Hearing Research 296 (2013) 13–24

Contents lists available at SciVerse ScienceDirect

Hearing Research

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

Research paper

The effect of overexpression of PGC-1α on the mtDNA4834 common deletion in a rat cochlear marginal cell senescence model

Xue-Yan Zhao [a,1], Jin-Li Sun [a,1], Yu-Juan Hu [a,1], Yang Yang [a], Wen-Juan Zhang [a], Yuan Hu [a], Jun Li [a], Yu Sun [a], Yi Zhong [a], Wei Peng [a], Hong-Lian Zhang [b,**], Wei-Jia Kong [a,c,d,*]

[a] Department of Otolaryngology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430022, PR China
[b] Department of Preventive Medicine and Public Health, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430022, PR China
[c] Institute of Otorhinolaryngology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430022, PR China
[d] Key Laboratory of Neurological Disease, Ministry of Education, PR China

ARTICLE INFO

Article history:
Received 2 May 2012
Received in revised form
17 October 2012
Accepted 7 November 2012
Available online 16 November 2012

ABSTRACT

Aging is a natural process usually defined as a progressive loss of function with an accumulation of senescent cells. The clinical manifestations of this process include age-related hearing loss (AHL)/presbycusis. Several investigations indicated the association between a mitochondrial common deletion (CD) (mtDNA 4977-bp deletion in humans, corresponding to 4834-bp deletion in rats) and presbycusis. Previous researches have shown that peroxisome proliferator-activated receptor-gamma coactivator-1α (PGC-1α) is a key regulator of mitochondrial biogenesis and energy metabolism. However, the expression of PGC-1α in the inner ear and the possible effect of PGC-1α on presbycusis are not clear. Our data demonstrated the distribution of PGC-1α and its downstream transcription factors nuclear respiratory factor-1 (NRF-1), mitochondrial transcription factor A (Tfam) and nuclear factor κB (NF-κB) in marginal cells (MCs) for the first time. To explore the role of PGC-1α in cellular senescence, we established a model of marginal cell senescence harboring the mtDNA4834 common deletion induced by D-galactose. We also found that PGC-1α and its downstream transcription factors compensatorily increased in our cell senescence model. Furthermore, the overexpression of PGC-1α induced by transfection largely increased the expression levels of NRF-1 and TFAM and significantly decreased the expression level of NF-κB in the cell senescence model. And the levels of CD, senescent cells and apoptotic cells in the cell model decreased after PGC-1α overexpression. These results suggested that PGC-1α might protect MCs in this cell model from senescence through a nuclear-mitochondrial interaction and against apoptosis. Our study may shed light on the pathogenesis of presbycusis and provide a new therapeutic target for presbycusis.

1. Introduction

Cell senescence is broadly defined as the physiological program of terminal growth arrest, which can be triggered by alterations of telomeres or by different forms of stress (Hayflick and Moorhead, 1961). Aging is a natural process usually defined as a progressive loss of function with the accumulation of senescent cells (Jeyapalan and Sedivy, 2008; Blagosklonny et al., 2010). It is accompanied by disturbances of organelle structures and functions, which are characterized by functionally impaired mitochondria (Ermini, 1976; Terman and Brunk, 2005). Age-related hearing loss (AHL), also known as presbycusis, is a diverse phenomenon, a key characteristic of mammalian aging and the most common auditory disorder in elderly people (Bielefeld et al., 2010). Because AHL is a multifactorial process involving genetic and environmental factors, the

Abbreviations: AHL, age-related hearing loss; PGC-1α, peroxisome proliferator-activated receptor-gamma coactivator 1α; MCs, marginal cells; EP, endocochlear potential; CD, common deletion; NRF-1, nuclear respiratory factor-1; Tfam, mitochondrial transcription factor A; NF-κB, nuclear factor κB; LSCM, laser-scanning confocal microscope; SA-β-gal, senescence associated β-galactosidase; TUNEL, terminal deoxynucleotidyl transferase (TdT)-mediated deoxyuridine triphosphate (dUTP) nick-end-labeling.

