Metabolic Labeling of Mucin-Induced Extracellular Vesicles Isolated from Suspension-Adapted Cell Culture

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Primary CNF Tools Used: Malvern NS300 Nanosight

Abstract:

Extracellular vesicles (EVs) are lipid-membrane-bound secreted nanoparticles which transport DNA, RNA, and proteins between cells and therefore have great potential as tools for disease diagnosis and treatment. The significance of the glycocalyx in EV biogenesis and function is largely unexplored, and the capacity to effect EV production and properties through rational manipulation of the glycocalyx remains poorly understood. We have previously demonstrated that overexpressing the transmembrane mucin glycoprotein Muc1 in the glycocalyx drives EV secretion in adherent MCF10A cells. Here, we expand these findings to suspension-adapted HEK293F cells and utilize metabolic labeling of inherent EV Muc1 biopolymer coatings as a proof-of-concept for engineering EVs with designed molecular payloads.

Summary of Research:

Extracellular vesicles (EVs) have rapidly garnered attention in biomedical engineering research for their ability to transport DNA, RNA and proteins, making them promising candidates as tools for disease diagnosis and treatment. The glycocalyx is a polymer meshwork of proteins, nucleic acids, and glycans which governs numerous intercellular interactions, but its role in regulating EV biogenesis and function remains poorly understood. It has been previously shown that engineering the glycocalyx via the overexpression of mucin can result in membrane morphologies which are favorable for the formation of EVs1. This report summarizes research from the last year characterizing "mucin-induced" EVs isolated from suspension-adapted cell culture and demonstrating the efficacy of bio-orthogonal labeling of EV mucin coatings as a strategy for engineering EV payloads. HEK293F cells were genetically engineered to express tetracycline-inducible Muc1 biopolymer. To induce Muc1 overexpression, cells were treated with 1 ug/mL doxycycline (dox) for 24 h.

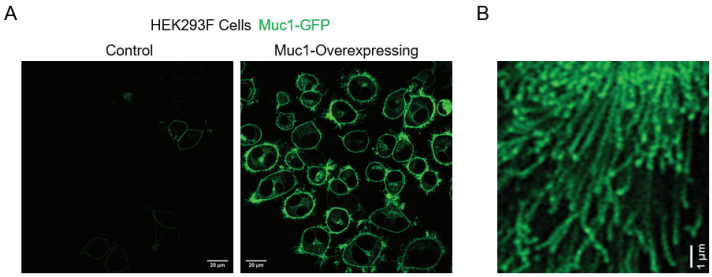

Figure 1: Induced expression of Muc1 biopolymer at the surface of HEK293F cells. A) Confocal fluorescence microscopy images of inducible Muc1-GFP expressed in engineered HEK293F cells. Cells samples were seeded onto poly-L-lysine coated glass-bottom dishes. Non-induced cells (Left) exhibit low leaky expression, while induced cells (Right) exhibit dramatic cell-surface Muc1 expression. Scale bar is 20 μm for both images. B) Pearled cell membrane tubules were observed on the surface of Muc1-overexpressing HEK293F cells. Individual pearls appear to be approximately 200-300 nm in diameter, consistent with structures observed in similarly engineered MCF10A cells. Scale bar is 1 μm.

Additionally, N-azidoacetylgalactosamine (GalNAz) was added to HEK293F culture media at a final concentration of 50 μM at the same time as dox induction. The cells were further incubated at 37 □, 5% CO2 for 2 d. EV-containing media was then harvested, and the EVs were isolated by PEG-enrichment according to an existing protocol2. EVs from non-induced cells and EVs from induced cells lacking GalNAz were used as negative controls. EV particle size and concentration were measured by nanoparticle tracking analysis (NTA) using the Malvern NS300 Nanosight.

Expression of cell-surface Muc1 biopolymer in induced HEK293F cells was verified by confocal fluorescence microscopy (Fig 1A). Notably, pearling membrane structures were observed similar to those previously reported on similarly engineered MCF10A cells, suggesting that cell-surface Muc1 could have a similar effect on EV secretion in different cell types (Fig 1B). Indeed, EV secretion was dramatically increased in Muc1-overexpressing HEK293F

cells compared to non-induced control (Figure 2A). Additionally, Muc1 expression resulted in a modest increase in median EV hydrodynamic diameter, consistent with Muc1 biopolymer coatings on the surfaces of mucin-induced EVs (Figure 2B). Metabolic incorporation of GalNAz into the glycans of EV Muc1 biopolymer coatings was confirmed by Western blot. Briefly, mucin-induced EVs from HEK293F cells with or without GalNAz incorporation were treated with DBCO-AzDye 568 dye (Click Chemistry Tools), allowing for bio-orthogonal click conjugation of fluorescent reporter to EVs containing GalNAz in their Muc1 surface coatings. Lysates from metabolically labeled and click-conjugated EVs were run on 7% Tris-Acetate SDS-PAGE gels. Western blot confirmed the presence of Muc1 in mucin-induced EV lysates, and further demonstrated successful bio- orthogonal conjugation of fluorescent dye specific to the EVs from HEK293F cells treated with GalNAz (Figure 3).

