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Revealing Mechanisms of Microvesicle Biogenesis in Breast Cancer Cells via *in situ* Microscopy

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Tumors secrete microvesicles (MVs) through direct shedding from the plasma membrane. When introduced into the extracellular matrix, MVs are believed to aid in tumor invasiveness [1]. Despite their importance, the mechanisms of MV biogenesis, as well as their ultrastructure and chemical cargos, are poorly understood. MV shedding is believed to occur through membrane bending of the cell [2]. Researchers have proposed that asymmetric molecular crowding on the lipid bilayer drives membrane bending [3]. One key structure that possesses such asymmetric crowding is the glycocalyx, a dense network of glycosylated proteins surrounding the exterior of most cells.

Here we used both room temperature and cryo-SEM and cryo-TEM to better understand the biogenesis of MVs from MCF10A-HAS3 (hyaluronan synthase 3) cells. Compared to more traditional approaches in which vesicles are isolated from the growth medium through centrifugation, here, cells are cultured directly on TEM grids, thereby reducing the risk of structural damage due to sample preparation. The adherence and densities of cells cultured on TEM grids were similar to those grown using standard methods. We determined that HAS3 cells contained tubular structures that extended from the cell surface. These tubules contained smaller vesicles, suggesting a mechanism of vesicle trafficking through pearling and potential actin dynamics. We find that vesicle expression is increased in HAS3 cells as compared to the non-malignant control. However, we observe a discrepancy in the particle diameter distributions of HAS3 vesicles obtained by nanoparticle tracking analysis (NTA) and those from direct inspection with cryo-TEM. A large peak at ~100 nm dominates the NTA data while the signal is distributed across other vesicle diameters in the cryo-TEM data. This may suggest that NTA not only captures vesicles but also biological debris present in the analyzed media. Cryo-TEM further shows that the majority of secreted HAS3 vesicles contain surface structure suggesting the presence of the glycocalyx. For HAS3 cells these surface features are not very pronounced. This is in stark contrast to the vesicles secreted by MCF10A-Muc1 cells. This difference may be due to the weak covalent linking of the glycocalyx in HAS3 vesicles. The presence of a glycocalyx confirms the MVs' origin from the plasma membrane.

Elucidating the biogenesis and morphology of MVs will ultimately lead to a better understanding of the key biophysical parameters that determine MV microstructure and bioactivity. Ultimately, such insight will be correlated with clinical diagnostic tools and possible interventions. [4]

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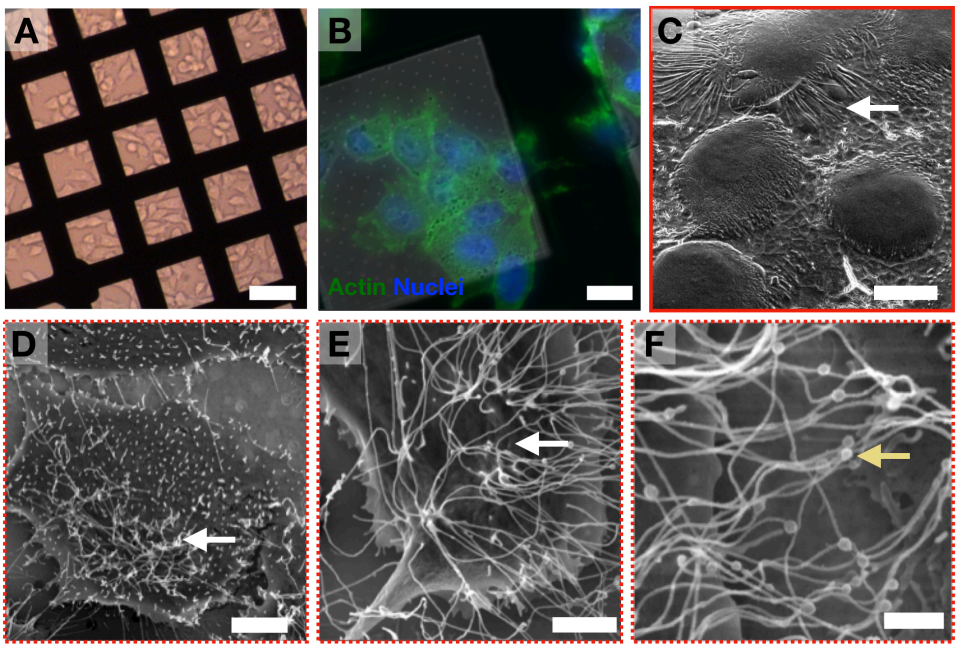

