Redox Biology 11 (2017) 365–374

Contents lists available at ScienceDirect

Redox Biology

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

Research Paper

The decay of Redox-stress Response Capacity is a substantive characteristic of aging: Revising the redox theory of aging

Jiao Meng[a], Zhenyu Lv[a,b], Xinhua Qiao[a,b], Xiaopeng Li[a,b], Yazi Li[a,b], Yuying Zhang[a], Chang Chen[a,b,c,*]

[a] National Laboratory of Biomacromolecules, Institute of Biophysics, Chinese Academy of Sciences, 15 Datun Road, Chaoyang District, Beijing 100101, China
[b] University of Chinese Academy of Sciences, 19 Yuquan Road, Shijingshan District, Beijing 100049, China
[c] Beijing Institute for Brain Disorders, 10 Xitoutiao, Youanmen, Beijing 100069, China

ARTICLE INFO

Keywords:
Oxidative stress
Aging
Redox
Redox-stress Response Capacity
RRC

ABSTRACT

Aging is tightly associated with redox events. The free radical theory of aging indicates that redox imbalance may be an important factor in the aging process. Most studies about redox and aging focused on the static status of oxidative stress levels, there has been little research investigating differential responses to redox challenge during aging. In this study, we used *Caenorhabditis elegans* and human fibroblasts as models to compare differential responses to oxidative stress challenge in young and old individuals. In response to paraquat stress, young individuals generated more ROS and activated signaling pathways including p-ERK, p-AKT and p-AMPKα/β. After the initial response, young individuals then promoted NRF2 translocation and induced additional antioxidant enzymes and higher expression of phase II enzymes, including SOD, CAT, GPX, HO-1, GSTP-1and others, to maintain redox homeostasis. Moreover, young individuals also demonstrated a better ability to degrade damaged proteins by up-regulating the expression of chaperones and improving proteasome activity. Based on these data, we propose a new concept "Redox-stress Response Capacity (RRC)", which suggests cells or organisms are capable of generating dynamic redox responses to activate cellular signaling and maintain cellular homeostasis. The decay of RRC is the substantive characteristic of aging, which gives a new understand of the redox theory of aging.

1. Introduction

Aging is characterized by the progressive deterioration of physiological functions, leading to impaired organism homeostasis and increased susceptibility to disease and death. Aging is considered a major risk factor for many diseases, including cancer, cardiovascular disorders, diabetes and neurodegenerative diseases. Many scientists have focused on the scientific topics of aging and longevity, and many theories, including the free radical theory of aging, the telomere theory and the Hayflick limit theory, have been proposed [1]. The free radical theory of aging proposed by Harman in 1956 has received widespread attention. This theory suggests oxidative stress is an important factor in age-associated diseases [2]. Specifically, oxidative stress levels increase with age due to an imbalance between reactive oxygen/nitrogen species production and antioxidant defenses. Many endogenous or exogenous stresses, including high glucose, high fat, hypoxia, ultraviolet (UV) or γ-rays, heat shock, osmotic pressure, and some cytokines, lead to oxidative stress. Some studies have provided evidence for a link between resistance to oxidative stress and longevity in a number of species, including yeast, *Caenorhabditis elegans*, Drosophila, and mice [3–6]. However, other findings suggest antioxidant over-expression does not extend lifespan in mice [7], whiles light increases in oxidative stress do extend lifespan [8]. Therefore, it is not clear whether reactive oxygen species (ROS)-induced damage is a major cause of aging. Recently, Manuel Serrano reviewed nine hallmarks of aging: genomic instability, telomere attrition, epigenetic alterations, loss of proteostasis, deregulated nutrient-sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion, and altered intercellular communication [9]. Among these, genomic instability and mitochondrial dysfunction are directly related to ROS, and the others demonstrate indirect relationships. Therefore, cellular redox regulation is a key focus of aging research.