* Corresponding author. Department of Otolaryngology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430022, PR China. Tel.: +86 27 85726900; fax: +86 27 85776343.
** Corresponding author. Tel.: +86 27 85726087; fax: +86 27 85776343.
E-mail addresses: zhl_bjk@whuh.com (H.-L. Zhang), entwjkong@yahoo.com.cn (W.-J. Kong).
[1] These authors contributed equally to this work.

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http://dx.doi.org/10.1016/j.heares.2012.11.007

etiology of this disease remains incompletely clarified. Presbycusis has been classified into several types according to the pathological findings (Schuknecht, 1964; Schuknecht et al., 1974), such as variable amounts of degeneration of the auditory receptors, neurons and the stria vascularis (Schuknecht and Gacek, 1993). Among all types of presbycusis, the strial presbycusis has attracted much attention because of its characteristic principles including age-related endocochlear potential (EP) declining, non-universality of EP declining, clear genetic basis, multiple causes, and the independent strial, organ of Corti, and neural pathology (Ohlemiller et al., 2006, 2010; Ohlemiller, 2009). One of the critical cells in generating EP is marginal cell (MC), which functions in taking up potassium via the Na+/K+ ATPase in an energy-intensive manner. Meanwhile, the marginal cell dysfunction is favored to be the origin of pathological changes in strial presbycusis (Schuknecht et al., 1974). The damage to stria vascularis is associated with a possible reduction in mitochondrial adenosine triphosphate (ATP) supply in the strial marginal cells (MCs) due to loss of the marginal cell processes (Spicer and Schulte, 2005). Considering the important role of MC in EP generation and its high demands of energy in metabolism, it might be an appropriate candidate for studying the etiology of metabolic presbycusis.

Mitochondria are semi-self-replicating organelles that harbor their own DNA. The nuclear genome encodes more than 95% of all proteins located in the mitochondria, whereas only 13 polypeptides (that are all subunits of the oxidative phosphorylation system, OXPHOS) are encoded by the mitochondrial genome (Cannino et al., 2007; Virbasius and Scarpulla, 1994). A specific kind of large-scale mtDNA deletion (mtDNA 4977-bp deletion in humans, corresponding to 4834-bp deletion in rats) has been shown to accumulate with age in a number of tissues of aged mammals (Fischel-Ghodsian et al., 1997; Ohlemiller, 2009). Because of the high incidence of this deletion, it is called the “common deletion” (CD). Several investigations have indicated that there was an association between presbycusis and acquired mitochondrial DNA (mtDNA) mutations/deletions, especially CD (Bai et al., 1997). However, the effect of the CD on presbycusis remains obscure. D-galactose (D-gal) is widely used to induce aging model of animals (Song et al., 1999; Kong et al., 2006b; Chen et al., 2010). Overdose of D-gal will allow aldose reductase to catalyze the accumulated D-gal into galactitol, which cannot be metabolized but will accumulate in the cell, resulting in osmotic stress and excessive reactive oxygen species (ROS) production (Cuatrecasas and Segal, 1966). More specifically, these ROS damage mtDNA, maybe resulting in the production of specific mtDNA deletions. In the previous study, we established a rat model associated with CD in the inner ear by injecting D-gal (Kong et al., 2006b), but a suitable cell senescence model of the cochlea for studying presbycusis in vitro was lacking until now.

