Biochemical and Biophysical Research Communications xxx (2018) 1–7

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Myeloid sirtuin1 deficiency aggravates hippocampal inflammation in mice fed high-fat diets

Kyung Eun Kim [a], Eun Ae Jeong [a], Jong Youl Lee [a], Chin-ok Yi [a], Kyung-ah Park [a], Zhen Jin [a], Jung Eun Lee [b], Tamas L. Horvath [c], Gu Seob Roh [a, *]

[a] Department of Anatomy and Convergence Medical Science, Bio Anti-aging Medical Research Center, Institute of Health Sciences, College of Medicine, Gyeongsang National University, Jinju, Gyeongnam 52727, Republic of Korea
[b] Department of Thoracic and Cardiovascular Surgery, Institute of Health Sciences, College of Medicine, Gyeongsang National University, Jinju, Gyeongnam 52727, Republic of Korea
[c] Program in Integrative Cell Signaling and Neurobiology of Metabolism, Section of Comparative Medicine, Yale University School of Medicine, New Haven, CT 06520, USA

ARTICLE INFO

Article history:
Received 17 March 2018
Accepted 6 April 2018
Available online xxx

Keywords:
Obesity
SIRT1
Lipocalin-2
Insulin resistance
Inflammation
Hippocampus

ABSTRACT

Chronic low-grade inflammation-induced insulin resistance is associated with neuroinflammation. Myeloid sirtuin1 (SIRT1) deficiency aggravates high-fat diet (HFD)-induced insulin resistance. However, the function of myeloid-specific SIRT1 in the hippocampus of obese mice is largely unknown. To address this question, we fed myeloid SIRT1 knockout (KO) mice a HFD for 40 weeks. We found that HFD-fed SIRT1 KO mice had increased insulin resistance and macrophage infiltration in adipose tissue than wild type (WT) mice. Levels of HFD-induced lipocalin-2 (LCN2) were lower in SIRT1 KO mice than in WT. HFD-induced hippocampal LCN2 expression was lower in HFD-fed SIRT1 KO mice than in WT. Hippocampal acetylation of nuclear factor-κB (NF-κB) and amyloid precursor protein levels were higher in HFD-fed SIRT1 KO mice than in HFD-fed WT mice. Taken together, our results suggest that targeted induction of the anti-inflammatory effects of SIRT1 and LCN2 may help prevent obesity-associated insulin resistance and neuroinflammation.

1. Introduction

A chronic high-fat diet (HFD) induces low-grade peripheral inflammation that promotes age-related pathologies (e.g., obesity, insulin resistance, and type 2 diabetes) and hippocampal inflammation that is associated with the development of neurodegenerative diseases, including Alzheimer's disease [1]. Nuclear factor-kappa B (NF-κB)-mediated signaling contributes to the development of insulin resistance and systemic inflammation [2]. It is unknown if a chronic HFD has a direct role in neuroinflammation development.

Sirtuin 1 (SIRT1), a member of the sirtuin family, participates in key cellular processes, including cellular survival, biological aging, and inflammation [3]. SIRT1 is also important for neuronal plasticity, cognitive function, and protection against aging-associated neuronal degeneration and cognitive decline [4,5]. Myeloid SIRT1 deletion promotes macrophage infiltration into insulin-sensitive organs and aggravates adipose tissue inflammation [6]. SIRT1 overexpression results in RelA/p65 deacetylation and reduced microglia-dependent amyloid-beta accumulation [7]. Resveratrol reverses metabolic derangement and hippocampal inflammation and corrects memory deficits in a HFD-fed mice [8]. However, it remains unclear whether myeloid-specific SIRT1 deletion in NF-κB signaling contributes to HFD-induced hippocampal inflammation.

Lipocalin-2 (LCN2) and other inflammatory factors such as cytokines and chemokines are released during neuroinflammation and obesity [9]. LCN2 has been found in the tissues of brains injured by ischemic stroke, spinal trauma, and neuroinflammation [10,11]. Without this inflammatory effect, LCN2-null mice experience increased disease severity and rapid progression to sepsis [12]. Because LCN2 deficiency is pro- or anti-inflammatory depending on the animal model [10,13], whether LCN2 is associated with chronic HFD-induced neuroinflammation remains unclear. Thus,

* Corresponding author. Department of Anatomy and Convergence Medical Science, College of Medicine, Gyeongsang National University, 15, Jinju-daero 816 Beon-gil, Jinju, Gyeongnam 52727, Republic of Korea.
E-mail address: anaroh@gnu.ac.kr (G.S. Roh).

https://doi.org/10.1016/j.bbrc.2018.04.044
0006-291X/

we sought to determine the role of a chronic HFD in neuroinflammation, in the context of SIRT1 and LCN2 function.

2. Materials and methods

2.1. Animals

SIRT1loxP/loxPLysM-Cre+ (KO) and SIRT1loxP/loxPLysM-Cre− (wild type, WT) mice were generated. Selective SIRT1 deletion in myeloid cells was confirmed, as previously described [14,15]. WT (n = 28) and KO mice (n = 28) of at least 6 weeks of age were fed (ad libitum) a standard laboratory chow diet (normal diet, ND) or a HFD (D12079B; Research Diets, New Brunswick, NJ, USA) for a total of 40 weeks. Experimental procedures were approved by the Animal Care Committee for Animal Research of Gyeongsang National University (approved study protocol GLA-150116-M0002) and performed in accordance with the National Institutes of Health Guidelines on the Use of Laboratory Animals.

