CHRD 2022: Abstract & Poster Submission Form

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Presenter Status

○ Undergraduate Students

○ Masters Student

◉ PhD Student

○ Post-Doctoral Fellows

○ Residents

○ Non-Trainee

Research Category

◉ Basic Science

○ Clinical

○ Community Health / Policy

Role in the project

☑ Design

☑ Perform Experiments

☑ Analyze Data

☑ Write Abstract

☐

Title

Cysteine Nitrosylation of Adenylyl Cyclase Isoform 6 and Forskolin Rescue in Hypoxic Pulmonary Hypertension of the Newborn

Background

Persistent pulmonary hypertension of the newborn (PPHN) is a catastrophic respiratory disease featuring hypoxemia and pulmonary vasoconstriction, causing 10-30% mortality. Therapies include nitric oxide (NO). The adenylyl cyclase (AC) signaling pathway causes vascular relaxation; we previously reported that activity of the dominant pulmonary arterial AC isoform, AC6, is inhibited after hypoxia in vivo or in vitro. By mutational analysis of AC6 cysteines, we identified C1004, found at the AC6-Gαs interface, is crucial for initiating AC6 activation. We inferred that C1004 may be required for Gαs to activate AC6. In this study we hypothesized that both hypoxia and NO promote cysteine thiol nitrosylation of AC6, decreasing its activity and impairing cAMP production, independent of its ability to be allosterically activated by forskolin. We propose that nitrosylation of C1004 under hypoxic conditions could prevent activation of AC6 by Gαs.

Objective

To determine which cysteine residue in AC6 is nitrosylated under hypoxic conditions

Methods

HEK cells stably overexpressing individual AC isoforms (AC 3, 5, 6, 7, 9) as well as cysteine-to-alanine mutants AC6C1004, AC6C1145 or AC6C447 were cultured in a normoxic (21% O2) or hypoxic (10% O2) incubator for 72 hours, with or without nitroso-donor S-nitrosocysteine (CysNO, 250μM) for 30 mins. Cells from all treatment groups were lysed for measurement of AC catalytic activity and detection of protein S-nitrosylation.

Results

C1004 is a conserved residue among AC isoforms. However, among the five AC isoforms studied, only AC6 is inhibited by hypoxia, leading to impaired cAMP production. Inhibition of AC6 in hypoxia is correlated with increased cysteine nitrosylation. The inhibited AC6 can be partially activated by forskolin.

Conclusion

We conclude that protein nitrosylation occurs in hypoxia, which uniquely inhibits activity of AC6. Forskolin partially reactivates AC6 activity and restores cAMP generation. These findings encourage the design of targeted forskolin derivatives that selectively activate AC6.

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