PROJECT TITLE	**Role of smooth muscle O-GlcNAcylation in regulation of cognitive dysfunction in Type 2 diabetes**
PRINCIPAL INVESTIGATOR	**Priya Raman**, Ph.D., Associate Professor, Biomedical Sciences
LOCATION	NEOMED, Rootstown

ABSTRACT OF PROJECT

Type 2 diabetes (T2D) negatively impacts cerebrovascular function and increases the risk of developing Alzheimer's disease-related dementia (ADRD). Growing literature supports the notion that dysregulated function of vascular smooth muscle cells (VSMC), a major cell type found in blood vessels that regulate cerebrovascular function, is a putative player in AD-related pathology and neurodegeneration. Hyperglycemia, a hallmark feature of T2D, increases O-GlcNAc transferase (OGT) signaling, a key regulator of protein O-GlcNAcylation. Increased O-GlcNAcylation, a ubiquitous posttranslational modification, correlates with adverse vascular remodeling and vascular dysfunction in T2D. However, it is unknown whether increased cerebrovascular O-GlcNAcylation contributes to cognitive dysfunction and AD pathology in T2D. The proposed project provides a unique platform to determine whether loss of VSMC-specific OGT-mediated O-GlcNAcylation inhibits cognitive dysfunction in T2D. For this, conditional VSMC-specific OGT knockout mice and wild-type controls (both sexes) on a high-fat high-sugar diet will be aged to 6 months, followed by a battery of behavioral tests to measure their cognitive, emotional, and sensorimotor behavior. The summer student working on this project will learn murine behavior phenotyping, including scoring of the behavioral tests, data analysis and data interpretation. The proposed studies will advance our understanding of how T2D contributes to cognitive dysfunction, with a focus on the regulatory role of cerebrovascular OGT-mediated O-GlcNAcylation, paving the way for the discovery of novel therapies to treat ADRD in diabetes.

BACKGROUND AND SIGNIFICANCE

Vascular pathology occurs in up to 50% of individuals with Alzheimer's disease-related dementia (ADRD) and frequently co-exists with AD-related neurodegenerative changes. Emerging evidence indicates that cerebrovascular dysfunction often precedes the onset of clinical symptoms and is a significant risk factor for the development of AD. Despite the importance of cerebrovascular dynamics in brain function, the molecular mechanism(s) that mediate cerebrovascular deficits that may lead to cognitive decline and AD pathology are poorly understood. Vascular smooth muscle cell (VSMC) is a major cell type found in blood vessels that regulate the vascular tone of the cerebral artery and are the primary controllers of cerebrovascular dynamics. Increasing evidence indicates that VSMCs have remarkable plasticity and readily transform into diseased phenotypes in response to pathogenic stimuli.

Type 2 diabetes (T2D) negatively impacts vascular function, which in turn may adversely affect brain health. T2D patients have an increased propensity for VSMC de-differentiation from 'quiescent' contractile to 'synthetic' proliferative phenotypes. Such proliferative VSMC phenotypes are linked to vascular disease progression and upregulation of multiple AD-related pathways. This supports the notion that dysregulated VSMC function may be a putative player in AD-related pathology and neurodegeneration in T2D. Hyperglycemia, a hallmark feature of T2D, increases protein O-GlcNAcylation, a ubiquitous post-translational modification (PTM) that plays a crucial role in cellular signaling and metabolism. Increased O-GlcNAcylation correlates with adverse vascular remodeling and vascular dysfunction in T2D. However, whether increased cerebrovascular O-GlcNAcylation contributes to cognitive dysfunction and AD pathology in T2D is unknown.

Using a novel transgenic mouse model lacking VSMC-specific OGT (a key regulator of O-GlcNAcylation), the proposed work is part of a larger research initiative to test the overarching hypothesis that elevated cerebrovascular O-GlcNAcylation promotes cerebral VSMC fate switch to diseased phenotypes, prompting cerebrovascular dysfunction, AD-related pathology, and cognitive dysfunction in T2D.

GOALS AND OBJECTIVES

Goal: We will investigate whether loss of VSMC-specific OGT-mediated O-GlcNAcylation inhibits cognitive dysfunction in high-fat, high-sugar diet-induced diabetic mice.

Objectives:

1) To compare the sensorimotor function and emotional reactivity of diabetic mice with intact OGT (sm*Ogt*^{WT}) vs. diabetic mice with VSMC-specific OGT loss-of-function (sm*Ogt*^{KO}).

2) To compare the attention and memory of diabetic sm*Ogt*^{WT} mice (with intact OGT) vs. diabetic sm*Ogt*^{KO} mice (with VSMC-specific OGT deletion).

