Engineered Extracellular Vesicles Transfer Mitochondria and pDNA to Increase Cellular ATP levels in Brain Endothelial Cells

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The oxygen and glucose deprivation in ischemic stroke results in a decrease in endothelial ATP levels and mitochondrial dysfunction leading to endothelial cell death. Therefore, the protection of endothelial mitochondrial function and endothelial cell growth by supplementing them with exogenous mitochondria and brain-derived neurotrophic factors (BDNF) is a viable strategy to increase endothelial cell survival under normoxic and hypoxic conditions. EVs are known to contain mitochondrial components as part of their innate vesicular cargo. Importantly, EVs can be engineered for the efficient delivery of exogenous nucleic acids and proteins. Therefore, we wanted to know if EVs can transfer functional mitochondrial into recipient endothelial cells and EVs can be engineered for the delivery of plasmid BDNF DNA (pBDNF) to increase endothelial BDNF levels under normoxic conditions. We hypothesized that engineered and/or naïve EVs derived from brain endothelial cells can increase endothelial cell survival under normoxic and hypoxic conditions.

In the present work, we isolated two EVs subpopulations, exosomes (EXOs) and microvesicles (MVs), from a human brain endothelial cell line. EXOs and MVs retained their characteristic particle diameters, zeta potentials, and membrane integrity during freeze/thaw cycles and post-storage. We demonstrated that EVs can be engineered for DNA delivery using a model luciferase pDNA. Naïve EVs showed about a four-fold increase in ATP levels in hypoxic primary human brain endothelial cells. The mitochondrial load of naïve EVs internalized into the recipient endothelial cells and colocalized with their mitochondrial network. Importantly, EVs increased the oxidative phosphorylation and glycolytic functions of recipient endothelial cells under normoxic and hypoxic conditions. We will continue to optimize the EV/BDNF complexes for endothelial BDNF supplementation during ischemia/reperfusion injury *in vitro*.