Submitter Name: Guoku Hu
Submitted email: guoku.hu@unmc.edu
PI Name: Shilpa Buch
PI email: sbuch@unmc.edu

Extracellular Vesicle-mediated small RNA delivery into the CNS

Guoku Hu[1], Ke Liao[1], Fang Niu[1], and Shilpa Buch[1]

[1]Department of Pharmacology and Experimental Neuroscience, University of Nebraska Medical Center

Background: Abuse of opioids has been reported to correlate with increased severity of central nervous system disease through a number of potential mechanisms including, but not limited to epigenetic regulation via long noncoding RNAs (lncRNAs).

Methods: Astrocyte-derived Extracellular Vesicles (ADEVs) were isolated using the standard differential ultracentrifugation method and were characterized using transmission electron microscopy, NanoSight, atomic force microscopy and western blot analyses. EVs were transfected with lncRNA-Cox2 siRNA using Exo-Fect Exosome Transfection Reagent and were labeled with PKH26. Groups of mice were intranasally administered labeled EVs dropwise with a micropipette and assessed for biodistribution using Xenogen IVIS 200 imager. Separate group of mice were administered either scrambled siRNA or lncRNA-Cox2 siRNA loaded EVs intranasally one hour prior to intraperitoneal injections of morphine twice a day. At seven days post morphine exposure, brains of mice were harvested for assessment of microglial functions by qPCR and immunostaining.

Results: morphine-ADEVs were effectively taken up by microglial endosomes, leading, in turn, to activation of TLR7 with a subsequent upregulation of downstream lncRNA-Cox2 expression, ultimately leading to impaired microglial phagocytosis. These findings were further validated *in vivo*, wherein inhibition of microglial phagocytic activity was also observed in brain slices isolated from morphine administrated mice compared with control mice. Additionally, we also showed that intranasal delivery of EVs containing lncRNA-Cox2-siRNA was able to restore microglial phagocytic activity in mice administered morphine.

Conclusion: These findings have ramifications for the development of EV-loaded RNA based therapeutics for the treatment of various disorders involving functional impairment of microglia.