2023 Summer
NEOSTAR Student Training in Geriatric Research Fellowship

LABORATORY SCIENCE PROJECT DESCRIPTION

1. Project Title: Disease-related changes in the distribution of cell-surface integrins in brain of Alzheimer's model mice
PI: Christine M. Crish, Assistant Professor
Location: Dept. of Pharmaceutical Sciences, NEOMED

2. Abstract: A major goal of our laboratory is to understand some of the earliest pathological mechanisms that contribute to Alzheimer's brain pathology. Decades prior to the first signs of cognitive deficits, tau pathology begins to accumulate in the brain, yet in fully symptomatic disease stages, spread of brain tauopathy closely correlates with progression of cognitive decline. There is a critical need to understand the factors fueling progression of tauopathy across these disease stages, and this defines the overall purpose of our research to identify early disease mechanisms that can be targeted to prevent onset of dementia.

A potential contributor to tauopathy progression involves the dysfunctional signaling of cell surface integrins. In the healthy adult brain, integrins are expressed ubiquitously and play important roles in neural plasticity. Recent studies have suggested that changes in expression levels and function of specific integrin subtypes may be associated with onset and spread of Alzheimer's brain pathology. Our lab has preliminary data showing that αVβ integrins (which are shown to directly bind tau) are dramatically upregulated in hippocampus of transgenic tauopathy model (htau) mice, and this increase occurred over a 5 month period as these animals aged from presymptomatic (5mo.) to symptomatic ages (10 mo.). The proposed project will work to determine which specific integrin subtypes (αVβ1, αVβ3, αVβ5, αVβ6 or αVβ8) are differentially regulated in htau mouse hippocampus and brainstem across disease stage and sex. Student fellows will be expected to work with brain tissue obtained from htau mice to conduct immunofluorescence assays to label αV integrin subtypes in target brain regions, and use microscopy to image and quantify integrin subtypes.

3. Significance:
Dysfunctional integrin signaling has been widely studied as a catalyst for various cardiovascular diseases and cancers, but its relevance to neurodegenerative disease is only just emerging. Integrins are highly accessible, druggable targets for which many modulating agents already exist and are even FDA-approved. Thus, our research proposes to grow the overall understanding of how aberrant integrin signaling contributes to tauopathy.

4. Goals & objectives:
Goal A: Prepare tissue for immunofluorescent assays.
Students will learn how to section fixed mouse brain sections on a microtome, store sections properly, and select region-specific sections that will be assayed.

Goal B. Conduct histological immunofluorescent labeling assays on brain tissue for specific integrin subtype targets. Students will learn the necessary steps on how to conduct antibody-based immunofluorescent assays including making stock laboratory solutions, calculating assay solution needs, and preparing assay incubation solutions. Students will be required to follow all assay steps to complete

assays from start to finish (often a two-day process). Students will then prepare labeled tissue on slides for microscopy analysis.

Goal C. **Perform microscopy and analyze images to determine integrin expression patterns**
Students will use microscopy to image immunofluorescent label of integrin subtypes and provide some level of quantitative analysis of expression patterns in hippocampus and/or brainstem.

5. Research methods

Immunofluorescence and Microscopy
The student will be trained to section fixed brain tissue coronally on a freezing sliding microtome. The student will then be trained to use multicolor immunofluorescence assays to visualize expression of integrin subtypes in hippocampus and brainstem regions of mice. We will compare integrin distribution between groups of age- and sex-matched Alzheimer's model htau mice and healthy control mice. The student will be trained to photograph brain sections using a Zeiss AxioZoom V16 epifluorescent macroscope equipped with a digital high-resolution camera and a computer guided motorized stage and Z-axis and an Axio Imager M2 epifluorescent microscope with a digital high resolution camera and Apotome structured illumination module for tissue requiring higher magnification. Each structure of interest will be imaged at under multiple channels to capture different labels from antibody staining. Images will be z-stacked, flattened with the extended depth of focus module of the Zen microscope software and converted to tiffs or jpegs for analysis. Students will then be trained how to identify brain regions and quantify integrin label using Image Pro software and prepare publication-quality micrograph images for presentation.

6. Proposed method of data analysis
We will use SPSS for IBM Statistical Software to analyze all data. The PI will directly guide the student fellow in the use of this program in order to calculate the applicable analyses if the student has no prior experience in statistical analysis. The student will also be required to generate figures and illustrations depicting important findings using Prism and Adobe Illustrator.

7. Outcomes of research findings
This project will generate fundamental data on integrin expression patterns in tauopathy, which has not been previously investigation. Knowledge in this area is critical because it will support future research that seeks to test novel pharmacological strategies to prevent or slow progression of dementia.

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2023 SUMMER RESEARCH FELLOW MENTORSHIP/TRAINING PLAN

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Training and site where research will be conducted
The student will perform the research at NEOMED in the C. Crish research lab and ancillary shared lab rooms on the fourth floor of RGE. The student accepted for this project will have an initial training phase that involves both web-based lab safety (EOHS online program). Students will not work directly with animals, just brain tissue obtained from animals, and therefore, will not be required to complete any CITI-training for working with live animals. Students will receive one-on-one skills-based

training with lab personnel. After these requirements are met, he or she will be directly trained by the PI (C. Crish) or senior lab staff on tissue preparation, assay conducting, microscopy, and analysis.

Resources available

The C.Crish Lab has access to all the resources necessary to train the summer fellow and enable them to carry out this work plan. The PI has a repository of brains collected from Alzheimer's and control mice across different disease stages/ages. The C. Crish lab owns a library of antibodies relevant to the proposed work, auxillary chemicals, laboratory supplies, and basic laboratory equipment (shakers, pipetters, incubators, etc) to conduct assays. The PI has access to all the required equipment, which is either part-owned by the PI, other colleagues in Pharmaceutical Sciences, or is core equipment of the Neurodegenerative Disease and Aging (NDA) research focus area which grants the PI free and unlimited use. The PI also owns statistical analysis software (SPSS) and image processing software (Adobe Creative Suite; Prism; Image Pro). C. Crish lab has dedicated lab bench space to accommodate lab staff and a dedicated desk/computer adjacent to the lab for use by research assistants.

Mentorship plan

The PI and student will have weekly one-on-one meetings to discuss the plan for data collection and analysis as well as to ensure that the project is moving forward at the correct pace. The PI has developed a workflow for all new lab assistants that details and tracks skills learned and their proficiency level, and this workflow will be employed for the student fellow as well.

The student will also attend the weekly C.Crish Lab research meetings to present and discuss their progress. The student fellow will work with the PI to assemble a research poster to present their data at the NEOMED OPRS summer fellowship presentation day.