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Isocitrate dehydrogenase 2 deficiency induces endothelial inflammation via p66sh-mediated mitochondrial oxidative stress

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ABSTRACT

Isocitrate dehydrogenase 2 (IDH2) is an essential enzyme in the mitochondrial antioxidant system, which produces nicotinamide adenine dinucleotide phosphate, and thereby defends against oxidative stress. We have shown that IDH2 downregulation results in mitochondrial dysfunction and reactive oxygen species (ROS) generation in mouse endothelial cells. The redox enzyme p66shc is a key factor in regulating the level of ROS in endothelial cells. In this study, we hypothesized that IDH2 knockdown-induced mitochondrial dysfunction stimulates endothelial inflammation, which might be regulated by p66shc-mediated oxidative stress. Our results showed that IDH2 downregulation led to mitochondrial dysfunction by decreasing the expression of mitochondrial oxidative phosphorylation complexes I, II, and IV, reducing oxygen consumption, and depolarizing mitochondrial membrane potential in human umbilical vein endothelial cells (HUVECs). The dysfunction not only increased mitochondrial ROS levels but also activated p66shc expression in HUVECs and IDH2 knockout mice. IDH2 deficiency increased intercellular adhesion molecule (ICAM)-1 expression and mRNA levels of pro-inflammatory cytokines (tumor necrosis factor [TNF]-α, and interleukin [IL]-1β) in HUVECs. The mRNA expression of ICAM-1 in endothelial cells and plasma levels of TNF-α and IL-1β were also markedly elevated in IDH2 knockout mice. However, p66shc knockdown rescued IDH2 deficiency-induced mitochondrial ROS levels, monocyte adhesion, ICAM-1, TNF-α, and IL-1β expression in HUVECs. These findings suggest that IDH2 deficiency induced endothelial inflammation via p66shc-mediated mitochondrial oxidative stress.

1. Introduction

Increased oxidative stress contributes to the development of inflammation, which is the main process in the development of vascular diseases and several age-related diseases [1]. Endothelial cells are located on the interior surfaces of blood vessels and play important roles in maintaining vascular homeostasis and preventing cardiovascular disease. Oxidative stress disrupts endothelial function, and impairment of the vascular endothelium plays an important role in the pathogenesis of systemic inflammation [2]. Mitochondria are dynamic organelles that generate adenosine triphosphate via oxidative phosphorylation (OXPHOS), mediate cellular redox homeostasis, and regulate programmed cell death in most eukaryotic cells. Although mitochondria in vascular endothelial cells are not present at a higher concentration compared with other cell types, they are the major sites of reactive oxygen species (ROS) production. Many studies have shown that mitochondrial ROS production in endothelial cells contributes to endothelial dysfunction and several cardiovascular risk factors [3].

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Increased ROS levels induce alteration of the mitochondrial membrane potential (MMP) as well as the release of inflammatory proteins and cytokines that cause cell injury or apoptosis [4]. Upon initiation of vascular inflammation, adhesion molecules on the endothelial cell membrane accumulate monocytes and macrophages, which participate in the inflammatory response. Therefore, mitochondrial dysfunction-induced endothelial ROS are potent stimulators in the promotion of vascular inflammation.

Mitochondrial NADP + -dependent isocitrate dehydrogenase 2 (IDH2) is a major source of mitochondrial NADPH production and promotes regeneration of reduced pools of glutathione by supplying NADPH to glutathione reductase or thioredoxin reductase [5]. Therefore, IDH2 plays an important role in mediating the redox status and protecting cells from oxidative stress-induced injury [6]. Animal studies have shown that IDH2 knockout (KO) mice developed heart failure and cardiac hypertrophy [7,8]. However, whether IDH2 deficiency-induced mitochondrial dysfunction stimulates endothelial inflammation remains unknown.

Mammalian ShcA encodes three isoforms, which are identified according to their molecular weights: p46shc, p52shc, and p66shc. Among them, only p66shc as a redox enzyme increases ROS levels by promoting the generation of ROS and inhibiting the expression of antioxidant enzymes [9]. Thus, p66shc is a sensor as well as an amplifier of oxidative stress [10]. Many studies have indicated that p66shc deficiency reduces oxidant levels and protects against ROS-induced endothelial dysfunction [11,12]. Furthermore, p66shc KO mice have been reported to be protected from age-dependent endothelial dysfunction diseases such as diabetes and hypercholesterolemia, to exhibit reduced inflammation [13,14]. Therefore, we hypothesized that p66shc, as a redox enzyme, may mediate IDH2 deletion-triggered ROS production and endothelial inflammation.

Our results showed that IDH2 deficiency promoted mitochondrial dysfunction, ROS production, and endothelial cell inflammation in human endothelial cells and IDH2 KO mice, and these effects were mediated by p66shc.

