FEBS Letters 585 (2011) 3360–3366

journal homepage: www.FEBSLetters.org

p53 deacetylation by SIRT1 decreases during protein kinase CKII downregulation-mediated cellular senescence

Seok Young Jang, Soo Young Kim, Young-Seuk Bae *

School of Life Sciences and Biotechnology, College of Natural Sciences, Kyungpook National University, Daegu 702-701, Republic of Korea

ARTICLE INFO

Article history:
Received 22 March 2011
Revised 8 September 2011
Accepted 13 September 2011
Available online 29 September 2011

Edited by Varda Rotter

Keywords:
p53 Acetylation
SIRT1
Protein kinase CKII
Senescence
Tumor suppression

ABSTRACT

Cellular senescence is thought to be an important tumor suppression process in vivo. We have previously shown that p53 activation is necessary for CKII inhibition-mediated cellular senescence. Here, CKII inhibition induced acetylation of p53 at K382 in HCT116 and HEK293 cells. This acetylation event was suppressed by SIRT1 activation. CKIIα and CKIIβ were co-immunoprecipitated with SIRT1 in a p53-independent manner. Maltose binding protein pull-down and yeast two-hybrid indicated that SIRT1 bound to CKIIβ, but not to CKIIα. CKII inhibition reduced SIRT1 activity in cells. CKII phosphorylated and activated human SIRT1 in vitro. Finally, SIRT1 overexpression antagonized CKII inhibition-mediated cellular senescence. These results reveal that CKII downregulation induces p53 stabilization by negatively regulating SIRT1 deacetylase activity during senescence.

Structured summary of protein interactions:
CKII Beta binds to **SIRT1** by pull down (View interaction)
CKII Beta physically interacts with **SIRT1** by pull down (View interaction)
SIRT1 physically interacts with **CKII Beta** and **CKII Alpha** by anti bait coimmunoprecipitation (View Interaction: 1, 2, 3)
CKII Beta physically interacts with **SIRT1** by two hybrid (View interaction)

1. Introduction

Normal primary cells withdraw from the cell division cycle after a finite number of divisions and enter irreversible growth arrest designated as replicative senescence, or more generally, cellular senescence [1]. Cellular senescence can also be induced by oxidative stress, DNA damage, and oncogenic activation [2–6]. Senescence is thought to be an important tumor suppression process in vivo. In a state of senescence, cells become enlarged, acquire a flattened shape, and express several senescence-associated markers such as senescent-associated β-galactosidase (SA-β-gal), cyclin-dependent kinase inhibitor $p21^{Cip1/WAF1}$, and tumor suppressor protein p53 [7–10]. p53 is a transcription factor that plays a major role in cell cycle regulation. It is activated in response to a variety of cellular stress signals, where upon it triggers cell cycle arrest or apoptosis depending on the circumstances. p53 activity is modulated by protein stability and post-translational modifications, including phosphorylation and acetylation [11].

The silent information regulator 2 (Sir2) is an NAD-dependent protein deacetylase that controls longevity in lower eukaryotes, such as budding yeast and nematode [12,13]. An increase in Sir2 activity extends the lifespan of these organisms. Mammals possess seven paralogs of the Sir2 gene, with SIRT1 being the most similar to Sir2. SIRT1 deacetylates not only histones (H1, H3, and H4), but also many non-histone proteins such as p53 [14]. It has been shown that SIRT1 is also important for various cellular functions, including apoptosis, differentiation, proliferation, and metabolism.