2.2. Metabolic assessments

We performed glucose tolerance tests as previously described [16]. Immediately before each mouse was humanely sacrificed at 46 weeks old, we measured fat mass using magnetic resonance imaging (EchoMRI; Echo Medical Systems, Houston, USA).

2.3. Measurement of metabolic parameters

We measured fasting glucose levels in serum samples using an enzymatic colorimetric assay (Green Cross Reference Laboratory, Yongin-si, South Korea). The homeostatic model assessment for insulin resistance (HOMA-IR) was calculated from the fasting serum glucose and insulin concentration. The serum insulin and LCN2 concentrations were measured using mouse insulin (Shibayagi Co., Gunma, Japan) and LCN2 (R&D Systems, MN, USA) enzyme-linked immunosorbent assay kits.

2.4. Real-time polymerase chain reaction

Total RNA (n = 4 mice/group) from the epididymal fat pad and hippocampus was isolated using TRIzol reagent (Invitrogen, CA, USA). Complementary DNA was then synthesized using a reverse transcription kit (Thermo Scientific, USA) according to the manufacturer's instructions. Real-time polymerase chain reaction (RT-PCR) was performed using the LightCycler 480 Instrument II (Roche, Mannheim, Germany) with TOPreal™ qPCR 2X PreMix (Enzynomics, Daejeon, Korea) and iQ™ SYBR Green Supermix (BIO-RAD, CA, USA). The PCR primers used for this study are presented in Supplementary Table 1. Expression was normalized to glyceraldehyde 3-phosphate dehydrogenase (GAPDH).

2.5. Histological analyses

For histological studies, samples of epididymal fat pad and pancreas tissues (n = 4 mice/group) were embedded in paraffin and cut into 5-μm sections. We performed terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) to measure the degree of adipose tissue apoptosis using an *in-situ* cell death detection kit (Roche Molecular Biochemicals, Mannheim, Germany), according to the manufacturer's instructions. For immunohistochemistry, sections of de-paraffinized epididymal fat and pancreas tissue were incubated (4 °C) overnight with the primary antibodies diluted in blocking solution (Supplementary Table 2). The histological staining and immunohistochemistry procedures were performed as previously described [15,16].

2.6. Protein extraction and Western blot analyses

To extract total proteins from frozen hippocampi, epididymal fat pads, and pancreata (n = 6 mice/group), tissues were homogenized in lysis buffer (#78510, Thermo Scientific, USA). Western blot analyses were performed using standard methods. The membranes were probed with primary antibodies (Supplementary Table 2). The protein bands were detected using enhanced chemiluminescence substrates (Pierce). The Multi-Gauge image analysis program (version 3.0; Fujifilm, Tokyo, Japan) was used for densitometric analyses of immunoblots. To normalize the protein level values, we used β-actin and α-tubulin as internal controls.

2.7. Statistical analyses

Between-group differences were assessed using one- or two-way analysis of variance (ANOVA) followed by post hoc analysis using the Bonferroni test (GraphPad Software Inc., San Diego, CA, USA). The results were expressed as mean ± standard error of the mean. A p-value <0.05 was considered statistically significant.

3. Results

3.1. Effects of a long-term HFD on body weights and insulin resistance in KO mice

To determine the role of myeloid-specific SIRT1 in the development of HFD-induced obesity, mice were fed either the ND or HFD for 40 weeks. Body weight gradually increased in HFD-fed WT and ND-fed or HFD-fed KO mice, compared with ND-fed WT mice (Supplementary Fig. 1A). Consistent with changes in body weight, we found that at 40 weeks, the fat mass was greater in ND-fed KO mice than in ND-fed WT mice. However, there was no difference between HFD-fed WT and KO mice (Supplementary Fig. 1B). During the development of chronic HFD-induced obesity, fasting blood glucose levels were higher in HFD-fed KO mice than in HFD-fed WT mice (Supplementary Fig. 1C). Compared with HFD-fed WT mice, HFD-fed KO mice had higher blood glucose levels in the glucose tolerance tests (Supplementary Figs. 1D and E). We observed a statistically significant increase in insulin resistance with reduced serum insulin levels and HOMA-IR in HFD-fed KO mice. This result indicated that development of insulin resistance was secondary to defective insulin secretion in the HFD-fed KO mice (Supplementary Figs. 1F–H). Taken together, these results indicate that myeloid SIRT1 deficiency aggravated insulin resistance after a 40-week HFD.

3.2. Effects of myeloid SIRT1 deficiency on macrophage infiltration in HFD-fed mice

Increased macrophage infiltration in adipose tissue is a hallmark of obesity-induced tissue inflammation and insulin resistance [6]. Myeloid SIRT1 promotes macrophage infiltration in adipose tissues after 12 or 24 weeks of HFD feeding [15,16]. The present findings also indicate that a chronic HFD promoted increased adipocyte size in HFD-fed WT mice. However, the adipocyte sizes were smaller in HFD-fed KO mice than HFD-fed WT mice (Fig. 1A and B). Because adipose tissue macrophages often surround and ingest dying or dead adipocytes to form crown-like structures (CLSs), we assessed macrophage infiltration into adipocytes by performing TUNEL analysis and counting CD68-positive CLSs (Fig. 1C–E). We found greater CLS and TUNEL-positive cell numbers in HFD-fed KO mice than HFD-fed WT mice. These results indicated that after 40 weeks of HFD feeding, myeloid SIRT1 deletion promoted massive macrophage infiltration in adipose tissues.

Please cite this article in press as: K.E. Kim, et al., Myeloid sirtuin1 deficiency aggravates hippocampal inflammation in mice fed high-fat diets, Biochemical and Biophysical Research Communications (2018), https://doi.org/10.1016/j.bbrc.2018.04.044