Measuring Thickness of Extracellular Vesicle Mucin Coatings Using Nanoparticle Tracking Analysis

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Principal Investigator(s): Dr. Matthew Paszek
User(s): Erik Chow

Affiliation(s): Department of Biomedical Engineering, Cornell University

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Contact: paszek@cornell.edu, ec829@cornell.edu

Primary CNF Tools Used: Malvern NS300 Nanosight

Abstract:

Extracellular vesicles (EVs) transport DNA, RNA, and proteins between cells and therefore have great potential as tools for disease diagnosis and therapeutics. 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. Here, we characterize the innate MUC1 surface coatings on these "mucin-induced" EVs and demonstrate the ability to bioengineer mucin biopolymer coatings through genetically encoded coating thickness.

Summary of Research:

Extracellular vesicles (EVs) have gained attention in numerous areas of biomedical engineering research — including disease pathogenesis and drug delivery, among others — for 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, 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 EVs [1]. This report summarizes research from the last year characterizing EV mucin coating thickness using nanoparticle tracking analysis (NTA).

MCF10A cells were genetically engineered to overexpress variable length, tetracycline-inducible MUC1 constructs with 0, 21, or 42 tandem repeats (TRs). Separately, cells expressing inducible MUC1 were genetically engineered for differential expression of glycosyltransferase or sialyltransferase enzymes to achieve variable MUC1 glycosylation. Specifically, GCNT1 overexpressing cells express MUC1 with more core II glycans; C1GALT1 KO cells express MUC1 with truncated glycans; or GNE KO cells express MUC1 with no glycans terminated by sialic acid. To induce MUC1 overexpression, cells were treated with 1 ug/mL doxycycline (Dox) for 24 h. Subsequently, cells were switched to serum-free media and cultured at 37 ., 5% CO2 for 15 h to 18 h. EV-containing media was harvested, and the EVs were isolated by PEG-enrichment according to an existing protocol [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.

Mucinase treatment of mucin-induced EVs resulted in a significant decrease in EV hydrodynamic radius (Figure 1). NTA of EVs from cells expressing variable-length MUC1 showed gradual increase in EV size correlated with mucin length, and significant increase in EV size was observed between EVs coated with MUC1 42xTR and those coated with MUC1 0xTR (Figure 2). Finally, mucin glycosylation had a measurable effect on EV coating thickness, with changes in EV size consistent with changes in parent cell glycocalyx thickness previously measured by scanning angle super-resolution microscopy [4] (Figure 3).

Conclusions and Future Steps:

These studies demonstrate that EV properties can be dramatically impacted by the glycocalyx. Overexpression

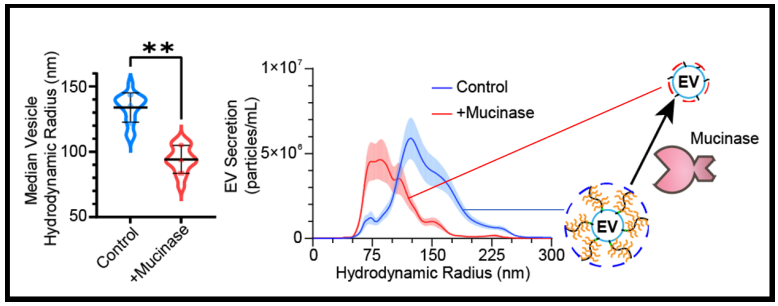

*Figure 1: Mucin-induced EVs have MUC1 surface coatings. Comparison of hydrodynamic diameter of EVs before and after treatment with StcE mucinase, (** = p < 0.01, Left). Size distributions of EVs from 1E7 cells before and after 100 nM StcE mucinase treatment measured by NTA. Plotted are the average particle concentrations +/-SEM from three independent experiments (Right).*

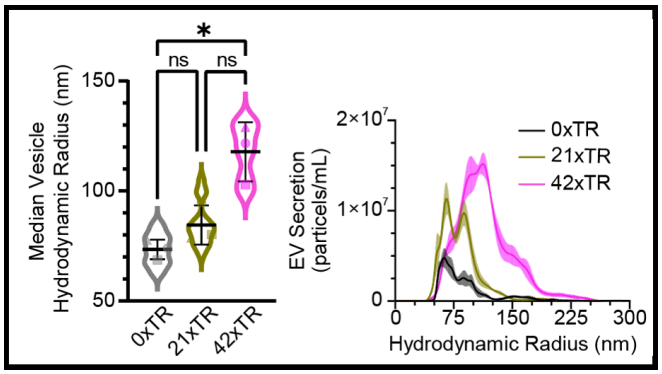

Figure 2: Expression of variable length mucins has a correlated effect on EV coating thickness. Comparison of median hydrodynamic radius of EVs from cells expressing MUC1 with 0-, 21-, or 42xTR (Left). Data were collected from three independent experiments (= p < 0.05). Size distributions of EVs. Plotted are average particle concentrations +/-SEM from three independent experiments (Right).*

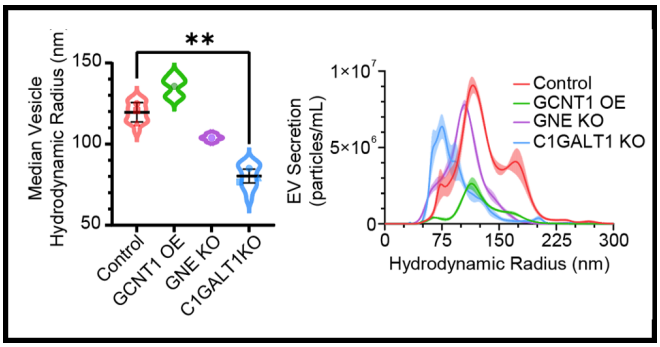

Figure 3: Differential MUC1 glycosylation changes EV coating thickness consistent with changes in parent cell glycocalyx thickness. Comparison of median hydrodynamic radius of EVs from GCNT1 overexpressing, GNE KO, or C1GALT1 KO cells across three independent experiments (= p < 0.05). Size distributions of EVs. Plotted are average particle concentrations +/-SEM from three independent experiments (Right).*

of MUC1 acts as a driver of EV release, and these EVs carry innate mucin surface coatings. Altogether, these data illustrate a synthetic biology approach to vesicle bioengineering by way of genetically encoded biopolymer coatings, which can be achieved either by direct manipulation of MUC1 biopolymer constructs or by genetic engineering of the EV parent cells to express mucins with varying glycosylation profiles. Future studies will assess the functional capabilities of mucin-coated EVs.

References:

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