Fig. 1: Cell morphology of MCF10-HAS3. Optical (A) and florescence light microscopy (B) images of HAS3 cells cultured on TEM grids. The high-intensity actin labeling in B is likely attributed to the proliferation of tubular filaments extending from the cell surface (C-E, white arrows). These filaments contain smaller vesicles (F, tan arrow), likely the result of pearling events. *Scale bars: 25 μm (A), 10 μm (B), 5 μm (C-D), 2 μm (E), 1 μm (F). Solid red box: cryo-SEM, dashed red boxes: RT-SEM.*

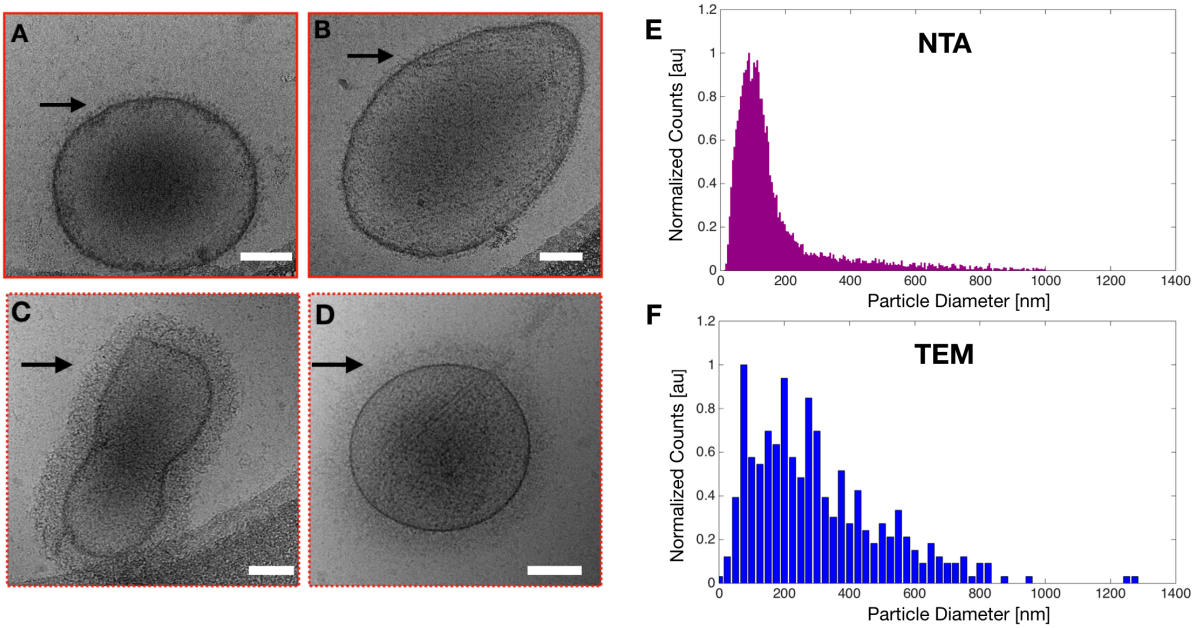

Fig. 2: Cryo-TEM of HAS3 (solid red box) and Muc1 (dashed red box) MVs. The surface of HAS3 MVs is coated with a thin layer of surface structure (A,B; black arrow) as compared to Muc1 MVs (C,D; black arrow). The difference might arise from the fact that the hyaluronic acid produced by HAS3, is not covalently linked to the membrane like for Muc1, and the glycocalyx may have sheared off during sample preparation. The particle distribution of HAS3 vesicles obtained from NTA (E) and TEM (F) show discrepancies, especially in the smaller diameter range. *Scale bars: 100 nm.*

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