Form 3

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

Date: 2019/4/26

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

Principal Applicant	
Institution	College of Biology, Hunan University
Position	Associate Professor
Name	Hong-Hui Wang

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

(Program No. 17007)

1. Research Title	The Role of Girdin in Glucose-stimulated Nephrin Trafficking and Insulin Secretion in Pancreatic Beta-cells
2. Purpose and Significance of the research project	Study mechanistic role of Girdin in high glucose induced nephrin-phosphorylation and endocytosis, in turn affecting insulin secretion in beta-cells. The expecting results may provide a novel pharmacological modulation of Girdin/Nephrin to facilitate pancreatic beta cell function.
3. Period of The Program	April 1, 2018 ～ March 31, 2019

4. Project Members

Name	Age	Gender	Institution/Department	Position	Role
(Principal Applicant) Hong-Hui Wang	38	M	Hunan University, College of Biology	Associate Professor	Project director
(Research Collaborators) Jihui Zheng	22	F	Hunan University, College of Biology	Graduate student	Cell Experiments
Kunli Zhao	25	F	Institute for Molecular and Cellular Regulation, Gunma University	Graduate student	Animal Experiments
Cong Chang	25	F	College of Biology, Hunan University	Graduate Student	Cell experiment
Hao Wang	37	M	Institute for Molecular and Cellular Regulation, Gunma University	Assistant Professor	Project uidance

※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

①. Study nephrin-phosphorylation, nephrin endocytosis and insulin secretion in MIN6 cells infected with lentivirus-mediated shRNA targeting Girdin.

②. Study nephrin-phosphorylation, nephrin endocytosis and insulin secretion in MIN6 cells infected with Adenovirus-mediated overexpression of Girdin WT or Girdin FA mutant (lacking its GEF function for Galphi3 activation);

③. TIRF microscopic analysis for exocytosis of insulin granules by using MIN6 cells or primary pancreatic beta cells after downregulation of Girdin.

④. Compare the phosphorylation level and subcellular localization of nephrin and Girdin in primary pancreatic beta-cells between db/m and db/db mouse.

⑤. Test potential small molecules to block Girdin mediated nephrin phosphorylation and endocytosis and apply the drugs to treat obesity db/db mouse and observe the insulin level and glucose in blood.

7. Research results:

We investigated the hypothesis that Girdin is the critical modulator of Nephrin phosphorylation and endocytosis in response to extracellular high glucose concentration, therefore regulating glucose-stimulated insulin secretion.

①. We verified the expression of Girdin in pancreatic β cells and partially colocalize with the actin cytoskeleton and insulin granules.

②. We found that Girdin and Nephrin correlate in the membrane fraction of pancreatic β cells. And we further confirmed the interaction in Nephrin and Girdin in pancreatic β cells and mouse islets.

③. The expression levels of nephrin and Girdin in islets isolated from db/db mice were reduced compared to islets isolated from normal mice.

④. High glucose stimulation accelerated nephrin phosphorylation and enhanced endocytosis in the mouse islet and pancreatic β cell line MIN6 and INS1cells.

⑤. Acute inhibition of phosphorylation signals by Src inhibitors impaired GSIS in pancreatic β cells and mouse islets and significantly inhibited the glucose-induced actin cytoskeleton remodeling.

⑥. Downregulation of Girdin decreased the glucose-caused phosphorylation of nephrin and inhibited GSIS in pancreatic β cells.

⑦. We also demonstrated that Girdin was a critical modulator of endocytosis of nephrin via Rac1 mediated actin-cytoskeleton remodeling, potentially promoting exocytosis of insulin vesicles.

These findings reveal the role of Girdin in insulin secretion and identify new regulatory pathways for insulin secretion, potentially contributing to targeted therapies to restore islet beta cell dysfunction.

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.

Girdin mediates phosphorylation and endocytosis of nephrin in glucose-stimulated insulin secretion (GSIS). Cong Chang, Kunli Zhao, Hao Wang, Hong-Hui Wang, Tetsuro Izumi. 2019, Manuscript in preparation.

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.

Girdin mediates phosphorylation and endocytosis of nephrin in glucose-stimulated insulin secretion (GSIS). Cong Chang, Kunli Zhao, Hao Wang, Hong-Hui Wang, Tetsuro Izumi. 2019, Manuscript in preparation.