Form 3

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

Date：2021/04/19

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 2020 as below.

（Program No. 20006 ）

1． Research Title	Glucose-gated DNA-nanodevice for glucose uptake in diabetic complications
2． Purpose and Significance of the research project	Design a Glucose-gated DNA-nanodevice that dynamically responds to diabetic blood glucose levels, and promoting glucose uptake by activating the AKT signaling pathway to restore normal blood glucose levels. The expecting results may provide a nongenetic receptor engineering for the treatment of diabetes.
3． Period of The Program	April 1, 2020 ～ March 31, 2021

4． Project Members

Name	Age	Gender	Institution/Department	Position	Role
（Principal Applicant）Hong-Hui Wang	40	M	Hunan University, College of Biology	Associate Pro-fessor	Project director
（Research Collaborators）Fang He	25	F	Hunan University, College of Biology	Graduate student	Cell Analysis
Meixia Wang	24	F	Hunan University, College of Biology	Graduate student	Animal Experiments

※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 the AKT signaling pathway was activated by HGF mimic by Western blot assay, and glucose uptake was analyzed by examining 2-deoxyglucose-6-phosphate (2DG6P) in myocyte, hepatocyte or lipocyte.
②. Design and synthesize a glucose-gated DNA-nanodevice.
③. Study the concentration and time dynamic range of activation of the AKT signaling pathway by glucose.
④. Use a glucose uptake kit to detect glucose uptake and metabolism in skeletal muscle, liver or adipose tissue in db/db mice.

7． Research results:

We designed a Glucose-gated DNA-nanodevice that dynamically responds to diabetic blood glucose levels, and promoted glucose uptake by activating the AKT signaling pathway to restore normal blood glucose levels.

①. We verified the AKT signaling pathway was activated by Glucose-gated DNA-nanodevice by In Cell Western assay (Fig.1). We found that AKT signaling pathways are activated when glucose levels exceed 8 mM.

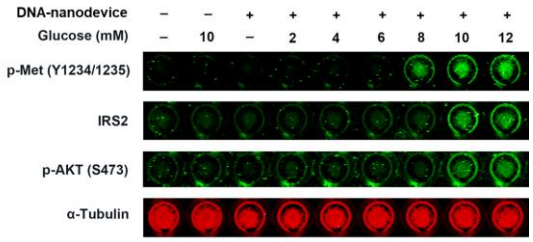

Fig.1, Glucose-triggered c-Met phosphorylation, IRS2 recruitment and AKT phosphorylation in the LO2 cells stimulated with DNA-nanodevices determined by in cell western.

②. We detected GLUT4 translocation in HEK293T cells transfected with a GLUT4-EGFP plasmids. We demonstrated that cells installed with DNA-nanodevice promoted GLUT4 transposition by immunofluorescence (Fig.2).

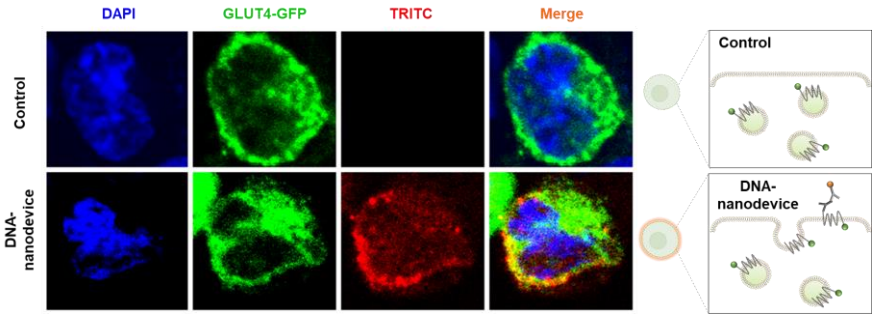

Fig.2, After HEK293T cells were transfected GLUT4-EGFP, GLUT4 transport to cell membrane under installed with DNA-nanodevice.

③. GLUT4 is one of the critical transporters promoting glucose uptake. We further found that cells installed with DNA-nanodevices promoted glucose uptake (Fig.3).

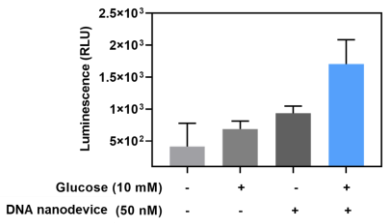

Fig.3, Glucose-gated DNA-nanodevice promoted glucose uptake.

④. The glucose tolerance test is the standard protocol for the diagnosis and monitoring of diabetes. We performed intraperitoneal glucose tolerance test (IPGTT) to confirm the effects of DNA-nanodevices in diabetic mice. We found that the capability of DNA-nanodevices in the improvement of glucose tolerance.

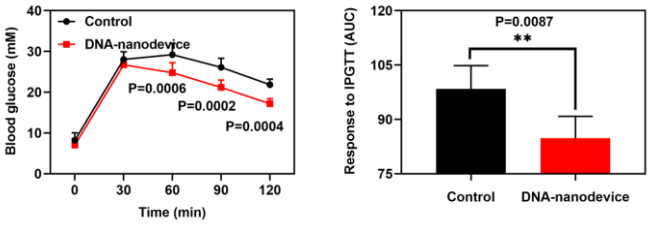

Fig.4, The BGLs in diabetic mice treated with the DNA-nanodevices rapidly decline after the peak of blood glucose, while the mice treated with the PBS decreased slowly within 120 min.

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

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. 2021, Manuscript in submission.

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.

Scan and Unlock: A Programmable DNA Molecular Automaton for Cell-Selective Activation of Ligand-Based Signaling. Zhang J, Qiu Z, Fan J, He F, Kang W, Yang S, Wang HH, Huang J, Nie Z.
Angew Chem Int Ed Engl. 2021 Mar 15;60(12):6733-6743.

③Enter the name of the conference, the date of the conference, and the title of the presentation of the conference.(up to 3 cases)
None

④Implementation status of information exchange with faculty members in charge of joint research.
The institutional collaboration agreement between IMCR and College of Biology Hunan university was established in April, 2016. Applicant and Dr. Hao Wang at Dr. Izumi's laboratory have started preliminary experiments and obtained some promising results to support future collaboration.