Redox Biology 14 (2018) 361–370

Contents lists available at ScienceDirect

Redox Biology

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

Research Paper

The dual role of poly(ADP-ribose) polymerase-1 in modulating parthanatos and autophagy under oxidative stress in rat cochlear marginal cells of the stria vascularis

Hong-Yan Jiang[1], Yang Yang[1], Yuan-Yuan Zhang, Zhen Xie, Xue-Yan Zhao, Yu Sun, Wei-Jia Kong*

Department of Otorhinolaryngology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430022, China

ARTICLE INFO

Keywords:
Oxidative stress
PARP-1
Parthanatos
Autophagy
Marginal cells
Glucose oxidase

ABSTRACT

Oxidative stress is reported to regulate several apoptotic and necrotic cell death pathways in auditory tissues. Poly(ADP-ribose) polymerase-1 (PARP-1) can be activated under oxidative stress, which is the hallmark of parthanatos. Autophagy, which serves either a pro-survival or pro-death function, can also be stimulated by oxidative stress, but the role of autophagy and its relationship with parthanatos underlying this activation in the inner ear remains unknown. In this study, we established an oxidative stress model in vitro by glucose oxidase/glucose (GO/G), which could continuously generate low concentrations of H_2O_2 to mimic continuous exposure to H_2O_2 in physiological conditions, for investigation of oxidative stress-induced cell death mechanisms and the regulatory role of PARP-1 in this process. We observed that GO/G induced stria marginal cells (MCs) death via upregulation of PARP-1 expression, accumulation of polyADP-ribose (PAR) polymers, decline of mitochondrial membrane potential (MMP) and nuclear translocation of apoptosis-inducing factor (AIF), which all are biochemical features of parthanatos. PARP-1 knockdown rescued GO/G-induced MCs death, as well as abrogated downstream molecular events of PARP-1 activation. In addition, we demonstrated that GO/G stimulated autophagy and PARP-1 knockdown suppressed GO/G-induced autophagy in MCs. Interestingly, autophagy suppression by 3-Methyladenine (3-MA) accelerated GO/G-induced parthanatos, indicating a pro-survival function of autophagy in GO/G-induced MCs death. Taken together, these data suggested that PARP-1 played dual roles by modulating parthanatos and autophagy in oxidative stress-induced MCs death, which may be considered as a promising therapeutic target for ameliorating oxidative stress-related hearing disorders.

1. Introduction

Age-related hearing loss (ARHL) or presbycusis is the most prevalent sensory disorder among elderly individuals. ARHL is characterized by a progressive, bilateral, inevitable decline of hearing sensitivity with age and impaired ability of speech discrimination, detection and localization of sound [1]. Three classic types of presbycusis have been defined according to the pathological syndrome: sensory, neural and metabolic/strial [2]. However, the mechanism involved in presbycusis remains ambiguous. A prevalent proposed mechanism of presbycus is oxidative stress [3]. In this theory, an imbalance between reactive oxygen species (ROS) production and antioxidant ability, plays fundamental roles in aging and age-related diseases, including presbycusis [4]. ROS accumulation can oxidative major macromolecules such as lipids, proteins, and DNA leading to cellular damage [5].

The degenerative changes of stria vascularis and consequent reduction of endocochlear potential (EP) are regarded as the hallmarks of strial presbycusis [2,6]. The stria vascularis, located at the lateral wall of the cochlea, which is critical for the maintenance of the EP at steady state, is consisted of several subtype of cells, including the stria marginal cell (MC), the intermediate cell and the basal cell [7]. The MC is identified as a primary cell type for exploring the etiology of strial presbycusis based on its unique characters. On one hand, MCs express an array of ion channels, pumps and Na, K-ATPase, which correlates with overall strial function and normal EP [8]. On the other hand, due to its location and plentiful mitochondria, stria MC is particularly vulnerable to oxidative attacks, which will lead to the decline of EP, disturbance of the cochlear function and subsequently elevation of hearing thresholds [8].

