Archives of Biochemistry and Biophysics 500 (2010) 203–209

Contents lists available at ScienceDirect

Archives of Biochemistry and Biophysics

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

Cigarette smoke-induced autophagy is regulated by SIRT1–PARP-1-dependent mechanism: Implication in pathogenesis of COPD

Jae-woong Hwang [a], Sangwoon Chung [a], Isaac K. Sundar [a], Hongwei Yao [a], Gnanapragasam Arunachalam [a], Michael W. McBurney [b], Irfan Rahman [a,*]

[a] Department of Environmental Medicine, Lung Biology and Disease Program, University of Rochester Medical Center, Rochester, NY, USA
[b] Ottawa Hospital Research Institute and Departments of Medicine and Biochemistry, Microbiology and Immunology, University of Ottawa, Ottawa, Canada

ARTICLE INFO

Article history:
Received 31 March 2010
and in revised form 14 May 2010
Available online 20 May 2010

Keywords:
SIRT1
PARP-1
Resveratrol
Cigarette smoke
Autophagy

ABSTRACT

Autophagy is a fundamental cellular process that eliminates long-lived proteins and damaged organelles through lysosomal degradation pathway. Cigarette smoke (CS)-mediated oxidative stress induces cytotoxic responses in lung cells. However, the role of autophagy and its mechanism in CS-mediated cytotoxic responses is not known. We hypothesized that NAD^+-dependent deacetylase, sirtuin 1 (SIRT1) plays an important role in regulating autophagy in response to CS. CS exposure resulted in induction of autophagy in lung epithelial cells, fibroblasts and macrophages. Pretreatment of cells with SIRT1 activator resveratrol attenuated CS-induced autophagy whereas SIRT1 inhibitor, sirtinol, augmented CS-induced autophagy. Elevated levels of autophagy were induced by CS in the lungs of SIRT1 deficient mice. Inhibition of poly(ADP-ribose)-polymerase-1 (PARP-1) attenuated CS-induced autophagy via SIRT1 activation. These data suggest that the SIRT1–PARP-1 axis plays a critical role in the regulation of CS-induced autophagy and have important implications in understanding the mechanisms of CS-induced cell death and senescence.

Introduction

Cigarette smoke (CS)[1] contains numerous oxidants/free radicals as well as chemical compounds that induce oxidative stress and are involved in the pathogenesis of lung and heart diseases. CS accelerates cell death and senescence both through direct mechanisms mediated by oxidants/free radicals as well as via generation of oxidants from inflammatory cells in chronic inflammatory diseases including chronic obstructive pulmonary disease (COPD) and cardiovascular co-morbidities [1,2]. Although there is emerging evidence that CS-mediated cell death and senescence increase the susceptibility to diseases, the exact mechanism by which cigarette smoking accelerates cell death and senescence remains unclear.

Autophagy is a fundamental cellular process that eliminates long-lived proteins and damaged organelles through a lysosomal degradation pathway, and has been suggested to have an essential function in maintaining cellular homeostasis [3]. The autophagic process is initiated by sequestering redundant cytoplasmic contents within double-membrane structures termed autophagosomes. The autophagosome fuses with a lysosome, and its contents are degraded and recycled [4]. Although autophagy occurs at basal levels in all cells to maintain cellular homeostasis, recent reports show that autophagy is also induced in response to environmental stresses, such as pathogen infections, starvation and oxidative stress [4–7]. Although, autophagy plays a protective role in overcoming the exogenous stress, prolonged and excessive autophagy can lead to cell death [8,9]. Failure to regulate autophagy has been implicated in pathogenesis of cancer, cardiovascular failure, immune disease, skeletal muscle atrophy and neurodegenerative disorders [10–17]. Recent studies have shown that increased autophagy occurs in lungs of patients with COPD and in lung cells of mouse exposed to CS [18,19]. However, the underlying mechanism for CS-induced autophagy was not studied.

Sirtuin 1 (SIRT1), the mammalian ortholog of yeast silent information regulator 2 (Sir2), is an NAD^+-dependent deacetylase which is shown to be an anti-inflammatory and anti-aging protein [20]. SIRT1 is involved in diverse physiological functions, including gene silencing, stress resistance, apoptosis, inflammation, senescence

* Corresponding author. Address: Department of Environmental Medicine, Lung Biology and Disease Program, University of Rochester Medical Center, Box 850, 601 Elmwood Avenue, Rochester, NY 14642, USA. Fax: +1 585 276 0239.
E-mail address: Irfan_Rahman@URMC.Rochester.edu (I. Rahman).

