Insulin-Like Growth Factor-1 Downstream Signaling is disrupted in the Lung by Preterm Birth and Prolonged Mechanical Ventilation of Preterm Lambs

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Background. Today, infants who are born as early as 22 weeks postconceptional age (term is 40 weeks) may survive if supported by antenatal steroids, postnatal surfactant replacement, mechanical ventilation, supplemental oxygen, antibiotics, and appropriate nutrition. These, and other, supports are required because of the sudden event of preterm birth, which is an abrupt change in environment. How does the abrupt change in environment dysregulate molecular pathways that are necessary for normal lung development during the canalicular stage to complete the alveolar stage? This is the question for which my summer research project will provide an answer.

Studies from Dr. Albertine's laboratory provide a number of insights that lead to my proposed project. These insights are made possible through the unique preterm lamb model of neonatal chronic lung disease (CLD; also called bronchopulmonary dysplasia) that his group created. His model manages preterm lambs that are delivered at the equivalent of about 29 weeks of gestation in humans, and are supported by contemporary clinical practices in neonatology. Preterm lambs that are endotracheal intubated and supported by intermittent mandatory ventilation (IMV; also called mechanical ventilation) develop evolving neonatal CLD. In comparison, preterm lambs that are supported by non-invasive high-frequency nasal ventilation (HFNV), do not develop neonatal CLD, and therefore serve as a positive-outcome control for chronically ventilated preterm lambs.

Insights that are specific to my project are focused on epigenetic mechanisms that contribute to neonatal CLD. One insight is that invasive IMV leads to genome-wide hypoacetylation of histone 3 in the lung. Coincidently, lung development and function are disrupted. Lung development is simplified, meaning that alveoli and alveolar capillaries do not form. Alveolar simplification prevents efficient exchange of respiratory gases, which together necessitate prolonged support with invasive IMV and oxygen-rich gas.

Another insight is that genome-wide hypoacetylation of histone 3 occurs in the lung of preterm lambs that are supported by invasive IMV and oxygen-rich gas. Recent experiments by Dr. Albertine's group show that preservation of genome-wide acetylation, by giving histone deacetylase inhibitors (either valproic acid or Trichostatin A) to preterm lambs supported by invasive IMV, improves lung development and respiratory gas exchange.

While juxtaposition of the hypoacetylation effect of invasive IMV alone versus treating preterm lambs with histone deacetylase inhibitors during invasive IMV provides an intriguing story line, the story line limited by not providing insight about gene-specific effects. To this end, current studies in Dr. Albertine's laboratory are using insulin-like growth factor -1 (IGF-1) as a prototypic epigenetically regulated gene in the lung. IGF-1 is being studied as a prototypic epigenetically regulated gene because IGF-1 directs lung development normally [5], IGF-1 mRNA expression is increased in the lung of preterm human infants who died with respiratory distress syndrome or neonatal CLD [2], and IGF-1 mRNA expression is epigenetically regulated.[3,4]. Together these results indicate that IGF-1 is an appropriate epigenetically regulated gene to analyze in the context of evolving neonatal CLD dysregulated in the lung of preterm lambs during invasive IMV but not non-invasive HFNV.

Dr. Scientist's laboratory is pursing these novel results by two approaches, one of which is the focus of my project. One approach, which is not the focus of my project, is identification of the effect of ventilation mode on nucleosome positioning at the 5-prime end of the IGF-1 gene. The purpose is to determine if nucleosome positioning determines initiation of transcription that may contribute to greater mRNA level of IGF-1 in the lung of preterm lambs that are supported by invasive IMV compared to non-invasive HFNV. Other potential mechanisms may be altered elongation of transcription and/or altered termination of transcription.