Poly(ADP-ribose) polymerase-1 (PARP-1) is an abundant nuclear

* Corresponding author.
E-mail address: entwjkong@hust.edu.cn (W.-J. Kong).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.redox.2017.10.002
Received 1 August 2017; Received in revised form 1 October 2017; Accepted 3 October 2017
Available online 07 October 2017

enzyme playing a prominent role in DNA damage repair, genomic integrity maintenance and execution of cell differentiation and death [9]. Various agents including ROS, ultraviolet (UV) light, alkylating agents inflicted DNA damage can activate PARP-1 [10,11]. Once PARP-1 is overactivated, it will results in rapid depletion of NAD + and ATP accompanied with excessive polyADP-ribose (PAR) polymer synthesis, ultimately leading to irreversible energy failure and detrimental effects, which have been implicated in different tissue types such as brain, liver, heart, lung, retina, kidney and skeletal muscle [12,13]. It has been well demonstrated that inhibiting PARP-1 overactivation improves the therapeutic efficacy in multiple disease models characterized by DNA damage, including ischemia, diabetes, shock, inflammation and cancer [14]. Besides, accumulated PAR polymers via PARP-1 activation can induce cell death through a mechanism defined as PARP-1-dependent cell death (parthanatos) by translocating from nucleus to mitochondria, where it binds to apoptosis-inducing factor (AIF) and triggers AIF translocation to the nucleus [15,16]. Parthanatos, a specific modality of cell death distinct from other types of cell death such as caspase-dependent apopotosis and necrosis is characterized by biochemical features including hyper-activation of PARP-1, excessive PAR polymer synthesis, mitochondrial depolarization and nuclear translocation of AIF [17]. Nuclear condensation and cellular propidium iodide (PI) positive staining after the initiation of parthanatos are its morphological features [18]. Pharmacological inhibition or genetic deletion of PARP-1 and AIF have been shown to abrogate parthanatos and ameliorate cell injury induced by oxidative stress in a variety of cell types like fibroblasts, neurons, and HeLa cells [18,19]. It has been reported that oxidative stress is able to induce autophagy, and autophagy contributes to sequestering oxidative biomolecules and organelles in turn [20]. Autophagy is a conserved self-degradation process in which aged, dysfunctional and/or damaged organelles and material are delivered to lysosomes for breakdown and then recycled by the cells [21]. It plays a crucial role for maintaining cellular homeostasis and health via removal of disposable or potentially harmful constituents, and the beneficial functions of autophagy have been observed in numerous cell types such as neurons, hepatocytes, T lymphocyte [22]. Although autophagy is generally considered as a cytoprotective mechanism against various stress stimuli, autophagy can mediate autophagic cell death in some rare cases [23]. Autophagy dysfunction is associated with several diseases such as neurodegeneration, infection, cancer, and heart diseases [24]. PARP-1 activation induced by DNA damage has been suggested to be involved in amplifying cytoprotective autophagy [25], and inhibition of PARP-1 causes the delay or suppression of pro-survival autophagy [26–28].

During normal aerobic metabolism, low concentrations of ROS are continuously generated in living organisms [29]. Among the different species of ROS, hydrogen peroxide (H_2O_2) is generated by nearly all sources of oxidative stress, and can cross cell membranes freely [30,31]. Though the rate of H_2O_2 generation varies for different types of cell, the production in vivo is continuous, with a steady-state level fluctuating within the range of 10^{-8} to 10^{-7} M [32]. In the majority of previous studies, H_2O_2 was added directly to the cells as a bolus, so cells were initially exposed to relatively high concentrations of H_2O_2 followed by a rapid reduction, because of the constant H_2O_2 consumption [33]. In contrast, glucose oxidase (GO), which could continuously generate low concentrations of H_2O_2 by catalyzing its substrate of glucose (G), was used to mimic continuous exposure to H_2O_2 in physiological conditions, and represented a superior method of H_2O_2 delivery.

