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NFATc3 deficiency protects against high fat diet (HFD)-induced hypothalamus inflammation and apoptosis via p38 and JNK suppression

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ABSTRACT

Hypothalamic inflammation and apoptosis cause neural injury, playing an important role in metabolic syndrome development. Nuclear Factors of Activated T cells (NFATc3) show many physiological and pathological effects. However, the function of NFATc3 in high fat diet (HFD)-induced hypothalamus injury remains unknown. The wild type (WT) and NFATc3-knockout (KO) mice were subjected to HFD feeding for 16 weeks to examine NFATc3 function in vivo. Astrocytes isolated from WT or KO mice were cultured and exposed to fructose (Fru) in vitro. The liver damage, hypothalamus injury, pro-inflammatory markers, NF-κB (p65), Caspase-3 and mitogen-activated protein kinases (MAPKs) pathways were evaluated. NFATc3 was significantly up-regulated in hypothalamus from mice challenged with HFD, and in astrocytes incubated with Fru. Both in vivo and in vitro studies indicated that NFATc3-deletion attenuated metabolism syndrome, reduced inflammatory regulators expression, inactivated NF-κB (p65), Caspase-3 and p38/JNK signaling pathway. Of note, we identified that promoting p38 or JNK activation could rescue inflammatory response and apoptosis in NFATc3-KO astrocytes stimulated by Fru. Together, these findings revealed an important role of NFATc3 NFATc3 for HFD-induced metabolic syndrome and particularly hypothalamus injury, and understanding of the regulatory molecular mechanism might provide new and effective therapeutic strategies for prevention and treatment of hypothalamic damage associated with dietary obesity-associated neuroinflammation and apoptosis.

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1. Introduction

Increasing evidence from human and animal studies indicates that consumption of a high-fat diet (HFD) results in metabolic disorders, such as insulin resistance, obesity, and systemic inflammation [1,2]. Recently, there was a study demonstrated that inflammation in the hypothalamic arcuate nucleus, a center of feeding regulation, develops 1–3 days after initiation of HFD feeding [3]. Additionally, diets rich in fat could result in neurodegeneration via induction of apoptosis in hypothalamus [4,5]. However, investigation of the essential molecular mechanism that contributes to the progression of hypothalamic damage and associated metabolic disorders is urgently required.

The nuclear factor of activated T cells (NFAT) family comprises five calcium/calcineurin-dependent transcription factors, including NFATc/2/c1, NFATp/1/c2, NFAT3/c4, NFAT4/c3, and NFAT5 [6,7]. NFAT proteins are expressed in most mammalian tissues, with the different members of the family being present in distinct but overlapping sets of cell types. NFATc3 is a predominant NFAT gene [8]. NFATc3-null mice were shown to have defects in skeletal muscle fiber number or size [9]. NFATc3 protein has been implicated in pathogenesis of various inflammatory pathologies [10]. For instance, NFATc3 transcriptionally regulates tumor necrosis factor-α (TNF-α) expression, contributing to acute lung injury progression induced by LPS [11]. NFATc3 has also been suggested to possess either pro-apoptotic or anti-apoptotic effects, relying on the physiologic and cellular context [12,13]. Both inflammation and apoptosis play critical roles in HFD-induced hypothalamus injury [3–5]. Hence, NFATc3 might feasibly function as a crucial regulator of the pathogenesis of hypothalamic damage induced by HFD.

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Nevertheless, revealing the actual effects and specific activity of NFATc3 needs further exploration.

The present research was conducted to investigate the regulatory role of NFATc3 in HFD-induced hypothalamus injury through deleting the expression of NFATc3 in vivo and in vitro. The findings demonstrated that NFATc3-knockout could markedly attenuated HFD-triggered insulin resistance, liver steatosis and importantly neuroinflammation and apoptosis, which was attributed to the reduced activation of NF-κB (p65), Caspase-3 and p38/JNK signaling pathways. Our results provided novel insights that NFATc3 functioned as a novel regulator in HFD-induced hypothalamic neural injury.

2. Materials and methods

2.1. Animals and treatments

WT mice and NFATc3-KO C57BL/6J male mice aged 6–8 weeks were purchased from Jackson Laboratory (Bar Harbor, ME). Neonatal ob/ob mice were obtained from HFK Bioscience Co., Ltd (Beijing, China). And all mice were fed in a temperature-controlled environment under a 12 h light/dark time cycle. A normal chow (NC) diet (D12450B, Research Diets Inc., USA) and HFD (D12492, Research Diets) were treated to mice continuously for 16 weeks. All mice had free access to food and water. Body weights were examined every week. All procedures were performed following the Regulations of Experimental Animal Administration issued by the Ministry of Science and Technology of the People's Republic of China. The Institutional Animal Care and Use Committee at the Renmin Hospital of Wuhan University (Wuhan, China) approved the animal study protocols. In the end of the study, eyeball bloods were harvested for biochemical research. The liver, visceral adipose tissue and brain samples were removed, and immediately fixed in 10% formalin or snap-frozen in liquid nitrogen and stored at −80 °C.

