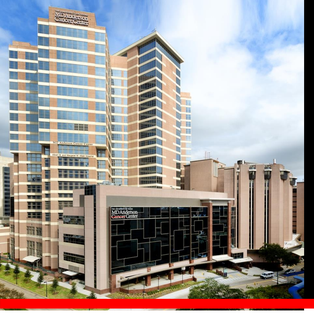

Golgi Apparatus in Airway Secretory Cells

Colin E. Chan, Oanh N. Hoang, Michael J. Tuvim and Burton F. Dickey

Department of Pulmonary Medicine, University of Texas MD Anderson Cancer Center

THE UNIVERSITY OF TEXAS MD Anderson Cancer Center

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Rationale

Mucus plays an important role in trapping foreign pathogens in the respiratory system. Mucus is formed through hydration of large, heavily glycosylated proteins called mucins, such as MUC5AC and MUC5B, along with other solutes such as salts. Mucin overproduction plays a key role in muco-obstructive diseases such as COPD. Preliminary data show that Golgi in airway epithelial cells appear dispersed.[1] The Golgi stacks which appears separate from the classical perinuclear ribbon are called outposts. We sought to elucidate how mucin is trafficked within the cell and packaged in the Golgi apparatus using immunofluorescence (IF) and electron microscopy (EM). Understanding mucin trafficking could offer therapeutic targets in the future to halt deleterious mucus production.

Methods

Naïve and IL-13 treated mouse lungs were studied using IF and EM. IL-13 was used to increase mucin production.

Immunofluorescence: Mouse and human lungs were fixed in 4% paraformaldehyde and paraffin embedded. Tissue samples were cut perpendicular to major airways in 5 µm sections and stained with Golgi antibodies. Images were taken with the Deltavision Deconvolution microscope.

Electron Microscopy: Mice lungs were harvested and fixed in 2.5% glutaraldehyde with post-fixation in OsO_4. The lung was then sectioned with a transverse cut and embedded in epoxy resin. Sections of 100nm were viewed on a Tecnai 12 transmission EM.

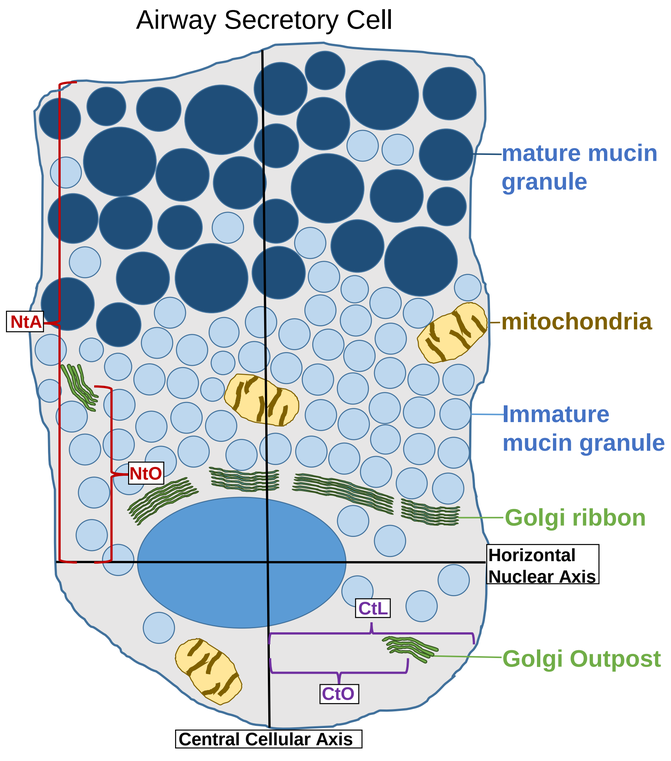

Fig 1. Representation of metaplastic airway secretory cell with quantitative parameters. Cartoon shows typical mouse airway secretory cell with classical perinuclear Golgi ribbon and two examples of dispersed Golgi outposts. Quantitative parameters include nucleus to apex (NtA), nucleus to outpost (NtO), central to lateral (CtL), and central to outpost (CtO).

