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

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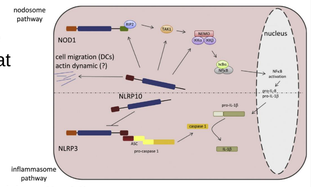
Introduction

House Dust Mites (HDM)

- One of the most common indoor allergens
- Comprised of 2 allergens in dry areas
  - *Dermatophagoides pteronyssinus* (*D. ptero*)
  - *Dermatophagoides farinae* (*D. fari*)
- 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 protease caspase-1 which leads to maturation of inflammatory cytokine IL-18



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) 32:6 or 34:6 by the enzyme ELOVL4

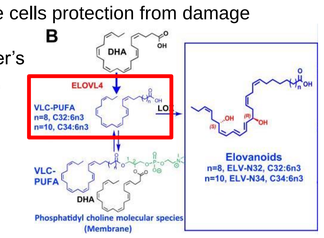


Figure 1: Biosynthesis of ELVs and precursors stemming from docosahexaenoic acid (DHA)

Objectives

Hypothesis: Do elovanoid precursors attenuate signaling responses of allergens? If so, which is the molecular mechanism?

Specific aims:

1. **Do HDM allergens affect the expression of the NLRP10 inflammasome and its related proteins?**
2. **Do ELV precursors 32:6 and 34:6 free fatty acids (FFAs) modulate the protein expression?**
3. **Does NLRP10 expression lead to IL-18 activation?**

Timelines

Non-silenced HNEpC growth timeline

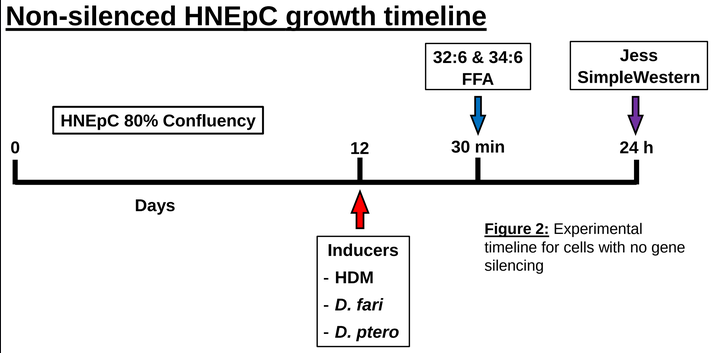


Figure 2: Experimental timeline for cells with no gene silencing

NLRP10-silenced HNEpC growth timeline

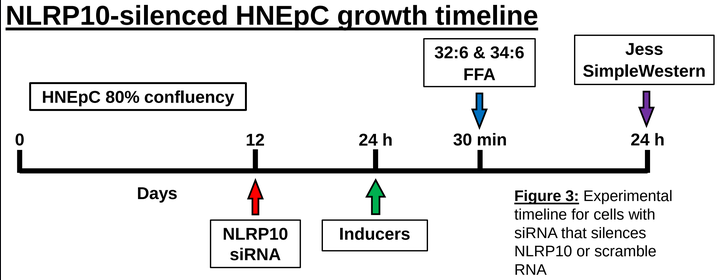
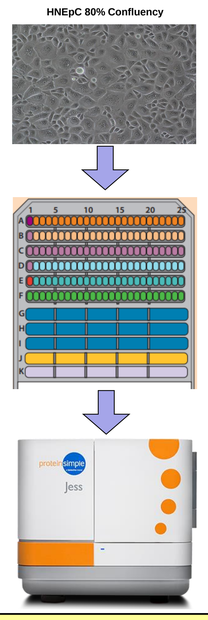


Figure 3: Experimental timeline for cells with siRNA that silences NLRP10 or scramble RNA

Methods



1. Human Nasal Epithelial Cells

- Cells are grown in 6-well plates to 80% confluency 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 500nM (final concentration)
- Cells are collected 1-day post-treatment

2. Western Blot plate loading

- 3µL (1.5µg) of cell sample added to a 230-12 kDa plate (Row A)
- 10µL (1/50th diluted) of 1° antibody added under respective cell sample (Row D)
- 10µL (neat) of 2° antibody added to match the animal that produced the 1° antibody (mouse, rabbit, or goat) (Row E)
- Remaining wells are loaded following the Jess immunoassay + 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

Figure 4: Stepwise procedure to collect protein expression data

Results- NLRP10

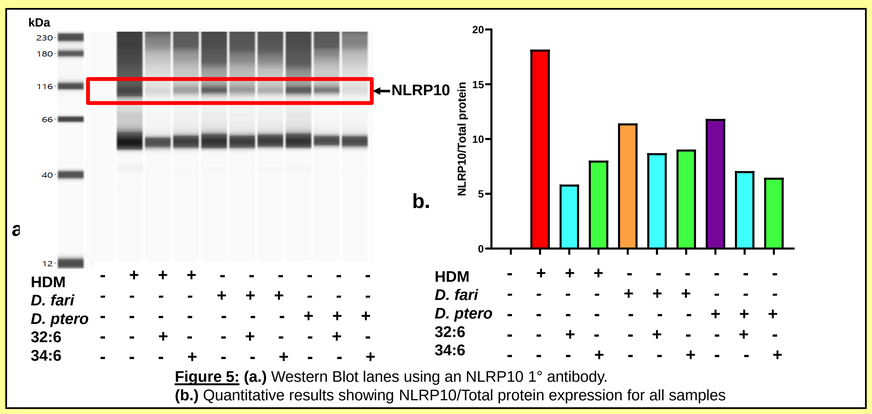


Figure 5: **(a.)** Western Blot lanes using an NLRP10 1° antibody.
(b.) Quantitative results showing NLRP10/Total protein expression for all samples

