Redox Biology 19 (2018) 339–353

Contents lists available at ScienceDirect

Redox Biology

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

Sirtuin 3-dependent mitochondrial redox homeostasis protects against AGEs-induced intervertebral disc degeneration

Yu Song[a,1], Shuai Li[a,1], Wen Geng[b,1], Rongjin Luo[a], Wei Liu[c], Ji Tu[a], Kun Wang[a], Liang Kang[a], Huipeng Yin[a], Xinghuo Wu[a], Yong Gao[a], Yukun Zhang[a], Cao Yang[a,*]

[a] Department of Orthopaedics, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430022, China
[b] Department of Ophthalmology, Shengjing Hospital, China Medical University, Shenyang 110004, China
[c] Department of Orthopaedics, First Hospital of Wuhan, Wuhan 430022, China

ARTICLE INFO

Keywords:
Mitochondrial redox homeostasis
Intervertebral disc degeneration
Sirtuin 3
Advanced glycation end products

ABSTRACT

Intervertebral disc (IVD) degeneration contributes largely to pathoanatomical and degenerative changes of spinal structure that increase the risk of low back pain. Apoptosis in nucleus pulposus (NP) can aggravate IVD degeneration, and increasing studies have shown that interventions targeting NP cell apoptosis can ameliorate IVD degeneration, exhibiting their potential for use as therapeutic strategies. Recent data have shown that advanced glycation end products (AGEs) accumulate in NP tissues in parallel with the progression of IVD degeneration and form a microenvironment of oxidative stress. This study examined whether AGEs accumulation aggravates NP cell apoptosis and IVD degeneration, and explored the mechanisms underlying these effects. We observed that the viability and proliferation of human NP cells were significantly suppressed by AGEs treatment, mainly due to apoptosis. Furthermore, activation of the mitochondrial apoptosis pathway was detected after AGEs treatment. In addition, the molecular data showed that AGEs could significantly aggravate the generation of mitochondrial reactive oxygen species and prolonged activation of the mitochondrial permeability transition pore, as well as the increased level of Bax protein and decreased level of Bcl-2 protein in mitochondria. These effects could be reduced by antioxidant (2-(2,2,6,6-Tetramethylpiperidin-1-oxyl-4-ylamino)-2-oxoethyl) triphenylphosphonium chloride (MitoTEMPO) and Visomitin (SKQ1). Importantly, we identified that impairment of Sirtuin3 (SIRT3) function and the mitochondrial antioxidant network were vital mechanisms in AGEs-induced oxidative stress and secondary human NP cell apoptosis. Finally, based on findings that nicotinamide mononucleotide (NMN) could restore SIRT3 function and rescue human NP cell apoptosis through adenosine monophosphate-activated protein kinase and peroxisome proliferator-activated receptor-γ coactivator 1α (AMPK-PGC-1α) pathway in vitro, we confirmed its protective effect on AGEs-induced IVD degeneration in vivo. In conclusion, our data demonstrate that SIRT3 protects against AGEs-induced human NP cell apoptosis and IVD degeneration. Targeting SIRT3 to improve mitochondrial redox homeostasis may represent a potential therapeutic strategy for attenuating AGEs-associated IVD degeneration.

1. Introduction

Intervertebral disc (IVD) degeneration and secondary pathological changes in the spinal structure contribute largely to low back pain, which ranks first among the five leading causes of years lived with disability [1]. As the principal intervertebral junction, the

Abbreviations: IVD, intervertebral disc; NP, nucleus pulposus; SIRTs, Sirtuins; AGEs, advanced glycation end products; NMN, nicotinamide mononucleotide; SKQ1, Visomitin; MitoTEMPO, (2-(2,2,6,6-Tetramethylpiperidin-1-oxyl-4-ylamino)-2-oxoethyl) triphenylphosphonium chloride; AMPK, adenosine monophosphate-activated protein kinase; PGC-1α, peroxisome proliferator-activated receptor-γ coactivator 1α; Cyt-c, Cytochrome c; SOD2, superoxide dismutase 2; TRX2, thioredoxin 2; TRXR2, thioredoxin reductase 2; VDAC, voltage-dependent anion channel; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; CCK-8, Cell Counting Kit-8; EdU, 5-Ethynyl-2'-deoxyuridine; TUNEL, TdT-mediated dUTP nick end labeling; MMP, mitochondrial membrane potential; mPTP, mitochondrial permeability transition pore; ROS, reactive oxygen species

