Form 3

Report for Joint/Usage Research Program for Endocrine/Metabolism (Fiscal Year 2018)

Date: 2019/5/20

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

Principal Applicant	
Institution	Endocrinology department, Beijing Tongren Hospital, Capital Medical University
Position	Professor and Director
Name	Jin-Kui Yang

We report on the results of joint research in fiscal 2018 as below.

(Program No. 18005)

1. Research Title	Biomarkers of Diabetes and its Complications
2. Purpose and Significance of the research project	Mitochondrial metabolism plays an essential role in the regulation of insulin release and glucose homeostasis. Evidence demonstrated that the angiotensin-converting enzyme 2 (ACE2) participates in the regulation of glucose metabolism, however, its role in mitochondrial metabolism remains unclear. The purpose of our study was to determine if ACE2 can regulate mitochondrial function in pancreatic b-cells.
3. Period of The Program	April 1, 2018 ～ March 31, 2019

4. Project Members

Name	Age	Gender	Institution/Department	Position	Role
(Principal Applicant) Jin-kui Yang	56	M	Endocrinology department, Beijing Tongren Hospital, Capital Medical University	Professor	Project director
(Research Collaborators) Jing Lu	36	F	Endocrinology lab, Beijing Tongren Hospital, Capital Medical University	Associate prfessor	Experimental executor
Jing-Yi Liu	28	F	Endocrinology lab, Beijing Tongren Hospital, Capital Medical University	Graduate student	Experimental executor
Miao-miao Zhao	25	F	Endocrinology department, Beijing Tongren Hospital, Capital Medical University	Graduate student	Experimental executor
Hao Wang	36	M	Molecular endocrinology and metabolism, IMCR, Gunma University	Assistant professor	Experimental executor

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

5. Collaborative Researcher of IMCR	Name of the Laboratory	Molecular Endocrinology and Metabolism	Name	Tetsuro Izumi

6. Research Plans

ACE2 knockout and db/db mice model will be used in this study. In each model, animals will be assigned into two groups, one group will inject with Ang-(1-7), while the other group will receive saline as control. Ang-(1–7) (100ng/kg/min) or equal amount of saline will be osmotically infused through micro pump for 4 weeks. IPGTT and IPITT will be performed at the end of the Ang-(1–7) treatment. Serum triglyceride, cholesterol, aspartate aminotransferase and alanine aminotransferase levels will be measured using enzymatic kits by automatic biochemical analyzer. Ang-(1–7) and Ang II in serum was measured by radioimmunoassay.

Metabolism regulation: Pancreatic islets will be isolated and liver, epididymal fat and brown adipose fat from Ang-(1–7) or saline treated animals will be collected for western blot analysis. Protein levels related to glucose transport; insulin secretion, oxidative stress as well as inflammation will be examined to study the effect of ACE2-Ang(1-7)-MAS axis on energy metabolism and its specific regulatory mechanism.

ROS Levels: Sections of optimum cutting temperature (OCT)-embedded liver epididymal fat and brown adipose fat tissues will be incubated with dihydroethidium (DHE) for 15 min at room temperature. The sections then will be analyzed by fluorescence microscopy.

ATP Content: In pancreases, pancreatic β cell will be extracted from the mouse islet. The dye dichlorofluorescin diacetate (DCF-DA) will be used to detect intracellular ROS production in pancreases. The fluorescence of this cell-permeable agent significantly increases after oxidation. The intensity of the fluorescence will be immediately read using a FACScan flow cytometer. Tissues and islets are lysed in a lysis buffer. The ATP contents are measured using ATP-Lite Assay Kit.

Mitochondrial function: Mitochondrial membrane potential (MMP) is the sensitive indicator of mitochondrial metabolism. Changes in MMP in pancreatic β cell will be investigated by a JC-1 kit, followed by FACS Calibur flow cytometer.

7. Research results:

We found that ACE2 over-expression restored glucose-stimulated insulin secretion (GSIS) and mitochondrial membrane potential (MMP) in the presence of H_2O_2 in INS-1 cells. PCR array demonstrated that ACE2 over-expression up-regulated 67 mitochondria-related genes in INS-1 cells. In pancreatic islets, ACE2 ablation attenuated intracellular calcium influx with a decrease in GSIS. Ace2-/y mice islets exhibited impaired mitochondrial respiration and lower production of ATP, along with decreased expression of genes involved in mitochondrial oxidation. In islets from db/db mice, ACE2 over-expression increased intracellular calcium influx and restored impaired mitochondrial oxidation, potentially causing an increase in GSIS. These results shed light on the potential roles of ACE2 in mitochondrial metabolism, moreover, may improve our understanding of diabetes.

8. Publications and/or Presentations resulting from Joint Research Program with IMCR.

①Please describe a list of publications in which the name of the collaborative researcher of IMCR appears and send one paper reprints of each publication to IMCR.

No

②Please describe a list of publications which include the description that the research is supported by Joint Research Program with IMCR and send one copy of each publication to IMCR.

No