The existence of a nuclear-mitochondrial interaction has been proposed in the process of many diseases, but the exact role of a nuclear-mitochondrial interaction in presbycusis was not well understood. Peroxisome proliferator-activated receptor-gamma coactivator 1α (PGC-1α), the member of peroxisome proliferators-activated receptor-gamma coactivator 1 (PGC-1) family, is an established integrator of nuclear and mitochondrial interactions (Puigserver et al., 1998; Puigserver and Spiegelman, 2003; Puigserver, 2005; Wu et al., 1999). The PGC-1α mRNA is expressed in tissues such as heart, skeletal muscle, brown fat, kidney, liver and brain, which have high energy demands and are rich in mitochondria (Lin et al., 2005). In these tissues, PGC-1α level is increased by signals that relay metabolic needs. For example, PGC-1α is induced in brown adipose tissue and skeletal muscle upon exposure to cold, in heart upon fasting and in skeletal muscle after prolonged physical exercise (Knutti and Kralli, 2001). However, the expression of PGC-1α in the inner ear has not yet been reported. In our previous studies, we established a mimetic aging rat model associated with CD in the central nervous system, inner ear and other peripheral tissues (Kong et al., 2006a, 2006b; Chen et al., 2010). Interestingly, we found that a remarkable increase in mtDNA replication resulting from increased Tfam level was involved in the accumulation of mtDNA deletion mutations in our aging rat model (Zhong et al., 2011). These results suggested that Tfam might play a role in presbycusis associated with CD. Tfam is an important downstream effector of PGC-1α and participates in nuclear-mitochondrial interactions (Bonawitz et al., 2006; Scarpulla, 2008a, 2008b; Mendelev et al., 2011), so we proposed that PGC-1α might play a role in presbycusis. The nuclear respiratory factor-1 (NRF-1) is involved in the transcriptional control of mitochondrial biogenesis through its interaction with PGC-1α (Virbasius et al., 1993) and is a transcriptional activator of the nuclear genes encoding mitochondrial transcription factor Tfam necessary for mtDNA transcription (Virbasius and Scarpulla, 1994; Choi et al., 2002). The nuclear factor κB(NF-κB) activation may act by controlling different set of genes and involving in related processes such as oxidative stress, inflammation, endothelial dysfunction, fibrosis, hypertrophy and apoptosis (Adler et al., 2007; Sen and Smale, 2010). The p65 subunit of NF-κB binds to PGC-1α , linking inflammation and metabolic disturbances in cardiac cells (Alvarez-Guardia et al., 2010). Thus, we studied two pathways (PGC-1α — NRF-1 — Tfam and PGC-1α — NF-κB) to explore the possible roles of PGC-1α in presbycusis.

In this study, we established a cell senescence model harboring CD induced by D-gal by using rat MCs and detected the distributions of PGC-1α and its downstream transcription factors in MCs. To further explore the possible role of PGC-1α in presbycusis with CD, we detected the relevant proteins expression levels, CD level, apoptosis and senescence associated β-galactosidase (SA-β-gal) staining after overexpressing PGC-1α in this MCs senescence model. Our results suggest that PGC-1α and its downstream transcription factors compensatorily increase in our cell senescence model and overexpression of PGC-1α might protect MCs from senescence through a nuclear-mitochondrial interaction and might antagonize MCs apoptosis, which may shed light on the disease etiology and provide a new therapeutic target for presbycusis.

2. Methods

The neonatal (3 days old) Sprague Dawley (SD) rats were obtained from the animal center of Tongji Medical College, Huazhong University of Science and Technology. The animal center of Tongji Medical College has qualification for raising and breeding animals. Animals were fed according to the standard protocols approved by the Laboratory Animal Management Statute of the China. All of the animal experiments followed the instructions of the Laboratory Animal Management Statute of China.

2.1. Primary culture and identification of MCs

Six neonatal SD rats were treated with 75% ethanol after receiving ethyl ether inhaled anesthesia. The bilateral temporal bones were removed and the stria vascularis from the apical turn to basal turn was dissected, chopped into 0.5 mm thick pieces and digested by 0.1% type II collagenase for 30 min at 37 °C. After the samples were centrifuged for 5 min at 800–1000 rpm, the cells were resuspended and planted into cell culture plates with Epithelial Cell Medium-animal (EpiCM-animal) (ScienCell, USA), which were placed in an incubator at 37 °C in 5% CO2 and 95% air. For identification of MCs, the expression of cytokeratin-18 (CK-18), which is the characteristic protein of MCs after purification (Kim