Biochimica et Biophysica Acta 1842 (2014) 164–174

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

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

Pro-inflammatory cytokines downregulate Hsp27 and cause apoptosis of human retinal capillary endothelial cells

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ARTICLE INFO

Article history:
Received 10 July 2013
Received in revised form 25 October 2013
Accepted 12 November 2013
Available online 16 November 2013

Keywords:
Hsp27
Retinal endothelial cell
Cytokine
ROS
Apoptosis

ABSTRACT

The formation of acellular capillaries in the retina, a hallmark feature of diabetic retinopathy, is caused by apoptosis of endothelial cells and pericytes. The biochemical mechanism of such apoptosis remains unclear. Small heat shock proteins play an important role in the regulation of apoptosis. In the diabetic retina, pro-inflammatory cytokines are upregulated. In this study, we investigated the effects of pro-inflammatory cytokines on small heat shock protein 27 (Hsp27) in human retinal endothelial cells (HREC). In HREC cultured in the presence of cytokine mixtures (CM), a significant downregulation of Hsp27 at the protein and mRNA level occurred, with no effect on HSF-1, the transcription factor for Hsp27. The presence of high glucose (25 mM) amplified the effects of cytokines on Hsp27. CM activated indoleamine 2,3-dioxygenase (IDO) and enhanced the production of kynurenine and ROS. An inhibitor of IDO, 1-methyl tryptophan (MT), inhibited the effects of CM on Hsp27. CM also upregulated NOS2 and, consequently, nitric oxide (NO). A NOS inhibitor, L-NAME, and a ROS scavenger blocked the CM-mediated Hsp27 downregulation. While a NO donor in the culture medium did not decrease the Hsp27 content, a peroxynitrite donor and exogenous peroxynitrite did. The cytokines and high glucose-induced apoptosis of HREC were inhibited by MT and L-NAME. Downregulation of Hsp27 by a siRNA treatment promoted apoptosis in HREC. Together, these data suggest that pro-inflammatory cytokines induce the formation of ROS and NO, which, through the formation of peroxynitrite, reduce the Hsp27 content and bring about apoptosis of retinal capillary endothelial cells.

1. Introduction

According to the International Diabetes Federation, there were 311 million people with diabetes worldwide in 2011 and this number is projected to increase to 552 million by 2030 [1]. One of the devastating long-term complications of this disease is diabetic retinopathy, which is the leading cause of visual impairment in working-class adults in the US and other developed countries. In both type 1 and type 2 diabetes, diabetic retinopathy becomes progressively worse with duration of the disease; it is estimated that after 20 years of diabetes, nearly 75% of patients show clinical signs of the disease [2].

Vascular, glial and neuronal abnormalities are the earliest changes in the diabetic retina [3,4]. Retinal capillary cells become increasingly permeable to macromolecules, leading to macular edema. The extracellular matrix of capillary cells becomes thicker and localized sacular microaneurysms develop on capillaries. Along with these changes, pericytes and endothelial cells, the two cell types in retinal capillaries, die early in the disease, causing formation of acellular capillaries that lead to local ischemia in the retina [reviewed in [5]]. A number of mechanisms have been put forward to explain these histopathological changes, including pro-inflammatory signals [reviewed in [6]]. Chronic low-grade inflammation appears to play a role in the pathogenesis of the disease [7,8].

Several pro-inflammatory cytokines, such as tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β) and interferon-γ (IFN-γ), are elevated in the diabetic retina [9–11]. These cytokines upregulate nuclear factor-kappaB (NF-κB), which, in turn, can promote the synthesis of cytokines [12]. Along with these changes, inflammatory markers, including vascular cell adhesion molecule-1 (VCAM-1), intracellular adhesion molecule-1 (ICAM-1), nitric oxide synthase-2 (NOS2), cyclooxygenase-2 (COX2), and monocyte chemotactic protein-1 (MCP-1), are upregulated in the diabetic retina [reviewed in [6]].

