Exploring the Relevance of Mucin-Induced Extracellular Vesicles in Therapeutics and Cancer

CNF Project Number: 2272-14
Principal Investigator(s): Dr. Matthew Paszek
User(s): Erik Chow

Affiliation(s): Department of Biomedical Engineering, Cornell University
Primary Source(s) of Research Funding: National Science Foundation Graduate Research Fellowship
Contact: paszek@cornell.edu, ec829@cornell.edu
Primary CNF Tools Used: Malvern NS300 Nanosight

Abstract:

Extracellular vesicles (EVs) have garnered increasing biomedical research focus due to their ability to transport DNA, RNA, and proteins. In a therapeutic context, EVs represent inherently biocompatible vessels for targeted delivery. However, in a disease context such as cancer, EVs become an avenue for disease pathogenesis and progression. In either context, 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 mucin glycoprotein MUC1 in the glycocalyx leads to a dramatic increase in the production of EVs [1]. Here we summarize our recent findings characterizing the physical properties of these mucin-induced EVs (MUC-EVs).

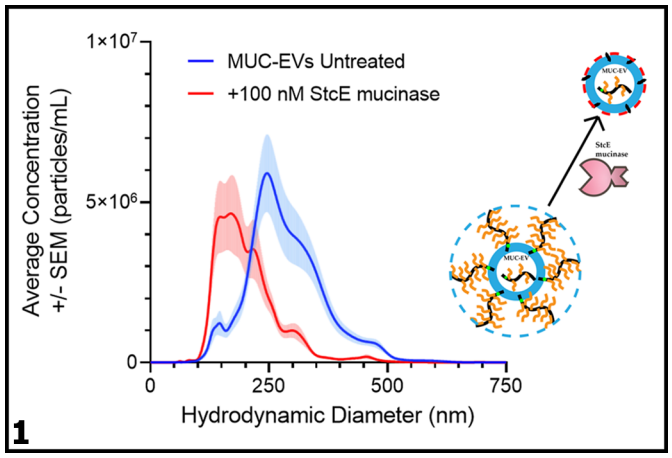
Summary of Research:

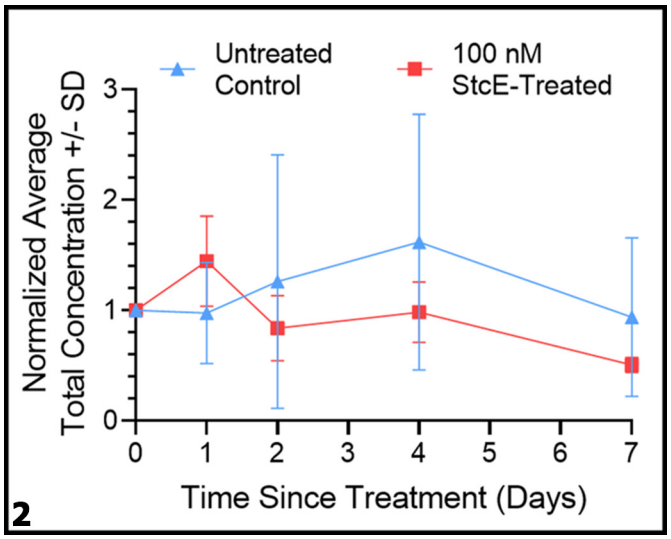
Extracellular vesicles (EVs) have quickly gained traction in numerous areas of biomedical engineering research — including disease pathogenesis and drug delivery, among others — due to their ability to transport DNA, RNA, and proteins. The glycocalyx is a polymer meshwork of proteins, nucleic acids, and glycans which dictates numerous intercellular interactions. However, the role of the glycocalyx in regulating intercellular communication by way of EVs 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 EVs [1]. This report summarizes research from the last year characterizing the effects of MUC1 overexpression on EV properties.

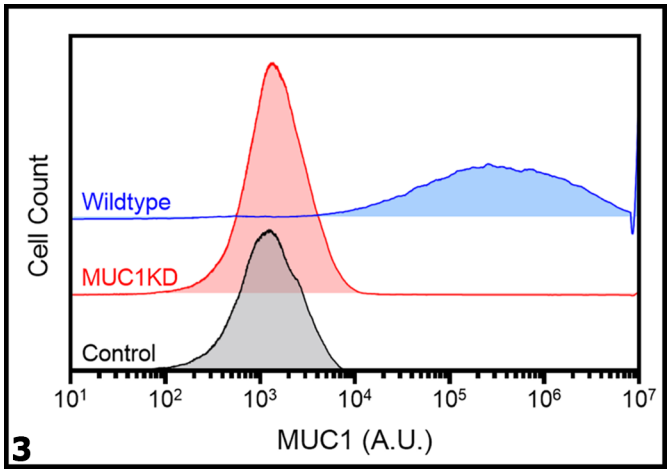
MCF10A cells were genetically engineered to over-express a tetracycline-inducible MUC1-construct. A single clone was then expanded and used as a workhorse cell line for this research, hereafter referred to as MCF10A-1E7 cells. To induce MUC1 overexpression, MCF10A-1E7 cells were treated with 1 μg/mL doxycycline (Dox) for 24 h. Uninduced MCF10A.1E7 cells were used as a negative control. Subsequently, the cells were switched to serum-free media and cultured at 37°C, 5% CO_2 for 15 h to 18 h. EV-containing media was harvested, and the EVs were isolated by PEG-enrichment [2]. EV mucin coatings were optionally removed by treatment with stcE mucinase [3], and EV sizes and concentrations were measured by nanoparticle tracking analysis (NTA) using the Malvern NS300 Nanosight.

