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

NLRP1 deficiency attenuates diabetic retinopathy (DR) in mice through suppressing inflammation response

Yan Li [a], Chang Liu [b], Xin-Shun Wan [c], Shao-Wei Li [a, b, *]

[a] Central South University, Changsha 410083, Hunan Province, China
[b] Beijing Aier-Intech Eye Hospital, Panjiayuan Plaza, No.12 Panjiayuan Nanli, Chaoyang District, Beijing 100021, China
[c] The Third Affiliated Hospital of Xinxiang Medical University, Xinxiang 453003, Henan Province, China

ARTICLE INFO

Article history:
Received 14 March 2018
Accepted 20 March 2018
Available online xxx

Keywords:
Diabetic retinopathy (DR)
NLRP1
Inflammation
VEGF
ASC

ABSTRACT

Diabetic retinopathy (DR) is the common cause of diabetic vascular complications. The NOD-like receptor (NLR) family, pyrin domain containing 1 (NLRP1), also known as NALP1, inflammasome is the first member of the NLR family to be discovered, playing an important role in inflammatory response. However, its effect on DR development has not been reported. In the study, the wild type (WT) and NLRP1$^{-/-}$ mice were injected with streptozotocin (STZ) to induce DR. The results indicated that NLRP1$^{-/-}$ significantly increased bodyweight reduction and decreased blood glucose levels induced by STZ. WT/DR mice exhibited higher levels of NLRP1 in retinas. NLRP1$^{-/-}$ ameliorated retinal abnormalities in DR mice using H&E staining. In addition, attenuated avascular areas and neovascular tufts were also observed in NLRP1$^{-/-}$/DR mice. The levels of pro-inflammatory cytokines in serum and retinas were highly induced in WT/DR mice, whereas being markedly reduced by NLRP1$^{-/-}$. In addition, vascular endothelial growth factor (VEGF) and Iba1 expressions induced by STZ in serum or retinas were significantly down-regulated in NLRP1$^{-/-}$/DR mice. Consistently, NLRP1$^{-/-}$ attenuated ASC and Caspase-1 expressions in retinas of DR mice. Compared to WT/DR group, NLRP1$^{-/-}$ markedly decreased retina p-nuclear factor-κB (NF-κB), interleukin-1β (IL-1β) and IL-18 levels. And similar results were confirmed in vitro that suppressing NLRP1/ASC inflammasome ameliorated inflammatory response in fructose-treated retinal ganglion cells. The results above indicated that the modulation of NLRP1 inflammasome might be a promising strategy for DR therapy.

1. Introduction

Diabetic retinopathy (DR) is a retinal microvascular disease, a leading cause of severe visual loss among the working-age population [1,2]. DR is a multifactorial disease and its pathogenesis is not fully understood. And some molecular mechanisms have been hypothesized to reveal the appearance of low-grade chronic inflammation in diabetic retina, evidenced by the elevation of TNFα, IL1β, IL-8 and MCP-1 [3].

The NLRP1, previously known as NALP1, inflammasome was the first member of the NLR family to be discovered. In vivo, NLRP1 could produce a functional Caspase-1-containing inflammasome to drive the inflammatory response [4]. Although present studies regarding NLRP1 functions are far scarcer than those described for other inflammasomes, a variety of immune inflammation diseases have been linked to mutations and polymorphisms in NLRP1 gene [5]. For instance, NLRP1 inflammasome was activated in Alzheimer's disease, along with higher amounts of the pro-inflammatory cytokines IL-1β and IL-18 [6]. In addition, NLRP1 protect against the development of spinal injury [7]. Recently, the inflammasome complex, which cleaves pro-IL-1β into secreted IL-1β via Caspase-1, could be activated in retinal pigment epithelial cells cultured under high glucose levels [8]. In spite of all of these findings, the role of NLRP1 in the retina during DR has not been fully understood.

Commonly used streptozotocin (STZ)-induced rodent models show rapid onset of hyperglycemia and several symptoms of DR, including retinal pericyte loss and capillaries, thickening of vascular basement membrane, and increased vascular permeability. In the present study, we calculated the effects of NLRP1 on inflammation in STZ-induced diabetic mice. The results indicated that suppressing NLRP1 attenuated DR in vivo and in vitro.

* Corresponding author. Beijing Aier-Intech Eye Hospital, Panjiayuan Plaza, No.12 Panjiayuan Nanli, Chaoyang District, Beijing 100021, China.
E-mail address: lishaowe.huang@foxmail.com (S.-W. Li).

https://doi.org/10.1016/j.bbrc.2018.03.148
0006-291X/

2. Materials and methods

2.1. Animals

40 male, 6–8-weeks old, C57/BL6 mice (Wild type, WT) and NLRP1-knockout (NLRP1$^{-/-}$) mice with C57BL/6 background were purchased from Jackson Laboratory (Bar Harbor, ME). Animals were housed in a room with a 12-h light/dark cycle with free access to food and water. Animals were randomly divided into four groups, including the non-diabetic control groups (WT/Con and NLRP1$^{-/-}$/Con) and the diabetic groups (WT/DR and NLRP1$^{-/-}$/DR). The body weight was monitored. The mice were intraperitoneally injected with 45 mg/kg STZ (Sigma, USA) after 4 h fast each day for 5 days consecutively and the non-diabetic control mice were only subjected to citrate buffer alone. Then, all mice were sacrificed via eyeblooding for collecting serum. The retina samples were harvested on 4 °C glacial table, frozen in liquid nitrogen and kept at −80 °C for further research. All experiments were performed according to the Ministry of Science and Technology of the People's Republic of China. The Animal Care and Use Committee of Central South University (Hunan, China) approved the animal study protocols.

