Biochemical and Biophysical Research Communications 437 (2013) 245–249

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

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

ERK1/2 regulates SIRT2 deacetylase activity

You Hee Choi [a,1], Hangun Kim [b,1], Sung Ho Lee [a], Yun-Hye Jin [a,*], Kwang Youl Lee [a,*]

[a] College of Pharmacy and Research Institute of Drug Development, Chonnam National University, Gwangju, South Korea
[b] College of Pharmacy and Research Institute of Life and Pharmaceutical Sciences, Sunchon National University, Sunchon, South Korea

ARTICLE INFO

Article history:
Received 11 June 2013
Available online 24 June 2013

Keywords:
SIRT2
ERK1/2
Protein level
Stability
Deacetylation

ABSTRACT

SIRT2 is a mammalian member of the Sirtuin family of NAD-dependent protein deacetylases. The function of SIRT2 can be modulated by post-translational modification. However, the precise molecular signaling mechanisms of SIRT2 and extracellular signal-regulated kinase (ERK)1/2 have not been correlated. We investigated the potential regulation of SIRT2 function by ERK1/2. ERK activation by the over-expression of constitutively active MEK increased protein levels and enhanced the stability of SIRT2. In contrast, U0126, an inhibitor of mitogen-activated kinase kinase, suppressed SIRT2 protein level. ERK1/2 interacted with SIRT2 exogenously and endogenously. Deacetylase activity of SIRT2 was up-regulated in an ERK1/2-mediated manner. These results suggest that ERK1/2 regulates SIRT2 by increasing the protein levels, stability and activity of SIRT2.

1. Introduction

There are seven Sir2 homologs in mammals (Sirt1–7). SIRT2 proteins are classified as class III histone deacetylases as they use NAD+ as a cofactor, unlike the classical class I and II histone deacetylases, HDAC I and II, which use zinc as a cofactor [1,2]. SIRT proteins also affect aging and metabolism, and are considered potential targets for pharmacologic interventions due to their vital functions [3]. SIRT1 can deacetylate non-histone proteins, including various transcription factors involved in growth regulation, stress responses, and endocrine signaling and it can suppress DNA damage-induced, p53-dependent apoptosis [4]. SIRT1 is the best characterized of the SIRT proteins; SIRT1 is involved in chromatin remodeling, gene silencing, and the DNA damage response. In contrast to SIRT1, little is known about the precise function of SIRT2. Human SIRT2 is a cytoplasmic protein [5] and a mammalian member of the Sirtuin family of NAD-dependent protein deacetylases [1,6]. It deacetylates lysine-40 of α-tubulin and co-localizes with microtubules and HDAC6 [7–9]. Foxo1 and Foxo3 are regulated by SIRT1 or SIRT2-mediated ubiquitination and degradation [10,11]. SIRT2 regulates nuclear factor-kappa B-dependent gene expression by the deacetylation of p65 Lys310 [12]. SIRT2-mediated protein deacetylation is an emerging key regulator in brain physiology and pathology [13].

Extracellular signal-regulated protein kinases 1 and 2 (Erk1/2) are members of the mitogen-activated protein kinase (MAPK) super-family that can mediate cell proliferation and apoptosis [14]. Extracellular stimuli such as growth factors, cytokines, mitogens, and hormones interact with a multimolecular complex of receptors such as receptor tyrosine kinases (RTKs), G protein-coupled receptors (GPCRs), and epidermal growth factor receptor (EGFR). MAPKs phosphorylate serine or threonine residues followed by a proline residue on many target substrates, and regulate cellular activities including gene expression, mitosis, embryogenesis, cell differentiation, movement, metabolism, and programmed cell death. At least four members of the MAPK family have been identified: ERK1/2, ERK5, and c-Jun-amino-terminal kinase (JNK). Activation of MAPK kinase (MEK)1/2 leads to the phosphorylation of threonine and tyrosine residues of ERK1/2 via the Thr-Glu-Tyr (TEY) recognition motif. ERK1 (44 kDa) and ERK2 (42 kDa) are homologous isoforms that share the same substrate specificities in vitro.

We have recently reported that SIRT2 interacts with 14-3-3 β/γ proteins and their interaction represents a novel negative regulatory mechanism for the function of p53 beside the well-characterized Mdm2-mediated repression [15]. Acetylation of SIRT2 by p300 attenuates its deacetylase activity [16]. SIRT2 down-regulation in HeLa cells can induce p53 accumulation via p38 MAPK activation-dependent p300 decrease, eventually leading to apoptosis [17]. Actually, SIRT1 and SIRT2 regulate the function of p53 through deacetylation. SIRT inhibitors induce cell death and p53 acetylation through targeting both SIRT1 and SIRT2 [18]. The deacetylation of p53 by SIRT1 decreases during protein kinase CKII downregulation-mediated cellular senescence [19]. SIRT1

* Corresponding authors. Fax: +82 62 530 2949 (Y.-H. Jin), +82 62 530 2939 (K.Y. Lee).
E-mail addresses: jinyune@hanmail.net (Y.-H. Jin), kwanglee@chonnam.ac.kr (K.Y. Lee).
[1] These authors contributed equally to the study.