Electron Microscope Data

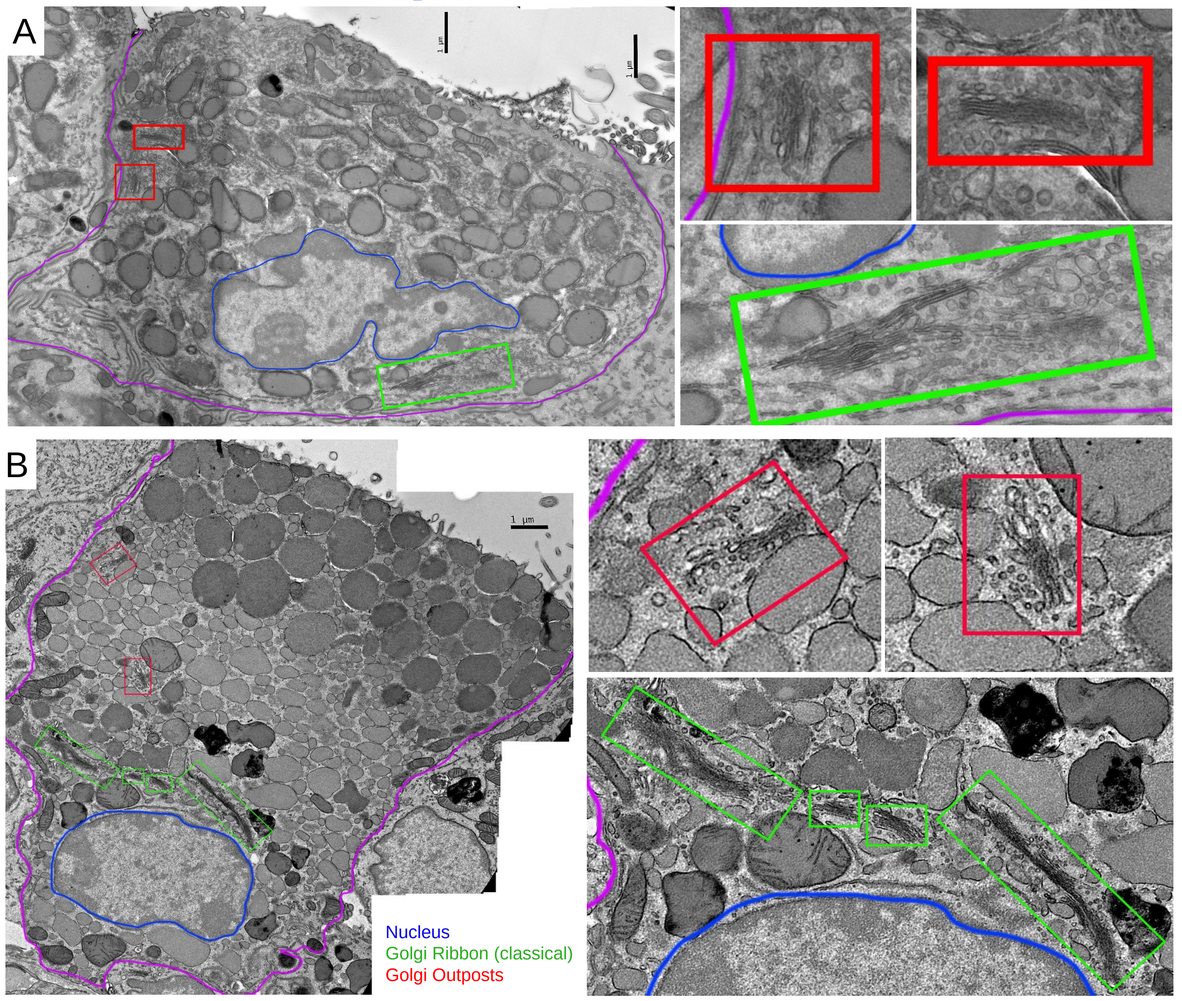

Fig 2. Electron microscopy of naïve and metaplastic mouse airway secretory cells with Golgi ribbon and outposts. (A) Naïve airway secretory cell shows the nucleus outlined in blue, the Golgi ribbon outlined in green, and two individually Golgi outposts outlined in red. (B) Metaplastic airway secretory cell is similarly annotated.