[1] *Abbreviations used*: CS, cigarette smoke; COPD, chronic obstructive pulmonary disease; SIRT1, sirtuin 1; FoxO3, forkhead box O3; NF-κB, nuclear factor-κB; PARP-1, poly(ADP-ribose)-polymerase-1; FBS, fetal bovine serum; DMEM/F-12, Dulbecco's modified Eagle's medium-Ham's F12; 3-AB, 3-aminobenzamide; HFL1, human fetal lung fibroblasts; CSE, cigarette smoke extract; TPM, total particulate matter; BSA, bovine serum albumin; HDAC, histone deacetylase; SAHA, suberoylanilide hydroxamic acid; mTOR, mammalian target of rapamycin; AMPK, AMP-activated protein kinase; ac-p53, acetylated p53.

0003-9861/$ - see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.abb.2010.05.013

and aging [21–24]. These physiological functions of SIRT1 are mediated by deacetylation of histones and several important transcription factors such as forkhead box O3 (FoxO3), p53 and nuclear factor-κB (NF-κB) [20,22,23,25–27]. SIRT1 activity is also regulated by NAD^+ depletion induced by oxidative stress or activation of the NAD^+-dependent enzyme poly(ADP-ribose)-polymerase-1 (PARP-1) [28,29]. It has recently been shown that SIRT1 regulates autophagy under calorie restriction/starvation [30]. Moreover, we have recently shown that SIRT1 levels/activity is decreased in response to CS exposure *in vitro* in macrophages and epithelial cells as well as in lungs of smokers and patients with COPD [20,24,28]. However, the role of SIRT1 and PARP-1 on CS-mediated autophagy is not known. Therefore, we hypothesized that SIRT1 plays an important role in regulating CS-mediated autophagy in lung cells. We studied the effect of CS on induction of autophagy in different lung cell types and macrophages *in vitro* and in mouse lung *in vivo*, and determined the role of SIRT1–PARP-1 axis in regulation of autophagy.

Materials and methods

Reagents

Penicillin–Streptomycin, L-glutamine and RPMI-1640 were obtained from Gibco BRL (Grand Island, NY). Fetal bovine serum (FBS) was obtained from HyClone Laboratories (Logan, UT). Dulbecco's modified Eagle's medium-Ham's F12 50:50 mixture (DMEM-F12) was purchased from Mediatech (Manassas, VA). Amphotericin B was purchased from Lonza (Walkersville, MD). Resveratrol was purchased from Biomol (Plymouth Meeting, PA). Sirtinol was procured from Sigma (St. Louis, MO). 3-Aminobenzamide (3-AB) was purchased from Calbiochem (La Jolla, CA).

Cell culture and treatments

Human bronchial epithelial cells (H292) and human fetal lung fibroblasts (HFL1) were obtained from American Type Culture Collection (Manassas, VA). H292 cells were cultured in RPMI-1640 supplemented with 10% FBS, 2 mM L-glutamine, 100 μg/ml penicillin and 100 U/ml streptomycin. HFL1 cells were cultured in DMEM-F12 supplemented with 10% FBS, 100 μg/ml penicillin, 100 U/ml streptomycin, and 1 μg/ml amphotericin B. Human bronchial epithelial cells (Beas-2B) were grown in DMEM-F12 supplemented with 5% FBS, 15 mM HEPES, 100 μg/ml penicillin, and 100 U/ml streptomycin. Human monocyte–marcophage cell line (Mono-Mac6), which was established from peripheral blood of patient with monoblastic leukemia, were grown in RPMI-1640 supplemented with 10% FBS, 2 mM L-glutamine, 100 μg/ml penicillin and 100 U/ml streptomycin, 1% nonessential amino acid, 1 mM sodium pyruvate, 1 μg/ml human holo-transferrin, and 1 mM oxaloacetic acid. The cells were incubated at 37 °C in a humidified atmosphere containing 7.5% CO_2 and 92.5% air. The cells were pretreated with resveratrol (10 μM), sirtinol (10 μM) or 3-aminobenzidine (3-AB, 1 mM) for 2 h before treated with cigarette smoke extract (CSE, 0.5–5%) for 24 h. To avoid induction of autophagy through the serum starvation pathway, all treatments were done in complete culture medium.

