) ($p<.001$). Insertion variants were strongly associated with African Americans (28/31), and deletions were associated with Caucasians (15/20) ($p<.001$). We then cloned fragments from a subset of 42 infants (22 African-American, 19 Caucasian, 1 Hispanic). Automated sequencing of the 32 variant alleles revealed 6 previously unreported variants (all insertions) that accounted for an unexpectedly high proportion (18/32) of variant alleles sequenced. Two different insertion variants shared agarose gel electrophoretic mobility (Mr~570 bps), as did 2 other insertion variants (Mr~600 bps). When analyzed by automated sequencing, these variants differed in motif insertion or deletion or in length of dinucleotide (CA) repeat. The C genotype at genomic position 1580, a C/T nonsynonymous, single nucleotide polymorphism in exon 4, was strongly associated with insertion ($28/31 = T$) ($p<.001$). We conclude that genotype at genomic position 1580 and race are associated with intron 4 variation. Characterization of intron 4 by agarose gel electrophoretic mobility alone may fail to detect important differences in genetic variation in intron 4 that contribute to risk for respiratory disease.