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Lamp-2 deficiency prevents high-fat diet-induced obese diabetes via enhancing energy expenditure

Mako Yasuda-Yamahara [a], Shinji Kume [a, *], Kosuke Yamahara [a], Jun Nakazawa [a], Masami Chin-Kanasaki [a], Hisazumi Araki [a], Shin-ichi Araki [a], Daisuke Koya [b], Masakzu Haneda [c], Satoshi Ugi [a], Hiroshi Maegawa [a], Takashi Uzu [a]

[a] Department of Medicine, Shiga University of Medical Science, Otsu, Shiga, Japan
[b] Department of Diabetology and Endocrinology, Kanazawa Medical University, Kahoku-Gun, Ishikawa, Japan
[c] Division of Metabolism and Biosystemic Science, Asahikawa Medical University, Asahikawa, Hokkaido, Japan

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ABSTRACT

Autophagy process is essential for maintaining intracellular homeostasis and consists of autophagosome formation and subsequent fusion with lysosome for degradation. Although the role of autophagosome formation in the pathogenesis of diabetes has been recently documented, the role of the latter process remains unclear. This study analyzed high-fat diet (HFD)-fed mice lacking lysosome-associated membrane protein-2 (lamp-2), which is essential for the fusion with lysosome and subsequent degradation of autophagosomes. Although lamp-2 deficient mice showed little alteration in glucose metabolism under normal diet feeding, they showed a resistance against high-fat diet (HFD)-induced obesity, hyperinsulinemic hyperglycemia and tissues lipid accumulation, accompanied with higher energy expenditure. The expression levels of thermogenic genes in brown adipose tissue were significantly increased in HFD-fed lamp-2-deficient mice. Of some serum factors related to energy expenditure, the serum level of fibroblast growth factor (FGF) 21 and its mRNA expression level in the liver were significantly higher in HFD-fed lamp-2-deficient mice in an ER stress-, but not PPARα-, dependent manner. In conclusion, a lamp-2-depenedent fusion and degradation process of autophagosomes is involved in the pathogenesis of obese diabetes, providing a novel insight into autophagy and diabetes.

1. Introduction

The number of patients with obese type 2 diabetes is growing, which is one of the biggest health issues worldwide [1]. Thus, clarification of the whole picture of the pathogenesis of type 2 diabetes is now urgently required to develop better therapies for patients. The complex integration of physiological processes such as inflammation, apoptosis, cell proliferation, differentiation, and metabolism is involved in the pathogenesis of diabetes, and the regulation of these processes is expected to become a new therapeutic target for diabetes [2–4]. Thus, the more detailed clarification of the relationship between a cellular physiological process and diabetes development may provide a better understanding of the pathogenesis of diabetes and lead to the development of novel therapies for diabetes.

Autophagy is an evolutionarily conserved intracellular catabolic process that allows for the degradation of proteins and organelles in lysosomes, and has been recently focused on as a new class of cellular process [5]. This system is essential to maintain intracellular homeostasis during starvation or other stress conditions [6–8]. During autophagy, *de novo* isolation membranes elongate and fuse while engulfing a portion of the cytoplasm within double-membrane vesicles (autophagosomes). There are three major steps in autophagy: autophagosome formation, fusion with lysosome, and eventual degradation (Sup. Fig. 1A) [6]. The step of autophagosome formation is tightly regulated by many proteins encoded by

Abbreviations: ATF4, activating transcription factor 4; ACO, acyl-CoA oxidase; BAT, brown adipose tissue; Cidea, cell death-inducing DNA fragmentation factor-α-like effector A; FGF21, fibroblast growth factor 21; HE, hematoxylin and eosin; HFD, high-fat diet; HSL, hormone-sensitive lipase; IPGTT, intraperitoneal glucose; IPITT, insulin tolerance test; Lamp-2, lysosome-associated membrane protein 2; MnSOD, manganese superoxide dismutase; MCAD, medium-chain acyl-CoA dehydrogenase; PPARα, peroxisome proliferator-activated receptor α; UCP-1, uncoupling protein-1; WAT, white adipose tissue.

* Corresponding author. Department of Medicine, Shiga University of Medical Science, Tsukinowa-Cho, Seta, Otsu, Shiga 520-2192, Japan.
E-mail address: skume@belle.shiga-med.ac.jp (S. Kume).

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autophagy-related (atg) genes, and lysosome-associated membrane protein-2 (lamp-2) is thought to be an essential protein for the fusion and degradation of autophagosomes with lysosomes [9–11].

Given that autophagy activity is regulated by certain intra- and extra-cellular metabolic and nutrient alterations [12], this physiological process is expected to be involved in the pathogenesis of diabetes [13]. In fact, several previous reports utilizing tissue-specific atg genes-knockout mice have shown that the impairment of autophagosome formation is associated with the pathogenesis of diabetes [14–17]. However, contrary to the step of autophagosome formation, there is still little experimental evidence indicating the role of fusion and/or degradation of autophagosomes in the pathogenesis of diabetes.