ROS may function as signaling molecules by covalently modifying specific cysteine residues in redox-sensitive target proteins. This process activates many signaling pathways, including JNK, p38MAPK, PI3K-Akt, and PKC [10], and regulates the expression of

* Corresponding author at: National Laboratory of Biomacromolecules, Institute of Biophysics, Chinese Academy of Sciences, 15 Datun Road, Chaoyang District, Beijing 100101, China.
E-mail address: changchen@moon.ibp.ac.cn (C. Chen).

http://dx.doi.org/10.1016/j.redox.2016.12.026
Received 21 December 2016; Accepted 23 December 2016
Available online 28 December 2016
2213-2317/

many important transcriptional factors such as AP-1, Nrf2, NF-kB, p53, and FOXO [11], which are involved in cell proliferation, metabolism, inflammation, tissue repair and many other life processes. ROS signaling pathway dysregulation may be linked to aging. In early studies, increases in SOD and CAT enzyme activities in the cytoplasm prolonged fruit fly lifespan, but these results have not been reproducible [12]. In addition, the over-expression of SOD and CAT does not extend lifespan in mice [7]. In contrast, loss of SOD extends lifespan in *C. elegans* [13]. Caloric restriction and the inhibition of insulin-like growth factor (IGF) signaling extend lifespan by increasing mitochondrial ROS in *C. elegans* [14,15]. During human fibroblast replication senescence, which is a common model of cell senescence, increased ROS due to hypoxia results in Hypoxia-inducible factors (HIFs) activation and an increase in the replicative lifespan of cells [16]. These results suggest moderate ROS production activates certain signaling pathways that promote longevity. Further support for the role of ROS-mediated signaling in aging is provided by studies involving the treatment of *C. elegans* with increasing doses of paraquat [17]. The increased superoxide levels resulting from the addition of low levels of paraquat extend the lifespan of wild-type worms, whereas at high doses of paraquat, lifespan is decreased. This biphasic pattern suggests a model in which *C. elegans* longevity is dependent on a balance between pro-survival ROS-mediated signaling and ROS toxicity. In addition, Kelvin J. A. Davies demonstrated the ability of mammalian cells (as well as bacteria and yeast) to transiently and reversibly adapt to mild oxidative stress by altering gene expression in a process that is sometimes called hormesis [18,19]. Therefore, the effects of ROS demonstrate a biphasic dependence on concentration.

Aging has been widely studied and is associated with redox imbalance. Most of these findings have involved comparing basal levels between young and old organisms. However, organisms are not static and will change according to their environment to achieve a new balance. Until now, few studies have focused on differences between the dynamic abilities of young and old individuals to response to stress. Although many people take for granted that the ability to response to challenge decreases during aging, there is little direct evidence. In the current study, we used *C. elegans* and a human fibroblast senescence cell model to compare differential responses to redox stress in young and old organisms. The results suggest old worms and senescent cells exhibit decreased abilities to generate ROS/RNS, decreased antioxidant capacities, and decreased abilities to ameliorate damage due to oxidative challenge.

2. Materials and methods

2.1. Cell culture and oxidative stress induction

Human fibroblasts (AG07095) is isolated from the skin of a 2 year old boy. The culture was initiated on 10/14/83 from explants of minced foreskin removed ante-mortem 4 h earlier. The cell morphology is fibroblast-like. The karyotype is 46, XY; normal diploid male. The cells we used were provided by the laboratory of Guanghui Liu and were cultured in Dulbecco's modified Eagle's medium (Gibco) supplemented with 10% fetal bovine serum (FBS) (Gibco), 100 units/ml penicillin (Hyclone), and 100 μg/ml streptomycin (Hyclone) at 37 °C in 5%CO_2. The cells were passaged from passage 25 to passage 43. The population doubling time was calculated using the following formula: $ln_2/(ln[A/A_0])/t$, where A=cell number at t=0, A_0=initial cell number, and t=time (hours since last passage). Five types of oxidative stress were induced in cells: 1) cells were treated with 100 μM paraquat (PQ) (Sigma) for 12 h, 24 h or 48 h; 2) cells were treated with lipopolysaccharide (LPS) (Sigma) combined with Interferon-gamma (IFN-γ) (Sigma) for 12 h or 24 h; 3) cells were exposed to hypoxic conditions (3% O_2) for 12 h; 4) cells were starved via incubation in Earle's balanced salt solution (EBSS) (Macgene) for 12 h or 24 h; and 5) cells were treated with 10 μM H_2O_2 (Sigma) for 12 h.