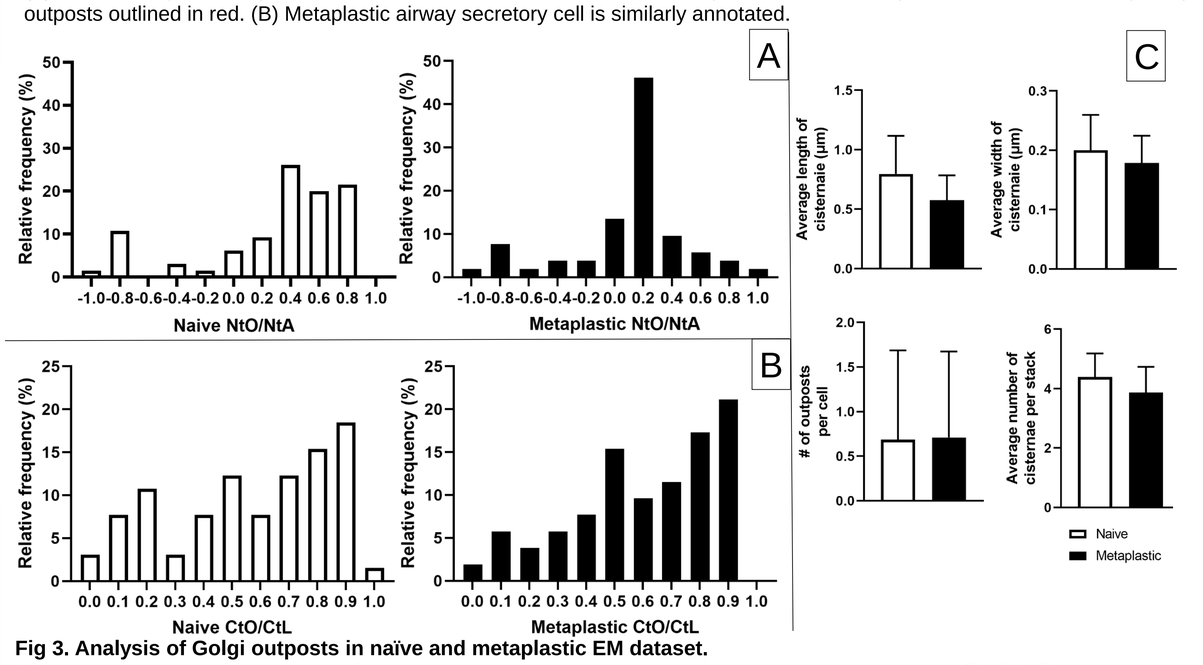

Fig 3. Analysis of Golgi outposts in naïve and metaplastic EM dataset. (A) Naive and metaplastic data of NtO/NtA as a measure of the basal to apical distribution of outposts. (B) CtO/CtL as a measure of central to lateral distribution of outposts. (C) Comparison of length, width of Golgi outposts along with the number of outposts per cell and the number of cisternae per stack in naïve and metaplastic dataset. Naïve cells, n = 95. Metaplastic cells, n = 76.

