Form 3

Report for Joint/Usage Research Program for Endocrine/Metabolism (Fiscal Year 2023)

Date：April 12, 2024

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

Principal Applicant	
Institution	Institute of Molecular and Cell Biology (IMCB), A*STAR
Position	PI; Assistant Professor
Name	Adrian Teo

We report on the results of joint research in fiscal year 2023 as below.

（Program No.　　）

1． Research Title	Protein translation in human insulin gene mutated beta cells
2． Purpose and Significance of the research project	In this proposal, we focus on the effects of heterozygous human INS gene mutations on protein translational status in both beta cell lines and human induced pluripotent stem cells (hiPSC)-derived beta-like cells.
3． Period of The Program	April 1, 2023 ～ March 31, 2024

4． Project Members

Name	Age	Sex	Affiliation	Position	Role
(Principal Applicant) Adrian Teo	41	M	Institute of Molecular and Cell Biology (IMCB), A*STAR, Singapore	Position：PI Degree：Ph.D. Acquisition date：2011.3	Project director
(Research Collaborators) Carmen Ching	25	F	IMCB	Research officer	Cell Analysis

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

5． Collaborating Researcher of IMCR	Name of Laboratory	Diabetes and Metabolic Disorders	Name	Jun Shirakawa

6． Research Plans

The insulin gene is translated as preproinsulin, consisting of the signal peptide, B-chain, A-chain, and C-peptide. The signal peptide is cleaved off in the endoplasmic reticulum (ER), forming proinsulin. Mutant proinsulin exhibits a dominant-negative effect by forming a complex with wild-type (WT) proinsulin, trapping it and decreasing insulin production. Moreover, mutant proinsulin has been shown to decrease beta cell mass by inducing ER stress.

We recently reported that the presence of ER stress, organelle changes and insulin processing defects, resulting in a decreased amount of insulin secreted but not the ability to secrete insulin. By 9 weeks of expression of mutant human INS, dominant-negative effects of mutant INS were evident and beta cell insulin secretory capacity declined. INS+/C109Y patient-derived beta-like cells and single-cell RNA-sequencing analyses then revealed compensatory upregulation in genes involved in insulin secretion, processing and inflammatory response (Diabetologia, 2021).

However, we still do not know the translational status in INS C109Y or G32V mutant beta cells. In this collaborative project, we will access the protein translational status in INS C109Y or G32V mutant beta cells by using in both beta cell lines and human induced pluripotent stem cells (hiPSC)-derived beta-like cells. We also plan to identify the target genes for translational changes by RNA-Seq of polysome fractions.

Nest, we will confirm the expression of candidate gene for translational regulation from polysome fraction by single-cell Western blotting of hiPSC-derived beta-like cells.

7． Research results:

Please describe the details of the contribution of the joint research with IMCR in obtaining the results.

1. Protein translation analysis in INS mutant beta cells.

Polysome profiling is commonly used to study translatomes and applies laborious extraction of efficiently translated mRNA (associated with >3 ribosomes) from a large volume across many fractions. To assess the global protein translational status, polysome profiling experiments was conducted in MIN6 cells stably transfected with WT and mutant human preproinsulin or INS C109Y mutant hiPSCs-derived pancreatic beta-like cells. The difference of protein translation was detected in mutant cells.

2. Identification of translational target genes by RNA-seq followed by polysome profiling.

Protein synthesis control pathways and post-transcriptional mechanisms involved in cell fate commitment are still being established. Genome-wide expression profiling has provided the possibility to investigate transcriptional changes during beta cell failure; however, a more accurate study regarding post-transcriptional regulation is required. Therefore, we isolated and performed high-throughput sequencing of ribosome-free and polysome-bound RNAs from INS mutant beta cells. The results showed that some specific genes related to ER stress were involved in protein translation in beta cells.

3. Confirmation of candidate gene for translational regulation from polysome fraction in islet cells at single cell resolution.

Single cell RNA-seq and Single cell Western blot will be performed in human islets and hiPSC-derived beta-like cells to check the expression of target genes, which were identified by RNA-Seq of polysome fraction in INS-mutant beta cells. The results showed beta-cell specific modification of identified candidate genes in hiPSC-derived islets.

８． Present status of academic conference presentations and research papers associated with the results of the joint research, and exchange of information on the joint research with the collaborating researcher at IMCR.
(As much as possible, please state papers that include the names of the collaborating researcher at IMCR or papers stating that the research was supported by the Joint Research Program with IMCR.
Regarding papers, please send a PDF file together with the report to the email address of the general affairs section of the Institute.) Office of General Affairs: kk-msomu4@ml.gunma-u.ac.jp

① Please list the publications that include the name of the collaborating researcher from IMCR and send a reprint of each publication to IMCR.
N/A

② Please list the publications that include a description that the research was supported by the Joint Research Program with IMCR and send a reprint of each publication to IMCR.
N/A

③ List up to 3 conferences (name of conference, date of conference, and title of the presentation).
N/A

④ Exchange of information exchange with collaborating researcher from IMCR (please list main points of communication).
We routinely discuss the experimental results (every month).