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

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BACKGROUND

- Bronchopulmonary dysplasia (BPD) is a chronic lung disease of infants that is associated with significant mortality and morbidity.
- Adenosine Monophosphate-Activated Protein Kinase (AMPK) was originally identified as the key player in maintaining cellular energy homeostasis.
- Additionally, AMPK regulates diverse metabolic and physiological processes and is dysregulated in major chronic diseases.
- There is strong evidence indicating that AMPK activation promotes angiogenesis and inhibits inflammation in the lungs of adult animals.
- However, the role of AMPK signaling in neonatal hyperoxic lung injury is not well understood.

HYPOTHESIS

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

MATERIALS AND METHODS

Neonatal C57BL/6J WT mice

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Vehicle | AMPK Agonist (AICAR)

Air for 14 days

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70% oxygen for 14 days

AMPKα Activation
•Immunoblotting Analysis
Alveolarization
•Lung Morphometry (Radial alveolar count & Mean linear intracepts)

Figure 1: Experimental design

RESULTS

Hyperoxia exposure increases AMPK-phosphorylation in neonatal WT mice

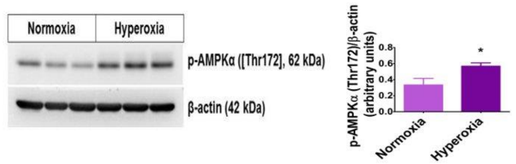


Figure 2: Expression of phosphorylated (p) AMPKα (pAMPKα [Thr172]) in neonatal murine lungs: Neonatal WT mice were exposed to normoxia or hyperoxia for 7 d and the lung protein was extracted to determine p-AMPKα (Thr172) protein expression using immunoblotting. p-AMPKα (Thr172) band intensities were quantified and normalized to β-actin band intensities. Data are expressed as mean ± SD (n=6/group). Significant differences between normoxia- and hyperoxia-exposed mice are indicated by *, p < 0.05 (t-test).

AICAR activates and Compound C inhibits AMPKα in neonatal murine lungs

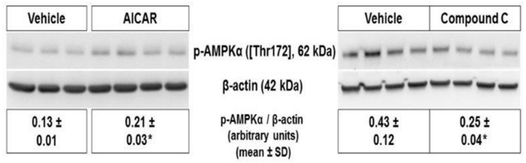
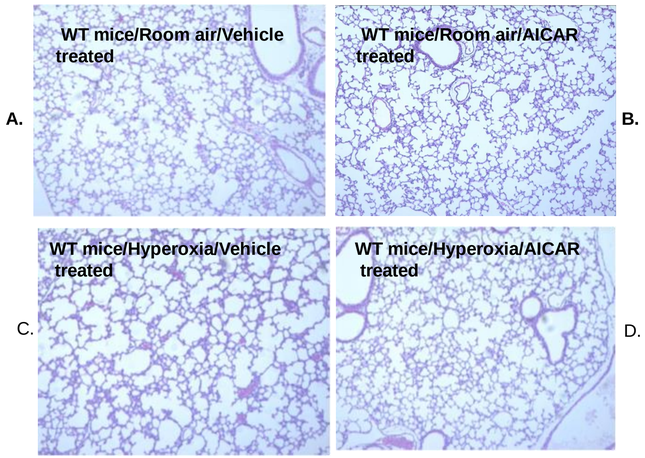


Figure 3: AICAR activates and Compound C inhibits AMPKα in neonatal murine lungs: Neonatal WT mice were injected i.p. with 1 mg/kg of AICAR (AMPK agonist), 25 mg/kg of compound c (AMPK inhibitor) or the vehicle daily for 7 d, and the lung p-AMPKα (Thr172) protein expression was determined using immunoblotting. p-AMPKα (Thr172) band intensities were quantified and normalized to β-actin band intensities. Data are expressed as mean ± SD (n=3-4/group). Significant differences between treatment groups are indicated by *, p < 0.05 (t-test).

AMPK Activation Attenuates Hyperoxia-Induced Alveolar Simplification in WT Mice



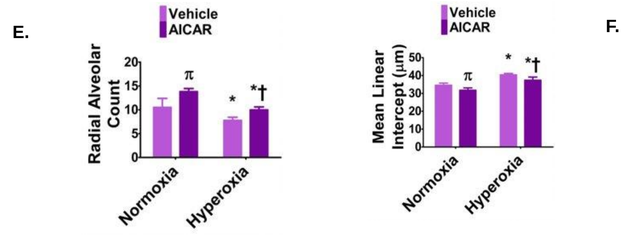


Figure 4:AMPK activation decreased hyperoxia induced alveolar simplicfication: Neonatal wild-type mice were injected i.p. with vehicle or 1 mg/kg of AICAR daily while being exposed to normoxia or hyperoxia (70 % O_2) for 14 d and the lung tissues were collected on PND14 for lung morphometry. A-D. Representative hematoxylin and eosin-stained lung sections from vehicle-treated (A and C) and AICAR-treated (B and D) mice exposed to normoxia (A and B) or hyperoxia (C and D). E-F. Alveolarization was quantified by determining RAC (E) and MLI (F). Data are expressed as mean ± SD (n=4-5/group). Significant differences between normoxia- and hyperoxia-exposed mice are indicated by *, p < 0.05. Significant differences between hyperoxia-exposed mice are indicated by †, p < 0.05. Significant differences between normoxia-exposed mice are indicated by π, p < 0.05 (ANOVA).

Hyperoxia Increases AMPKα in Neonatal Human Pulmonary Microvascular Endothelial cells (HPMECs)

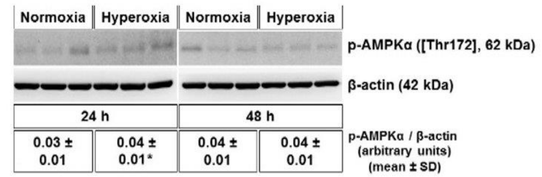


Figure 5: Expression of phosphorylated (p) AMPKα (p-AMPKα [Thr172]) in neonatal HPMECs: Neonatal HPMECs were exposed to normoxia or hyperoxia for 48 h and the lung protein was extracted to determine p-AMPKα (Thr172) protein expression using immunoblotting. Data are expressed as mean ± SD. Significant differences between normoxia- and hyperoxia-exposed mice are indicated by *, p < 0.05 (ANOVA).

CONCLUSIONS

- AMPKα activation mitigates hyperoxia-induced experimental BPD in neonatal mice.

FUTURE DIRECTIONS

- Use of endothelial specific AMPKα knockout mice to demonstrate if AMPKα activation will be sufficient to decrease hyperoxia induced BPD.
- Determine the mechanisms by which endothelial AMPKα modulates lung development and injury.
- Determine the interaction between angiotensinogen (AGT) and AMPKα in neonatal lung injury.

ACKNOWLEDGEMENT

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