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D-galactose induces premature senescence of lens epithelial cells by disturbing autophagy flux and mitochondrial functions

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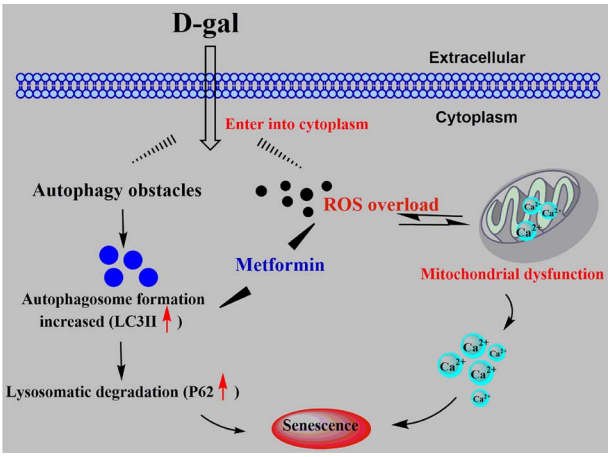
GRAPHICAL ABSTRACT



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ABSTRACT

Cataract is the leading cause of blindness with an estimated 16 million people affected worldwide. D-galactose (D-gal) is a reducing sugar that widely distributed in foodstuffs, and studies show that D-gal could promote cataract formation by damaging nature lens epithelial cells (LECs). However, the underlying mechanism is unclear. In our present study, D-gal resulted in premature senescence of LECs, which was confirmed by determining the β-galactosidase activity, cell proliferative potential and cell cycle distribution, though apoptosis of LECs was not observed. We also verified that D-gal induced the impairment of autophagy flux by measuring the expression of LC3II and P62. Meanwhile, we found that D-gal induced mitochondrial dysfunctions of LECs through increasing reactive oxygen species (ROS), reducing ATP synthesis and mitochondrial potential (MMP), enhancing the concentration of cytoplasm Ca^{2+} and permeability transition pore (mPTP) opening. Metformin, as a potential

Abbreviations: D-gal, D-galactose; LECs, lens epithelial cells; HCQ, hydroxychloroquine; ROS, reactive oxygen species; VADC, voltage dependent anion channel; mPTP, mitochondria permeability transition pore; MMP, mitochondrial membrane potential; NAC, N-acetylcysteine; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; MAPK, mitogen-activated protein kinase; ATP, Adenosine Triphosphate; TXNIP, Thioredoxin-interacting protein; SIRT1, sirtuin 1; DOX, doxorubicin; Met, metformin; Rapa, rapamycin; AGEs, advanced glycation end products

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anti-aging agent, suppressed the senescence of LECs by restoring autophagy flux and mitochondria functions. Nevertheless, the antioxidant N-acetylcysteine (NAC) scavenged ROS significantly but was not efficient in preventing LECs from premature senescence. Our data suggests that restoring autophagy activity and improving mitochondrial functions may be a potential strategy for the prevention of LECs senescence-related cataract.

1. Introduction

Cataract is the leading cause of global blindness owing to the limitations of drug treatment and severities of aging population (Brian and Taylor, 2001; Harding, 2001). Lens epithelial cells are highly specialized epithelial cells located in lens anterior capsule, the injury of which significantly increases the risk of cataract formation (Thylefors et al., 1995; Leske et al., 1991). It is widely believed that oxidative stress is a key mediator contributes to cataractogenesis. Healthy lens maintain the structural proteins of lens in a reduced state by its defense system. However, in the aging lens, protection and repair mechanisms slowly deteriorate or become ineffective to counteract the oxidant (Liu et al., 2013). Considerable evidence has supported that advanced glycation end products (AGEs) formation due to oxidant reactions dramatically accelerated in cataractogenesis (Ahmed, 2004).

D-galactose (D-gal) is a reducing monosaccharide abundant in milk products and other non-dairy foodstuffs such as fruits and vegetables. Unlike other sugars (such as glucose), D-gal has been implicated in various age-related disorders such as Alzheimer's disease, cataracts, aging and chronic inflammation (Gao et al., 2016; Abdul Nasir et al., 2014; Ruan et al., 2014). D-gal is reported to form AGEs through the non-enzymatic reaction of glycation, activating the oxidative stress and protein cross-linking in lens, and subsequently accelerate the progress of cataract (Ullah et al., 2015; Linetsky et al., 2014). At physiological conditions, D-gal is ultimately metabolized to glucose through galactokinase (GK) and galactose-L-phosphate uridylyl, and GK-deficient individuals are susceptible to cataracts. However, high concentration of D-gal is conversed to galactitol through activating aldose reductase (AR) instead. Accumulated galactitol badly affects the physiological environment of lens, leading to cataract (Ai et al., 2000; Wu et al., 2008; Suryanarayana et al., 2003). Besides, D-gal shows higher affinity with AR than glucose, which make D-gal more offensive in producing sugar cataract than glucose (Jyothi et al., 2011; Wu et al., 1993). Nevertheless, the related mechanism of cataract associated with LECs damages due to D-gal exposure remains ambiguous.