2.2. Cells and culture

The retinal ganglion cells were obtained and cultured as previously described [9]. Finally, cells were grown on poly D-lysine-coated petri dishes. Half of the media was changed every third day. To knockdown NLRP1 and ASC, cells were transfected with NLRP1-specific siRNA (Santa Cruz, USA), ASC-specific siRNA (Santa Cruz) or negative control (siCon) using lipofectamine 2000 (Invitrogen, USA) following the manufacturer's instructions for 24 h. Then, cells were subjected to fructose (5 mM, Sigma Aldrich) for another 24 h.

2.3. Oral glucose tolerance test (OGTT) analysis

Mice were fasting for 6 h. Then, all mice were treated with 20% glucose dissolved in saline orally. The tail-vein blood was collected at 0, 30, 60, 90 and 120 min after glucose treatment, and the blood glucose levels were measured with o-toluidine reagent (Sigma).

2.4. Quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was isolated from retina tissues and cells using TRIzol (KeyGen Biotech, Nanjing, China) and real-time RT-PCR was conducted by Q-PCR machine (Applied Biosystem, USA) for mRNA expression. PCR was performed as previously described [10]. Primer sequences for the targeting genes were exhibited in Supplementary Table 1.

2.5. Western blot analysis

Mouse retinas and cells were homogenized in modified RIPA buffer (KeyGen Biotech). The protocol was performed as previously described [10]. Primary antibodies used in our study were exhibited in Supplementary Table 2.

2.6. Enzyme-linked immunosorbent assay (ELISA)

Serum IL-1β, TNF-α, and IL-6 levels were detected using the commercial ELISA kits (R&D Systems, Inc., USA).

2.7. H&E and VEGF staining

The eye samples were enucleated and dipped in 4% paraformaldehyde overnight at room temperature, and embedded in paraffin. Serial sections (5-μm thickness) were cut and stained with hematoxylin and eosin (H&E). VEGF (1:200, Abcam) was incubated to the sections for 30 min at room temperature. Subsequently, the sections were washed with PBS and incubated with biotin-conjugated goat anti-rabbit IgG secondary antibody (KeyGen Biotech) and avidin–biotin peroxidase complex (ABC reagent) for 30 min.

2.8. Iba1 immunofluorescent analysis

Eyes were fixed in 4% paraformaldehyde for 24 h at 4 °C and infiltrated with sucrose 25% (w/v). For immunofluorescence analysis, we followed the protocol detailed [11]. The rabbit anti-Iba1 and p-NF-κB (1:200, Abcam) antibody in blocking solution was used for incubation. Staining was observed with an inverted laser confocal microscope.

2.9. Fluorescein angiography

Mice were anesthetized by the use of ketamine and xylazine. The eyes were dilated using 0.125% atropine. Each animal was held on its side on the microscope platform. The right eye was rinsed using 2% Methocel gel (Fluka, Italy). After color fundus photography was carried out, fluorescein was used for fundus fluorescein angiography evaluation via IP injection.

2.10. TUNEL analysis

Apoptosis in vitro was measured using a one-step TUNEL Apoptosis Assay KIT (Roche, Germany) following the kit's manual. The images were captured with a Nikon ECLIPSE Ti microscope (Nikon, Japan).

2.11. Electroretinogram (ERG) assays

Animal ERG was recorded as described earlier [12]. ERG signals were amplified and digitized with a 2 μV resolution at 1 kHz. The implicit time and wave amplitude were recorded.

2.12. Periodic acid-schiff (PAS) analysis

Eyes of mouse were fixed in 4% paraformaldehyde overnight and the PAS staining was performed as previously described [13]. Finally, the retinal vessels were observed under a microscope.

2.13. Statistical analysis

Results were expressed as mean ± SEM. Statistical analyses were performed using GraphPad PRISM (version 6.0; Graph Pad Software, USA) by ANOVA with Dunnet's least significant difference post-hoc tests. A $p < 0.05$ was considered significant.

3. Results

3.1. NLRP1 expression is increased in retina of diabetic mice

First, we found that STZ-induced diabetes led to a significant decrease of body weight and increase of blood glucose levels compared to the WT/Con group. NLRP1 ablation markedly up-regulated body weight of mice and down-regulated blood glucose levels compared to the WT diabetic mice (Fig. 1A and B). Fig. 1C and D indicated that in DR mice, NLRP1$^{-/-}$ significantly reduced serum insulin and glucagon levels in STZ-treated mice. NLRP1 deletion attenuated STZ-induced insulin resistance using OGTT (Fig. 1E).

Please cite this article in press as: Y. Li, et al., NLRP1 deficiency attenuates diabetic retinopathy (DR) in mice through suppressing inflammation response, Biochemical and Biophysical Research Communications (2018), https://doi.org/10.1016/j.bbrc.2018.03.148