Protein kinase CKII (formerly known as casein kinase II) (CKII), a ubiquitous serine/threonine kinase, plays a significant role in the control of cell proliferation and transformation [15–17]. The holoenzyme of CKII is a heterotetramer composed of two catalytic (α and/or α′) and two regulatory (β) subunits. We previously demonstrated that CKII activity is downregulated at the transcriptional level in both senescent human lung fibroblast

Abbreviations: CKII, protein kinase CKII (also known as casein kinase II); DRB, 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole; HDAC, histone deacetylase; MBP, maltose binding protein; NOX, NADPH oxidase; PBS, phosphate-buffered saline; ROS, reactive oxygen species; SA-β-gal, senescent-associated β-galactosidase; Sir2, silent information regulator 2; PMSF, phenylmethylsulfonyl fluoride; SDS, sodium dodecyl sulfate; siRNA, small-interfering RNA

* Corresponding author. Fax: +82 53 943 2762.
E-mail address: ysbae@knu.ac.kr (Y.-S. Bae).

0014-5793/$36.00
doi:10.1016/j.febslet.2011.09.027

IMR-90 cells and aged rat tissues. CKII inhibition by CKII inhibitors or CKIIα small-interfering RNA (siRNA) induces premature senescence in IMR-90 cells [18]. In addition, silencing of the *CKIIα* and *CKIIα′* genes during cellular senescence is mediated by DNA methylation [19]. Studies designed to examine the mechanism through which CKII inhibition contributes to the development of senescence have indicated that p53 is involved in the development of senescence induced by CKII inhibition in HCT116 human colon cancer cells [20]. Superoxide anion generation by NADPH oxidase (NOX) activation is the upstream mediator of p53 stabilization in cells made senescent by CKII inhibition [21]. However, we cannot rule out the possibility that CKII downregulation also mediates p53 stabilization by another mechanism. Here, we provide evidence that CKII downregulation can stabilize p53 via inhibition of SIRT1 deacetylase activity in cells and that, conversely, exogenous SIRT1 delays CKII inhibition-mediated senescence.

2. Materials and methods

2.1. Cell culture and transfection

Human colon cancer HCT116 and human embryonic kidney (HEK) 293 cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum at 37 °C in 5% CO_2. To establish SIRT1-overexpressing cells, cells were transfected with pECE-Flag-SIRT1 by Lipofectamine (Invitrogen, Carlsbad, CA) as described by the manufacturer.

2.2. Preparation of cell extracts

For Western blotting, cells in 100-mm dishes were washed with ice-cold phosphate-buffered saline (PBS), collected by scraping with a rubber policeman, and lysed in 100 μl of ice-cold RIPA buffer [50 mM Tris–HCl (pH 8.0), 150 mM NaCl, 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate (SDS), 0.5 mM phenylmethylsulfonyl fluoride (PMSF), 1 μg/ml of aprotinin, 1 μg/ml of leupeptin, and 1 μg/ml of pepstatin]. For measurement of deacetylase activity, cells were lysed by sonication in lysis buffer [50 mM Tris–HCl (pH 8.0), 20 mM NaCl, 1 mM $MgCl_2$, 1 mM EDTA, 1% Nonidet P-40, 0.5 mM PMSF, 1 μg/ml of aprotinin, 1 μg/ml of leupeptin, 1 μg/ml of pepstatin, 1 mM sodium orthovanadate, 1 mM sodium pyrophosphate, and 4 mM *p*-nitrophenyl phosphate]. The particulate debris was then removed by centrifugation at 12000×*g*. The volumes of the supernatants were adjusted for equal protein concentration.

2.3. Immuno-blotting

Proteins were separated by polyacrylamide gel electrophoresis in the presence of SDS and then transferred by electrophoresis to a nitrocellulose membrane. The membrane was blocked with 5% (w/v) non-fat, dry skim milk in TBST [20 mM Tris–HCl (pH 7.4), 150 mM NaCl, 0.05% Tween 20] for 2 h and then incubated with specific antibodies in 1% (w/v) non-fat, dry skim milk for 1 h. The membrane was washed three times in TBST and then analyzed with the ECL system (Amersham Pharmacia Biotech, Korea). Some membranes were stripped in stripping buffer [2% SDS, 100 mM β-mercaptoethanol, and 50 mM Tris–HCl (pH 7.0)] at 50 °C for 1 h with gentle shaking and then reprobed with anti-β-actin antibody as a control for protein loading. Anti-p53, -CKIIα, -SIRT1, and -β-actin antibodies were obtained from Santa Cruz Biotechnology Inc. (Santa Cruz, CA). Anti-acetyl p53 K382 antibody was obtained from Cell Signaling Technology (Danvers, MA).

