

Exosomes/miRNA as nano-neurological therapy mediate recovery after stroke and neural injury
Michael Chopp, USA

The ability to regulate and modulate intercellular communication may provide the basis for the treatment of neurological injury, neurodegenerative diseases and stroke. Exosomes are small (30-100 nm) endosomal generated particles consisting of a complex lipid membrane and contain proteins, RNAs, mRNAs and microRNAs (miRNAs). Nearly all cells generate exosomes, and these small lipid containers are ubiquitous in biological systems and provide an intercellular communications network which regulate cellular function. Exosomes mediate intercellular communication by transferring proteins, lipids, and genomic materials including mRNAs and miRNAs between source and target cells.

In this presentation, I will describe our work on the treatment of stroke, traumatic brain injury and diabetic peripheral neuropathy with exosomes, with a focus on the transfer of microRNA (miRNA) content within the exosomes to recipient cells. miRNAs are 20-25 nucleotide non coding RNA which regulate gene translation. They act as major molecular switches and are post transcriptional regulators of protein production, and they can simultaneously impact multiple molecular pathways and signaling within cells. We have found that cell-based therapies promote neurological recovery and promote neurovascular remodeling by transferring exosomes to recipient cells. Thus, we have harvested exosomes by means of ultracentrifugation or using biochemical methods from a variety of cells, and directly employed these exosomes by intravenous administration for stroke and TBI to promote neurological recovery. By labeling the exosomes with fluorescent markers we have shown that intravascular administration of these exosomes pass the blood brain barrier and enter into parenchyma cells. The content of these exosomes, as noted, consists of proteins, miRs and mRNAs. Downstream molecular targets of specific miRs known to be transferred into parenchyma cells have been shown to affect their molecular targets, thus demonstrating that the harvested exosomes transfer miRs to parenchyma cells and thereby affect the molecular downstream targets.

In vitro studies using microfluidic chambers can be employed to give insight into how exosomes promote neurological recovery. Microfluidic chambers are compartmental structures where neuronal soma and axons are located in separate compartments. The microfluidic device permits distal axons to grow into the axonal compartment after passing 450µm long microgrooves that connect the cell body and axonal compartments. We demonstrate that exosomes placed either on the somal or the axonal compartment significantly promote axonal outgrowth. This exosome enhanced outgrowth can also be inhibited by using siRNA to block Argonaut proteins, such as Ago2. Ago2 protein is a component of the RNA induced silencing complex (RISC), and is the key regulator of miRNA function by mediating the activity of miRNA-guided mRNA cleavage or translational inhibition. The majority of miRNAs in exosomes are bound to Ago2. Reduction of Ago2 in exosomes abolishes axonal outgrowth. Very importantly, the content of exosomes can be tailored to contain specific miRNAs. By transfecting exosomes source cells with specific genes or using siRNA on exosomal parental source cells, we can respectively, upregulate or reduce miRNA content within exosomes derived from parental cells. Using microfluidic chambers and vascular angiogenic experiments we demonstrated that

targeting specific miRs will impact specific physiological events. For example, when parental mesenchymal stromal cells (MSCs) were transfected with a miR-17-92 cluster plasmid, exosomes harvested from the MSCs exhibited enriched levels of the miR-17-92 cluster. Applying these exosomes to either the somal or axonal compartments of the microfluidic chamber significantly increased neurite outgrowth. Thus, tailored exosomes can deliver their selective cargo miRNAs into and activate their target signals in recipient neurons.

We have performed extensive preclinical studies on the therapeutic use of exosomes for stroke, traumatic brain injury, diabetic peripheral neuropathy, multiple sclerosis and dementia. For example, our data demonstrate that treatment of embolic stroke with exosomes derived from MSCs or other progenitor cells one or more days after stroke onset significantly promotes neurological recovery compared to control populations. Treatment with exosomes also concomitantly enhanced neurovascular plasticity, promoted neurogenesis, angiogenesis, and oligodendrogenesis. Similarly, treatments of experimental traumatic brain injury, peripheral neuropathy, and neurodegenerative models using exosomes harvested from a variety of cells, as well as harvested exosomes tailored to contain specific miRs, were shown to enhance neurological recovery along with neurovascular plasticity. Thus, we are developing a novel therapy, nano-neurological therapy, utilizing the body's nano-lipid containers, exosomes, which contain and can be loaded with proteins, miRs and genetic instructions, to promote neurological recovery.