Mitochondria are the major source of intracellular ROS, but also the targets of ROS, which makes them more susceptible to kinds of endogenous and exogenous stressors (Droge, 2002). Studies reported that excessive ROS could promote cataract formation by disrupting the redox state in lens (Pendergrass et al., 2010). Besides, mitochondria play an important role in cells metabolism including apoptosis, aging and autophagy (Chan, 2006; Nunnari and Suomalainen, 2012). It's well known that basal autophagy is necessary for recycling and degrading the injured or dysfunctional cellular organelles and proteins (Mizushima et al., 2010). More recently, the oxidative stress (free radical) theory of aging proposes that the damaged macromolecules are accumulated in elderly due to the decreased autophagy activity (Zhong et al., 2012; Xia et al., 2017). Emerging evidences also suggest that autophagy dysfunction is related to aging and various age-related diseases, the dynamic process of autophagy general declines with advancing age (Levine and Klionsky, 2004). Thus, the aging-like phenotypes of LECs observed in preliminary experiments prompted us to hypothesis that the impairment of mitochondrial functions and autophagy activity (the autophagy flux) may be involved in the toxicity of D-gal.

In the present study, we exposed LECs to D-gal in order to investigate the toxic mechanism underlying the LECs damages *in vitro*. We found that D-gal could induce premature senescence of LECs through impairing autophagy flux and disturbing mitochondrial functions. We also determined that metformin was efficient to alleviate senescence by restoring autophagy flux and mitochondrial functions. However, the anti-oxidant NAC eliminated the ROS markedly but failed in alleviating the premature senescence of LECs, indicating that D-gal was required for the production of ROS in LECs, whereas, ROS scavenging was not necessary for the restoration of D-gal related premature senescence.

2. Materials and methods

2.1. Cell culture

Human lens epithelium cell line (LECs) HLE-B3 was recipient from Laboratory of Molecular Genetics of Eye Disease, West China Hospital. The cells were cultured in DMEM supplemented with 10% fetal bovine serum (FBS), penicillin (100 U/mL) and streptomycin (100 mg/mL) in a humidifed incubator of 5% CO_2 at 37 °C.

2.2. MTT and giemsa staining

MTT assay was performed (Sigma, USA) for cell viability. Suspended cells were seeded into 96-well plates at a density of 5×10^3 cells/well. After adherence overnight, cells were treated with different concentrations of D-gal (25–225 mM) for 12, 24 and 48 h respectively. At the endpoint, 0.5 mg/mL MTT was incubated for further 3 h, and the SpectraMax M5 microplate reader (Molecular Devices, LLC, Sunnyvale, CA, USA) was used to record the absorbance at 490 nm. The morphology of cells that treated under same conditions was assessed by Giemsa stain kit (Sangon Biotech Co., Ltd, Shanghai, China). Firstly, cells were washed with iced phosphate buffered saline (PBS, Solarbio Science and Technology Co., Ltd, Beijing, China) and fixed in solution A for 5 min, then fixed cells were dyed in a mixed solution of A and B (1:5; V:V) for 10 min in room temperature. Finally, cells were washed with PBS for 3 times and the images were captured under light microscope (uX71; Olympus Corp., Tokyo, Japan).

2.3. Cell cycle distribution

Cells were treated with various concentrations of D-gal for 48 h and harvested, then cells were washed with PBS for 3 times, final fixed using 75% ethanol at 4° C overnight. After centrifugation at 800*g* for 5 min, cells were stained using a cell cycle analysis kit (Jiangsu Keygen Biotech Corp, Ltd, Nanjing, china). Briefly, DNA was stained with propidium iodide (PI) solution (50 μg/mL) according to the manufacturer's instructions. The cell cycle distribution was analyzed using a flow cytometer (Becton Dickinson, USA). Finally, the Flow Jo software (version 7.6.1) was used for data analysis.

2.4. Determination of mitochondrial swelling

The mitochondria were divided firstly according to the previously described protocol (Drahota et al., 2014). Mitochondria were suspended in the swelling medium (125 mM sucrose, 65 mM KCl, 5 mM succinate, 10 mM HEPES and 1 mM KH2PO4, pH 7.2). After 1 min of incubation, various concentrations of D-gal were added to the mitochondria suspension respectively. The decrease in the absorbance for 520 nm was measured at 6 s intervals and sustained for 5 min. The extent of swelling was estimated as the difference of the optical density at the beginning and the end point measurement.