* Correspondence to: Department of Orthopaedics, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, No. 1277 Jiefang Avenue, Wuhan 430022, Hubei, China.
E-mail address: caoyangunion@hust.edu.cn (C. Yang).
[1] These authors contributed equally to the study.

https://doi.org/10.1016/j.redox.2018.09.006
Received 26 April 2018; Received in revised form 3 September 2018; Accepted 5 September 2018
Available online 06 September 2018

fibrocartilaginous property of IVD allows it to buffer the different patterns of mechanical stress, in which the corresponding redistribution of gelatinous NP tissue plays an important role [2]. The resident NP cells are the main executors that control the extracellular matrix metabolism in NP tissue and the collagen II and proteoglycan they produce are the main molecules that maintain the gelatinous property of NP tissue [3,4]. The increased NP cell death partially due to apoptosis may contribute to the metabolic disorders of extracellular matrix, which was seen along with IVD degeneration [4,5]. Furthermore, increasing studies indicated that the interventions targeting NP cell apoptosis could alleviate the metabolic disorders and IVD degeneration [6,7]. Therefore, more investigation on NP cell apoptosis and the target interventions may not only increase the pathogenetic knowledge of IVD degeneration, but also provide potential therapeutic strategy.

Recently, increasing evidence has verified the presence of oxidative stress and increased concentrations of oxidation products in aged and degenerated IVDs [8–10]. Additionally, oxidative stress and subsequent mitochondrial dysfunction participate in the intrinsic pathway of cellular apoptosis, and their roles have been confirmed in NP cell death and IVD degeneration induced by various risk factors [11–13]. Although various interventions targeting oxidative stress and mitochondrial dysfunction have shown beneficial effects on NP cell apoptosis and IVD degeneration [14,15], the detailed mechanisms underlying these disorders are not clear.

Sirtuins (SIRTs) comprise a class of NAD^+-dependent deacetylases with seven members, which share the same conserved NAD^+-binding site and a Sir2 catalytic core domain, and regulate a wide variety of biological functions [16]. Among them, the SIRT3, SIRT4, and SIRT5 members are mainly localized in the mitochondria, with SIRT3 being the best characterized and possessing robust deacetylase activity that is closely related to maintaining mitochondrial redox homeostasis and functional integrity [17]. Impaired SIRT3 function has been found in various diseases associated with oxidative stress and mitochondrial dysfunction, including cardiac hypertrophy, acute kidney injury, and osteoarthritis [18–20]. In addition, nicotinamide mononucleotide (NMN), as a key NAD^+ precursor in mammals, could normalize the NAD^+/NADH ratio and its administration have shown protective effect on oxidative stress-related diseases through manipulating the activity of SIRT members [21–23]. Previously, the studies by our group and others have demonstrated that the accumulation of advanced glycation end products (AGEs) in NP tissues could induce an oxidative microenvironment and mitochondrial dysfunction, which were closely related to IVD degeneration [24–26]. However, the role of SIRT3 in this process and the related mechanism in regulating the survival and proliferation of NP cells have not been examined.

In this study, we reported that in vitro AGEs treatment suppressed the survival and proliferation of human NP cells through induction of oxidative stress and mitochondrial dysfunction, in which the impairment of SIRT3 function and mitochondrial antioxidant network play an important role. The suppression of AMPK/PCG-1α induced by AGEs was involved in this process. Furthermore, NMN supplementation exhibited a protective effect on AGEs-induced NP cell apoptosis and IVD degeneration, partly through the restoration of SIRT3 function and mitochondrial redox homeostasis. Our discoveries provide novel insights into the mechanism of oxidative stress and apoptosis in NP cells, with therapeutic implications for treating IVD degeneration.

