Form 3

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

Date: 2019/04/10

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

Principal Applicant	
Institution	University of California San Francisco
Position	Associate Professor
Name	Shingo Kajimura

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

(Program No. 17008)

1. Research Title	Histone code analysis of brown and beige adipose cells
2. Purpose and Significance of the research project	The combinations of various histone marks during brown, white, and beige adipose cell biogenesis is mostly unrevealed. We seek to elucidate the combinations of histone codes in brown, white and/or beige adipose cells.
3. Period of The Program	April 1, 2018 ～ March 31, 2019

4. Project Members

Name	Age	Gender	Institution/Department	Position	Role
(Principal Applicant) Shingo Kajimura	41	M	UCSF Diabetes Center	Associate Professor	Project leader
(Research Collaborators)					

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

5. Collaborative Researcher of IMCR	Name of the Laboratory	Laboratory of Epigenetics and Metabolism	Name	Takeshi Inagaki

6 . Research Plans

Epigenetic regulatory factors are recently reported to regulate brown, white and beige adipose cell fate and their function. We revealed that EHMT1 which is a H3K9 methyltransferase regulates biogenesis of brown and beige adipocytes (Ohno et al. *Nature* 2013) and the group of Prof. Inagaki in IMCR, Gunma University revealed that a H3K9 demethylase JMJD1A regulates brown adipocyte function (Abe et al. *Nat. Commun.* 2015). Thus, histone modifications mediate brown and beige adipose cell biogenesis. It is known that histone modifications regulate gene expression either positively or negatively. The bivalent modified histone signature of both positive and negative mark keeps active genes poised for future activation. However, the combinations of various histone marks during brown, white, and beige adipose cell biogenesis is mostly unrevealed. In the current study we seek to elucidate the combinations of histone codes in brown, white and/or beige adipose cells. Beige pre-adipocytes, pre-brown adipocytes or pre-white adipocytes originated from mouse/human are differentiated into beige or brown adipocytes, respectively, using the method which we previously reported (Shinoda et al. *Nat Med* 2015, Abe et al. *Nat Commun.* 2015). Core histones are isolated from the cell cultures using the histone purification kit (Active motif). Histone H3 tail is digested from the purified histones using restriction enzyme and applied to the mass spectrometry analysis for determining the combinations of multiple histone marks.

7 . Research results:

We have established a novel screening technique to determine histone modifications using mass spectrometric analysis in the previous fiscal year. However, the protocol did not allow us to identify histone mono-methylation because the pretreatment of histone tail with propionic acid in our protocol made it indistinguishable from butyrylation. By adding a minor modification employing 13C-propionic acid for the pretreatment, we successfully identified the mono-methylation of histone H3 tail. Using the modified protocol, we determined novel combinations of posttranslational modifications which occur on a single histone tail, while the data require the further confirmation. Based on the collaboration with Prof. Inagaki, we have published a manuscript entitled "Histone demethylase JMJD1A coordinates acute and chronic adaptation to cold stress via thermogenic phospho-switch" (Nature Communications 19;9(1):1566) in 2018.

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.

Abe Y., Fujiwara Y., Takahashi h., Matsumura Y., Sawada T., Jiang S., Nakaki R., Uchida A., Nagao N., Naito M., **Kajimura S.**, Kimura H., Osborne T.F., Aburatani H., Kodama T., **Inagaki T.***, Sakai J.* (2018) Histone demethylase JMJD1A coordinates acute and chronic adaptation to cold stress via thermogenic phospho-switch *Nature Communications* 19;9(1):1566.

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.

N/A