Philadelphia College of Osteopathic Medicine

Annual Progress Report: 2012 Formula Grant

Reporting Period


Formula Grant Overview

The Philadelphia College of Osteopathic Medicine received $14,266 in formula funds for the grant award period January 1, 2013 through December 31, 2016. Accomplishments for the reporting period are described below.

Research Project 1: Project Title and Purpose

Functional Roles of IMP Isoforms in Axon Regeneration by Localizing Specific mRNAs – Axon degeneration is a common pathway leading to neurological deficits after spinal cord and nerve injuries. However, therapeutic approaches to enhance axon regeneration are very limited. Our recent studies point to the functional importance of localizing mRNAs into neuronal axons and subsequently translating them into proteins. The purpose of this project is to investigate molecular mechanisms underlying axon regeneration. We will focus on insulin-like growth factor-II mRNA-binding protein (IMP) isoforms which are known to localize specific mRNAs into regenerating axon. This study will advance our knowledge of the regenerative process and lead to effective therapeutic strategies for axon regeneration.

Anticipated Duration of Project

1/1/2013 – 12/31/2016

Project Overview

It becomes increasingly clear that axons are capable of localizing mRNA and locally synthesizing proteins. Their functional significance has been demonstrated by several lines of evidence. Using in vitro models for axon regeneration, many mRNAs are found to be targeted and translated within regenerating axons following injury. Insulin-like growth factor-II mRNA-binding protein (IMP) 1 is a well-studied mRNA binding protein reported to localize several mRNAs into axons and regulate local translation. Our recent findings further indicate that IMP1 plays an important role in facilitating axon regeneration by transporting multiple mRNA cargos into the axons.

IMP isoforms have conserved RNA binding domains, suggesting that they are most likely to target similar mRNAs. IMP1 expression in the nervous system is hardly detectable after birth, whereas IMP2 expression is sustained throughout life. Our preliminary data reveals that the short
variant of IMP2 promotes axon outgrowth in vitro. Therefore, we hypothesize that IMP2 plays a significant role in axon regeneration by localizing specific mRNAs. We will test this hypothesis in two specific aims to determine whether IMP2: 1) can localize specific mRNAs in the regenerating axons and 2) plays a role in axon regeneration. We will use injury-conditioned dorsal root ganglion (DRG) neuron culture and nerve allograft models. We will perform fluorescent in situ hybridization and immunofluorescence to visualize the localization, high resolution fluorescence microscopy and digital imaging methods to analyze mRNA localization, and immunoprecipitation followed by real-time PCR to examine mRNA targets for IMP2. Electroporation and viral transduction approaches will be applied to manipulate IMP2 expression. Established quantification methods will be used to evaluate axon regeneration after injury. This novel study will provide a new avenue to manipulate axon regrowth and improve axon regeneration.

Principal Investigator

Mei Xu, MD, PhD
Assistant Professor
Philadelphia College of Osteopathic Medicine
4170 City Avenue
Philadelphia, PA 19131

Other Participating Researchers

None

Expected Research Outcomes and Benefits

Recent insights into the functional role of mRNA localization and local translation in axon regeneration have inspired our exploration of its clinical relevance. A mechanism of local protein synthesis requires localization of mRNAs by their binding proteins from cell body to axon terminals. Members of insulin-like growth factor-II (IGF-II) mRNA-binding protein (IMP) have been identified to localized specific mRNAs (e.g. β-actin). Our previous study indicates that IMP1 plays an important role in facilitating axon regeneration by transporting multiple mRNA cargos into the regenerating axons. Our study also reveals that local protein synthesis occurs in both the peripheral axons after nerve injuries and the central axons after spinal cord injury. But the former seems more extensive than the latter, consistent with a restrained regenerative capacity of the central axons. It has been proven that both developing and mature axons are capable of localizing and locally synthesizing proteins. However, IMP1 expression diminishes in the nervous system after birth, whereas IMP2 exhibits a substantial level at all stages of life. Being isoforms of the IMP family, IMP1 and IMP2 are likely to have common mRNA targets. In addition, our pilot data shows IMP2 alternative splice product can enhance axon growth in both motor and cortical neurons. Further investigation of role of IMP2 in axon regeneration will add our knowledge of regenerative medicine. This project will offer an opportunity to develop the potential therapeutic target used to enhance neurological recovery after spinal cord and nerve injuries.
Summary of Research Completed

During this reporting period, we have established and optimized the primary dorsal root ganglion (DRG) neuron culture from embryonic, postnatal and adult mice. We have found different culture conditions for embryonic, postnatal and adult DRG neurons. For example, we used 10ng/ml nerve growth factor (NGF) in DMEM/F-12 with 10% horse serum for embryonic DRG; whereas we used N1 supplement in DMEM/F-12 with 10% horse serum for both postnatal and adult DRG culture (Figure 1). We successfully electroporated the DRG neurons with pmaxGFP®. About 60% of survival neurons were found GFP+ after electroporation (Figure 2A & 2B).

We also performed immunostaining on the cultured DRG neurons. We have found extensive IMP2 expression in the DRG culture from the embryonic, postnatal and adult mice. Its expression is strong in neurons, but weak in Schwann cells (Figure 2B’). In the embryonic and postnatal DRG neurons, intense IMP2 staining is also detected in the entire axon, including their growth cones.

Now we are working on the injury-conditioned DRG culture. We have conducted 2 pilot experiments to optimize the culture conditions. Nerve crush surgery was performed on the sciatic nerve of a mouse at the mid-thigh level before its bifurcation. After surgery, the mouse survived for 7 days before it was euthanized for primary DRG neuron culture, which is called injury-conditioned DRG culture. We were able to pool neurons from 2 mice to get enough for morphological studies.

Primary Postnatal DRG Neuron Culture from thyl-YFP Mouse

Figure 1. The dissociated DRG neurons from thyl-YFP mouse of postnatal 16 days old. The DRG were dissected, dissociated and plated on the 24-well culture plate. At 1 DIV, extensive YFP+ processes can be found for most of the neurons (A, B). Schwann cells are occasionally seen, but they do not express YFP.
Figure 2. IMP2 expression in the adult DRG neurons transfected with pmaxGFP®. The adult DRG neurons were transfected with pmaxGFP® by Nucleofector™. At 1 DIV, a significant number of neurons express GFP after transfection as shown by two examples (A, B). Some of the neurons were further immunostained for IMP2. The YFP+ neuron (B) shows strong staining for IMP2 (B'). IMP2 expression is also found in the Schwann cells (arrow in B'). Staining is absent in the control (A’) which is also expressing YFP after pmaxGFP® transfection (A).