2. Materials and methods

2.1. Study design and Ethics statement

Human NP tissues were obtained from patients who underwent intervertebral fusion surgery due to lumbar spinal stenosis, lumbar disc herniation or idiopathic scoliosis. Correspondingly, the patients' medical records were also collected and magnetic resonance images were used to evaluate the IVD degenerative level according to Pfirrmann MRI-grade system [27]. Once isolated, the NP tissue samples were handled according to their intended use. Generally, 14 human NP tissues collected from 6 males and 8 females, aged 16–64 years (mean: 48.6 years) were preserved for immunohistochemical and western blotting analysis. Among them, five tissues evaluated as Grade II were from patients with idiopathic scoliosis; three evaluated as Grade III from patients with lumbar disc herniation; three evaluated as Grade IV and three evaluated as Grade V from patients with lumbar spinal stenosis and lumbar disc herniation. All of them were separated into two parts, immersed in RNAlater Stabilization Solution and frozen in liquid nitrogen for protein and RNA analysis, fixed using buffered formaldehyde fixation (4%, pH 7.4) and embedded in paraffin for histological analysis. Specially, the NP tissues from idiopathic scoliosis patients were generally evaluated as Grade II and also regarded as healthy, another three of which were preserved to isolate NP cells for the further in vitro experiments. Further, we assessed the IVD degenerative events in vivo using rat tail disc degeneration model.

All experimental protocols involving human IVD, including medical records, NP tissue collection and analysis, NP cell isolation and interventions, were approved by the Ethics Committee of Tongji Medical College, Huazhong University of Science and Technology (NO. S214). The animal experiments were performed following a protocol approved by the Animal Experimentation Committee of Huazhong University of Science and Technology.

2.2. Isolation and culture of human NP cells

Three human NP tissues used for NP cell isolation were described as above. Hank's balanced salt solution was used for NP tissue transportation, and subsequent cell isolation was performed as described previously [28], by plating and expanding the cells at 37 °C and 5% CO_2 in Dulbecco's modified Eagle's medium with the F12 nutrient mixture containing 15% fetal bovine serum (Gibco, Waltham, MA, USA) and 1% penicillin/streptomycin (Invitrogen). Cells from the second passage were identified using fluorescently labeled antibody for NP cell markers [29] (CD24, ab31622; KRT18, ab215839; Abcam, Cambridge, UK) and used in further experiments. In in vitro experiments, NP cells were exposed to an equal volume of phosphate-buffered saline (PBS) or AGEs (100 μg/ml or 200 μg/ml buffered using PBS; Merck Millipore, Darmstadt, Germany) for 0, 12, 24, or 36 h. Cells were either pre-treated with MitoTEMPO (5 μM; Sigma, Shanghai, China) or SKQ1 (20 nM; MedChemExpress, New Jersey, USA) for 2 h and then treated with PBS or AGEs, or directly co-cultured with PBS or AGEs in combination with NMN (100 μM), A-769662 (50 μM), Compound C (50 uM; MedChemExpress) or RAGE antibody (10 μg/ml; R&D Systems, Minneapolis, USA). To knock down SIRT3 expression, cells were transfected for 48 h with 100 nM SIRT3 small-interfering RNA (siRNA) or scrambled siRNA (GenePharma, Shanghai, China) using Lipofectamine 2000 (Invitrogen) and immediately stimulated with PBS or AGEs. In addition, lentiviral plasmid containing the SIRT3 expression (Lenti-SIRT3) vector and flanking sequence control (Lenti-vector) were purchased from GeneChem (Shanghai, China) and transduced into human NP cells following the manufacturer's instructions. Transfection efficacies were detected by western blotting and the cells were further cultured for 3 days, and passaged for subsequent experiments.

2.3. Assessment of human NP cell viability and proliferation

Cell viability assessment was performed as described previously [30], using a Cell Counting Kit-8 (CCK-8; CK04, Dojindo, Japan). After treatment with PBS or AGEs, CCK-8 solution (10 μl) was added to each well, and the cells were further cultured for 4 h at 37 °C. Absorbance in the wells was measured at 450 nm using a spectrophotometer (BioTek, Winooski, VT, USA).

NP cell proliferation was also detected by 5-ethynyl-2′-deoxyuridine (EdU) incorporation (C10310-3; Ribobio, Guangzhou, China). The