Free Fatty Acid Elovanoid Precursors Modulate Allergen-induced NLRP10 Inflammasome Expression in Human Nasal Epithelial Cells

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Introduction

House Dust Mites (HDM)
• One of the most common indoor allergens
• Comprised of 2 allergens in dry areas: Dermatophagoides pteronyssinus (D. pteron)
• Allergens responsible for allergic rhinitis, allergic asthma, and airway obstruction

NLRP10
• Nucleotide oligomerization domain (NOD)-like receptor protein that lacks a leucine-rich repeat domain like other NOD-like receptors
• Pro-inflammatory protein that forms an inflammasome complex
• Inflammasome complex leads to activation of other proteins such as pro tease caspase-1 which leads to maturation of inflammatory cytokine IL-1β

Elovanoids (ELVs)
• Pro-homeostatic lipid mediators that provide cells protection from damage
• ELVs have shown potential therapeutic benefits in experimental models of Alzheimer’s disease, age-related macular degeneration, stroke, and allergic rhinitis
• Derived from free omega-3 very-long-chain polyunsaturated fatty acids (VLC PUFA)

Methods

1. Human Nasal Epithelial Cells
• Cells are grown in 6-well plates to 80% confluent in 2mL of Airway Epithelial Cell Growth Medium
• siRNA is added 12 days after growth for NLRP10 silencing at 0.1nM (final concentration)
• Inducers are added the next day after gene silencing or after 12 days of growth without silencing at 30µg/mL
• 30 minutes after adding the inducers, 32:6 and 34:6 FFA are added at 500µM (final concentration)
• Cells are collected 1-day post-treatment

2. Western Blot plate loading
• 3µL (1µg) of cell sample added to a 230-12 kDa plate (Row A)
• 10µL (150° diluted) of 1° antibody added under respective cell sample (Row B)
• 10µL (neat) of 2° antibody added to match the animal that produced the 1° antibody (mouse, rabbit, or goat) (Row C)
• Remaining wells are loaded following the Jess immunoassay plus total protein with RePlex™ guidelines

3. Jess ProteinSimple Western Blot
• Plate is put into the Jess machine allowed to run following the computer program
• Protein expressions are collected and quantified

Results - NLRP10 Silencing

Figure 2: Experimental timeline for cells with no gene silencing

Figure 3: Experimental timeline for cells with siRNA that silences NLRP10 or scrambled RNA

Results - NLRP10

Figure 4: Stepwise procedure to collect protein expression data

Results - Caspase-1 & IL-18

Figure 5: Quantitative results showing NLRP10/Total protein expression for all samples

Figure 6: (a) Western Blot lanes using an NLRP10 1° antibody (lanes 2-9) and an IL-18 1° antibody (lanes 10-17) in HDM and NLRP10 silenced samples.
(b) Quantitative results showing NLRP10/Total protein expression for samples using an NLRP10 1° antibody.
(c) Quantitative results showing IL-18/Total protein expression for samples using an IL-18 1° antibody.

Conclusions and References

Conclusions
1. HDM, D. fari, or D. pteron upregulate expression of NLRP10 inflammasome, leading to upregulation of caspase-1 and IL-18 in HNEpC. HDM was superior to D. fari, and D. pteron alone.
2. The ELV precursors 32:6 and 34:6 FFA can offer HNEpC protection from inflammation through downregulating the expressions of NLRP10, caspase-1, and IL-18.
3. Silencing NLRP10 through an NLRP10 specific siRNA results in a decrease in IL-18 production controls and in HDM challenged cells.
4. These findings might open avenues to potential therapeutic exploration for allergies and asthma.

References

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