In this study, we thus examined the role of lamp-2-mediated autophagosome-lysosomal fusion and degradation process in the pathogenesis of obese diabetes, using lamp-2-deficient (lamp-2$^{y/-}$) mice. The present results show that lamp-2 deficiency in mice prevents the development of high-fat diet (HFD)-induced obese type 2 diabetes accompanied with increasing energy expenditure. These results suggest that lamp-2-mediated autophagosome-lysosome degradation process is involved in the pathogenesis of obese diabetes.

2. Materials and methods

2.1. Animal models

All procedures were performed in accordance with the guidelines of the Research Center for Animal Life Science of Shiga University of Medical Science. All experimental protocols were approved by the Gene Recombination Experiment Safety Committee and the Research Center for Animal Life Science at Shiga University of Medical Science.

2.2. Lamp-2$^{y/-}$ mice fed a high-fat diet

Lamp-2$^{y/-}$ mice were crossbred with C57BL/6 mice at least five times. Eight-week old of male lamp-2$^{y/-}$ mice and control lamp-2$^{y/+}$ mice were fed either a 60% HFD or a normal die (ND) for 8 weeks and maintained on a 12-h light/12-h dark cycle and provided food and water. A ND and a HFD were purchased from Research Diets (New Brunswick, NJ).

2.3. Blood and urinary analysis

Blood glucose concentrations were measured using a Glutest sensor (Sanwa Kagaku, Nagoya, Japan). Plasma insulin was measured by ELISA (Morinaga Institute of Biological Science, Tokyo, Japan). Serum leptin (Morinaga Institute of Biological Science, Tokyo, Japan), free T3 (Cusabio Biotech, Wuhan, China) and FGF21 (Millipore, Billerica, MA) were also measured by ELISA.

2.4. Intraperitoneal glucose and insulin tolerance test (IPGTT and IPITT)

Mice were fasted for 8 h followed by intraperitoneal glucose injection (1 g/kg body wt), and were fasted for 3 h followed by intraperitoneal insulin injection (0.75 IU/kg body wt). The AUC was calculated for statistical analysis.

2.5. Histological analyses

Immunohistochemistry was performed as previously described [18]. The sample sections were stained with hematoxylin and eosin (HE), antibodies to F4/80 (Serotec, Oxford, UK) and uncoupling protein-1 (UCP-1) (Sigma, St. Louis, MO). Cell size and count analysis of WAT and BAT were calculated using Image-Pro Plus 7.0 (Media Cybernetics, Bethesda, MD, USA).

2.6. RNA extraction and quantitative real-time PCR

The mRNA levels were assessed by real-time PCR. Total RNA was isolated using the TRIzol protocol and cDNA was synthesized by reverse transcriptional PCR as described [19]. Primer sets were shown in Sup. Table1.

2.7. Analysis of energy balance

Activity, food consumption, oxygen consumption, and energy expenditure were assessed in a metabolic monitoring system (CLAMS: Columbus Instruments) for 6 days (5 days of acclimation followed by 24 h of measurement) employing 16- 20-week-old of male lamp-2$^{y/-}$ mice and lamp-2$^{y/+}$ mice fed a ND or a HFD. Locomotor activity was measured by counting the number of infrared beam breaks on x- and z-axes during the measurement period.

2.8. Protein extraction and western blot analysis

Western blot analysis was performed as previously described [19]. The membranes were incubated with the antibodies to phospho-eIF2a (Ser51), eIF2a, activating transcription factor 4 (ATF4) (Cell Signaling Technology, Beverly, MA), and β-actin (Sigma, St. Louis, MO). The blots were visualized using an enhanced chemiluminescence detection system (Perkin Elmer Life Science, Boston, MA).

2.9. Statistical analysis

Results are expressed as mean ± SEM. Analysis of variance with subsequent Tukey's test was used to determine significance of differences in multiple comparisons. A P-value <0.05 was considered statistically significant.

3. Results

3.1. Fusion of autophagosome with lysosome was impaired in tissues of lamp-2-deficient (lamp-2$^{y/-}$) mice

First, we examined autophagosome accumulation in various tissues from control lamp-2$^{y/+}$ and lamp-2$^{y/-}$ mice by electron microscopic analysis. This analysis showed a number of autophagic vacuoles in various metabolic tissues such as liver and skeletal muscle from lamp-2$^{y/-}$ mice compared to lamp-2$^{y/+}$ mice (Sup. Fig. 1B), as previously reported [11]. We further analyzed autophagosome accumulation by the crossbreeding lamp-2$^{y/-}$ mice with a GFP-LC3 transgenic mouse model generated to monitor autophagy activity (Sup. Fig. 1C). In tissue sections from this transgenic line, autophagosomes can be observed as green dots emitted by GFP-LC3 proteins localized on autophagosome membranes [20]. In lamp-2$^{y/+}$ mice, few GFP-LC3-dots were observed in several organs such as liver, skeletal muscle and heart under ad libitum feeding, and 36-h fasting induced autophagy activation in various organs (Sup. Fig. 1D). In contrast, many green dot spots were observed in various tissues even under feeding condition in lamp-2$^{y/-}$ mice and this increased more after 36-h fasting (Sup. Fig. 1D). These results indicated that lamp-2 deficiency impaired autophagosome fusion with lysosome in various tissues, and that this experimental model was appropriate to analyze the role of autophagosome-lysosome fusion and degradation process in