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Idh2 deficiency accelerates renal dysfunction in aged mice

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ABSTRACT

The free radical or oxidative stress theory of aging postulates that senescence is due to an accumulation of cellular oxidative damage, caused largely by reactive oxygen species (ROS) that are produced as by-products of normal metabolic processes in mitochondria. The oxidative stress may arise as a result of either increased ROS production or decreased ability to detoxify ROS. The availability of the mitochondrial NADPH pool is critical for the maintenance of the mitochondrial antioxidant system. The major enzyme responsible for generating mitochondrial NADPH is mitochondrial $NADP^+$-dependent isocitrate dehydrogenase (IDH2). Depletion of IDH2 in mice (*idh2*$^{-/-}$) shortens life span and accelerates the degeneration of multiple age-sensitive traits, such as hair grayness, skin pathology, and eye pathology. Among the various internal organs tested in this study, IDH2 depletion-induced acceleration of senescence was uniquely observed in the kidney. Renal function and structure were greatly deteriorated in 24-month-old *idh2*$^{-/-}$ mice compared with wild-type. In addition, disruption of redox status, which promotes oxidative damage and apoptosis, was more pronounced in *idh2*$^{-/-}$ mice. These data support a significant role for increased oxidative stress as a result of compromised mitochondrial antioxidant defenses in modulating life span in mice, and thus support the oxidative stress theory of aging.

1. Introduction

Aging is an inevitable biological process characterized by a general decline in various physiological functions [1]. Although numerous theories have been proposed to explain the progressive and deleterious changes characteristic of aging, the free radical or oxidative stress theory has been among the most widely accepted theories in aging research [2]. The theory proposes that the physiological declines associated with aging are due to an accumulation of oxidative damage to cellular macromolecules over the life span of the organism [3].

Reactive oxygen species (ROS) are ubiquitously generated in living cells, and mitochondria are the major site for the production of ROS within the cell [4]. During normal mitochondrial respiration, electrons escape from the electron transport chain and combine with oxygen to form the superoxide anion radical and other ROS [5]. Under normal physiological conditions, cell viability and function are critically dependent on the continued balance between mitochondrial ROS formation and removal [6]. Oxidative stress, defined as a pathological state characterized by increased ROS production or decreased ability to detoxify ROS, plays a causative role in tissue injury in many disease conditions, and in aging [4].

ROS are eliminated through an effective but complex network of defense mechanisms in mitochondria, and the ultimate antioxidant capacity of mitochondria is determined by the supply of reducing potentials [7]. The availability of the mitochondrial NADPH pool is critical for the maintenance of glutathione (GSH)-dependent mitochondrial antioxidant defense systems [8], as well as the mitochondrial thioredoxin system, which includes thioredoxin 2 and thioredoxin reductase 2 [9]. Thus, mitochondrial NADPH is a fundamentally important molecule in the defense against ROS. We previously proposed that mitochondrial $NADP^+$-dependent isocitrate dehydrogenase (IDH2) is one of the primary NADPH producers in the mitochondria, and thereby plays a critical role in mitochondrial antioxidant defense [10,11].

Since mitochondria are assumed to play a major role in the formation of superoxide radicals and are suggested to contribute to aging, and IDH2 is indispensable for the maintenance of the

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mitochondrial redox balance, we explored in this study the possibility that the depletion of IDH2 activity regulates senescence by altering intracellular levels of ROS. Here, we report that a defect in *idh2* gene expression in mice triggers oxidative stress and accelerates the degeneration of multiple age-sensitive traits.

2. Materials and methods

2.1. Animals

Experiments were performed using 24-month-old male C57BL/6 mice with two genotypes: wild-type (WT) $idh2^{+/+}$ mice and knockout $idh2^{-/-}$ mice, generated by breeding and identified by PCR genotyping, as previously described [12]. The mice were housed in microisolator rodent cages at 22 °C with a 12 h light/dark cycle and allowed free access to water and standard mouse chow. All surgeries were performed under anesthesia induced by a mixture of zoletil and xylazine in PBS, and all efforts were made to minimize suffering. All procedures were conducted according to animal experimental procedures approved by the Animal Care and Use Committee of the Kyungpook National University.

2.2. Analysis of mouse plasma

We obtained serum samples from 24-month-old male mice under retro-orbital vein plexus blood collection for measurement of creatinine and blood urea nitrogen (BUN). Plasma creatinine and BUN concentrations were measured with a VITROS 250 Chemistry Analyzer (Johnson & Johnson, Rochester, NY, USA) according to the manufacturer's protocol.

