**Title: Neural Stem Cell-Derived Exosome Targeting of Injured Intestinal Neurons is Integrin Dependent**

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**Category: Basic Research**

**Objective:** The aim of this study was to investigate the roles of integrins in targeted exosome delivery to enteric neurons.

**Abstract:**

**Background:** Necrotizing Enterocolitis (NEC) is a GI emergency that occurs predominantly in premature newborns. We have previously shown that stem cell therapy protects the intestines and the enteric nervous system (ENS) from experimental NEC. In addition, we have shown that stem cell derived exosomes, extracellular nano-sized vesicles that are secreted from most cells, also protect the intestines from experimental NEC. However, very little is known about the mechanisms used by exosomes to target injured enteric neurons. Integrins are an important extracellular matrix component, and integrin subunits αV and β1 have been reported to be involved in neural migration and proliferation during the development of central nervous system (CNS) and the ENS. The aim of this study was to investigate the roles of integrins in targeted exosome delivery to enteric neurons.

**Hypothesis:** Exosome targeting of injured neuronal cells is integrin dependent.

**Methods:** Neural stem cells (NSC) were harvested from the small intestine of three day old rat pups. NSC-released exosomes were collected and purified using ultracentrifugation. The intestinal longitudinal muscle myenteric plexus layer (LMMP) was dissected from rat pup intestine and mixed LMMP cells were cultured and exposed to oxygen-glucose deprivation (OGD). NSC-derived exosomes targeting the cultured mixed LMMP cells were analyzed by immunocytochemistry (ICC). The integrin inhibitor RGD was added to cultured mixed cells to examine the effects of integrin in exosome internalization.

**Results:** Under non-injury conditions, NSC-derived exosomes target uninjured NSC but not neurons. After OGD injury, NSC-derived exosomes target injured intestinal neuronal cells. NSC-derived exosomes reduce the number of apoptotic cells during OGD injury as demonstrated by ICC of cleaved caspase 3. In the presence of the integrin inhibitor RGD, there was decreased NSC-exosome internalization to injured neuronal cells.

**Conclusion:** NSC-derived exosomes target injured neuronal cells, and exosome internalization is integrin dependent.