Extracellular vesicles generated by Chinese hamster ovary cells under normal and stressed conditions facilitate large-scale, dynamic exchange of proteins and RNAs

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Chinese Hamster Ovary (CHO) cells are the most widely used mammalian cell host in the biopharmaceutical industry for producing therapeutic proteins. CHO cells dynamically produce and uptake extracellular vesicles (EVs), and through these EVs, they exchange proteins and RNAs at a large scale. EVs are nano-sized membrane-derived vesicles that are a major mechanism of cell-to-cell communication. Despite the importance of EVs in CHO-culture physiology there is a limited understanding of the role CHO EVs play in culture. The dynamic exchange of CHO EV production and cellular uptake was captured quantitively and qualitatively with flow cytometry and correlative confocal microscopy/scanning electron microscopy (SEM), tracking the associated protein and RNA cargo of the EVs. Using flow cytometry and the fluorescent cell stain, SYTO RNASelect, to label RNA cargo, the exchange of fluorescent EVs between cells was quantified over 24 hours, observing over 95% of cells in culture participated in the EV exchange network. Correlative confocal/SEM imaging identified specific EV exchange events where EVs are observed at the surface of a target cell prior to uptake. This native cell communication and protein and RNA exchange mechanism mediated by EVs in suspension culture suggests that the close proximity of cells may result in prolific cellular exchange.

The widespread exchange of CHO EVs, and the associated exchange of proteins and RNAs, among cells in culture is hypothesized to result in a collective regulation of the cellular state. EVs are highly enriched in small regulatory RNAs, notably microRNA (miRNA), relative to the parent cell. To understand how the miRNA content in CHO EVs changes with stress (ammonia or osmotic stress) compared to non-stressed, exponential phase cultures, we used RNA sequencing of the parent cells, MPs, and exosomes. The miRNA landscape in cultures (cells, MPs, exosomes) exposed to ammonia or osmotic stress was highly enriched in the let-7 family of miRNAs. The changing miRNA landscape of EVs exposed to stress (ammonia, osmolarity) conditions indicates a dynamic gene regulation mechanism for cells in culture to homogenize cellular state and behave as a community in response to environmental stressors. Gene ontology analysis of the genes and pathways targeted by these highly enriched miRNAs suggest their profound physiological role in regards to cell proliferation and survival, and their potential to be used for strain engineering.

Beyond the native mechanisms of CHO EVs in culture, applications of CHO EVs as cargo delivery vehicles of plasmid DNA and proteins for gene editing was developed. Current challenges of delivering functional CRISPR/Cas9 protein and single guide RNAs (sgRNAs) to cells in vitro and in vivo include targeting specific cell types, modularity of the cargo-carrying vehicle, and off-target gene editing effects. EVs have been shown to have cell-specific targeting properties due to the presence of native surface moieties. To generate a non-viral approach for a targeted CRISPR/Cas9 delivery vehicle, hybrid liposome-EVs were developed combining the endogenous loading mechanisms and targeted delivery abilities of EVs with the nucleic acid and Cas9 encapsulation of liposomes (i.e. Lipofectamine 2000) and lipid nanoparticles (i.e.
Lipofectamine CRISPRMAX). Delivery of plasmid DNA through the hybrid EVs to CHO cells in culture resulted in functional expression of the recombinant fluorescent protein in 35% of cells after 24 hours of culture. Delivery of CRISPR/Cas9 machinery as a protein as opposed to Cas9 mRNA or DNA reduces the burden on host cell machinery and eliminates the risk of uncontrolled Cas9 expression. EV hybrids carrying Cas9-GFP (Cas9 protein fused to GFP), cocultured with CHO cells resulted in Cas9-GFP delivery to 60% of cells in culture and the Cas9-GFP protein was observed with confocal microscopy to colocalize with the nucleus. Colocalization of Cas9-GFP and the nucleus indicates that CHO EV hybrids are an effective delivery vehicle for gene editing machinery. Through this platform, Cas9 is delivered as a protein, minimizing the long-term risks associated with delivering Cas9 as plasmid DNA such as integration and continued expression.