2.3. Renal histology analysis

For histological analysis, kidney samples were fixed in PLP solution (4% paraformaldehyde, 75 mM L-lysine, 10 mM sodium periodate). PLP-fixed kidneys were washed with PBS three times for 10 min each time, embedded in paraffin, and cut into 4 μm thick sections using a microtome (RM2165, Leica, Bensheim, Germany). Sections were mounted on Fisherbrand Superfrost Plus microscope slides (Thermo Fisher Scientific Inc. MA, USA) and stained with periodic acid-Schiff (PAS; Sigma, St. Louis, MO, USA) following the standard protocol. To stain, slides were deparaffinized with xylene and then rehydrated with 100%, 95%, and 80% ethanol and deionized water. The slides were immersed in periodic acid solution for 5 min at room temperature and washed in several changes of distilled water. Next, the slides were immersed in Schiff's reagent for 15 min at room temperature and washed in tap water for 5 min. The slides were then counterstained with hematoxylin solution, Gill No. 3 (Sigma, St. Louis, MO, USA). Next, the slides were dehydrated and mounted with mounting media (Vector Laboratories, Burlingame, CA, USA). To evaluate collagen deposition, 4 μm sections were deparaffinized and rehydrated with xylene and 100%, 95%, and 80% ethanol and deionized water. The sections were stained with picro-sirius red (PSR) (Sigma, St. Louis, MO, USA) for 1 h at room temperature and washed in three changes of acidified water. The slides were then dehydrated and mounted using mounting media. Micrographic images were acquired using a Nikon Microphot-Fx (Nikon Inc. Melville, NY, USA). Regions of collagen deposition in PSR-stained kidney sections were measured using the image analysis program i-solution (Image & Microscope Technology Inc. Daejeon, Korea). For hematoxylin and eosin (H&E) staining, 4-μm sections were stained with H&E according to the standard protocol. Slides containing lung sections were stained sequentially with hematoxylin Gill No. 3, bluing solution, and eosin Y by gently shaking at room temperature.

2.4. Immunohistochemistry

Immunohistochemical staining for MCP-1 was performed as follows: the paraffin sections of renal tissues were dewaxed, washed in PBS, drained, and incubated overnight at 4 °C, with anti-MCP-1 as the primary antibody. After washing, the sections were incubated for 40 min at room temperature with Alexa Fluor 488-conjugated secondary antibody (Invitrogen, Carlsbad, CA, USA). Images for immunofluorescence staining were taken using an Axiovert 40 CFL microscope (Carl Zeiss Ag, Oberkochen, Germany).

2.5. Immunoblot analysis

Total protein extracts were separated on 10–15% SDS-polyacrylamide gels and transferred to nitrocellulose membranes. The membranes were incubated with specific primary antibodies overnight at 4 °C, and immunoreactive antigen was detected using horseradish peroxidase-labeled secondary antibodies and an enhanced chemiluminescence detection kit (GE Healthcare, Buckinghamshire, UK).

2.6. Statistical analysis

All statistical analysis was performed using Student's *t*-test for comparison of two groups. P values < 0.05 were considered as statistically significant.

3. Results and discussion

Depletion of IDH2 in mice was found to shorten life span (Fig. 1A) and accelerate the degeneration of multiple age-sensitive traits, such as hair grayness, skin pathology, and eye pathology (Fig. 1B). Young $idh2^{-/-}$ mice were indistinguishable from WT littermates, but long-term follow-up revealed a striking premature aging phenotype beginning at approximately 6 months of age. Previously, we demonstrated that 10-month-old $idh2^{-/-}$ mice exhibited a reduction in body weight compared with WT mice and that this difference resulted from lowered adipocyte hypertrophy [12]. Consistent with this result, 24-month-old $idh2^{-/-}$ mice exhibited lower body weight compared with WT mice, despite there being no significant difference in body weight between the two genotypes from birth to 6 months (Fig. 1C). Among the various internal organs of 24-month-old mice tested, IDH2 depletion-induced acceleration of senescence was uniquely observed in kidney. The kidney damage presented as hydronephrosis, congestion and dilation of tubules, and loss of brush border and nuclei in tubular cells [13]. Macroscopic evaluation revealed significant hydronephrosis in the kidney and an increase in absolute kidney weight compared to WT mice in 24-month-old $idh2^{-/-}$ mice (Fig. 1D).

We first examined how *idh2* deficiency affects kidney function under normal conditions of senescence. The levels of serum creatinine (Cr), which is used to measure the glomerular filtration rate (GFR), and BUN are the most commonly used markers of renal function [14]. Serum levels of Cr and BUN were greater and creatinine clearance was lower in $idh2^{-/-}$ mice than in $idh2^{+/+}$ mice at 24 months of age (Fig. 2A and Fig. 2B), indicating that *idh2* deficiency was associated with the renal dysfunction. Histological examination showed that kidneys in both WT and $idh2^{-/-}$ mice at 8 weeks of age were normal (Fig. 2C). However, consistent with the results for functional damage, senescence resulted in more severe damage to kidneys of $idh2^{-/-}$ mice than WT mice; kidneys of 24-month-old $idh2^{-/-}$ presented with congestion and dilation of tubules, loss of brush border and nuclei in tubular cells, and accumulation of interstitial cells (Fig. 2C-E). Monocyte chemoattractant protein-1

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