Immunofluorescent Data

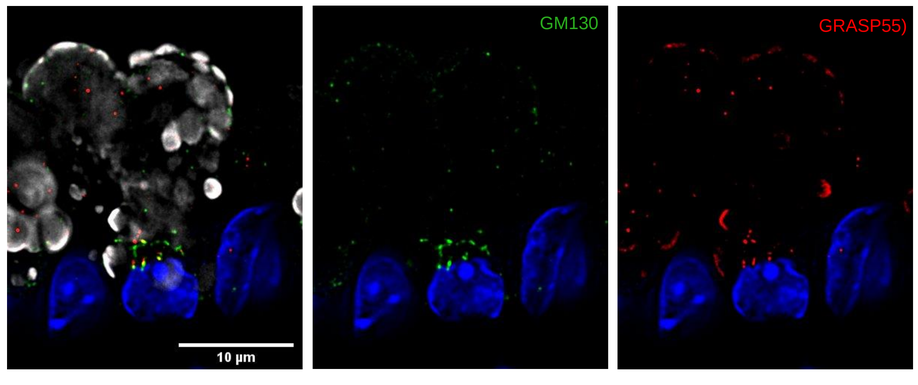

Fig 4. IF of metaplastic mouse with cis and trans Golgi markers. Metaplastic mouse airway stained with antibodies for cis Golgi (GM130), trans Golgi (GRASP55), club cell secretory protein (CCSP) and DAPI. Images show condensed Golgi stacks near the nucleus along with scattered outposts. CCSP differentiates secretory from ciliated cells.

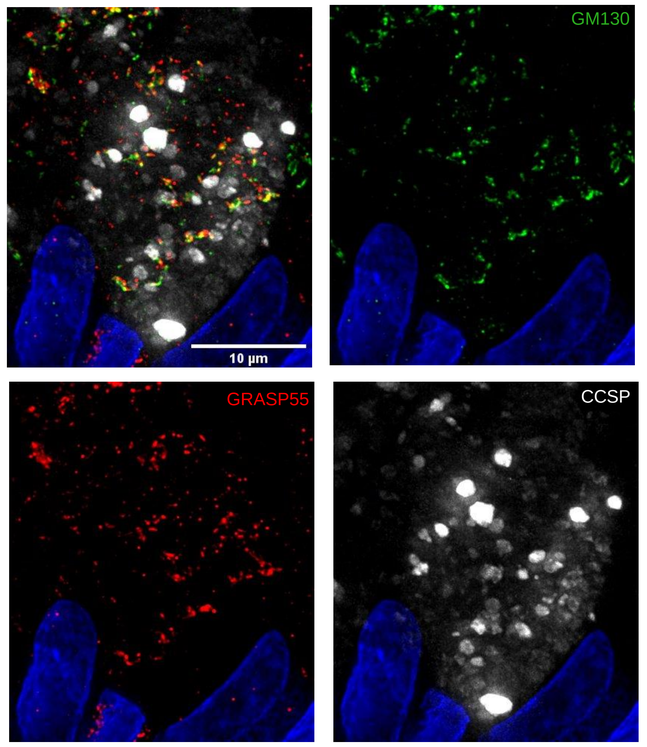

Fig 5. IF of human lung tissue with cis and trans Golgi markers. Human lung tissue stained with antibodies for cis Golgi (GM130), trans Golgi (GRASP55), club cell secretory protein (CCSP) and DAPI. Notable differences between human and mouse lung samples are the aspect ratio, with the human tissue having much taller cells. Additionally, there are much more uniformly and disparately distributed cis and trans markers.

Conclusions

Within airway secretory cells, the Golgi apparatus show a different organization compared to the classical perinuclear Golgi. For one, the perinuclear Golgi ribbon in secretory cells is extremely long, and secondly, the Golgi appears to stretch farther out towards the apex of the cell and along the lateral cell walls. In addition, evidence of small individual cisternae stacks in EM along with distinct punctate in IF imply the presence of several Golgi outposts throughout the cell. These observations of a unique Golgi apparatus architecture can suggest many possible things. One of which could be that the need for a high volume of glycosylated protein in the form of mucin would dictate the need for a larger and more robust Golgi machinery. Another possibility is that the large mucin protein (>10MDa) would mean trafficking mucin throughout the cell is impractical, thus requiring the need to synthesize and glycosylate the mucins in place before exocytosis.

References

1) Tuvim, Michael J et al. “Synaptotagmin 2 couples mucin granule exocytosis to Ca2+ signaling from endoplasmic reticulum.” *JBC* vol. 284,15 (2009): 9781-7.