Introduction

• Bronchopulmonary dysplasia (BPD) is a multifactorial chronic lung disease of infants that is associated with significant mortality and morbidity.

• AMPK was originally identified as the key player in maintaining cellular energy homeostasis.

• AMPK regulates diverse metabolic and physiological processes and is dysregulated in major chronic diseases.

• There is strong evidence supporting the effect of AMPK on angiogenesis and reduction of lung inflammation in adults.

• The effect of AMPK signaling on the lung and heart function in neonatal hyperoxic injury remains unknown.

Hypothesis

• AMPKα signaling mitigates hyperoxia-induced experimental BPD in neonatal murine lungs.

Abstract

Bronchopulmonary dysplasia (BPD) is the most common chronic lung disease of preterm infants and hyperoxia is a major risk factor for this disease. Hypoxiopathologically, BPD is characterized by alveolar simplification (faster and fewer alveoli). Our studies showed that hyperoxia exposure increases lung adenosine monophosphate-activated protein kinase alpha (AMPKα) activation in neonatal mice. Whether this alteration is a compensatory or contributory phenomenon in hyperoxia-induced experimental BPD is unknown. Therefore, we hypothesized that lung AMPKα activation protects against hyperoxia-induced experimental BPD in neonatal mice.

Materials & Methods

A C57Bl/6J wild-type (WT) male and female mice pups were housed in air (21% FiO2, normoxia) or 70% O2 (hyperoxia) for 14 d while being injected intraperitoneally (i.p.) with the AMPK agonist, 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR), or the vehicle daily through postnatal days (PND) 1 to 14. Lung tissues were harvested on PND7 or PND14 to determine lung AMPKα activation in neonatal lungs, respectively. AMPKα activation was determined by immunoblotting, whereas alveolar development was evaluated by radial alveolar counts (RAC) and mean linear intercepts (MLI).

In Vitro Experimental Design

A C57Bl/6J wild-type (WT) male and female mice pups were housed in air (21% FiO2, normoxia) or 70% O2 (hyperoxia) for 14 d while they are injected intraperitoneally (i.p.) with the AMPK agonist, aminoimidazole-4-carboxamide ribonucleotide (AICAR), or the vehicle daily through postnatal days (PND) 1 to 14. Lung tissues were harvested on PND7 or PND14 to determine lung AMPKα activation in neonatal lungs, respectively. AMPKα activation was determined by immunoblotting, whereas alveolar development was evaluated by radial alveolar counts (RAC) and mean linear intercepts (MLI).

Results

• AMPKα signaling increases hyperoxia-induced experimental BPD in neonatal mice.

• There is strong evidence supporting the effect of AMPK on angiogenesis and reduction of lung inflammation in adults.

• The effect of AMPK signaling on the lung and heart function in neonatal hyperoxic injury remains unknown.

• AMPKα activation protects against hyperoxia-induced experimental BPD in neonatal mice.

Conclusions

• AMPKα signaling mitigates hyperoxia-induced experimental BPD in neonatal mice.

Future Directions

• Use of endothelial specific AMPKα knockout mice to demonstrate if AMPK signaling will be sufficient to lessen hyperoxia induced BPD.

• Determine the mechanistic role of endothelial AMPKα in lung development and injury.

• AMPK activation correlates inversely with angiogenesis (AGT) mRNA levels. Testing the interactions between these two molecules is unknown in neonatal lung.

References


• J. Omura et al., Protective Roles of Endothelial AMPK Activation: Interactions between Angiogenesis and Inflammatory Hyperoxia-Induced Pulmonary Hypertension in Mice. Circulation research 119, 179 (Jul, 2016).

Texas Pediatric Society Electronic Poster Contest

Protective Role of Adenosine Monophosphate-Activated Protein Kinase Alpha in Hyperoxia-Induced Experimental Bronchopulmonary Dysplasia

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Figure 1: Experimental design

Figure 2: Expression of phosphorylated (p) AMPKα (pAMPKα) (Thr172) in neonatal murine lungs. Neonatal WT mice were treated with normoxia or hyperoxia for 7 d and the lung protein was extracted to determine p-AMPKα (Thr172) protein expression using immunoblotting. p-AMPKα (Thr172) band intensities were quantified and normalized to β-actin band intensities. Data are expressed as mean ± SD (n=4-5/group). Significant differences between normoxia- and hyperoxia-exposed mice are indicated by *, p < 0.05 (t-test).

Figure 3: A. RADIAL ALVEOLAR COUNT AND B. MEAN LINEAR INTERCEPT IN WT MICE EXPOSED TO 70% O2 FOR 14 D. (A) VEHICLE TREATED, (B) AICAR TREATED

Figure 4: QUANTIFICATION OF ALVEOLARIZATION: A. RADIAL ALVEOLAR COUNT IN WT MICE EXPOSED TO NORMOXIA (21% O2) OR HYPEROXIA (70% O2) FOR 14 D. (A). VEHICLE TREATED, (B) AICAR TREATED

Figure 5: QUANTIFICATION OF ALVEOLARIZATION: A. RADIAL ALVEOLAR COUNT IN WT MICE EXPOSED TO NORMOXIA (21% O2) OR HYPEROXIA (70% O2) FOR 14 D. (A). VEHICLE TREATED, (B) AICAR TREATED

Figure 6: Quantiﬁcation of alveolarization: A. Radial alveolar count in wild type mice exposed to hyperoxia (70% O₂) for 14 d. (A). Vehicle treated, (B) AICAR treated

Figure 7: A. Hyperoxia increased AMPKα in fetal human pulmonary microvascular endothelial cells (HPMECs). B. AMPKα knockdown reduces hyperoxia increased AMPKα expression in HPMECs.