# Analyzing the Effects of Hyaluronic Acid through Enzymatic Digestion in Cancer Cell-Derived Extracellular Vesicles

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Extracellular vesicles (EVs) are nano-sized particles involved in intercellular signaling thought to be secreted by all cells. Differences in surface glycosylation of EVs have been observed in distinct biodistribution patterns. Hyaluronic acid (HA) is known to be upregulated in braintropic MDA-MB-231 EVs in comparison to parental EVs. The purpose of this project was to determine if digestion of HA alters the function of braintropic MDA-MB-231 EVs. Tangential flow filtration (TFF) was used to isolate EVs from both cell-lines. The EVs were then characterized by zeta-potential, nanoparticle tracking analysis (NTA), protein concentration by bicinchoninic acid assay (BCA), and surface glycan expression by Enzyme-Linked Immunosorbent Assay (ELISA). Native EVs were then treated with hyaluronidase (HYAL) for HA removal. Control groups included untreated and ΔH, which underwent the heat treatment accompanying enzymatic digestion. All EV groups were labeled with DiI, a lipophilic fluorescent dye, and human brain microvascular endothelial cells (hBMEC) were treated with the EV populations. An hBMEC uptake assay was conducted to determine whether MDA-MB-231 derived EVs were taken up. It was expected that higher levels of braintropic EVs would be taken up than parental EVs and HYAL-treated braintropic EVs; however, there was no significant difference in uptake between untreated controls. Trans-endothelial electrical resistance (TEER) was performed on the EV-treated hBMEC cells, but no relationship was established between HA expression and EV braintropic function. The exact role of surface glycans on EVs may have implications in the mechanisms of site-specific metastasis that are yet unknown; further research is needed.