2.2. Astrocytes isolation and culture

Astrocytes from hypothalamus of WT or NFATc3-KO mice were prepared as previously described [14]. The isolated cells were cultured in plates and incubated in the initial experiments with 5 mM fructose (Fru) (Senbo Biology Co., Ltd., Xi'an, China) for 24 h in DMEM (Gibco, USA) containing 10% fetal bovine serum (Hyclone, USA) and 100 KU/L penicillin and 100 mg/L streptomycin at 37 °C, 5% CO_2. Both p38 activator of Dehydrocorydaline chloride (DHC) and JNK activator of Anisomycin (ANS) were purchased from MedChemExpress (USA).

2.3. Western blot analysis

Cellular or nuclear proteins were extracted from hypothalamus tissue samples and astrocytes. Protein extracts (20–40 μg) were subjected to SDS-PAGE and transferred to a PVDF membrane (Millipore, USA), followed by incubation with primary antibodies (Supplementary Table 1) and secondary antibodies (Abcam, USA) for visualization.

2.4. Real time-quantitative PCR (RT-qPCR) analysis

Total mRNA was isolated and then cDNA synthesized. Next, RT-qPCR was conducted using SYBR Green (Invitrogen, USA). The mRNA expressions of the targeting genes (Supplementary Table 2) were normalized to GAPDH levels.

2.5. Immunofluorescent analysis

Frozen brain sections and cells were stained with anti-mouse NFATc3 (Santa Cruz, USA), Iba-1 and/or GFAP (Abcam, USA) or Alexa Fluor 488-conjugated goat anti-mouse IgG (Abcam).

2.6. Biochemical analysis

Fasting insulin contents were measured with an ELISA (Millipore) at the end of the experiment. Plasma contents of triglyceride (TG) and total cholesterol (TC) were assessed using commercial kits (Beyotime Biotechnology, Shanghai, China). ELISA kits for serum measurements of monocyte chemoattractant protein-1 (Mcp-1), keratinocyte-derived cytokine-1 (Cxcl-1), interleukin (IL)-18, and IL-1β were from R&D Systems (USA). ELISA kit to test TNF-α contents in serum was purchased from eBioscience (USA).

2.7. Oral glucose tolerance testing (OGTT) analysis

Mice were subjected to an intraperitoneal injection of glucose (2 g/kg body weight). Blood samples were collected from tail vein after glucose administration for 0, 15, 30, 60, 90 and 120 min. Blood glucose levels were measured uisng o-toluidine reagent (Sigma, USA).

2.8. Immunohistochemical analysis

The paraffin-embedded liver sections were stained with hematoxylin and eosin (H&E), while the frozen liver sections were stained with Oil Red O [15]. Immunohistochemistry staining was performed to determine the expression profile of F4/80 (Abcam) in adipose tissue sections (5-μm thickness) [16]. Adipocyte diameter was calculated using ImageJ software (version 1.32, US National Institutes of Health, USA) [17].

2.9. Apoptosis determination

An annexin V-fluorescein isothiocyanate-propidium iodide (PI) apoptosis detection kit (KeyGen Biotech, Nanjing, China) was used to determine cell apoptosis following the manufacturer's instructions.

2.10. TUNEL analysis

Apoptosis of frozen brain tissue sections was determined using a commercially available fluorescent TUNEL kit (KeyGen Biotech) following the manufacturer's protocols.

2.11. Statistical analysis

All data in this study are expressed as the mean ± SEM, and were calculated using Graphpad Prism software (version 5.0b; Graph Pad Software, USA) through a one-way ANOVA with Dunnet's least significant difference post hoc tests, or two-way repeated measures ANOVA with Bonferroni post-tests. $P < 0.05$ was regarded as statistically significant.

3. Results

3.1. NFATc3 expression is increased in HFD-induced hypothalamus

NFATc3 mRNA and protein expressions were markedly up-regulated in murine hypothalamus after 16 weeks of HFD feeding (Fig. 1A). The gene and protein levels of NFATc3 in the hypothalamus of ob/ob mice gradually increased from 2 to 12 weeks (Fig. 1B).

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