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Characterization of p75NTR Interactions and Associated Effects on Neurodegeneration Associated with Parkinson's Disease

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RCEU 2023 Project Proposal

Project Title

Characterization of $p75^{NTR}$ Interactions and Associated Effects on Neurodegeneration Associated with Parkinson's Disease

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I. Project Description

Parkinson's disease (PD) is a neurodegenerative disorder involving the progressive deterioration and death of neurons that communicate using the neurotransmitter dopamine. During the early stages of PD, the deterioration of these neurons begins at the tips of their axons, and in a process termed axonal degeneration, the axons become dysfunctional and fragmented. The dopaminergic neurons also begin to undergo oxidative stress, a condition in which toxic and reactive molecules accumulate and disrupt numerous cellular functions. The progression of this damage leads to death of the neurons, ultimately resulting in a variety of debilitating motor and cognitive impairments in PD patients. Unfortunately, current treatment options for PD do not impact final outcomes on cognition and mortality. Thus, there is an urgent need for research that reveals the molecular underpinnings of neurodegeneration and elucidates new treatment targets.

The p75 Neurotrophin Receptor ($p75^{NTR}$) is a protein that has been implicated in neurodegeneration associated with a variety of pathological conditions. However, whether $p75^{NTR}$ regulates neurodegeneration associated with PD remains poorly understood. We recently discovered that oxidative stress activates $p75^{NTR}$ signaling in dopaminergic neurons by stimulating cleavage of the receptor into multiple signaling fragments. The student working on this project will use a combination of cell culture and mouse models to evaluate how activation of $p75^{NTR}$ in this manner influences PD-related axonal degeneration, as well as to elucidate the molecular mechanisms that regulate oxidative stress-induced $p75^{NTR}$ cleavage.

II. Student Duties, Contributions, and Outcomes

a. *Specific Student Duties:* The student will dedicate approximately 30 hours per week to this project during a 10-week period of the summer. The student will review research articles to gain a stronger understanding of the area of interest of the laboratory, as well as gain additional conceptual training through participation in weekly meetings with the project mentor, bi-weekly journal club meetings, and bi-weekly group laboratory meetings. The student will interact with the project mentor and laboratory peers to learn cell culture, protein estimation, western blot analysis, cryosectioning, immunostaining, and fluorescence microscopy. Using these methods, the student will conduct experiments in pursuit of the following objectives:

1. Characterize the Expression Profiles of $p75^{NTR}$ Interactors in Dopaminergic Neurons and Evaluate Their Effects on $p75^{NTR}$ Signaling: Cultured dopaminergic neurons will be analyzed by western blot for expression of $p75^{NTR}$ coreceptors, including TrkA, TrkB, TrkC, and sortilin. The effects of any detected coreceptors on $p75^{NTR}$ signaling will then be evaluated. Cultured LUHMES cells will be pretreated with a pharmacological coreceptor inhibitor (or a vehicle solution as a control condition) and then subjected to oxidative stress by exposure to the neurotoxin 6-hydroxydopamine (6-OHDA). Lysates will then be assessed for $p75^{NTR}$ fragments by western blot analysis to determine the effects of coreceptor inactivation on oxidative stress-induced $p75^{NTR}$ signaling.

2. Determine the Effects of $p75^{NTR}$ on Axonal Degeneration Associated with Parkinson's Disease. To model axonal degeneration associated with PD in mice, intrastriatal administration

of 6-OHDA will be performed. This procedure will be conducted using mice with normal genomes (wildtype mice) and mice lacking the gene for $p75^{NTR}$ ($p75^{NTR}$-/- mice) to determine whether $p75^{NTR}$ regulates the axonal degeneration. Immunostaining of striatal tissue slices for tyrosine hydroxylase (an enzyme in dopaminergic axons) and fluorescence microscopy will be performed to quantify the axonal fragmentation induced by 6-OHDA administration.

The student will conduct statistical analyses of collected data and contribute to data interpretation, as well as generation of graphs and figures. The student will also participate in scientific discussions and present research findings in both weekly 1-on-1 meetings with the mentor, as well as bi-weekly laboratory meetings with all members of the Kraemer lab. The student will describe the research project and the impact of findings in a written report.

b. *Tangible Contributions:* The student associated with this project will obtain qualitative and quantitative data. The mentee will also create a poster featuring the data and will have the opportunity to present the poster at the Southeastern Neurodegenerative Disease Conference (held in late September annually) or a similar regional conference. The student will write a report on the findings of the study in the format of a primary research article. Data from rigorously executed experiments will be featured in a manuscript that will be submitted to a national or international, peer-reviewed journal with an impact factor of greater than 3.0.

c. *Specific Outcomes:* Through the aforementioned activities, the mentee will gain a stronger understanding of concepts in the fields of neuroscience and molecular biology; develop an ability to design experiments using appropriate control conditions; learn to manage data and critically analyze data using statistical approaches; acquire an enhanced ability to articulate scientific concepts in written and oral form, and gain experience discussing scientific ideas in a formal environment while attending a regional scientific conference.

III. Student Selection Criteria: Students must have completed a course in cell biology. To ensure that the student can benefit from significant progress during the 10-week project period, preference will be given to applicants with previous molecular biology research experience.

IV. Project Mentorship: Mentorship will be provided through individual meetings held with the mentee each Monday, as well as via biweekly group laboratory meetings held on Fridays. Such meetings involve group discussions about the purpose of our ongoing experiments and our research methodologies. I also foster discussions about responsible conduct of research, planning experiments with appropriate control conditions and blinded designs, and objectively interpreting data. The mentee will also engage in discussions of assigned research articles via bi-weekly journal club meetings. I will personally meet with the student in the lab multiple times throughout each week to train them in conducting assays, as well as have the student collaborate with other experienced lab members to foster peer learning. The mentee will gain instruction in scientific writing as they prepare a project report and receive feedback about report drafts. They will also learn to create a scientific poster and, after practicing their conference presentation with the mentor, receive advice about preparing and delivering effective audiovisual presentations.

Safety and Contingency Plan:

Safety: As required of all members of my laboratory, the student associated with this project will be required to complete training courses in Laboratory Safety, Biological Safety, Bloodborne Pathogens, and Hazardous Waste Management, all of which are offered by the Office of Environmental Health and Safety at UAH. Since our laboratory conducts research using mice, the student will also be required to complete training courses in Animal Biosafety, as well as Responsible Conduct of Research, via the CITI Program. Students will also be provided with a laboratory emergency plan and trained in safety measures as documented in the Chemical Hygiene Plan of the laboratory.

Contingency: If circumstances related to the COVID-19 pandemic or other circumstances restrict the student from meeting in-person with the mentor the following changes will be implemented:

1.) 1-on-1 meetings, journal club meetings, and group laboratory meetings will be held in a virtual format using Zoom.
2.) In-person demonstrations of research methods will be substituted with assigned videos demonstrating how specific research techniques are performed.
3.) If feasible and safe, the student will be allowed to independently perform research techniques in the lab while the mentor provides guidance in real-time via Zoom.
4.) The goals of the project will be adapted to feature a greater emphasis on remote data analysis using Microsoft Excel, ImageJ, and Graphpad Prism, as well as literature review related to $p75^{NTR}$ signaling mechanisms and the influence of neurotrophins on dopaminergic neurodegeneration.

Prior Awardees: Dr. Bradley Kraemer has no prior RCEU awardees and is applying to the RCEU program for the first time.