Final Report for the Twenty-fourth (2017) Research Grant from Mizutani Foundation for Glycoscience

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4. Title of the Proposed Project (do not exceed 80 typewriter spaces):

Molecular basis of brown fat development and function by glycosylation

5. Abstract of the proposed project

Obesity develops when energy intake chronically exceeds total energy expenditure. Adipose tissue serves as a central regulator of energy balance; white adipose tissue (WAT) functions mainly in the storage of excess energy, whereas brown adipose tissue (BAT) dissipates energy in the form of heat and functions as a defense against hypothermia and obesity. Recent studies identified a “recruitable” form of thermogenic adipocytes, termed beige adipocytes that emerge within subcutaneous WAT in response to certain external cues, such as chronic cold exposure and exercise, often referred to as the “browning” of white fat. Importantly, our recent studies, along with others, indicate that adult human BAT is primarily composed of the recruitable beige adipocytes. Because of its inducible nature and the relevance to adult humans, beige adipocytes have recently attracted much attention as a new therapeutic target for obesity and obesity-related metabolic diseases, such as type 2 diabetes. To further explore the biological roles of human beige adipocytes, we recently identified secretory molecules from human beige adipocytes, so-called “batokine”, many of which are highly glycosylated. Of particular interest, our preliminary study suggests that a batokine, sCNTFRα, has N-linked glycosylation, indicating that beige adipocyte-selective glycosylation is critical for the function of sCNTFRα in the regulation of beige adipocyte development and glucose homeostasis. To test the hypothesis, we aim to characterize the function of sCNTFRα in whole-body energy homeostasis. We further aim to determine the global glycan repertoire of human batokines.

Progress Report

1) To determine the physiological role of sCNTFRα in whole-body glucose homeostasis.

Our preliminary study indicates that overexpression of sCNTFRα by administrating adenovirus via the tail vein significantly improved systemic glucose tolerance under a diet-induced obese condition. Because adenovirus administration potentially causes pleiotropic effects in mice, including inflammatory responses in the liver, we have generated a transgenic mouse line expressing sCNTFRα driven by an adipose tissue-selective promoter *Fabp4* promoter/enhancer. We found that the transgenic mice expressed significantly higher levels of sCNTFRα both in the inguinal WAT and epididymal WAT (Fig. 1A, B). Furthermore, we confirmed that sCNTFRα protein was significantly increased in the adipose tissue of *Fabp4*-sCNTFRα transgenic mice (Fig. 1C), suggesting that these transgenic mice would serve as an alternative animal model to study the role of sCNTFRα in whole-body energy homeostasis.

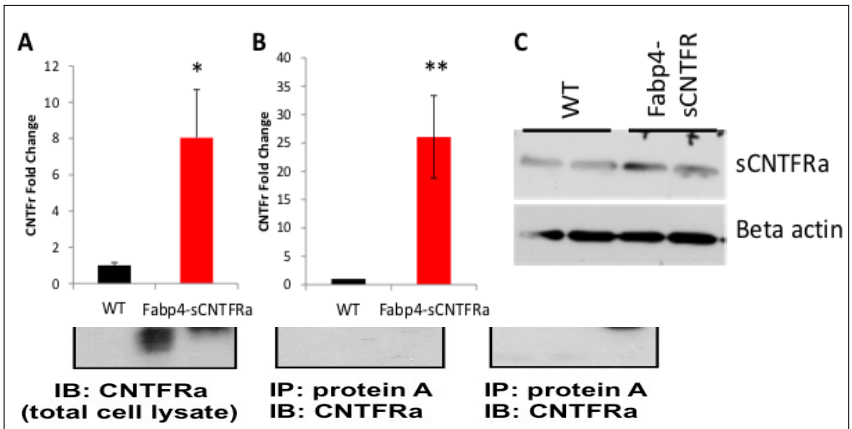

Fig.1. Generation of fat-specific transgenic mice expressing sCNTFRa. A) mRNA expression of sCNTFRα in the inguinal WAT. n=5-9, * P<0.05. **B)** mRNA expression of sCNTFRα in the epididymal WAT. n=5-9, ** P<0.01. **C)** Protein expression of sCNTFRα in the

Next, we examined if sCNTFRα stimulates the thermogenic program in the WAT, *i.e.,* beige adipocyte biogenesis. To this end, we harvested WAT depots from wild-type controls and *Fabp4*-sCNTFRα transgenic mice for RNA extraction and subsequent analysis of brown/beige-fat selective genes, such as *Ucp1*. As shown in Fig. 2, we found that *Ucp1*, a brown/beige fat-specific marker, was significantly increased in the WAT of *Fabp4*-sCNTFRα transgenic mice. The data support the hypothesis that sCNTFRα expression stimulates beige adipocyte biogenesis.

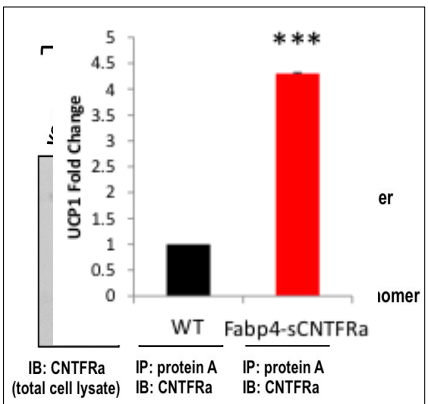

Fig.2. sCNTFRa stimulates the thermogenic program in WAT. mRNA expression of *Ucp1* in the

