Form 3

Report for Joint/Usage Research Program for Endocrine/Metabolism (Fiscal Year 2022)

Date：(Year)/(Month)/(Day)

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 2022 as below.

（Program No. 22-3-1 ）

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, 2022 ～ March 31, 2023

4． Project Members

Name	Age	Sex	Affiliation	Position	Role
(Principal Applicant) Adrian Teo	39	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	24	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.

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 analysis was conducted in MIN6 cells stably transfected with WT and mutant human preproinsulin or INS C109Y mutant hiPSCs-derived pancreatic beta-like cells. And some translational changes were detected.

2. Identification of translational target genes by polysome profiling
We isolated polysome fraction in WT and mutant human preproinsulin or INS C109Y mutant hiPSCs derived pancreatic beta-like cells. We performed qPCR analysis of ribosome-free and polysome-bound RNAs from INS mutant beta cells and detected some candidate genes.

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@jimu.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).