Genetically Encoded Platform for Mucin-Induced Extracellular Vesicle Production

CNF Project Number: 2272-14

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Primary Source(s) of Research Funding: National Science Foundation Graduate Research Fellowship

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Primary CNF Tools Used: Malvern NS300 Nanosight

Abstract:

Extracellular vesicles (EVs) are critical in mediating intercellular communication. Because of the diverse nature of their cargoes — which include DNA, coding and noncoding RNA, and proteins — and their innate biocompatibility, EVs have quickly become a prominent focus in numerous biomedical engineering applications, including diagnostics, drug delivery, and targeted therapies. One largely unexplored area is the significance of the glycocalyx in EV biogenesis and function. Specifically, the capacity to rationally manipulate the glycocalyx to produce vesicular materials for biomedical applications 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 efforts to develop and characterize a genetically encoded platform for the production of these so-called mucin-induced EVs (MUC-EVs).

Summary of Research:

Extracellular vesicles (EVs) have been shown to carry a wide range of cargoes, including DNA, coding and non-coding RNAs, and proteins. Because of the diverse nature of their cargoes, and their innate biocompatibility, EVs have quickly gained traction in numerous areas of biomedical engineering research, including disease pathogenesis, diagnostics, drug delivery, and targeted therapies. The glycocalyx is a polymer meshwork of proteins, nucleic acids, and glycans which dictates numerous intercellular interactions. However, the capacity for the production of rationally designed EVs through engineering of the glycocalyx 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 a genetically encoded platform for increased generation of so-called "mucin-induced" EVs with tunable size characteristics and surface coatings.

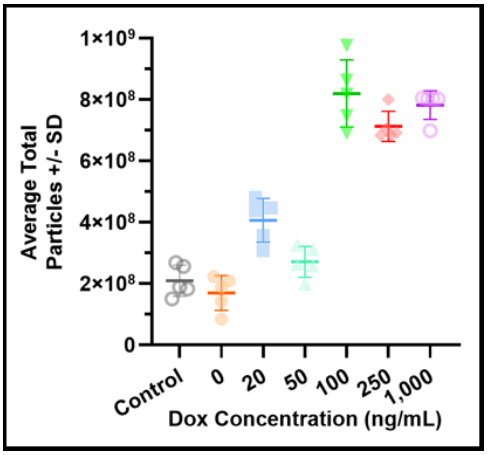

Figure 1: Tunable mucin-induced EV production. EV particle concentrations measured by NTA from MCF10A-rtTA cells (Control) and MCF10A-1E7 cells induced with a ranged of Dox concentrations. Plotted are the average reported total particle concentrations +/-SD from five video recordings.

To engineer the glycocalyx, MCF10A cells were genetically engineered to overexpress a MUC1-mOxGFP construct on the cell membrane. A single clone was expanded and used as a workhorse cell line for this

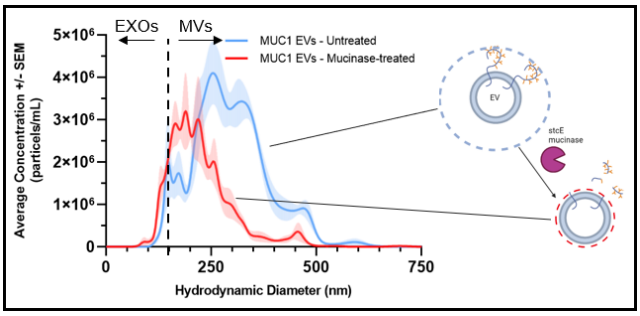

Figure 2: Mucin-induced EVs have removable mucin coatings. EV size distributions measured by NTA from MCF10A-1E7 derived EVs either untreated (Control) or treated with 100nM stcE mucinase. Histograms represent the average reported size from five video recordings.

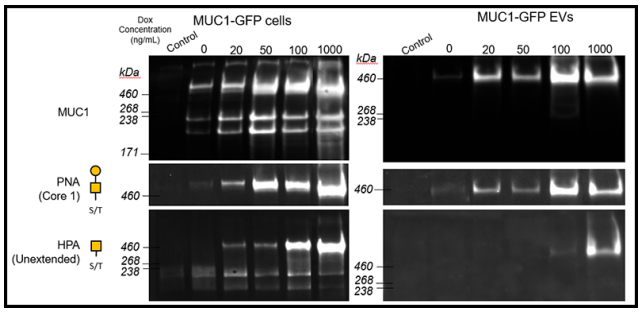

Figure 3: EVs from MCF10A-1E7 cells have similar mucin coating characteristics to their parent cells. Lectin blots comparing MUC1 glycosylation patterns in mucin-induced EVs and the cells from which they were derived.

research, hereafter referred to as MCF10A-1E7 cells. Expression of MUC1-mOxGFP in MCF10A-1E7s was tied to a tetracycline-inducible promoter, and cells were treated with doxycycline (Dox) for 24 h at a concentration of either 0.1 µg/mL or 1 µg/mL to induce MUC1-mOxGFP overexpression. MCF10A cells engineered with only the promoter but no MUC1-mOxGFP construct, hereafter referred to as MCF10A-rtTA cells, were used as a negative control. After Dox treatment, the cells were switched to serum-free media and cultured at 37°, 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 illustrates the dose-dependent production of mucin-induced EVs based Dox titration. These data illustrate that tunable EV production in MCF10A-1E7 cells is achieved using our genetically encoded platform. Additionally, mucin-induced EVs were found to carry their own mucin coatings, as supported by Figure 2. Treatment of mucin-induced EVs with mucinase resulted in an overall decrease in EV size, consistent with the cleavage of MUC1 from the EV surface.

Figure 3 shows lectin staining of the mucins isolated from mucin-induced EVs. The EV-derived mucins show both similar expression and glycosylation patterns to cell-surface mucins from MCF10A-1E7 cells, suggesting that glycocalyx engineering at the cellular level can be used to tune mucin-induced EV coatings.

Conclusions and Future Steps:

Altogether, these data demonstrate that EV production and properties can be controlled by engineering the glycocalyx of cells. Further experiments are needed to explore the applications of engineered EV mucin coatings. Additionally, future experiments will strive to reliably segregate exosomes and microvesicles in order to more precisely study the exosome and microvesicle characteristics.

References:

[1] Shurer, C. R., Kuo, J. C.-H., Roberts, L. D. M., Gandhi, J. G., Colville, M. J., Enoki, T. A., Pan, H., Su, J., Noble, J. M., Hollander, M. J., O'Donnell, J. P., Yin, R., Pedram, K., Möckl, L., Kourkoutis, L. F., Moerner, W. E., Bertozzi, C. R., Feigenson, G. W., Reesink,

H. L., and Paszek, M. J. (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., Hurwitz, S. and Meckes, D. ExtraPEG: A Polyethylene Glycol-Based Method for Enrichment of Extracellular Vesicles. Sci Rep 6, 23978 (2016).