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Degradation of Airway Secretory Cell Mucin Granules Is Dependent on Lysosome Activity

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Summer Undergraduate Research Program

Degradation of Airway Secretory Cell Mucin Granules Is Dependent on Lysosome Activity

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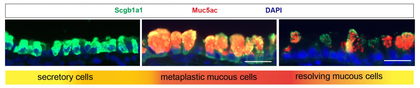
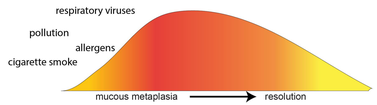
Background and hypothesis

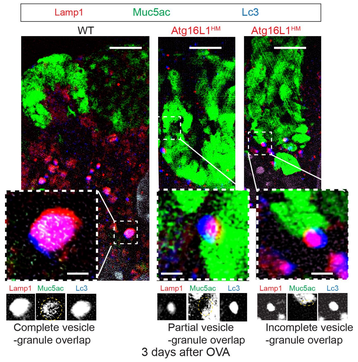
- Inflammatory airway diseases such as COPD and asthma are associated with expansion of secretory cell populations through mucous cell metaplasia and mucin hypersecretion, causing airway obstruction and symptoms of cough and shortness of breath.
- How secretory cells remove excess mucin granules during resolution of mucous metaplasia is not well understood.



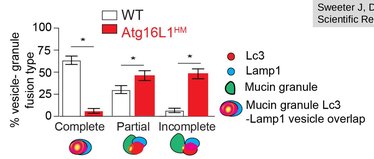


- **Hypothesis:** Elimination of excess mucin granules depends on direct lysosome-mediated degradation.

Mucin granules fuse with Lamp1 labeled lysosome structures in secretory cells in an Atg16L1-dependent manner

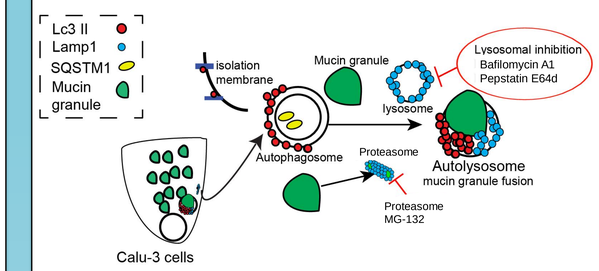


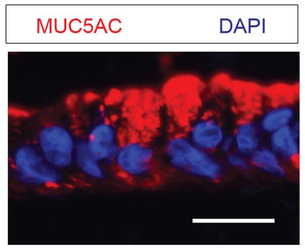
Sweeter J, Dickinson JD, et al Scientific Reports 2021



Representative SR-SIM images from WT and Atg16L1HM mouse airways at day 3 of resolution from OVA-mediated mucous metaplasia with LC3 (blue), Lamp1 (red), and Muc5ac (green) detected by lectin UEA-1 (green). Scale bar = 5 microns. Inserts show each magnified image of fusion event and have scale bar = 1 micron. Individual channels are shown below insert with gold dashed circle showing area of overlap. Quantification of fusion events among Lc3 and Lamp1 vesicles and mucin granules shown in graph: N= 3 Atg16L1HM mice with 26 distinct secretory cells and 81 fusion events, N=5 WT mice with 24 distinct secretory cells with 104 fusion events. Significant difference denoted by * using unpaired Student T-test for fusion events between WT and Atg16L1HM mice.

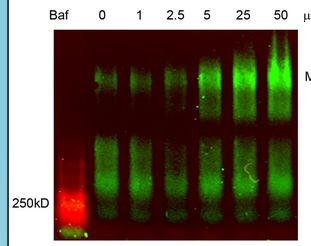
Potential pathways for mucin granule degradation

