PILOT AWARDS – FISCAL YEAR 2016

Institute on Aging Pilots

1. Elucidating Opportunities for Improving the Field Triage and Quality of Hospital Care for Injured Older Adults

PI: Mucio Kit Delgado, MD, MS, FACEP

Abstract: The purpose of this pilot grant application is to catalyze the collaboration and extramural funding of three young investigators at Penn from fields of emergency medicine, anesthesiology and critical care, and trauma surgery who mutually share a goal of reducing the burden of traumatic injury on older adults. Injury is the leading cause of loss of independence among the elderly and accounts for over 880,000 hospitalizations with 24,000 of these ending with in-hospital deaths. While it is well recognized that emergency medical services (EMS) field triage and transport to a designated trauma center has been shown to reduce mortality from major injuries by at least 25% in patients under the age of 55, similar benefits have not been observed for older adults. It is unknown why there is a discrepancy in the overall benefit of trauma center triage for younger vs. older adult major trauma patients. The persistence of this knowledge gap is problematic given: 1) current triage guidelines consider age >55 as indication alone for triage to a trauma center despite absence of evidence that this approach is beneficial; and 2) the majority of injured elderly adults prefer to be transported by EMS to their local community hospital. The objective of this application is to determine whether the benefit of triage to a trauma center in this population is heterogeneous according to patient characteristics and whether differences in the quality of care following complications explains why trauma center care has not been as beneficial for older adults as it has been for younger adults. To carry out this project, the PI has also recruited an establish health economist and expert in emergency care research who has previously linked EMS, hospital, and vital statistics records in the state of New Jersey. It is expected that this will be the first study to potentially identify which injured older adult benefit from triage to a trauma center and that this will provide the preliminary data needed for the investigators to secure large-scale extramural grants aimed at identifying ways to improve the acute care for injured older adults.

2. Role of Alpha Synuclein Arginylation in Prevention of Neurodegeneration

PI: Anna S. Kashina, PhD

Abstract: Neurodegenerative disorders affect one in every four older Americans, costing billions of dollars in health care every year and significantly worsening the quality of life. These highly destructive diseases are characterized by abnormal accumulation of misfolded proteins in the brain that lead to neuronal loss and progressive neurodegeneration. Among those proteins, a major role belongs to alpha synuclein, a protein that prominently accumulated in the brain of Parkinson's disease patients and has also been shown to participate in other forms of neurodegeneration. The mechanisms that trigger abnormal misfolding and intracellular accumulation of alpha synuclein are not fully understood. Our preliminary data demonstrate that alpha synuclein serves as highly efficient and specific target of protein arginylation, an emerging posttranslational modification that regulates protein-protein interactions and targets a subset of proteins for ubiquitin-dependent degradation. We find that arginylation specifically targets alpha synuclein in the normal brain via a novel side-chain mechanism that modifies internal Glu residues of the synuclein sequence. Moreover, mice with knockout of arginyltransferase Ate1 exhibit symptoms of neurodegeneration that progresses with age and our preliminary tests suggest that alpha synuclein accumulates abnormally in the brain of these mice. This proposal will test our hypothesis that side chain arginylation constitutes a novel regulatory mechanism that prevents abnormal accumulation of alpha synuclein in the normal brain, and that impairments in arginylation lead to neurodegenerative disorders. We propose the following specific aims: (1) to test the hypothesis that alpha synuclein arginylation prevents its misfolding and facilitates its turnover; (2) to test the hypothesis that alpha synuclein arginylation prevents its accumulation in the normal brain; and (3) to test the hypothesis that a reduction of alpha synuclein arginylation correlates with brain aging and neurodegeneration in human patients. If successful, these experiments will outline a novel pathway that regulated alpha synuclein through side chain agrilyation, protects the brain from neurodengeration and maintains normal brain health.

3. Role of HYPK Protein in Huntington's Disease

PI: Ronen Marmorstein, PhD

Abstract: The goal of this project is to determine the molecular role of the HYPK protein in Huntington's disease (HD). Huntingtin (Htt) yeast two-hybrid protein K (HYPK) is a subunit of the NatA N-terminal protein acetyltransferase (NAT) complex, one of the six metazoan N-terminal acetyltransferase (NATs) that differ in their substrate specificities and subunit composition. NatA acetylates N-terminal Ser, Ala, Thr, Val and Gly residues and contains three protein subunits, the Naa10p catalytic subunit, and two regulatory subunits, Naa15p and HYPK. The molecular role of HYPK in the NatA complex is unclear, although HYPK interacts with Htt and diminishes Htt polyglutamine aggregation associated with the pathogenesis of HD1,2. The N-terminus of Htt contains the sequence ATL, suggesting that Htt is a NatA substrate. However, the involvement of NatA and HYPK in HD has not yet been explored. We hypothesize that the binding of HYPK to NatA modulates the N-terminal acetylation of Htt, thus implicating both NatA and HYPK in the pathophysiology of HD. Consistent with this hypothesis, knockdown of either the Naa10p catalytic subunit of NatA or HYPK in HeLa cells leads to an increase in Htt aggregation. We propose to test this hypothesis through the following specific aims: (1) Determine the molecular role of HYPK in NatA-neduated acetylation of Htt, and (2) Characterize the aggregation properties of Htt as a function of NatA acetylation. These studies will represent the first model where N-terminal acetylation reduces protein aggregation for neurodegenerative disease resistance and will provide novel avenues for treating HD and possibly other protein misfolding neurodegenerative disorders.

