Disseminating Glycocalyx Biopolymer-Induced Microvesicle Shedding through Nanoparticle Tracking Analysis and Cryo-Electron Microscopy

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Primary CNF Tools Used: Malvern NS300 NanoSight

Abstract:

Cancer-derived microvesicles (MVs) have been linked to cancer progression through their ability to propagate an oncogenic phenotype in normal cells. However, the mechanism of their biogenesis is not clearly established. The biogenesis of MVs is inherently a mechanical process where direct vesicle shedding from the plasma membrane requires physical bending. Through scanning electron microscopy, we have found that biopolymers within the sugar-rich coating on the plasma membrane known as the glycocalyx contribute induce membrane bending. Through nanoparticle tracking analysis (NTA), we also have found that molecular crowding within the glycocalyx drives membrane bending for microvesicle biogenesis. Lastly, we show that using nanoparticle tracking analysis in tandem with cryo-transmission electron microscopy (cryo-TEM) allows us to validate and visualize microvesicles and other extracellular vesicle sub-types to better understand mechanisms of biogenesis and shedding.

Summary of Research:

Theory has predicted membrane instabilities and formation of spherical structures in the presence of molecular membrane surface crowding. Remarkably, we have found that increased expression of cancer-associated biopolymers, including mucin-1 (Muc1), induce membrane instabilities as well as drive formation and shedding of spherical microvesicles through conventional scanning electron microscopy (SEM), nanoparticle tracking analysis (NTA) using the Malvern NS300 NanoSight, and cryo-transmission electron microscopy (cryo-TEM). These results suggest that increased glycocalyx biomass might be a general mechanism for MV upregulation. Together, our data suggests that large, cancer associated glycoproteins and glycopolymers, such as Muc1, may perturb cellular communication in cancer through regulation of microvesicle biogenesis. Understanding these physical processes could lead to new therapies that target the mechanical basis of MV biogenesis and cancer.

Together, these experiments and theories describe an entropic mechanism through which the glycocalyx can strongly influence the plasma membrane shapes. We also find that the plasma membrane in these cells become unstable following inhibition of actin polymerization. These results suggest that the cytoskeleton counterbalances the entropic pressure exerted by cell surface glycopolymers and dynamics play a role in formation and release of microvesicles. Given that glycosylation changes dramatically and in tandem with cell fate transitions, and that the pool of monomers for construction of glycocalyx polymers is tightly coupled to specific metabolic programs, our work raises the intriguing possibility that the glycocalyx may serve as a conduit linking physical morphology to specific physiological and diseased states.

References:

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[3] Shurer, et al., ACS Biomater. Sci. Eng., 2017, 4, 2, 388-399.

[4] Shurer, et al., in resubmission, 2018.

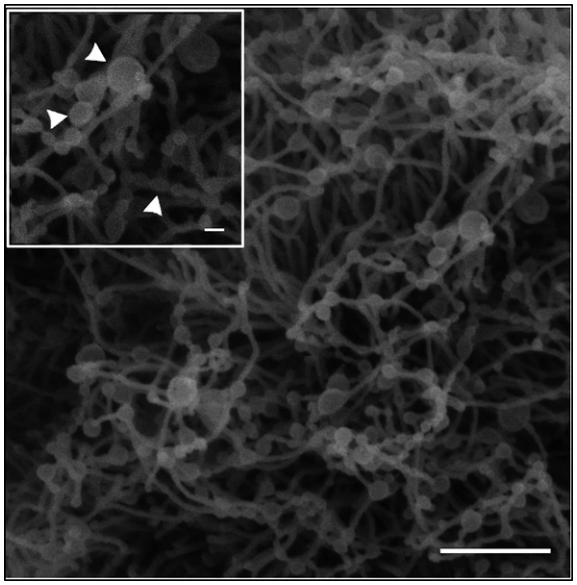

Figure 1: Nanoparticle tracking analysis shows that increased Mucin-1 (Muc1) expression induces membrane tubules and instabilities on the surface of MCF10A cells. (Inset; top left) Microvesicles (indicated by white arrows) present on the membrane tubes (scale bars: 200 nm).

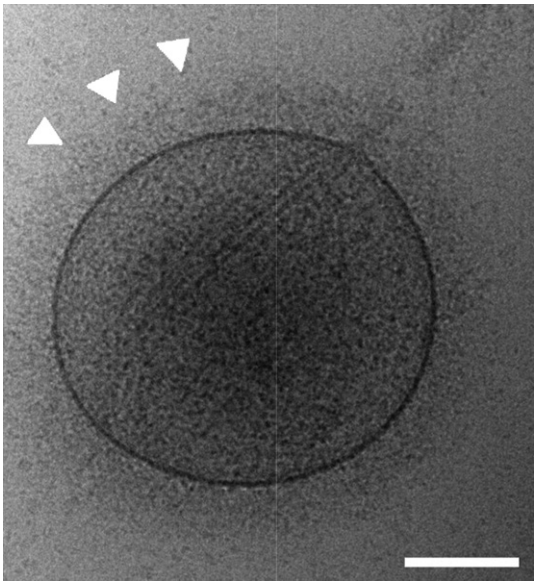

Figure 3: Cryo-TEM of a microvesicle shed from Muc1 MCF10A cells. The ultrastructure of the glycocalyx is indicated by white arrows (Scale bar: 100 nm).

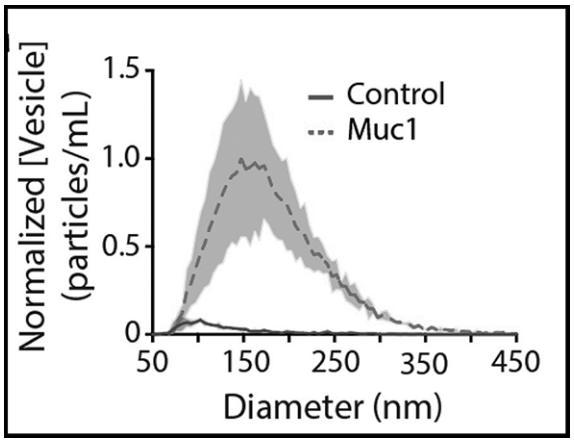

Figure 2: Increased expression of Muc1 induces microvesicle shedding in MCF10A cells compared to control MCF10A cells as shown in nanoparticle tracking analysis (n=3).

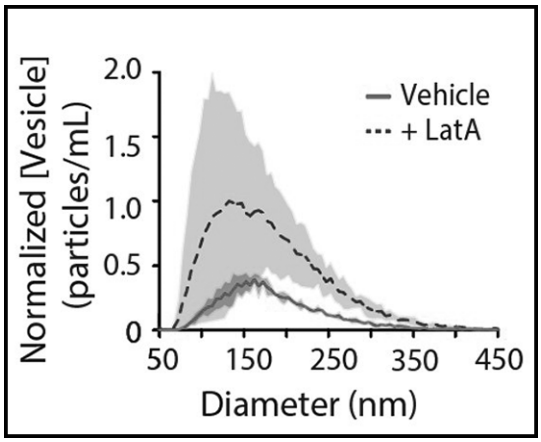

Figure 4: Destabilization of actin through 10 uM of Latrunculin A treatment in cells with increased Muc1 expression treatment shows enhanced microvesicle shedding compared to control Muc1 cells (n=3).