Biochemical and Biophysical Research Communications 444 (2014) 662–669

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Sirt3 controls chromosome alignment by regulating spindle dynamics during mitosis

Byung-Soo Choi, Ji Eun Park, Chang-Young Jang *

Research Center for Cell Fate Control, College of Pharmacy, Sookmyung Women's University, Seoul 140-742, Republic of Korea

ARTICLE INFO

Article history:
Received 21 January 2014
Available online 1 February 2014

Keywords:
Sirt3
Spindle assembly checkpoint
Chromosome alignment
Spindle dynamics

ABSTRACT

Sirt3, one of mammalian sirtuins is a prominent mitochondrial deacetylase that controls mitochondrial oxidative pathways and the rate of reactive oxygen species. Sirt3 also regulates energy metabolism by deacetylating enzymes involved in the metabolic pathway related with lifespan. We report here a novel function of Sirt3 which was found to be involved in mitosis. Depletion of the Sirt3 protein generated unaligned chromosomes in metaphase which caused mitotic arrest by activating spindle assembly checkpoint (SAC). Furthermore, the shape and the amount of the spindles in Sirt3 depleted cells were abnormal. Microtubule (MT) polymerization also increased in Sirt3 depleted cells, suggesting that Sirt3 is involved in spindle dynamics. However, the level of acetylated tubulin was not increased significantly in Sirt3 depleted cells. The findings collectively suggest that Sirt3 is not a tubulin deacetylase but regulates the attachment of spindle MTs to the kinetochore and the subsequent chromosome alignment by increasing spindle dynamics.

© 2014 Elsevier Inc. All rights reserved.

1. Introduction

Sirtuins consist of seven deacetylase family (Sirt1–Sirt7) which has NAD^+-dependent protein deacetylase and/or ADP-ribosyltransferase activities [1]. Mammalian sirtuins have been identified as homologs of SIR2 (silent information regulator 2), a histone deacetylase involved not only in the positive effects of caloric restriction on the longevity of lifespan but in gene expression by chromatin silencing [2]. Whereas histone deacetylase removes an acetyl group from histone, sirtuins deacetylates a variety of proteins including transcription factors and metabolic enzymes [3]. Therefore, sirtuins are crucial factors responsible for metabolic and oxidative stress and are related to diseases associated with aging [4]. It has been reported that Sirt1, Sirt6, and Sirt7 mainly localize in the nucleus, Sirt2 is in the cytoplasm, and Sirt4 and Sirt5 in the mitochondria. Sirt3 primarily localizes in the mitochondria but translocates from the nucleus to the mitochondria under cellular stress conditions [5].

Individual sirtuins have specific substrates in different metabolic or signaling pathways. Sirt1 deacetylates lysine 9 in histone H3 (H3K9), lysine16 in histone H4 (H4K16), and nonhistone proteins involved in metabolism, cell growth, apoptosis, adaptation to caloric restriction, and tumorigenesis [6,7]. Sirt2 is a histone deacetylase and controls acetylation of histone H4 lysine 16 (H4K16Ac) [8]. Sirt3, a mitochondrial deacetylase, controls global mitochondrial lysine acetylation and acts as a tumor suppressor by maintaining mitochondrial integrity and metabolism [9]. Sirt4 negatively regulates glutamate dehydrogenase and decreases the effect of calorie restriction in some cell types [10,11]. Sirt5 controls the urea cycle by deacetylating carbamoyl phosphate synthetase 1 [12] and also has a protein lysine desuccinylase activity and a protein lysine demalonylase activity [13]. Sirt6 is also histone deacetylase and acts as a tumor suppressor by controlling cancer metabolism [14]. Sirt7 regulates the transcription of rRNA genes by interacting with RNA polymerase I [15]. Several sirtuins such as Sirt1 and Sirt2 are involved in the mitotic process. Sirt1 is associated with chromosome instability in mitosis [16]. Sirt2 has tubulin deacetylase activity in addition to histone deacetylase activity and regulates chromosome condensation and spindle dynamics [17]. During mitosis, Sirt2 interacts with various mitotic structures such as centrosome, mitotic spindle, and midbody in order to maintain genome integrity.

