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SCIENCE DIRECT
Free Radical Biology & Medicine 40 (2006) 2175–2182
www.elsevier.com/locate/freeradbiomed

Original Contribution

Silent information regulator 2 (SIRT1) attenuates oxidative stress-induced mesangial cell apoptosis via p53 deacetylation

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Received 17 November 2005; revised 3 February 2006; accepted 21 February 2006
Available online 20 March 2006

Abstract

Oxidative stress-induced apoptosis of renal glomerular cells is an important factor for the development of various kidney diseases. Identification of molecules that modulate this process could lead to the development of new strategies for preventing kidney diseases. In this study, we evaluated whether mammalian silent information regulator 2 (SIRT1), which has been recently identified as a cell survival factor countering various stressors, is a key regulator of oxidative stress-induced mesangial cell apoptosis. Morphological features of apoptotic cell death (nuclear condensation) and the expression of biochemical proapoptotic markers [cleavages of caspase-3 and poly (ADP-ribose) polymerase (PARP)] were assessed in murine mesangial cells (MMCs) exposed to hydrogen peroxide (H_2O_2). H_2O_2 increased mesangial cell apoptosis, predominantly through p53 activation by acetylation, which is a posttranscriptional modification for p53 activation. H_2O_2-induced apoptosis was significantly attenuated in *SIRT1*-overexpressing MMCs, but enhanced in *SIRT1*-knockdown MMCs. Although SIRT1 did not affect H_2O_2-mediated phosphorylation of mitogen-activated protein (MAP) kinase, it interacted with p53 and inhibited H_2O_2-mediated p53 acetylation but not phosphorylation in MMCs. Our results indicate that SIRT1 can prevent oxidative stress-induced apoptosis through p53 deacetylation in mesangial cells. Upregulation of SIRT1 may provide a new strategy for preventing kidney glomerular diseases.

Keywords: Apoptosis; p53; Oxidative stress; Silent information regulator 2; SIRT1; Mesangial cell; Renal diseases; Free radical

Introduction

Apoptosis is a distinct form of cell death that is observed under various physiological and pathological conditions. Glomerular cell apoptosis is observed in human and experimental kidney diseases such as diabetic nephropathy, hypertensive nephrosclerosis, and glomerulonephritis, and is considered to be involved in the progression of these diseases [1–4]. Therefore, preventing glomerular cell apoptosis may lead to the prevention of various kidney diseases.

Oxidative stress regulates a broad array of signal transduction pathways that regulate various biological processes including gene expression, cell growth, differentiation, and apoptosis [5]. Oxidative stress has been reported to contribute to the development of various kidney diseases [6–8]. Furthermore, oxidative stress caused by various stressors such as high glucose, angiotensin II, and TNF-α enhances apoptosis of cultured mesangial cells [4,9–14]. However, the precise regulatory mechanisms of oxidative stress-induced apoptosis of mesangial cells remain to be defined. Several investigators have attempted to prevent the

Abbreviations: BSA, bovine serum albumin; ECM, extracellular matrix; DAPI, 4,6-diamidino-2-phenylindole; DMEM, Dulbecco's modified Eagle's medium; MAP, mitogen-activated protein; MMCs, murine mesangial cells; PARP, poly (ADP-ribose) polymerase; PBS, phosphate-buffered saline; PMSF, phenylmethylsulfonyl fluoride; SIRT1, silent information regulator 2.

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doi:10.1016/j.freeradbiomed.2006.02.014

development of kidney diseases by using antioxidants [15–17]. However, the effects of these antioxidants are controversial. Thus, it is hoped that the identification of new molecules that can modulate oxidative stress-induced renal cell damage could lead to the development of therapeutic strategies for preventing the development of various kidney disease.

Recently, mammalian Silent information regulator 2 homolog (SIRT1) was identified as a cell survival factor against DNA damage [18–20]. SIRT1 functions as a class III histone deacetylase, with its deacetylase activity depending on intracellular NAD^+ concentrations [21,22]. This protein, through its deacetylase activity, regulates a wide array of cellular processes, including gene silencing, rDNA recombination, and life-span elongation under various stress conditions [20,23]. Calorie restriction and some polyphenols, known as antioxidants, are reported to increase deacetylase activity of SIRT1, resulting in mammalian cell survival and increased longevity [24,25]. Thus, SIRT1 can be regarded as a key regulator of cell defense and survival under various stress conditions including oxidative stress [20,26–29].