Experimental Design: We will use conditional VSMC-specific *Ogt* knockout mice developed in our lab by crossing *Ogt-floxed* mice with a tamoxifen-inducible VSMC-restricted Cre driver mouse (*Itga8-CreER*^{T2}), expressing CreER^{T2} under the control of the mouse alpha integrin 8 (*Itga8*) promoter; all mice will be on C57Bl6 background. Here, tamoxifen-induced Cre recombination triggers conditional VSMC-restricted *Ogt* deletion. Age and sex-matched sm*Ogt*^{WT} and sm*Ogt*^{KO} mice will be placed on a high-fat, high-sugar (HFHS) diet to induce diabetes and aged to 6 months. For the study duration, body weight and non-fasted blood glucose levels will be measured monthly. Two weeks before the study endpoint, mice will be subjected to Intraperitoneal Glucose Tolerance and Insulin Tolerance Tests (IPGTT, IPITT) at one-week intervals. At 6 months of age (study endpoint), mice will be used for behavioral studies as outlined below. This will be followed by animal harvests for blood and tissue collection for molecular studies.

The above-described animal experiments are currently ongoing in our lab and we anticipate completion of the HFHS diet feeding, body weight and blood glucose monitoring as well as GTT and ITT measurements, prior to initiation of the summer fellowship. Due to the restricted time frame of this fellowship, the summer student will focus on behavioral tests outlined below in a subset of mice, and will conduct scoring of the behavioral data, followed by data analysis. The summer student will also participate in animal harvest procedures, including the collection of blood and tissue samples for future analysis.

INVESTIGATIVE METHODS TO BE USED

A battery of behavioral tests will be used to measure cognitive function, including the Barnes maze that measures spatial memory, an Object Recognition test that measures both attention and memory, and the Y-maze that measures habituation and working memory. In addition, basic tests of sensorimotor function (beam traversal and spontaneous activity) and emotional reactivity (elevated plus maze) will be used to ensure that any detected differences in the cognitive tests are not due to motor impairments and/or enhanced fear. Tests will be implemented in the same order (**challenging beam, spontaneous activity, elevated plus maze, Y-maze, object recognition, and Barnes maze**) for each cohort of mice and at the same time of day. No more than two tests will be performed in one day and male and female testing will be performed separately to prevent potential interference with behavioral performance by pheromones. Videos taken during testing will be scored by experimenters blinded to genotype information and when multiple scorers are needed, they will have an inter-rater reliability of ~95%.

We will collaborate with Dr. Sheila Fleming on these studies. Dr. Fleming has considerable experience with murine behavior phenotyping and currently has these tests set up and available in her laboratory with established protocols. My lab has been working with Dr. Fleming for the past 5 years, and we have a senior graduate student who is routinely conducting murine behavioral studies.

PROPOSED METHOD OF DATA ANALYSIS

All data sets will be checked for normality and homogeneity of variance, followed by appropriate statistical analyses (parametric or non-parametric). For parametric data, statistical significance will be analyzed by one-way or two-way ANOVA followed by Tukey HSD pos-hoc or unpaired two-tailed Student's t-test, as appropriate. For non-parametric data, Kruskal-Wallis followed by Dunn's post-hoc or Mann Whitney U/Wilcoxon Sign Rank tests will be used, as appropriate; $p \leq 0.05$ is considered statistically significant.

SIGNIFICANCE OF ANTICIPATED FINDINGS

Expected Outcome. We predict that VSMC-specific loss of *Ogt* and O-GlcNAcylation will halt cognitive dysfunction in diabetic sm*Ogt*^{KO} vs. sm*Ogt*^{WT} mice (with intact *Ogt*).

Impact. The proposed studies will provide key pilot data in support of our hypothesis and validate the experimental feasibility of studies planned in a future R01 application, aiming to explore the mechanism(s) by which metabolic syndrome contributes to AD-related cognitive decline and cerebrovascular dysfunction.

SUMMER RESEARCH FELLOW TRAINING/MENTORING PLAN

The proposed study will offer research and training opportunities for two students, and we anticipate that these students will work concurrently on this project. Therefore, we request support for two summer students for this study.

Plan for Training/Mentoring: The summer research fellow(s) will be supervised and mentored by Dr. Priya Raman. During the first 2-3 weeks of the program, the students will receive hands-on training from Dr. Raman's graduate student, who is skilled in murine behavior phenotyping. Upon demonstration of adequate independence, the summer fellows will be expected to run independent experiments under close supervision by Dr. Raman and her team. Dr. Raman will meet with the summer students weekly to discuss the proposed research question, relevant scientific literature, and progression of experiments and data and will also provide necessary guidance on the approaches proposed in the project. Dr. Raman will be responsible for student training in all aspects of this project, including data analysis, graphical presentations, interpretation of data and poster preparation and presentation. The summer fellows will also receive training in reading the scientific literature relevant to the project. Dr. Raman is a member of the HBVD and DOM RFAs and the summer students will be expected to participate in weekly group meetings organized by the HBVD and DOM RFAs. These meetings will develop the student's research horizons and enhance his/her scientific presentation and perception skills. At the end of the training period, the students will be expected to submit a brief report summarizing the project and results and present their data during NEOMED's Annual Poster Day.

Description of Resources available: The summer students will have access to Dr. Raman's laboratory, Dr. Fleming's behavioral test equipment and other departmental core facilities, as needed for completion of the proposed studies. The summer fellows will also have access to graphing and imaging software, as needed.

Site where the research will be conducted: This project will be conducted in Dr. Raman's laboratory and Dr. Fleming's behavioral suite at NEOMED.