2.4. Maltose binding protein (MBP) pull-down assay and immunoprecipitation

An MBP pull-down assay was performed by incubating amylose-agarose beads with MBP-CKII and cell lysates in 200 μl of binding buffer [20 mM Tris–HCl (pH 7.4), 150 mM NaCl, 1 mM PMSF]. The reaction was allowed to proceed for 1 h while rocking at 4 °C. For immunoprecipitation, cell lysates were pre-cleared with normal mouse or rabbit IgG along with protein A Sepharose (Amersham Biosciences, Piscataway, NJ) for 1 h at 4 °C. The supernatant was then incubated with anti-CKII or anti-SIRT1 antibody along with protein A Sepharose with mixing for 12 h at 4 °C. The beads were collected by centrifugation and washed three times with PBS.

2.5. Yeast two-hybrid assay

The reporter strain *Saccharomyces* cerevisiae HF7c (MATa ura3-52 *his*3-200 ade2-101 lys2-801 trpl-901 leu2-3112 gal4-542 gal80-538 LYS2::$GAL1_{UAS}$-$GAL1_{TATA}$-HIS3 URA3::$GAL4_{17mers(x3)}$-$CyC1_{TA-TA}$-lacZ) was co-transformed with various combinations of hybrid plasmids containing a DNA-binding domain or a transcriptional activation domain. Transformants were plated on synthetic medium lacking tryptophan and leucine. After 4 days of growth, the transformants were cultured on selective medium lacking tryptophan, leucine, and histidine but including 1 mM 3-amino-triazole, after which they were incubated for 4 days at 30 °C. Interactions between the hybrid proteins were monitored by growth on selective media.

2.6. RNA interference

The siRNA for CKIIα was 5′-UCAAGAUGACUACCAGCUGdTdT. The siRNA for the negative control was 5′-GCUCAGAUCAAUACGGAGAdTdT. Both siRNAs were transfected into cells using Lipofectamine (Invitrogen) as described by the manufacturer. Five hours after transfection, the medium was changed, and the cells were grown for another 3 days before being harvested or stained for SA-β-gal.

2.7. Purification of CKII and SIRT1 phosphorylation by CKII

CKII holoenzyme and CKII subunits tagged with MBP were expressed in *Escherichia coli* and purified as described previously [22,23]. Phosphorylation of human SIRT1 (BIOMOL International, Plymouth Meeting, PA) by CKII was carried out in a reaction mixture containing 20 mM Tris–HCl (pH 7.5), 100 mM KCl, 10 mM $MgCl_2$, 1 mM DTT, 1 mM EGTA, 100 μM [γ-^{32}P]ATP, and 1 μg of human SIRT1 in a total volume of 30 μl. The reactions were initiated by the addition of purified CKII and incubated for 15 min at 30 °C. The samples were then separated on a 10% SDS–polyacrylamide gel. The gel was stained with Coomassie blue, dried, and subjected to autoradiography.

2.8. Measurement of histone deacetylase (HDAC) and SIRT1 activity

HDAC activity was assessed with the HDAC Colorimetric Assay/Drug Discovery Kit (BIOMOL International) according to the manufacturer's instructions. Briefly, cell extracts and Color de Lys™ substrate were added to the wells of a microtiter plate and incubated at 37 °C for 15 min. HDAC inhibitor trichostatin A and Color de Lys™ developer were added to stop the HDAC reaction. The mixture was incubated at 37 °C for 10 min and read on a microtiter-plate reader (PerkinElmer, Waltham, MA) at 405 nm. The enzyme activity of SIRT1 was measured with the SIRT1 Fluorimetric Drug Discovery Kit (BIOMOL International) based on Fluor de Lys–SIRT1