Results- Caspase-1 & IL-18

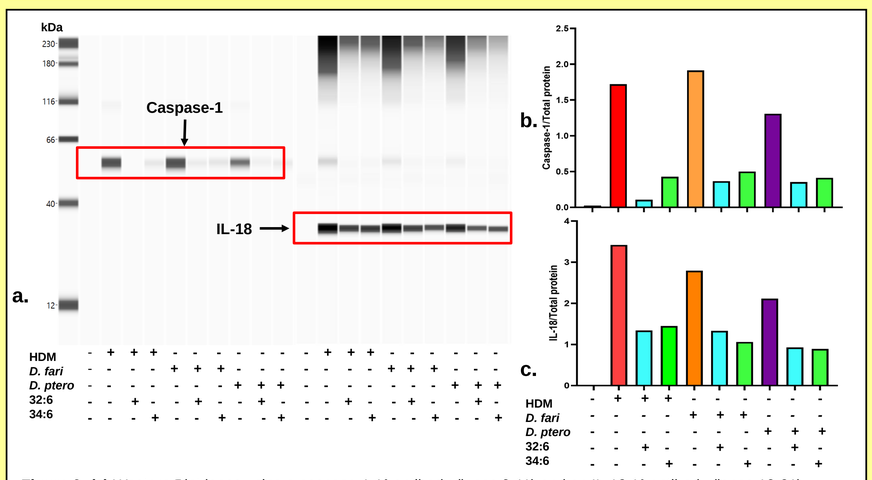


Figure 6: **(a)** Western Blot lanes using a caspase-1 1° antibody (lanes 2-11) and an IL-18 1° antibody (lanes 12-21).
(b) Quantitative results showing caspase-1/Total protein expression for samples using a caspase-1 1° antibody
(c) Quantitative results showing IL-18/Total protein expression for samples using an IL-18 1° antibody

Results- NLRP10 Silencing

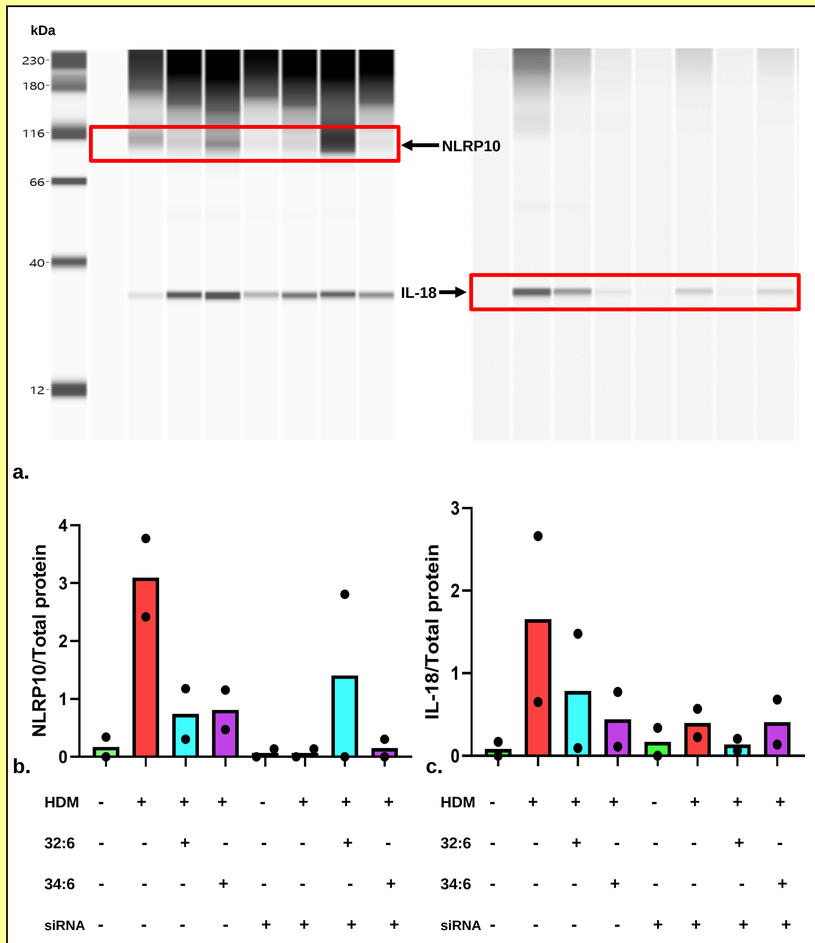


Figure 7: **(a.)** Western Blot lanes using an NLRP10 1° antibody (lanes 2-9) and an IL-18 1° antibody (lanes 10-17) in control and NLRP10 silenced samples.
(b.) Quantitative results showing NLRP10/Total protein expression for samples using an NLRP10 1° antibody.
(c.) Quantitative results showing Il-18/Total protein expression for samples using an IL-18 1° antibody.

Conclusions and References

Conclusions

1. HDM, *D. fari*, or *D. ptero* upregulate expression of NLRP10 inflammasome, leading to upregulation of caspase-1 and IL-18 in HNEpC. HDM was superior to *D. fari*, and *D. ptero* alone.
2. The ELV precursors 32:6 and 34:6 FFA can offer HNEpC protection from inflammation though 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 in 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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