Preparation of cigarette smoke extract

Research grade cigarettes 2R4F were obtained from the Kentucky Tobacco Research and Development Center at the University of Kentucky (Lexington, KY). These cigarettes contain 11.7 mg of total particulate matter (TPM), 9.7 mg of tar, and 0.76 mg of nicotine per cigarette. CSE was prepared by bubbling smoke from one cigarette into 10 ml serum-free media at a rate of one cigarette/min as described previously [31–33]. The pH of the CSE was adjusted to 7.4, and was sterile-filtered through a 0.45 μm filter (25 mm Acrodisc; Pall Corporation, Ann Arbor, MI). CSE preparation was standardized by measuring the absorbance (OD: 1.00 ± 0.05) at a wavelength of 320 nm. The pattern of absorbance (spectrogram) observed at 320 nm showed very little variation between different preparations of CSE. CSE was freshly prepared for each experiment and diluted with culture media supplemented with 10% FBS immediately before use. Control medium was prepared by bubbling air through 10 ml serum-free media, adjusting pH to 7.4, and sterile-filtered as described above.

Transfection

For the autophagy assays, H292 cells were plated on chamber slides and transfected with 1 μg of GFP-LC3 expression construct, a kind gift of Dr. Tamotsu Yoshimori (Research Institute for Microbial Diseases, Osaka University, Osaka, Japan) [34], using lipofectamine™ 2000 (Invitrogen, CA) according to the manufacturer's protocol. Images were captured using a fluorescent microscope (BX51, Olympus Optical, Tokyo, Japan).

Immunoblotting

Whole cell extracts were separated on a 6.5–12% sodium dodecyl sulfate–polyacrylamide gel by electrophoresis. Separated proteins were transferred onto nitrocellulose membranes (Amersham, Arlington Heights, IL), and blocked for 1 h at room temperature with 5% bovine serum albumin (BSA) (Sigma–Aldrich). The membranes were then probed with specific primary antibodies of LC3, β-actin (Sigma–Aldrich), SIRT1, acetylated p53 on lysine 382, GAPDH (Cell Signaling Technology, Beverly, MA) or p53, poly-(ADP-ribose) (Santa Cruz Biotechnology, Santa Cruz, CA) at 4 °C for overnight. After three washing steps, the levels of protein were detected by probing with secondary anti-rabbit or anti-mouse antibody linked to horseradish peroxidase for 1 h, and bound complexes were detected using the enhanced chemiluminescence method (Perkin–Elmer, Waltham, MA). Equivalent loading of the gel was determined by quantification of protein as well as by reprobing membranes for β-actin or GAPDH. ImageJ software (Version 1.41, National Institutes of Health, Bethesda, MD) was used for gel band quantitative densitometric analysis.

Animals and cigarette smoke exposure

SIRT1 heterozygous knockout ($Sirt1^{+/-}$) mice [35] and wild-type mice of genetic background 129/SvJ were bred and maintained under specific pathogen-free condition in the vivarium facility of the University of Rochester. These SIRT1 deficient mice and WT littermates were housed in the vivarium facility of the University of Rochester with a 12 h light/dark cycle (light on at 6:00 am). All animal procedures were approved by the Committee on Animal Research at the University of Rochester. In brief, mice were exposed to CS using research grade cigarettes 2R4F according to the Federal Trade Commission protocol (1 puff/min of 2 s duration and 35 ml volume) with a Baumgartner-Jaeger CSM2072i automatic CS generating machine (CH Technologies, Westwood, NJ). Mainstream CS was diluted with filtered air and directed into the exposure chamber. The smoke exposure (TPM in per cubic meter of air) was monitored in real-time with a MicroDust Pro-aerosol monitor (Casella CEL, Bedford, UK) and verified daily by gravimetric sampling. The smoke concentration was set at a value of ~300 mg/m^3 TPM by adjusting the flow rate of the diluted medical air, and the level of carbon monoxide in the chamber was 350 ppm [36]. Mice (n = 4 per group) received two 1 h exposures (1 h apart) daily