4. Neural Correlates of Cognitive Fatigue and Blue Light Treatment in Older Adults

PI: Hengyi Rao, PhD

Abstract: Fatigue is a common neuropsychiatric complaint and can be defined as difficulty initiating or sustaining voluntary physical, mental, and social activities. Older people suffer significantly more from fatigue symptoms than younger individuals, yet both the neural basis for fatigue and the mechanisms of action of anti-fatigue treatments remain poorly understood. Using functional magnetic resonance imaging (fMRI), we have successfully examines the neural correlates of fatigue in healthy adults (age between 21-50 years) after prolonged cognitive workload and/or sleep loss. Our data demonstrates that fatigue may be associated with altered resting brain function in the default mode network (DMN), thalamus, and fronto-parietal attention network. However, it remains unknown if fatigue will affect the aged brain in the same manner. Therefore, this project aims to collect pilot data to further examine the neural correlates of cognitive fatigue in n=12 cognitively intact older adults (age >= 65 years). We also propose to evaluate the efficacy of blue light, a stimulus that signals circadian daytime, for reducing fatigue and enhancing brain function. We hypothesis that cognitive fatigue will be associated with reduced resting brain function in the DMN, thalamus, and fronto-parietal attention network in older adults, while blue light therapy will reduce fatigue symptoms by mitigating the brain function changes in these networks. We primarily seek funding to cover the cost for MRI scans as well as partial salary support for the PI and a postdoctoral researcher who will carry out the proposed work. This multidisciplinary project t will help the early career PI who seeks to enter research fields on aging with his expertise in functional brain imaging, sleep, and fatigue research. This project is expected to yield key preliminary data for future NIH/NIA grant applications examining the neural bases underlying fatigue and its therapy in healthy older individuals as well as in those suffering from neurodegenerative disorders.

5. Regeneration of the Intervertebral Disc using Notochord-Derived Cells and Mesenchymal Stem Cells

PI: Lachlan J. Smith, PhD

Abstract: Lumbar intervertebral disc degeneration, a condition ubiquitous amongst the aging population, is a cascade of cellular, structural and mechanical changes that is strongly implicated as a cause of low back pain. There is a critical need for new therapies for disc degeneration that both alleviate painful symptoms, and restore disc structure and mechanical function by directly addressing the underlying biological causes. One area of active research is to use stem cell based therapies, such as MSCs, to regenerate the central nucleus pulposus (NP). A central challenge, however, is that unlike cartilage and bone cells, NP cells are derived from the embryonic notochord, not the mesenchyme. The overall objective of this pilot study is to establish the optimal cell phenotype for regeneration of the intervertebral disc using a novel in vivo mouse model. We hypothesize that

notochord-derived cells (NDC's), the embryonic progenitors of NP cells, possess the ideal properties for disc regeneration. Specifically, we hypothesize that NDCs will survive upon delivery to the degenerate disc, differentiate towards an NP cell-like phenotype, and will exhibit enhanced ability to regenerate the disc compared to adult mesenchymal stems cells (MSCs). We propose the following aims: (1) Investigate the survival and phenotypic stability of NDCs in an in vivo mouse model of intervertebral disc degeneration; and (2) Compare the regenerative potential of NDCs from different stages of embryonic development to adult MSCs in an in vivo mouse model of intervertebral disc degeneration. We will use an established mouse caudal disc injury model of degeneration, which recapitulates key characteristics of human disc degeneration. Novel in vivo MRI and fluorescent optical imaging will be used to assess disc condition, and cell survival and migration, respectively. Regenerative effects of injected cells will be assessed post mortem in terms of disc composition, mechanical function, degeneration grade and expression of key matrix and phenotypic markers. The results of this pilot study will establish the ideal cell characteristics for intevertebral disc regeneration. Our ultimate goal is to recapitulate these characteristics using translationally appropriate cells such as MCSs or IPSCs.

6. Transcription Errors in Age-related Neurodegenerative Diseases

PI: Marc Vermulst, PhD

Abstract: Transcription is required for every biological process inside a cell. Although most transcripts are generated faithfully from their DNA template, errors do occur form time to time. How these errors affect cellular function is unknown. To answer this question, we monitored yeast cells that were genetically engineered to display error-prone transcription. To our surprise, we discovered that these cells suffer from a profound loss in proteostasis, which sensitizes them to the expression of genes that are associated with protein-folding diseases in humans. For example, transcription errors accelerated the aggregation of a peptide that is implicated in Alzheimer's disease (A-beta 1-42), amyotrophic lateral sclerosis (TDP-43), and Huntington's disease (Htt103). We further discovered that the error rate of transcription increases with age in yeast, suggesting that transcription errors reduce proteostasis particularly in aging cells. Together, these results suggest that transcription errors represent a novel, basic biological process that exacerbates cellular aging and disease. To determine the importance of these findings for human aging and disease, it will be important to repeat these experiments in human cells. We propose to do this using human cell lines that are genetically engineered to display error prone transcription. We will then use these cells to determine the effect of transcription errors on the toxicity and aggregation of proteins associated with age-related neurodegenerative diseases. These experiments have the potential to identify a novel, mechanistic link between aging and disease, and greatly enhance our understanding of age-related pathology.