Abbreviations: HREC, human retinal endothelial cells; IDO, indoleamine 2,3-dioxygenase; MT, 1-methyl-DL-tryptophan; NOS, nitric oxide synthase; ROS, reactive oxygen species; TNF-α, tumor necrosis factor-α; IL-1β, interleukin-1β; IFN-γ, interferon-γ; VCAM-1, vascular cell adhesion molecule-1; ICAM-1, intracellular adhesion molecule-1; MCP-1, monocyte chemotactic protein-1; COX2, cyclooxygenase-2; L-NAME, N^{ω}-nitro-L-arginine methyl ester hydrochloride; TEMPOL, 1-oxyl-2,2,6,6-tetramethyl-4-hydroxypiperidine; HG, high glucose; CM, cytokine mixture; CM-H_2DCFDA, 5-(and-6)-chloromethyl-2′,7′-dichlorodihydro-fluorescein diacetate; Ac-DEVD-AFC, N-acetyl-Asp-Glu-Val-Asp-7-amido-4-trifluoromethylcoumarin; DETA NONOate, (*Z*)-1-[2-(2-aminoethyl)-*N*-(2-ammonioethyl)amino]diazen-1-ium-1,2-diolate; NO, nitric oxide; ONOO, peroxynitrite; HSF-1, heat shock factor-1

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http://dx.doi.org/10.1016/j.bbadis.2013.11.011

Several studies have provided direct evidence for a role of inflammation in diabetic retinopathy. These include a demonstration that pharmacological suppression of inflammation leads to inhibition of ICAM-1 expression and leukostasis (attachment of leukocytes to endothelial cells, a characteristic feature of inflammation) and that the absence of TNF-α leads to suppression of blood retinal barrier breakdown in diabetic retina [13,14]. In addition, the inhibition of caspase-1, which activates IL-1β, inhibits capillary degeneration [15], and the inhibition of NF-κB leads to inhibition of ICAM-1 and vascular endothelial growth factor-A production in the diabetic retina [16]. Finally, the inhibition of COX2 and NOS2 blocks capillary cell death and reduces leukostasis and blood retinal barrier breakdown, respectively, in the retinas of diabetic rodents [17,18].

Despite clear evidence of an increased inflammatory response in diabetic retinopathy, the source(s) of inflammatory cytokines in the retina are unclear. While capillary cells can produce cytokines in small amounts, it is believed that retinal glia, Muller cells and microglia, as well as retinal pigmented epithelial cells, are capable of producing these cytokines. Cellular stresses, such as endoplasmic reticulum stress and stress imposed by ROS, promote the synthesis of cytokines [8,19]; however, it remains unclear whether these cells synthesize inflammatory cytokines in response to hyperglycemia in diabetes or through hyperglycemia-driven processes. Nevertheless, there is a clear link between inflammation and capillary cell death in experimental diabetic retinopathy and in cultured retinal capillary cells. Despite these advances, the exact mechanism by which inflammation brings about apoptosis of retinal capillary cells remains unknown. It has been suggested that ROS, produced from activated NADPH oxidase and leakage of electrons from the mitochondrial electron transport chain, and nitric oxide, produced from upregulated NOS, are involved, but the actual mechanism is largely unknown. We also demonstrated that IFN-γ upregulates indoleamine 2,3-dioxygenase (IDO) through the JAK/STAT pathway in human retinal endothelial cells, leading to the formation of kynurenines, which spontaneously produce ROS [20].

Small heat shock proteins are ubiquitously present in cells and they protect cells from stresses through their chaperone, anti-apoptotic and anti-inflammatory activities. This family is composed of more than 10 members [21], among which αA- and αB-crystallin and Hsp27 are present in retinal cells [22–25]. Both αB-crystallin and Hsp27 have been implicated in angiogenesis [26,27]. Despite these findings, whether there are alterations in their levels in capillary cells in diabetes is unknown. In this study, we investigated the effects of pro-inflammatory cytokines and high glucose on Hsp27 expression and present data to show that downregulation of this protein leads to apoptosis of human retinal endothelial cells.

