THE microRNA LANDSCAPE OF THE EXTRACELLULAR VESICLES GENERATED BY CHINESE HAMSTER OVARY CELLS UNDER NORMAL AND STRESSED CONDITIONS

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Large scale exchange of proteins and RNAs among Chinese hamster ovary (CHO) cells in culture is a result of the dynamic production and uptake of extracellular vesicles (EVs), both membrane-derived microparticles (MPs) and endosome-derived exosomes. To visualize the dynamic production and cellular uptake of CHO EVs, and the associated protein and RNA exchange, correlative confocal microscopy and scanning electron microscopy was used to interrogate fluorescently labeled CHO cells. Using flow cytometry and an RNA green fluorescent cell stain, the exchange of cellular RNA between CHO cells through EV exchange was tracked and quantified. This hitherto underappreciated native cell communication and protein/RNA material exchange mechanism mediated by EVs in suspension culture suggests that the close proximity of cells may result in prolific cellular exchange[1].

EVs are highly enriched in small regulatory RNAs, notably microRNA (miRNA), relative to the parent cell. The widespread exchange of EVs, and the associated exchange of proteins and RNA, among cells in culture is hypothesized to result in a collective regulation of the cellular state. To understand how the miRNA content in CHO EVs changes with stress (ammonia or osmotic stress) and culture age compared to non-stressed, exponential phase cultures, we used RNA sequencing of the parent cells, MPs, and exosomes followed by quantitative PCR (qPCR). The top five most abundant miRNAs (miR-92a, miR-23a, miR-21, miR-25, let-7c) identified with RNA sequencing in CHO MPs were tracked over a 9-day batch culture to determine how specific miRNAs change throughout culture. 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. Computational analysis of the genes and programs targeted by these highly enriched miRNAs suggest their profound physiological role, and their potential to be used for strain engineering. Four of the highly enriched miRNAs from stressed and non-stressed cultures were reintroduced to stationary phase cells as synthetic miRNA mimics or inhibitors. The findings from these experiments suggest that these selected enriched miRs are involved in cell viability and apoptosis.

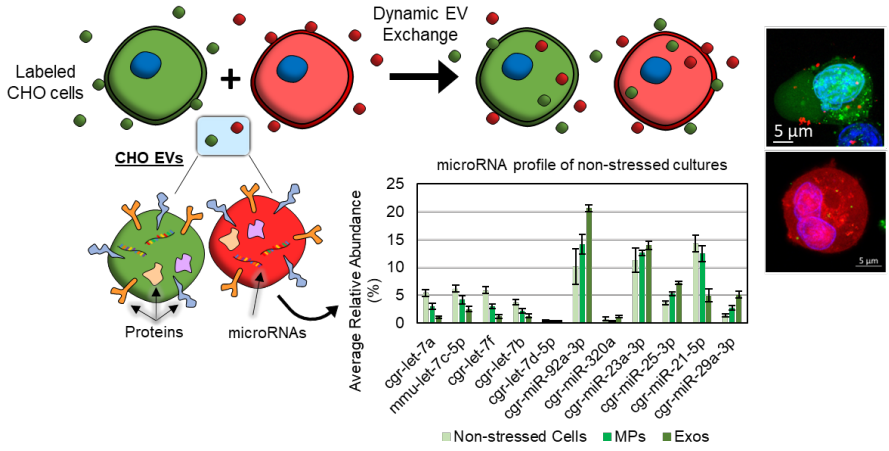

Figure 2: Dynamic production and uptake of CHO EVs results in widespread exchange of proteins and RNAs. The microRNA landscape of CHO cells, MPs, and exosomes from non-stressed, exponential phase cultures was determined by RNA sequencing.

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(1) Belliveau, J.; Papoutsakis, E. T. Extracellular vesicles facilitate large-scale dynamic exchange of proteins and RNA among cultured Chinese hamster ovary and human cells. *Biotechnol Bioeng* 2022, *119* (5), 1222-1238. DOI: 10.1002/bit.28053