Progression of mitosis consists of dynamic processes such as nuclear envelop breakdown, centrosome maturation, spindle nucleation from centrosome, formation of bipolar spindles, chromosome congression, and chromosome segregation [18]. It is viral to ensure proper mitotic progression for normal cell division [19]. Cells alerts these processes with the spindle-assembly checkpoint (SAC) which is activated by misaligned chromosomes and

* Corresponding author. Fax: +82 2 710 9871.
E-mail address: cyjang@sookmyung.ac.kr (C.-Y. Jang).

http://dx.doi.org/10.1016/j.bbrc.2014.01.124
0006-291X/© 2014 Elsevier Inc. All rights reserved.

abnormal spindle orientation and as a result arrests defective mitotic cells in metaphase [20]. The SAC monitors the capture of kinetochore by spindle MTs and the tension between inter-kinetochores by bi-oriented mitotic spindles. When spindle MTs capture all kinetochores and form bi-orientation, SAC is inactivated by releasing SAC proteins such as Mad2 and BubR1 from kinetochores and consents to initiate anaphase onset. In this regard, the SAC prevents mis-segregation of chromosomes and aneuploidy which can cause tumorigenesis.

Sirt3 has been identified as a cell division gene through time-lapse microscopy, because depletion of Sirt3 causes misaligned chromosomes and mitotic cell death [21]. This report prompted us to investigate the precise mitotic function of Sirt3 and its mechanism during mitosis. Depletion of Sirt3 caused unaligned chromosome which was not attached by spindle MTs and activated SAC to arrest cells in metaphase. Furthermore, depletion of Sirt3 increased the stability of spindle MTs, which, in turn, increased spindle polymerization. Thus, we can conclude that Sirt3 is a new mitotic regulator that controls chromosome alignment by regulating the stability of mitotic spindles in mitosis.

2. Materials and methods

2.1. Antibodies

The full-length Sirt3 gene was subcloned into the pCMV-Tag 5 vector with a C-terminal myc tag (Stratagene, USA). Sirt3H248Y mutant in pCDNA4-Myc-HisA vector was purchased from Addgene (UAS).

Anti-Sirt3 and anti-Tpx2 antibodies were purchased from GeneTex (USA). Rabbit antibodies against Mad2 and BubR1 were described previously [22]. Anti-Hsp90 antibody was purchased from Santa Cruz Biotechnology (USA). Anti-acetylated tubulin antibody was purchased from Sigma (USA). Anti-β-tubulin E7 monoclonal antibody was obtained from the Developmental Studies Hybridoma Bank (USA).

2.2. Cell culture and transfection

HeLa cells were cultured in Dulbecco's modified Eagle's medium (DMEM, WelGENE Inc.) supplemented with 10% fetal bovine serum (FBS, Invitrogen), penicillin (100 units/mL) and 100 μg/mL streptomycin (Invitrogen). The cells were maintained at 37 °C in a humidified atmosphere containing 5% CO_2. siRNAs were synthesized by Bioneer, Inc. (South Korea). The sequence targeting Sirt3 was 5′-GUCCAUAUCUUUUUCUGUGTT-3′. The control siRNA (siGL2) was 5′-CGTACGCGGAATACTTCGATT-3′. siRNAs were transfected into HeLa cells using DharmaFect 1 (Dharmacon, Inc.). DNA transfection was performed using Lipofectamine 2000 (Invitrogen, USA) as instructed by the manufacturer.