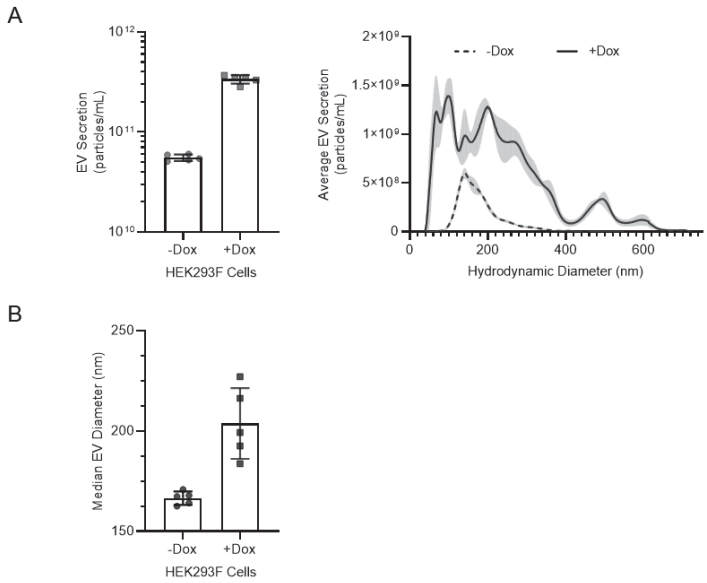

Figure 2: Nanoparticle tracking analysis of mucin-induced EVs isolated from HEK293F cells. A) Comparison of EV secretion from HEK293F cells with low (-Dox) and high (+Dox) Muc1 expression. Bar plot (Left) depicts the average +/- SD from 5 technical replicates. Histogram (Right) shows the average secretion +/- SEM from 5 technical replicates for vesicles ranging in size from 0 to 750 nm. B) Comparison of median hydrodynamic diameter (HDD) of EVs from HEK293F cells with low (-Dox) or high (+Dox) Muc1 expression. Bars represent the average +/- SD from 5 technical replicates.

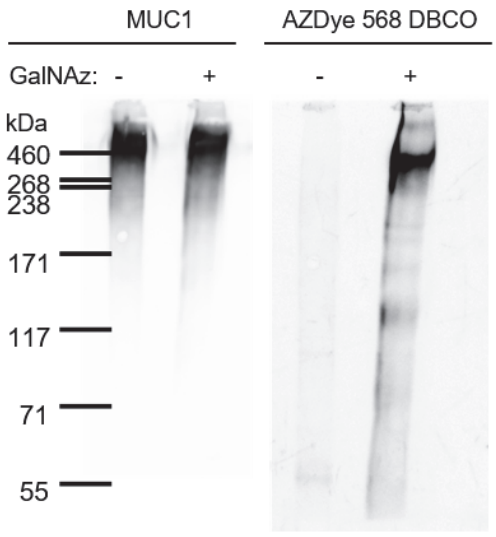

Figure 3: Bio-orthogonal click conjugation of mucin-induced EVs. Western blot detection of Muc1 (Left) and DBCO-AzDye 568 (Right) in lysates of mucin-induced EVs with or without N- azidoacetylgalactosamine (GalNAz) metabolically incorporated into the glycans of EV Muc1 biopolymer coatings.

Conclusions and Future Steps:

These studies reinforce the role of the glycocalyx in EV secretion by demonstrating that the glycocalyx mucin Muc1 drives EV secretion in different cell types. These mucin-induced EVs carry innate mucin biopolymer surface coatings. EV mucin coatings can be further engineered using a combination of endogenous and exogenous modifications to design EV payloads, as demonstrated by the successful bio-orthogonal conjugation of fluorescent reporter molecules to metabolically labeled EVs.

References:

[1] Shurer, C. R. et al. (2019). Physical Principles of Membrane Shape Regulation by the Glycocalyx. Cell, 177(7), 1757–1770. https://doi.org/10.1016/j.cell.2019.04.017

[2] Rider, M. et al. (2016). ExtraPEG: A Polyethylene Glycol-Based Method for Enrichment of Extracellular Vesicles. Sci Rep 6, 23978. https://doi.org/10.1038/srep23978.