2. Materials and methods

1-Methyl-DL-tryptophan, DETA NONOate [2,2′-(hydroxynitrosohydrazino)bis-ethanamine], L-NAME (N^{ω}-nitro-L-arginine methyl ester hydrochloride), 1-oxyl-2,2,6,6-tetramethyl-4-hydroxypiperidine (TEMPOL), DL-kynurenine, and D-glucose were obtained from Sigma-Aldrich Chemical Co. LLC (St. Louis, MO, USA). TNF-α and IL-1β were obtained from Invitrogen (Grand Island, NY). IFN-γ was obtained from R and D systems (Minneapolis, MN). N-Acetyl-Asp-Glu-Val-Asp-7-amido-4-trifluoromethylcoumarin (Ac-DEVD-AFC) was from BD Biosciences, San Jose, CA. The peroxynitrite donor, 3-morpholinosydnonimine (SIN-1) was obtained from Acros Organics, NJ. Peroxynitrite was from Calbiochem, EMD Biosciences, San Diego, CA and 5-(and-6)-chloromethyl-2′,7′-dichlorofluorescein diacetate, acetyl ester (CM-D_2DCFDA) was from Molecular Probes, Eugene, OR. All other chemicals were of analytical grade.

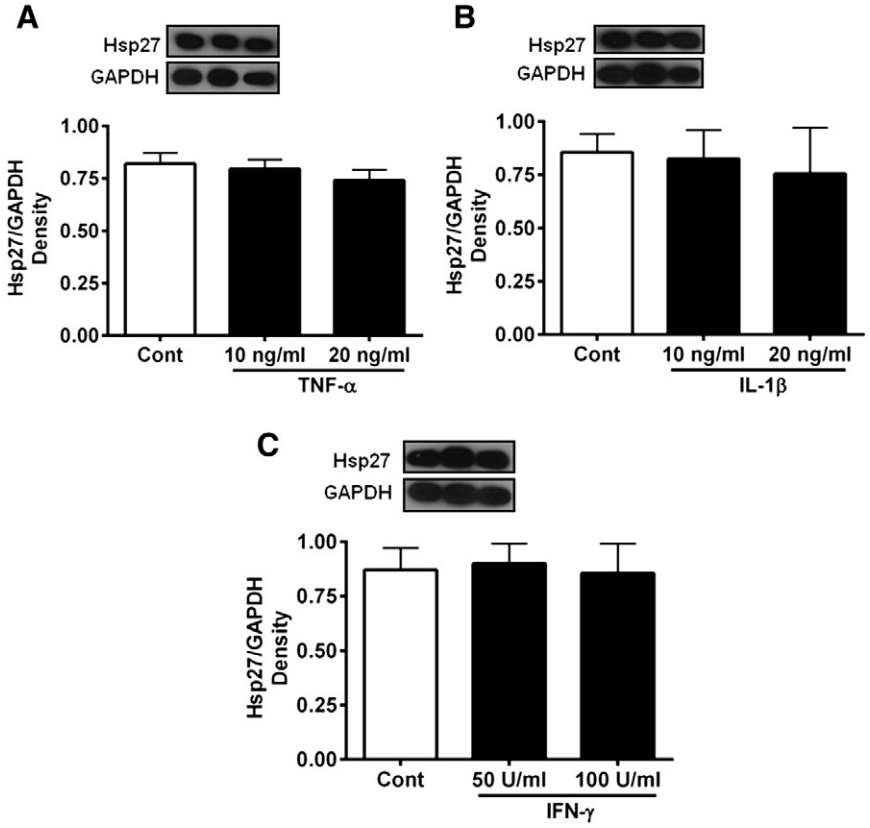

Fig. 1. Individual cytokines do not affect Hsp27 levels in HREC. HREC cultures were incubated with 10 and 20 ng/mL TNF-α (A), 10 and 20 ng/mL IL-1β (B), and 50 and 100 U/mL of IFN-γ (C) for 48 h. Hsp27 was measured by Western blotting and densitometry. The bars represent means ± SD of three independent experiments. GAPDH was used as the loading control. Cont = Control.