Penn Alzheimer's Disease Core Center (ADCC) Pilots

7. microRNA Changes in Alzheimer's disease Brain and CSF by Small RNA Sequencing

PI: Yuk Yee Leung, PhD

Abstract: Alzheimer's disease (AD) affects millions worldwide, yet no effective early diagnosis or therapies are available. Recent discoveries of functional small non-coding RNAs (sncRNAs), especially microRNAs (miRNAs), have greatly increased our understanding of neurodegenerative diseases and their role in AD using blood, cerebrospinal fluid (CSF), or autopsy brain. Most of these however focus on studying a few miRNA targets at a time. With the development of next generation sequencing (NGS) technologies such as small-RNA sequencing (smRNA-seq), we can 1) discover novel sncRNAs; 2) study many classes of sncRNAs at a time; 3) obtain a comprehensive list of putative sncRNAs as possible candidates for AD biomarkers. Results from smRNA-seq on autopsy brains may reflect the neuropathology in AD brains, the gold standard of AD diagnosis. Validation is challenging because postmortem findings should be confirmed in vivo, and CSF is a promising source to meet this requirement. New experimental and bioinformatics approaches are needed for analyzing smRNA-seq data and prioritizing circulating sncRNA candidates for validation.

Innovation and approach: We will demonstrate the feasibility of using NGS to find circulating CSF miRNAs and other sncRNAs biomarkers. We have assembled an interdisciplinary collaboration with several labs at UPenn SOM, including Wang (bioinformatics), Schellenberg (AD Genetics), CNDR/PMC (clinical and molecular pathology of AD), Gregory (RNA) and NGSC (sequencing). The specific aims are:

Specific Aim 1: Perform smRNA-seq on CSF from three groups of subjects (15 samples each): cognitive normal, AD with ApoE e4 carriers and AD without ApoE e4 carriers. Fresh CSF will be obtained upon lumbar puncture at Penn PMC. Will will refine the protocol to maximize RNA isolation from these samples for sequencing using HiSeq2500 high output mode.

Specific Aim 2A: Develop a customized bioinformatics pipeline to prioritize AD-relevant miRNAs for validation. The pipeline will integrate public genomics databases including functional genomics data from brain, functional annotation on miRNA-mRNA target pairs, and molecular biology knowledge of AD neuropathology. Together with the CSF smRNA-seq data, our pipeline will rank each miRNA for its propensity in contributing to AD pathology.

Specific Aim 2B: Validate top miRNA candidate biomarkers on sequenced subjects and replicate on a new cohort of AD and CN subjects. We will also study their relationships with A-beta 1-42 and tau proteins.
We expect our proposed approach, which combines both experimental and bioinformatics techniques, will help us develop novel circulating sncRNA biomarkers. The same approach has the potential for other translational research needs, such as accelerating drug target discovery for AD and other neurodegenerative diseases. Deliverables include 1) smRNA-seq data from CSF; 2) a bioinformatics pipeline to identify AD-relevant sncRNAs will be developed and can be applicable to other diseases; 3) qRT-PCR results on validated miRNAs will be shared. Results will be submitted to the public NIA Genetics of Alzheimer's Disease Storage Site (NIAGADS) and available to investigators at Penn and the general research community.

8. Genetic Risk Factors of Primary Age Related Tauopathy

PI: Corey T. McMillan, PhD

Abstract: The primary risk factor for developing Alzheimer's disease (AD) is age: an estimated 4% of aging individuals < 65 years old and 40% of aging individuals > 80 years old will ultimately develop AD. The neuropathological criteria for AD include the co-occurence of neurofibrillary tau tangles (NFT) and amyloid plaque (Aß) burden. However, there are now several reports of aging individuals who at autopsy have substantial NFTs in the absence of Aß. In fact, nearly all older adults > 50 years of age have NFTs and therefor a new neuropathological consensus diagnosis term called "primary age-related tauopathy" (PART) has been proposed to capture these individuals that range from normal to demented cognitive status. While several genetic risk factors have been proposed for AD, the genetic risk factors of PART remain unknown. It is critical to identify biomarkers and risk factors associated with PART so that patients can be accurately screened for emerging trials of tau-related disease-modifying agents. Also, as we enter an era of precision medicine in Neurology it is becoming increasingly important to identify genomics factors that may influence outcome of therapeutic intervention. In this proposal we will investigate a candidate risk factor, a genetic variant in the bridging integrator 1 (BIN1) gene, that has previously been associated with risk of AD, hippocampus pathology, and NFTs. Specifically, we will use neuroimaging (Aim 1) and neuropathological assessments (Aim 2) to investigate associations between BIN1 polymorphism and candidate genetic mechanisms that contribute to APRT in the aging population.