The other approach, which is the focus of my project, is testing the hypothesis that increased IGF-1 in the lung leads to alveolar simplification and poor respiratory gas exchange. Accordingly, two experiments have been performed. One experiment is to treat preterm lambs supported by invasive IMV with an IGF-1 receptor antagonist to prevent increased expression (mRNA and protein) of IGF-1 in the lung. Treatment is daily for 3 days, at which time the lungs are analyzed. The other experiment is to treat preterm lambs supported by non-invasive HFNV with an IGF-1 receptor agonist to increase expression (mRNA and protein) of IGF-1 in the lung. Treatment also is daily for 3 days, at which time the lungs are analyzed. Five preterm lambs have been done for each experiment, using optimized doses of each reagent.

Results of the two experiments indicate that IGF-1 causes alveolar simplification and poor respiratory gas exchange. That is, IGF-1 receptor antagonism during invasive IMV leads to better alveolar formation and respiratory gas exchange, even though the preterm lambs are supported by invasive IMV. Conversely, IGF-1 receptor agonism during non-invasive HFNV leads to alveolar simplification and poor respiratory gas exchange, even though the preterm lambs were supported by non-invasive HFNV.

My project will specifically focus on downstream molecular players that are normally triggered by IGF-1 signaling. The rationale for my project is that immunoblot, immunohistochemistry, and quantitative real time RT-PCR results indicate that invasive IMV upregulates proliferative genes and downregulates apoptotic genes in the lung of preterm lambs [6]. The opposite occurs in the lung during non-invasive HFNV [6]. Also, our new results show that a marker of cell proliferation, proliferating cell nuclear antigen (PCNA), is upregulated when IGF-1 ligand-receptor binding occurs. Other studies show that IGF-1 downstream signaling includes upregulation of JAK/STAT [7]. My project will measure protein abundance and mRNA levels of pro-proliferation genes in the lung of preterm lambs that are supported either by invasive IMV alone or non-invasive HFNV plus IGF-1 receptor agonist compared to preterm lambs that are supported by non-invasive HFNV alone or invasive IMV plus IGF-1 receptor antagonist.

Objective. The purpose for this proposed study is to quantify protein and mRNA levels of pro- and anti-proliferative genes in the lung of preterm lambs.

Hypothesis. Pro-proliferative protein and mRNA levels will be greater when IGF-1 signaling is triggered in the lung of preterm lambs. That is, more pro-proliferative protein and mRNA levels will be in the groups of preterm lambs that are supported either by invasive IMV alone or non-invasive HFNV non-invasive HFNV plus IGF-1 receptor agonist compared to non-invasive HFNV alone or invasive IMV plus IGF-1 receptor antagonist.

Design/Methods. Lung tissue will be used from four groups of preterm lambs. The groups are (1) invasive IMV along (bad lung outcomes), (2) non-invasive HFNV alone (good lung outcomes), (3) invasive IMV plus IGF-1 receptor antagonist (better outcome than invasive IMV alone), and (4) non-invasive HFNV plus IGF-1 receptor agonist (worse lung outcome than non-invasive HFNV). All of the preterm lamb studies are completed. The regimens of

the treatment compounds are optimized (n=4). My project will use standard methods for Dr. Scientist's laboratory to quantify levels of mRNA and protein, as well as localize the mRNAs and proteins in lung tissue sections [1,6]. The methods that will be used are quantitative real-time RT-PCR, immunoblot, in situ hybridization, and immunohistochemistry. Reagents in hand are for sheep IGF-1 mRNA and protein, as well as JAK/STAT3 ,TGF-β, c-Myc, Bax, p53 and caspase 3.

Expected Results. We expect to find more proliferative gene product levels in the lung of preterm lambs that are supported by invasive IMV alone or non-invasive HFNV non-invasive HFNV plus IGF-1 receptor agonist compared to non-invasive HFNV alone or invasive IMV plus IGF-1 receptor antagonist. If we get to the apoptotic gene candidates, we expect to find the opposite results.

Limitations. My project uses a candidate-gene approach. Justification is that we have supporting data for each of the candidate genes. However, an -omics approach is attractive, although its cost is prohibitive.

References

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