Form 3

Report for Joint/Usage Research Program for Endocrine/Metabolism (Fiscal Year 2020)

Date：2021/3/6

To Director of Institute for Molecular and Cellular Regulation, Gunma University

Principal Applicant	
Institution	University of Minnesota
Position	Assistant Professor
Name	Emilyn Alejandro

We report on the results of joint research in fiscal 2020 as below.

（Program No. 20007）

1． Research Title	**Role of nutrient-driven O-GlcNAc-posttranslational modification in pancreatic exocrine and endocrine islet development**
2． Purpose and Significance of the research project	Type 2 diabetes (T2D) is the most common chronic disease affecting human health. We propose that OGT (O-GlcNAc Transferase), a nutrient-sensor expressed at a very high level in β-cells, has key developmental regulatory properties and the ability to integrate signaling networks to regulate β-cell plasticity in response to insulin demand and nutrient stress. OGT is the sole enzyme adding a single O-GlcNAc post-translational modification (O-GlcNAcylation) onto proteins to orchestrate and fine-tune glucose metabolism, and β-cell growth and maintenance of identity under stress responses to nutrient changes and hormonal cues. We hypothesize that OGT tightly controls the O-GlcNAcylation state of downstream targets, including Pdx1, to promote β-cell development and function. Thus, our long-term goal is to define the mechanisms of how OGT integrates signaling networks impinging on β-cell plasticity (development and identity) to promote functional β-cells. To establish the molecular mechanisms of how OGT regulates pancreas and β-cell development, we aim to delineate the impact O-GlcNAc modification on key proteins, such as Pdx1, that is essential for pancreas development. Pdx1 (pancreatic and duodenal homeobox 1) is necessary for pancreatic development, including β-cell maturation and function. We recently determined that OGT is required for pancreas development as genetic deletion of OGT in pancreatic progenitors caused pancreas agenesis. In the current grant, our aim is to assess whether genetic reconstitution of Pdx1 rescues pancreas agenesis induced by OGT loss. This grant is significant because it will show the central role of OGT in β-cell biology and will open new horizons for therapies for patients with diabetes. Moreover, this grant will strengthen a newly formed collaboration between the labs of Dr. Emilyn Alejandro at the University of Minnesota, USA and Dr. Yoshio Fujitani, Gunma University in Japan.

3. Period of The Program	April 1, 2020 ～ March 31, 2021

4. Project Members

Name	Age	Gender	Institution/Department	Position	Role
(Principal Applicant) Emilyn Alejandro	40	F	University of Minnesota Medical School, Integrative Biology and Physiology, Twin Cities	Assistant Prof	Principal Investigator
(Research Collaborators) Yoshio Fujitani	54	M	Lab of Developmental Biology & Metabolism Institute for Molecular & Cellular Regulation	Professor	Collaborator

※If additional space is required, attach a separate sheet.

5. Collaborative Researcher of IMCR	Name of the Laboratory	Lab of Developmental Biology & Metabolism Institute for Molecular & Cellular Regulation	Name	Dr. Yoshio Fujitani

6. Research Plans

Drs. Yoshio Fujitani and Emilyn Alejandro have established an ongoing collaboration two years ago. Dr. Fujitani has generated the transgenic animal harboring the Pdx1 overexpression and has shared this model with Alejandro. Her laboratory has identified that nutrient sensor O-GlcNAC Transferase (OGT) is critical for pancreatic beta-cell development and function. Genetic deletion of OGT in pancreatic progenitors or in beta-cells causes pancreas agenesis or beta-cell failure respectively. Beta-cell failure in OGT deficient cells is in part due to loss of Pdx1. For the funding period of April 2020-March 2021, the Alejandro lab aimed to test whether Pdx1 reconstitution in OGT-deficient cells will rescue pancreas agenesis or mitochondrial function. We also planned to have Dr. Alejandro visit IMCR, Gunma University to discuss new data and to prepare manuscripts for publication in person, where Dr. Fujitani will serve as a co-author. Dr. Alejandro was expected to give a seminar on her research in diabetes to the research community. Faculty members of the IMCR members at Gunma University were expected to meet with Dr. Alejandro one-on one and discuss research in progress and possible collaborations. Moreover, Dr. Alejandro had also intended to meet with trainees (graduate and post-doctoral fellows) working with Dr. Fujitani in IMCR, Gunma University.

7. Research results:

Unfortunately, due to COVID19 pandemic and the closure of labs, Dr. Alejandro was not able to visit Gunma University in Japan during 2020-2021. However, progress was made in the Alejandro lab and manuscripts are being prepared for two publications (Mohan *et al*, and Wong *et al*). During this funding period, Dr. Fujitani had provided edits to a manuscript we intend to submit for publication by the end of April 2021.

8. Publications and/or Presentations resulting from Joint Research Program with IMCR.
Exchange of information on joint research with faculty members.

We anticipate two publications in which Dr. Fujitani, our collaborator researcher of IMCR will be included. The abstract of one manuscript we intend to submit to *Diabetes* by end of April 2020 is below.

1. **O-GlcNAc transferase (OGT) in pancreatic β-cells regulates mitochondrial biogenesis and function via diabetes susceptibility gene Pdx1**

Ramkumar Mohan[1], Seokwon Jo[1], Amber Lockridge[1], Deborah A. Ferrington[2], Kevin Murray[3], Arthur Eschenlauer[3], Ernesto Bernal-Mizrachi[4,5], Yoshio Fujitani[6], and Emilyn U. Alejandro[1*]
[1]Department of Integrative Biology & Physiology, [2]Department of Ophthalmology and Visual Neurosciences, [3]University of Minnesota Informatics Institute, University of Minnesota Medical School, Minneapolis, Minnesota, USA. [4]Miami VA Healthcare System and [5]Division Endocrinology, Metabolism and Diabetes, University of Miami, Miami, Florida. [6] Institute for Molecular & Cellular Regulation, Gunma University, Gunma, Japan.

Pancreatic β-cells release insulin in response to high glucose levels, and they rely on the mitochondria for energy needed for insulin synthesis and secretion. O-GlcNAc transferase **(**OGT), a nutrient-sensor sensitive to glucose flux, is highly expressed in the pancreas. However, the role of OGT in the mitochondria of β-cells is unexplored. Here, we aimed to identify the role of OGT in mitochondrial function in β-cells. Constitutive deletion of OGT (βOGTKO) or inducible ablation in mature β-cells (iβOGTKO) causes distinct effects on mitochondrial morphology and function. Islets from βOGTKO, but not iβOGTKO, mice display swollen mitochondria without cristae, reduced glucose-stimulated oxygen consumption rate (OCR), and ATP production. Mechanistically, alleviating ER stress by genetic deletion of C/EBP homologous protein (*Chop*) did not rescue the mitochondrial dysfunction in βOGTKO mice. By candidate and unbiased quantitative proteomics, we identified altered islet proteome between βOGTKO and iβOGTKO mice. Pancreatic and duodenal homeobox 1 (Pdx1) was reduced in in βOGTKO islets and identified as top upstream regulator based on differentially altered proteins in βOGTKO lysates. Pdx1 over-expression increased insulin content and improved mitochondrial morphology and function in βOGTKO islets. These data underscore the essential role of OGT in regulating β-cell mitochondrial morphology and bioenergetics. In conclusion, OGT couples nutrient signal and mitochondrial function to promote normal β-cell physiology.