2. Materials and methods

2.1. Cell culture and transfection

HUVECs were obtained from Clonetics (San Diego, CA, USA) and cultured in endothelial growth medium-2 (Lonza, Walkersville, MD, USA). Subconfluent, proliferating HUVECs were used at passages 4–8. U937 monocytes were obtained from Clonetics and cultured in RPMI 1640 media (Walgene, Inc., Daegu, South Korea). HUVECs were transfected with human IDH2 siRNAs (sense, CAGUAUGCCAUCCAGAAGA, and antisense, UCUUCUGGAUGGCAUACUG) (Bioneer, Deajeon, South Korea) and negative control siRNA using Lipofectamine 2000 reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions.

2.2. Mouse studies

All animal experiments were approved and carried out in accordance with the guidelines of the Animal Care Committee of Chungnam National University (CNU-00084). The animals used in this study were IDH2$^{-/-}$ germ-line KO mice, and their congenic background strain (C57BL/6J) served as the WT (IDH2$^{+/+}$) control. Animals were raised under controlled lighting (12-h light/dark cycle) and temperature (24 ± 1 °C) with free access to food and water. The IDH2 KO mice were a donation from Kyungbook National University (School of Life Sciences, College of Natural Science, South Korea).

2.3. Antibodies and immunoblotting

The following antibodies were used: anti-IDH2 (Abcam, Cambridge, UK); anti-ICAM-1 and mitochondrial dynamics proteins anti-Mfn1, anti-Mfn2 (Santa Cruz Biotechnology, Santa Cruz, CA, USA), anti-OPA1, anti-DRP1, and anti-total p66shc (BD Biosciences, Franklin Lakes, NJ, USA), OXPHOS complex subunits proteins anti-NDUFA9, anti-SDHA, anti-UQCRC2, anti-COX4 and anti-ATP5A1(Invitrogen), loading control anti-Tom20 (Cell Signaling, Beverly, MA, USA), anti-β-actin from Sigma-Aldrich (Darmstadt, Germany). Immunoblotting was performed as previously described [6].

2.4. Determination of mitochondrial ROS

Cells were transfected with negative control or IDH2 siRNAs for 48 h and the relative mitochondrial ROS levels in endothelial cells were detected by MitoSOX red (Invitrogen) as previously described [6].

2.5. Determination of mitochondrial membrane potential (MMP)

Changes in MMP following transfection with negative control or IDH2 siRNAs were detected using TMRE dye (Invitrogen, Cat. No. T-669), which is suitable for mitochondrial labeling of live cells. TMRE fluorescence intensity is indicative of the MMP. TMRE (100 nM) was added to each sample and the cells were incubated in complete medium for 15 min at 37 °C. The fluorescence intensity was detected using the Fluoroskan Ascent fluorescence reader at excitation and emission wavelengths of 530 and 590 nm, respectively.

2.6. Measurement of DNA fragmentation

The effect of IDH2 deficiency on the DNA fragmentation was measured using the Cell Death Detection ELISA kit (Roche Applied Science, Indianapolis, IN, USA) according to the manufacturer's instructions.

2.7. Oxygen consumption rate (OCR)

HUVECs were transfected with negative control or IDH2 siRNAs for 48 h, and the OCR was performed using the Seahorse XF-24 analyzer (Seahorse Bioscience, Santa Clara, CA, USA) as described previously [11].

2.8. Quantitative polymerase-chain reaction (qPCR)

Total RNA from HUVECs or lung endothelial cells were isolated using a protocol described previously [15]. Primers were as follows: IDH2, forward, 5′GAAGGTGTGCGTGGAGAC-3′, and reverse, 5′-CCGTG GTGTTCAG GAAGT-3’; ICAM-1, forward, 5′-AGAGGTTGAACCCCACAG TC-3′, and reverse, 5′-TCTGGC TTCGTCA GAATCAC-3’; p66shc, forward, 5′-TCCGGAATGAG TCTCTGTCA-3′, and reverse, 5′-GAAGGAGCACAGGGTAGTGG-3’; TNF-α, forward, 5′-CCCA GGGACCTCTCTCTAATCA-3′, and reverse, 5′-AGCTGCCCCTCAGCTTGAG-3’; and IL-1β, forward, 5′-CCCAGGGACCTCTC TCTA ATCA-3′, and reverse, 5′-AGCTGCCCCTCAGCTTGA G-3’. 18S ribosomal RNA and GAPDH were used as controls.

2.9. Mitochondrial fragmentation

Following transfection with IDH2 siRNA for 48 h, cells were stained with 10^{-7} M MitoTracker Red FM (Cell Signaling, MA, USA) for 30 min to visualize mitochondria. Stained cells were washed three times with Hank's balanced salt solution (HBSS). Images were obtained using a confocal microscope.

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