Based on these findings, we hypothesized that upregulation of SIRT1 prevents the development of glomerular kidney diseases through its cell survival effect. To test this hypothesis, we investigated the effects of SIRT1 on mesangial cell survival under oxidative stress.

Materials and methods

Reagents and antibodies

Dulbecco's modified Eagle's medium (DMEM) was purchased from GIBCO Life Technologies (Grand Island, NY). Anti-SIRT1 antibody was purchased from Upstate Cell Signaling (Lake Placid, NY). Anti-p53, anti-acetylated p53 (Lys382), anti-phospho p53 (Ser15), anti-cleaved caspase-3 (ASP175), anti-PARP (poly (ADP-ribose) polymerase, anti-phosho-p44/42 MAPK, anti-phospho-SAPK/JNK, anti-phospho-p38, anti-SAPK/JNK, and anti-p38 antibody were purchased from Cell Signaling Technology (Beverly, MA). Protein A/G agarose and anti-ERK2 antibody was purchased from Santa Cruz Biotechnology (Santa Cruz, CA), lipofectamine reagent from Invitrogen (Carlsbad, CA), and hydrogen peroxide (H_2O_2) from Kanko Chemical Co. (Tokyo, Japan). 4,6-Diamidino-2-phenylindole (DAPI) was purchased from Dojido (Kumamoto, Japan).

Cell culture

SV40-transformed murine mesangial cell line (MES 13 cells) was purchased from the American Type Culture Collection (Rockville, MD) and cultured at 37°C in 5% CO_2 atmosphere in DMEM supplemented with 10% fetal calf serum, penicillin (100 U/ml), streptomycin (100 μg/ml), L-glutamine (2 mM), and glucose (100 mg/dl). Cells were incubated with or without various concentrations of H_2O_2, as an oxidative stress, for the indicated periods.

Analysis and quantitation of apoptosis

Cells treated with or without H_2O_2 were stained with DAPI for 5 min at room temperature and then examined under a fluorescence microscope (BX61; Olympus, Tokyo, Japan). The staining was performed in triplicate for each group, and 30 random fields (containing about 600 nuclei) were studied in each replicate. Apoptosis was defined as nuclear condensation, and the results were expressed as percentage apoptotic cell number to total number of nuclei per field. Apoptosis was also confirmed by assessing the amounts of cleaved caspase-3 and cleaved PARP, as a biological marker of apoptosis, by immunoblot analysis.

Immunoprecipitation and immunoblot analysis

Cells were lysed in ice-cold lysis buffer (50 mM Hepes [pH 7.2], 150 mM NaCl, 1 mM EDTA, 20 mM NaF, 2 mM sodium orthovanadate, 1% (v/v) Triton X-100, 10% (v/v) glycerol, 1 mM PMSF, 10 mM sodium butyrate, and 1% aprotinin) and cleared by centrifugation at 15,000 rpm for 10 min at 4°C. Immunoprecipitations were carried out by adding the appropriate antibodies plus protein A/G Sepharose beads, followed by incubation at 4°C for 4 h. The immunoprecipitates were washed extensively, resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and transferred to polyvinylidene fluoride membranes (Immobilon, Bedford, MA). The membranes were incubated with the appropriate antibodies, washed, and incubated with horseradish peroxidase-coupled secondary antibodies (Amersham, Buckinghamshire, UK). After washing, the blots were visualized by using an enhanced chemiluminescence detection system (Perkin Elmer Life Science, Boston, MA).

RNA interference

The small interference RNA (sRNAi) for p53 (5′-AAUGAGGCCUUAGAGUUAAAGGAUG-3′) and control (5′-UUCUCCGAACGUGUCACGU-3′) were purchased from iGENE therapeutics (Tsukuba, Japan) and Qiagen (Tokyo, Japan), respectively. Cells on 6-well plates were transfected with *p53* sRNAi or control sRNAi using the TransIT-TKO reagent (Madison, WI). After 24 h, cells were treated with or without H_2O_2. The level of p53 expression was determined by immunoblot analysis.

Retroviral infection

The pBABE, pBABE-*SIRT1*, pSUPERretro, and pSUPERretro-*SIRT1* sRNAi vectors were kind gifts from L. Guarente (Massachusetts Institute of Technology, Cambridge, MA). Retroviral infection was performed as described previously [30]. HEK293T cells were transfected with pBABE, pBABE-*SIRT1*, pSUPERretro, and pSUPERretro-*SIRT1* sRNAi by using lipofectamine reagent. At 48 h after transfection, the media containing retroviruses were collected, centrifuged, and transferred to murine mesangial cells treated by polybrene