Considering the association of PARP-1 with both autophagy and parthanatos, it is justified to speculate that they are interconnected via PARP-1 or downstream molecules of PARP-1 activation. Since the relationship between parthanatos and autophagy, and the functional role of autophagy in MCs remain unreported, in this study, we used GO/G to mimic the states of oxidative stress in vivo. We found that GO/G stimulated parthanatos and autophagy in MCs via PARP-1 activation. We also investigated the correlation between GO/G-induced parthanatos and autophagy, and found that autophagy inhibition by 3-Methyladenine (3-MA) exacerbated parthanatos, demonstrating a pro-survival role for autophagy in response to GO/G in MCs.

2. Materials and methods

2.1. Isolation, culture and identification of primary MCs

All experimental procedures in this study were in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and approved by Ethics Committee of Huazhong University of Science and Technology. Isolation and culture of primary MCs have been described in our previous report [34]. Briefly, stria vascularis tissue, which was isolated from neonatal (3 days old) Sprague Dawley (SD) rats, was dissected, minced and then followed by digestion with 0.1% collagenase II (Sigma-Aldrich, St. Louis, MO, USA) for 30 min at 37 °C. Then the samples were centrifuged for 5 min at 800 g and plated in Epithelial Cell Medium-animal (EpiCM-animal, ScienCell, USA) at 37 °C in a humidified incubator with 5% CO2. The identification of primary MCs by cytokeratin-18, a characteristic molecule of MCs [35,36], was performed as described previously [37].

2.2. Cell viability assay

After the MCs fused into a monolayer, cells were resuspended and plated in 96-well culture plates at 1×10^4 cells per well in quintuple for the CCK-8 colorimetric assay (Dojindo, Tokyo, Japan). In brief, after incubating for 48 h, the MCs were treated for 4 h with different concentrations of GO (0 U/L, 10 U/L, 20 U/L, 30 U/L, 40 U/L, 50 U/L, 60 U/L, 70 U/L, 80 U/L, 100 U/L) (Sigma-Aldrich) in the fresh EpiCM-animal medium supplemented with 5 mM G (Sigma-Aldrich). Then the medium was replaced with 100 μl fresh complete growth medium and 10 μl of CCK-8 solution. After incubation for 2 h at 37 °C in the dark, the absorbance of per well was recorded at 450 nm using a microplate reader (Bio-Tek, Colmar, France).

2.3. Measurement of ROS production

2′,7′-dichlorofluorescin diacetate (DCFH-DA, Sigma-Aldrich) which can be oxidized into highly fluorescent dichlorofluorescein (DCF) in the presence of ROS, was used to determine the intracellular ROS level. Briefly, after exposed to GO/G for indicated incubation time, the treated cells were harvested and incubated with DCFH-DA (20 μM) for 20 min in the dark at 37 °C. Then, the MCs were washed three times with DMEM/F12 (Hyclone, Logan, UT, USA). The fluorescence signals were recorded using flow cytometry (Olympus, Tokyo, Japan), and mean fluorescence intensity was calculated with GraphPad Prism 5.0 software.

2.4. Cell treatment and transfection

The MCs were incubated with 60 U/L GO for 4 h in the fresh EpiCM-animal medium supplemented with 5 mM G to induce oxidative stress. The GO/G (0 U/L GO/5 mM G) group was considered as a control. For achieving autophagy inhibition, 3-MA (10 mM, 1 h) (Sigma-Aldrich) was applied to MCs prior to GO/G treatment.

The recombinant adenoviruses for interference vector of PARP-1 (Ad-PARP-1-RNAi) and control vector (Ad-Con) were purchased from GeneChem (Shanghai, China). MCs transfection was performed as described previously [37]. A multiplicity of infection (MOI; 30) for Ad-Con and Ad-PARP-1-RNAi was used. Briefly, MCs were cultured in EpiCM-animal medium containing Ad-PARP-1-RNAi or Ad-Con for 4 h. Then the medium was replaced with fresh EpiCM-animal medium and transfected MCs were incubated for 48 h before subsequent experiments.