Duquesne University

Annual Progress Report: 2009 Formula Grant

Reporting Period

July 1, 2013 – December 31, 2013

Formula Grant Overview

The Duquesne University received $121,663 in formula funds for the grant award period January 1, 2010 through December 31, 2013. Accomplishments for the reporting period are described below.

Research Project 1: Project Title and Purpose

Implementation of an Asthma Program to Improve Asthma Identification and Education in Children - The purpose of the Asthma Program is to develop and implement a method to increase the identification of children with undiagnosed or uncontrolled asthma; to develop and evaluate educational interventions that teach caregivers and children with asthma how to properly control their disease state; and to encourage active healthy lives. The goal of the Asthma Program is to improve health outcomes and quality of life for children suffering from asthma.

Duration of Project

1/1/2010 - 6/30/2013

Summary of Research Completed

This project ended during a prior state fiscal year. For additional information, please refer to the Commonwealth Universal Research Enhancement C.U.R.E. Annual Reports on the Department's Tobacco Settlement/Act 77 web page at http://www.health.state.pa.us/cure.

Research Project 2: Project Title and Purpose

Engineering Aβ-selective Enzymes for Treatment of Alzheimer's Disease - This project involves approaches aimed at engineering metalloendoproteinases to specifically cleave and clear Aβ peptide as a potential therapeutic approach for treatment of Alzheimer's Disease (AD). Dysfunctional metabolism leading to excess quantities of Aβ that is produced at normal levels throughout life may be the causative agent in many, if not most, cases of AD. Neprilysin, neprilysin-2 and insulin degrading enzyme have been shown to cleave Aβ *in vivo* or *in situ.* However, these enzymes are promiscuous with respect to substrate specificity and proteolyze other (neuro) peptides involved in critical physiological responses. The nano-engineering studies proposed here offer the opportunity to produce Aβ-selective enzymes that may be safely

employed in subsequent gene transfer studies aimed at treating AD.

Duration of Project

1/1/2010 - 12/31/2013

Project Overview

Protein engineering offers the opportunity to design proteins with altered functions, via site-directed mutagenesis of regions targeted towards catalytic and/or substrate-binding sites as determined by atomic resolution structural studies. We propose two specific aims in order to engineer Aβ-selective enzymes for treatment of Alzheimer's Disease (AD):

Aim 1: Utilize peptidomic methods to profile substrates of neprilysin, neprilysin-2 and insulin degrading enzyme. These mass spectrometric methods will also be used in Aim 2 to similarly profile substrates of mutagenzied enzymes, and identify cleavage sites in Aβ.

Aim 2: Engineer a more selective form of these metalloendoproteinases with increased specificity solely towards Aβ to limit aberrant catabolism of other natural targets of the enzyme using phage display methods.

The nanoengineering of any of these three peptidases to safely express enzymatic activity will be a novel therapeutic tool in the treatment of AD by acting selectively to catabolize Aβ for requisite proof-of-concept data essential to integrate these studies in the *future* for preclinical trials. In addition, this systems biology approach using three similar, but unique, endoproteinases will provide data regarding enzyme design and basic information regarding targeted nanosculpting of protein activity for other aging disorders. This generalizable approach, wherein a protease is modified to more selectively, and specifically, degrade amyloidogenic substrates, may also be exploited to combat other amyloidoses, and provide a major tool for reducing plaque burden. *Overall, enzymes that cleave and clear* Aβ *can be protective, but these same enzymes also cleave other target peptides, and their use in treating this disease might have potentially lethal side effects.* Our successful implementation of nanoengineered enzymes will produce novel therapeutics that may be safe and efficacious agents for treating Alzheimer's Disease.

Principal Investigator

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Other Participating Researchers

Marc J. Glucksman, PhD - employed by Rosalind Franklin University in Chicago

Expected Research Outcomes and Benefits

Successful engineering of an Aβ-selective neprilysin, neprilysin2 and/or insulin-degrading enzyme would provide an important therapeutic tool in combating Alzheimer's Disease. Our ultimate aim is to develop an effective degradative enzyme that safely and specifically only targets neurotoxic Aβ to strategically combat AD. The approach pioneered in this project may have a value-added feature in that engineering of a more specific form of an endogenous enzyme with specificity for the pathological protein component in the amyloid deposit may prove to be of general use for other amyloidogenic diseases or protein misfolding diseases. Since the accumulation of amyloids appears to be a common pathological function in degenerative disease, the design and re-engineering of catabolic enzymes to specifically target a given amyloidogenic peptide/protein may have broad therapeutic utility.

Summary of Research Completed

As described in previous reports, we have primarily focused our latter work on novel computational studies to better understand the substrate-binding site of the enzyme, while we have continued to conduct experimental studies as part of undergraduate training. As part of these studies we have published a comprehensive review entitled "Neprilysin Inhibitors Provide Insight into the Specificity of the Enzyme and its Potential for Alzheimer's Disease Therapy" in Frontiers of Drug Design and Discovery. This review examines the structural and biochemical characterization of neprilysin-inhibitor interactions in order to provide insight into metalloendoproteinase function and is now in press. This review was submitted in the previous year, but revisions and communication with the journal were conducted during this reporting period. We have also submitted a manuscript describing our completed modeling studies that assess binding modes of Aβ in the active site of neprilysin (these studies were completed during this reporting period, and included the computational studies described therein). This manuscript, "β-amyloid and neprilysin computational docking studies identify critical residues implicated in binding specificity" was submitted to an ACS journal, The Journal of Chemical Information and Modeling (submitted 9/2013). The submission was considered to be acceptable with minor revisions, and the revised version will be submitted in the coming weeks. The successful publication of these studies, supported by our CURE grant, are expected to support future efforts at soliciting extramural funding and provided guidance in directing our on-going mutagenic studies of neprilysin (and other metalloendoproteinases) so that we may engineer a more selective form of these enzymes via phage display studies.