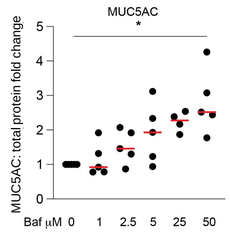




Calu-3 model cell line

Inhibition of lysosome acidity increases secretory mucin, MUC5AC, levels

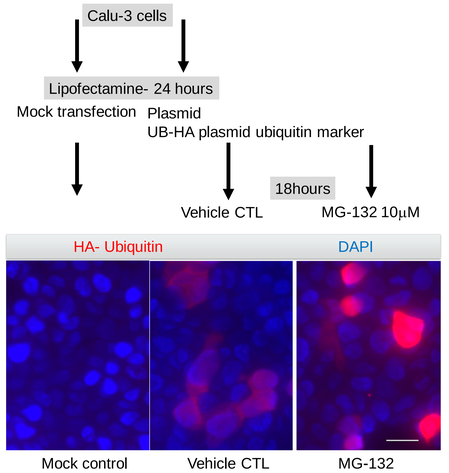


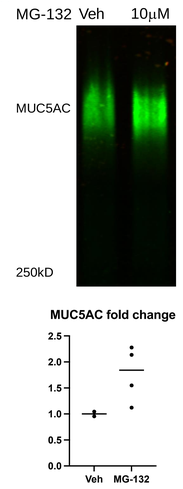


Bafilomycin A1 dose response

Calu-3 cells were treated with Baf in a dose-dependent manner for 18 hours. Cell lysates were collected for immunoblotting for secretory mucin, MUC5AC. Lysates were homogenized without detergent, underwent electrophoresis on 0.8% agarose gel and were transferred to nitrocellulose membrane for antibody detection. Significant difference denoted by * using Kruskal Wallis with Dunn test for multiple comparisons, with each comparison to vehicle control. N=5 per condition

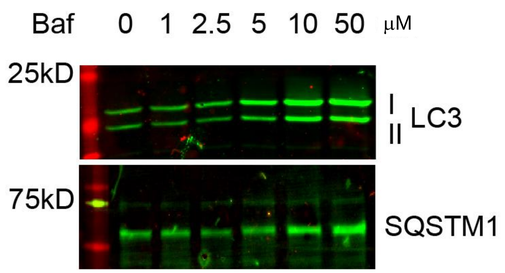
Inhibition of proteosome function





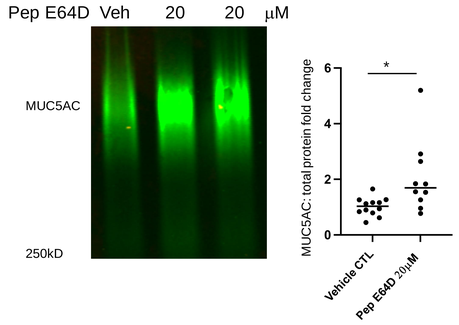
Calu-3 cells were transfected with lipofectamine-3000 +/- 3.5 μg of plasmid. After 24hr, media was changed and cells were treated with +/- MG-132 10 μM or vehicle for 18hr. Cells were harvested for mucin blot for MUCAC. A second group was fixed with 4% PFA for 10 minutes. After washing, cells were blocked with 5% donkey serum and stained for Hemagglutinin (HA).

Inhibition of lysosome function increases autophagosome markers LC3-II and SQSTM1



Bafilomycin A1 (Baf) inhibits lysosome function by blocking vacuolar-type H+-ATPase on the lumen of the lysosome to raise pH and reduce lysosome function. Calu-3 cells were treated with Baf in a dose-dependent manner for 18 hours. Cells were collected for immunoblotting for autophagosome proteins, LC3, and SQSTM1.

Inhibition of lysosome enzyme function increases secretory mucin, MUC5AC, levels



Pepstatin E64D (Pep E64) inhibits lysosome enzyme function (namely Cathepsin B function.) Calu-3 cells were treated with 20μM Pep E64 for 6 hours. Cells were then collected for immunoblotting for secretory mucin, MUC5AC. Significant difference denoted by * using Mann-Whitney N=10 and 12 per respective condition.

Conclusion

- Mucin granules fuse with Lamp1+ and LC3+ vesicles in an Atg16L1-dependent fashion.
- Inhibition of lysosome function increases secretory mucin, MUC5AC levels.
- Inhibition of proteasome function with MG-132 was inconclusive. May increase MUC5AC levels. MG-132 may have some off-target effects that are non-specific.

Future Directions

- Need to optimize transfection conditions to ensure MG-132 is inhibiting proteasome.
- Investigate whether mucin granules are ubiquitinated for degradation in the proteasome.
- Determine if mucin granules can be degraded directly to the lysosome, independent of autophagy-related proteins.

Funding Acknowledgment

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