Figure 1 shows that mucin-induced EVs (MUC-EVs) are themselves coated in MUC1. Treatment of mucin-induced EVs with StcE mucinase resulted in an overall decrease in EV size consistent with the removal of MUC1 from the EV surface [4]. The stability of MUC-EVs stored at 4°C was evaluated by repeatedly recording NTA measurements of MUC-EV samples with or without mucinase treatment over the span of seven days. Non-mucinase-treated MUC-EV stability was dramatically improved over MUC-EVs which had their MUC1 coatings cleaved by StcE mucinase, as shown in Figure 2, with only mucinase-treated EVs decaying to 50% of their original concentration within seven days. This suggests that MUC1 overexpression enhances EV stability through the generation of EVs with MUC1 surface coatings.

Recognizing the MUC1 overexpression is a hallmark of numerous solid-tumor cancers, EV production was analyzed from KPL-1, a MUC1-overexpressing breast cancer cell line. A MUC1 knockdown cell line (KPL-1 MUC1KD) was generated by lentiviral delivery of small hairpin RNA (shRNA) with reduced MUC1 expression validated by flow cytometry shown in Figure 3. EVs were harvested from wild-type KPL-1 and KPL-1 MUC1KD using the same method as described above. Figure 4 demonstrates that MUC1 knockdown dramatically attenuated the release of EVs from KPL-1 cells.







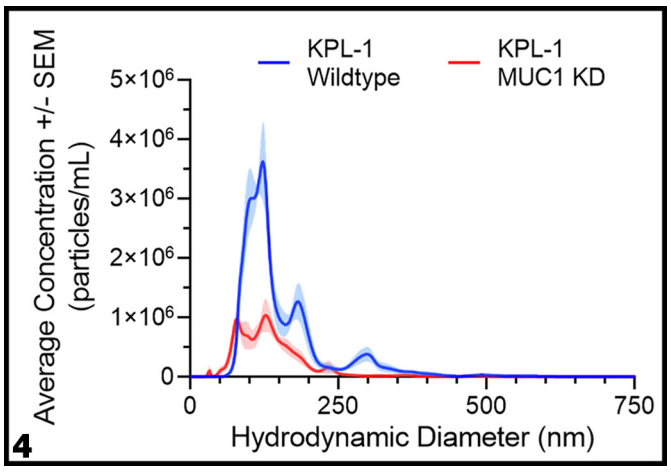


Figure 1: Induced biogenesis of MUC1-coated EVs. *Size distributions of EVs from MCF10A.1E7 cells before and after 100 nM StcE mucinase treatment measured by NTA. Plotted are the average particle concentrations +/-SEM from three independent experiments.*
Figure 2: MUC1 coatings improve EV stability. *Total particle concentrations of EVs from MCF10A-1E7 measured by NTA. EVs were either untreated (Control) or treated with 100 nM stcE mucinase. Plotted are the average total particle concentrations +/-SD from three independent experiments as a percentage of the concentration at Day 0. Dotted line represents 50% EV decay.* **Figure 3: Validation of MUC1 knockdown in engineered KPL-1 cells.** *Wildtype KPL-1 and KPL-1 MUC1KD cells were fixed with 4% PFA for 15 min at room temperature, incubated with mouse anti-MUC1 (HPMV) primary antibody for 30 min at room temperature, and finally incubated with goat anti-mouse IgG secondary antibody conjugated with AlexaFluor™ 647 for 30 min at room temperature. Fluorescence histograms shown were acquired using an Attune™ NxT flow cytometer. KPL-1 wildtype cells fixed and stained only with secondary antibody were used as a negative control.*
Figure 4: MUC1 knockdown attenuates release of EVs from KPL-1 breast cancer cell line. *Size distributions of EVs from wildtype KPL-1 and KPL-1 MUC1KD measured by NTA. Plotted are the average particle concentrations +/-SEM from five technical replicates.*

Conclusions and Future Steps:

Altogether, these data demonstrate that EV properties can be dramatically impacted by the glycocalyx. Overexpression of MUC1 acts as a driver of EV release, and these EVs may be imparted with mucin coatings which improve stability and could enhance their role as long-range delivery vehicles of therapeutic or oncogenic agents. Further studies are needed to characterize MUC-EV cargoes and to explore the applications of engineered EV mucin coatings.

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.

[3] Malaker, S.A., et al. (2019). The mucin-selective protease StcE enables molecular and functional analysis of human cancer-associated mucins. PNAS, 116(15), 7278-7287. https://doi.org/10.1073/pnas.1813020116.

[4] Park, S., et al. (2022). Mucins form a barrier against immune cell attack. bioRxiv. https://doi.org/10.1101/2022.01.28.478211.