2.3. Immunofluorescence

HeLa cells on coverglasses were fixed with methanol at −20 °C for 30 min. Alternatively, cells were extracted with the BRB80-T buffer (80 mM PIPES, pH 6.8, 1 mM $MgCl_2$, 5 mM EGTA and 0.5% Triton X-100) and then fixed with 4% paraformaldehyde for 15 min at room temperature. The fixed cells were then permeabilized and blocked with PBS-BT (1 × PBS, 3% BSA, and 0.1% Triton X-100) for 30 min at room temperature. Coverslips were then incubated in primary and secondary antibodies diluted in PBS-BT. Images were acquired using an LSM image examiner (Carl Zeiss, Germany) under a Zeiss LSM510 confocal microscope and 63× oil immersion lens. Some images were acquired with AxioVision 4.8.2 (Carl Zeiss) under a Zeiss Axiovert 200 M microscope using a 1.4 NA plan-Apo 100× oil immersion lens and a HRm CCD camera. Deconvolved images were obtained using AutoDeblur v9.1 and AutoVisualizer v9.1 (AutoQuant Imaging).

2.4. Live cell image

For time-lapse microscopy, HeLa cells stably expressing GFP-H2B were cultured in Leibovitz's L-15 medium (Invitrogen) supplemented with 10% fetal bovine serum (Invitrogen) and 2 mM L-glutamine (Invitrogen). Cells were placed into a sealed growth chamber heated to 37 °C and observed on a Zeiss Axiovert 200 M microscope with a 20× lens. Images were acquired every three minutes for five hours with AxioVision 4.8.2 (Carl Zeiss).

For FLIP, HeLa cells stably expressing GFP-α-tubulin were transfected with a control or siSirt3 and placed in a sealed growth chamber heated to 37 °C. Cytoplasmic GFP-α-tubulin was photobleached with a laser and images were acquired at 0.632 s intervals for 337.459 s with ZEN (Carl Zeiss) under a LSM 700 confocal microscope (Carl Zeiss) with a 40× lens. 10 half-spindles from 10 metaphase cells in each transfection were analyzed by measuring the absolute GFP-α-tubulin fluorescence intensity in a defined circular area contained entirely within each half-spindle. Fluorescence intensities for each half-spindle were normalized to their maximum intensity at the beginning of the time lapse and the 10 normalized datasets were averaged to generate a single trace for each transfection.

2.5. Measurement of intracellular ROS levels

The 2′,7′-dichlorofluorescein diacetate (DCFH-DA) was used to monitor the intracellular ROS levels. After transfection with Sirt3 siRNA, cells were plated in 96 well plates at a density of 5×10^5 cells/well. DCFH-DA was added to transfected cells at a final concentration of 5 μM and cells were incubated for 30 min at 37 °C. Fluorescence intensity was measured using microplate reader at an excitation wavelength of 485 nm and an emission wavelength of 530 nm.

3. Results and discussion

3.1. Sirt3 controls mitotic progression

It has been suggested that Sirt3 may be a mitotic regulator due to the fact that its depletion induces mitotic cell death and metaphase alignment problems [21]. To investigate whether Sirt3 has mitotic functions, human HeLa cells were transfected with siRNA against Sirt3 (Fig. 1A and B). As shown in Fig. 1A, Sirt3 showed no subcellular localization on the mitotic structures such as mitotic spindle, the centrosome, midbody, the kinetochore. Consistent with previous reports, however, depletion of Sirt3 with siRNA not only generated unaligned chromosomes but altered the shape of spindles and the amount of spindles. Quantitative analysis of mitotic progression indicated that sirt3 depleted cells were arrested in metaphase (Fig. 1C). Among metaphase cells, more than 30% of the cells contained unaligned chromosomes (Fig. 1D), indicating that Sirt3 is involved in chromosome congression to the mitotic equator. Furthermore, removal of Sirt3 increased the density of spindles (Fig. 1E) and resulted in an abnormal spindle shape (Fig. 1B), suggesting that Sirt3 controls spindle formation and stability. Because reactive oxygen species (ROS) generated by depletion of mitochondrial Sirt3 could directly affect mitosis, we analyzed ROS level and mitotic defects in Sirt3 depleted cells with or without antioxidant, N-acetylcysteine (NAC). As expected, ROS caused by Sirt3 depletion was decreased with NAC treatment (Fig. 1F). However, una-

Download English Version:

https://daneshyari.com/en/article/10756501

Download Persian Version:

https://daneshyari.com/article/10756501

Daneshyari.com