Subsequently, we examined if transgenic expression of sCNTFRα sufficiently improved systemic glucose homeostasis *in vivo*. To this end, wild-type mice and sCNTFRα transgenic mice were fed a high-fat diet for 8-12 weeks and then, glucose tolerance was examined by glucose tolerance test (GTT). To our surprise, we did not observe statistically significant improvement in glucose tolerance by transgenic expression of sCNTFRα. In the cohort on a high-fat diet for 8-12 weeks, there were nearly identical glucose tolerance between wild-type mice and *Fabp4*-sCNTFRα transgenic mice (Fig. 3A and B). In an independent cohort on a high-fat diet for 11 weeks, we also did not observe an improvement in glucose tolerance (Fig. 3C). A possible explanation is that transgenic expression of sCNTFRα is not high enough to achieve the expression level that requires systemic changes *in vivo*. Although a modest expression of sCNTFRα was able to stimulate thermogenic program in the WAT, but it might not to alter whole-body glucose homeostasis *in vivo*.

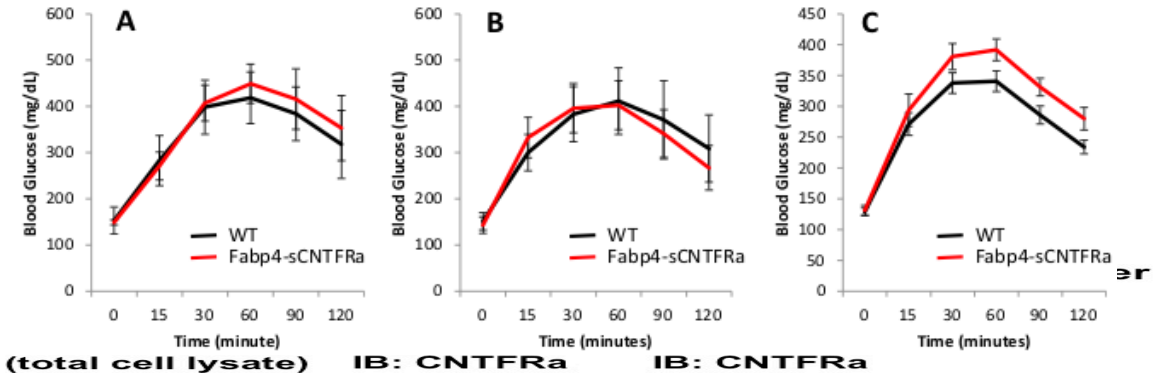

Fig.3. Glucose tolerance in *Fabp4*-sCNTFRa transgenic mice. A) Wild-type (WT) and *Fabp4*-sCNTFRα transgenic mice were fed a high-fat diet for 8 weeks. n=7. **B)** mice were on a high-fat diet for 12 weeks. n=7. **C)** Mice were on a high-fat diet for 11 weeks. n=7-8. 1.5mg/kg of glucose was

Accordingly, we took an alternative approach to increase circulating levels of sCNTFRα while avoiding pleiotropic effects of adenovirus injection. To do so, we generated Fc-fusion protein of sCNTFRa for the use *in vivo*. The extracellular domain of protein containing the biologically active site was linked to the immunoglobulin Fc-region, which allowed for easy purification by Protein A-sepharose and for increasing the half-life of the protein *in vivo*. As shown in Fig.4,

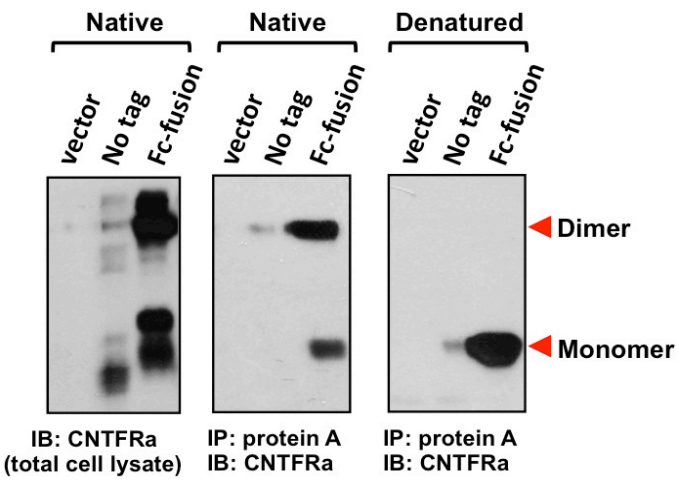

Fig.4. Fc-fusion protein of sCNTFRa. sCNTFRa was fused to the human Fc-domain. Expression vectors for non-tagged and Fc-fusion protein were expressed in HEK293 cells. Conditioned media were applied to IP and/or

we have been able to generate Fc-sCNTFRa fusion protein in mammalian cells (HEK293 cells), such that glycosylation patterns remained conserved like endogenous sCNTFRa.

As a continuous effort to test the original hypothesis, we first plan to optimize the treatment conditions, including doses and kinetics of the fusion proteins in mice. Subsequently, we plan to administer the Fc-fused protein into diet-induced obese mice and test their effects on beige adipocyte biogenesis, energy expenditure, and whole-body glucose homeostasis.

2) To characterize human batokine glycomes

We aim to determine the adipose tissue-selective glycan repertoire of human batokines by a high-density lectin microarray. Through collaboration with Dr. Tateno at the National Institute of Advanced Industrial Science and Technology (AISTT), we have analyzed the glycosylation profiles of secretory molecules from human beige adipocytes and white adipocytes. A robust glycosylation was observed in many beige-derived secretory molecules as compared to white-derived molecules. Accordingly, we are currently developing the new ELISA system to quantify glycosylated batokines in human serum. This study will potentially lead to identification of novel circulating biomarkers for human BAT.