2.2. C. elegans strains and induction of oxidative stress

The *C. elegans* strains used in this study were Bristol N2, *Pmyo3::HyPer*, *hsp4$_{pr}$::gfp*, *hsp6$_{pr}$::gfp*, *lgg-1::gfp* and *sqst-1::gfp*. Bristol N2 and *Pmyo3::HyPer* were obtained from the Caenorhabditis Genetics Center (CGC) at the University of Minnesota, USA. The *lgg-1::gfp* and *sqst-1::gfp* strains are gifts from Hong Zhang lab of Chinese Academy of Sciences [20]. The *hsp4$_{pr}$::gfp* [21] and *hsp6$_{pr}$::gfp* strains [22] are gifts from Ying Liu lab of Peking University with the permission of Cole M. Haynes professor. The *hsp4pr::gfp* and *hsp6pr::gfp* strains are UPR^{ER} and UPR^{mt} reporter transgenic worms, and the GFP expression is mediated by hsp4 and hsp6 promoter, separately. All strains were maintained at 20 °C in nematode growth medium (NGM) seeded with the *Escherichia coli* OP50 feeding strain. To obtain the Day 12 old worms, the C. elegans were transfered to the NGM plates with 100 μM 5-fluoro-2′-deoxy-β-uridine (FudR, Sigma) at L4 stage. To induce oxidative stress, worms were cultured on NGM plates containing 0.1 mM PQ for 1 d. Fluorescent proteins were visualized using a confocal laserscanning microscope (LSM750) (Carl Zeiss).

2.3. β-galactosidase staining

Cells were fixed and stained using a Senescence β-Galactosidase Staining Kit according to the manufacturer's protocol (Cell Signaling). The cells were incubated in β-galactosidase staining solution at 37 °C overnight in a dry incubator in the absence of CO_2 before they were examined under a microscope to assess blue color development.

2.4. Immunofluorescentstaining

Prior to immunofluorescent staining, cells were cultured on a confocal plate for 24 h. Then, the cells were fixed in 4% paraformaldehyde for 20 min, washed three times with PBS, and permeabilized with 0.2% Triton X-100/PBS (PBST) for 10 min. After washing with PBS, the cells were blocked by incubating with 5% BSA at room temperature for 2 h. Cells were immunostained by incubating with an anti-Nrf2 antibody (1:200, Santa Cruz) overnight at 4 °C. After washing, immunoreactive proteins were visualized following incubation with a fluorescein isothiocyanate (FITC)-conjugated goat anti-rabbit IgG antibody (Zhongshanjinqiao Corp.). Nuclei were stained with Hoechst (Santa Cruz). The cells were then observed using a confocal laserscanning microscope (LSM750) (Carl Zeiss).

2.5. Monodansylcadaverine (MDC) staining and autophagy detection with a commercial kit

The presence of autophagy was assessed using the fluorescent dye MDC (Sigma). Following treatment, cell media were aspirated and replaced with HBSS. MDC (0.01 mM) was added, and the cells were incubated for 20 min in the dark at 37 °C. The cells were then washed with PBS. MDC staining was visualized using a confocal laserscanning microscope (LSM750) (Carl Zeiss). The level of autophagy was also assayed using a CYTO-ID® Autophagy detection kit (ENZ-51031-0050) from Enzo Life Sciences, Inc. This kit measures autophagic vacuoles and monitors autophagic flux in lysosomally inhibited live cells using a novel dye that selectively labels accumulated autophagic vacuoles. The process was operated according to the protocol from the kit.

2.6. Lentivirus infection

A lentiviral vector expressing mRFP-GFP-LC3 was purchased from Addgene. HEK293T cells were transfected with this vector together with the packaging plasmids pMD2. G and psPAX2 (Addgene). Virus-containing supernatants were harvested at 48 and 72 h after transfection, filtered with a 0.45-μm PVDF membrane (Millipore), and then