Form 3

Report for Joint/Usage Research Program for Endocrine/Metabolism (Fiscal Year 2017)

Date:(Year)/(Month)/(Day)

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

Principal Applicant	
Institution	College of Biology, Hunan University
Position	Professor
Name	Haijun Tu

We report on the results of joint research in fiscal 2017 as below.

(Program No.)

1. Research Title	In Vivo Two-photo imaging of synapse changes in depression mice
2. Purpose and Significance of the research project	**Depression is a leading cause of death and disability, and the treatment options for the disease are limited. However, the synaptic dynamics in the process of depression remains elusive. We aim to using two-photo *In vivo* imaging to trace the synapses change in depression mice.**
3. Period of The Program	April 1, 2017 ～ March 31, 2018

4. Project Members

Name	Age	Gender	Institution/Department	Position	Role
(Principal Applicant) Haijun Tu	42	Male	College of Biology, Hunan University	Professor	PI, project director
(Research Collaborators) **Akiko Hayashi-Takagi**		Female	IMCR, Guma University	Professor	Co-PI
Wei Jiang		Male	College of Biology, Hunan University	PhD Candidate	MCAO model, experiment conductor, Key Participants

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

5. Collaborative Researcher of IMCR	Name of the Laboratory	Biomedical Neuroscience Lab	Name	Akiko Hayashi-Takagi

6 . Research Plans

By screening for GluR1 protein (synapse marker protein) aptamer using cell based Systematic evolution of ligands by exponential enrichment (cell-SELEX),through injecting GluR1 aptamers (FITC modification) into cortex neuron in depression mice, two-photo *In vivo* imaging to trace GluR1 which reflect synapse change .

7 . Research results:

In order to identify molecules that involve in pathological machinery of neural injury and dysfunction in cerebral ischemia, we performed proteomic profile of focal cerebral ischemic brain homogenate by coomassie blue staining
and mass spectrometry as compared with that of sham mice, and identified Eno1 is a potent molecule that involves in the process of neuronal death in cerebral ischemia.

Clinically, the time window within 6 hours of human cerebral ischemic stroke is critical for the therapy and the outcomes of the disease. To investigate the dynamic molecules of the cerebral ischemia by using middle cerebral artery occlusion (MCAO) of mouse as a model system, firstly, we define the time window 0-4 hours of MCAO as the early stage of cerebral ischemia. Secondarily, the MCAO early stage is subdivided into the beginning phase (0-2 hours of ischemia) and the later phase (2-4 hours of ischemia).

To test if the expression of enolase 1 (Eno1), Enolase 2 (Eno2), and one of the postsynaptic markers, AMPA receptor subunit GluR1, are dynamically altering during the pathological process in the MCAO early stage, we performed immune blotting with ischemic brain homogenate, and found that MCAO ischemia *in vivo*, as well as cultured neuron treated with Oxygen-Glucose Deprivation (OGD) *in vitro*, stimulates to increase Eno1, Eno2, and GluR1 expression in the beginning phase of the MCAO early stage, but down-regulate Eno1, Eno2, and GluR1 expression level in the later phase of MCAO early stage. These data suggest that acute cerebral ischemia or OGD induces to enhance some neuronal trophic genes expression to support cell survival in the ischemia condition.

To test the expression pattern of Enolases, we performed immunostaining in mouse brain slices and in cultured neurons. We found that Eno1 was enriched in neuron and localized to soma and dendrite. To check if Enolases play a role of survival signals in ischemia, we overexpressed Eno1 in cultured neurons and found that Eno1 overexpression in hippocampal neurons protected spine loss and dendrite damages during OGD. Taken together, our data suggest a protective role of Enolases in neural injury of ischemia.

We also found that there are two sites of phosphorylation in the amino acid sequence of Enolases by using bioinformatics analysis, suggesting that Enolse phosphorylation may play a role in the enzymatic activity of Enolases.

The project is still in process for this collaborative project was a complete new project. We still need to perform the project cooperatively.

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.

Not yet. Hopefully, we could get collaborative publication in this year.

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.

Not yet. Hopefully, we could get collaborative publication in this year.