Ask1 Inhibition Prevented Mitogen-Induced Human Airway Smooth Muscle Growth In Chronic Obstructive Pulmonary Disease

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Rationale: Chronic obstructive pulmonary disease (COPD) is the third leading cause of death worldwide and poses enormous economic and social burden. While there is no cure for COPD and the existing medication does not affect disease progression. COPD pathogenesis involves structural changes in the lung collectively known as airway remodeling. Increased airway smooth muscle (ASM) mass and ASM thickness is part of overall structural changes observed in COPD, and is correlated with severity of the disease and has been found to negatively impact lung function. Thus there is clear unmet clinical need for finding new therapies for COPD which can target airway remodeling and disease progression. Apoptosis signal-regulating kinase 1 (ASK1) is a ubiquitously expressed MAPK kinase kinase (MAP3K), activated by various stress stimuli, including ROS, TNF-α, and LPS. ASK1 affects multiple cellular functions, including cell survival, differentiation, and the innate immune response and has been reported to be involved in the pathogenesis of various human diseases. However, the role of ASK1 in airway remodeling is not established. In this study we aimed to determine the effects of ASK1 inhibition on ASM growth and pro-mitogenic signaling using ASM cells from well-established COPD patients.

Methods and Results: It is known that ASM cells from COPD patients have greater proliferative capacity to variety of mitogens *in vitro*. We used human lung tissue samples and primary human ASM cells obtained from COPD patients and healthy controls. Western blotting studies demonstrated expression of ASK1 in human lung tissues and primary human ASM cells. Pre-treatment of human ASM cells with highly selective (IC_{50}:14 nM) and orally available ASK1 inhibitor; TCASK10 resulted in a dose-dependent reduction in mitogen (FBS, 10%; PDGF and EGF; 10 ng/ml, 72 hours)-induced ASM growth as measured by CyQuant assay. Furthermore, the role of ASK1 in the regulation of ASM growth was established using gene-silencing experiments wherein ASK1 siRNA prevented mitogen-induced human ASM cell growth. Immunoblotting revealed that the anti-mitogenic effect of ASK1 inhibition or silencing is mediated by JNK and p38 MAP kinase-signaling pathways evident by reduced phosphorylation of downstream effectors JNK1/2 and p38MAP kinase respectively with no effect on ERK1/2 signaling.

Conclusions: Collectively, these findings establish the anti-mitogenic effect of ASK1 inhibition and identify a novel pathway that can be targeted to reduce or prevent excessive ASM mass in COPD.

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