0006-291X/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2013.06.053

activation by resveratrol ameliorates cisplatin-induced renal injury through deacetylation of p53. p53 rescue through HDM2 antagonism suppresses melanoma growth and potentiates MEK inhibition [20]. The Raf/MEK/ERK pathway regulates the subcellular localization of p53 and the relative contribution of transcription-dependent and transcription-independent pathways in p53-mediated apoptosis [21]. SIRT2 regulates the signaling mechanism of p53. However, the precise regulatory mechanism between histone deacetylase, SIRT2, and ERK1/2 function is unclear.

In this study, we examined whether ERK1/2 signaling is involved in the regulation of the histone deacetylase in SIRT2 function. We found that SIRT2 is regulated by post-translational modification and that ERK1/2 activation by constitutively active MEK increased protein levels and the stability of SIRT2, On the contrary, U0126, an inhibitor of MEK, decreased protein levels of SIRT2. Also, constitutively active MEK interacts with SIRT2 in exogenous and endogenous. Finally, the deacetylase activity of SIRT2 was up-regulated by ERK1/2 signaling. These results suggest that ERK1/2 regulates the SIRT2 through increased protein levels, stability, and deacetylase activity of SIRT2.

2. Materials and methods

2.1. Plasmids, antibodies, and reagents

Myc-tagged or GFP-tagged SIRT2 wild type was constructed in a cytomegalovirus promoter-derived mammalian expression vector (pCS4-Myc or green fluorescence protein GFP), MEKEE (constitutively active form) or MEKAA (dominant negative form). For inhibition of MEK activity, 10 mM stock solution of U0126 (Cat #662005; Calbiochem), was used at 10 μM final concentration. Antibodies against Myc (9E10; Roche Applied Science), GFP (G1544; Santa Cruz Biotechnology), SIRT2 (H-95; Santa Cruz Biotechnology), α-Tubulin (B-5-1-2, Sigma–Aldrich), acetylated-tubulin (Sigma–Aldrich), MEK1/2 (Cell Signaling Technologies), and ERK1/2 (Santa Cruz Biotechnology) were used.

2.2. Cell culture and transient transfection

HEK 293 human embryonic kidney epithelial cells were cultured at 37 °C and 5% CO_2 in Dulbecco's Minimal Essential Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) with 100 units/ml penicillin and 100 μg/ml streptomycin. DMEM, FBS, and antibiotics were purchased from Invitrogen. Transient transfection was performed using the Effectene (QIAGEN) or the calcium phosphate-mediated method. Total amounts of transfected plasmids in each group were equalized by the addition of an empty vector.

2.3. Immunoblotting and immunoprecipitation

For immunoblotting, transfected HEK 293 or C2C12 cells were harvested after washing with ice-cold phosphate buffered saline (PBS) and lysed in an ice-cold lysis buffer [25 mM HEPES (pH 7.5), 150 mM NaCl, 1% NP-40, 0.25% sodium deoxycholate, 10% glycerol, 25 mM sodium fluoride (NaF), 1 mM EDTA, 1 mM Na_3VO_4, 250 μM phenylmethylsulfonyl fluoride (PMSF), 10 μg/ml leupeptin, and 10 μg/ml aprotinin]. After centrifugation, supernatants containing 30 μg of total protein were subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) and the resolved proteins were transferred to a polyvinylidene fluoride (PVDF) membrane. For immunoprecipitaion, the supernatants of cell lysates were incubated with appropriate antibodies and protein A or G-Sepharose beads. Lysate supernatants or immunoprecipitated proteins were electrophoretically resolved and transferred to PVDF membranes. Proteins were visualized using appropriate primary antibody followed by horseradish peroxidase-coupled secondary antibody (Amersham Biosciences). Blots were developed with the enhanced chemiluminescence (ECL) solution (Amersham Biosciences). Signals were detected and analyzed by a LAS4000 luminescent image analyzer (Fuji Photo Film).

2.4. Protein stability assay

HEK 293 cells were co-transfected with Myc-tagged Sirt2, MEKEE, or MEKAA expression vectors. After 24 h, cells were exposed to fresh medium. Transfected cells were incubated at defined times and treated with 40 μM cycloheximide (CHX) and harvested with lysis buffer as described above. The protein levels were analyzed by immunoblotting using the anti-Myc antibody.

2.5. In vitro tubulin deacetylation assay

HEK 293 cells transfected with Myc-Sirt2, MEKEE, or MEKAA were and treated with U0126. After 2 days, the transfected cells were harvested after washing with ice-cold PBS and lysed in an ice-cold lysis buffer [25 mM HEPES (pH 7.5), 150 mM NaCl, 1% NP-40, 0.25% sodium deoxycholate, 10% glycerol, 25 mM NaF, 1 mM EDTA, 1 mM Na_3VO_4, 250 μM PMSF, 10 μg/ml leupeptin, and 10 μg/ml aprotinin]. After centrifugation, supernatants containing 30 μg of total protein were subjected to SDS–PAGE and the resolved proteins were transferred to a PVDF membrane. Acetylated tubulin was visualized by Western blotting using antisera specific for acetylated tubulin [6].

3. Results

3.1. SIRT2 expression is affected by ERK1/2 activation

To investigate the role of the histone deacetylase SIRT2 and ERK1/2, we first examined whether ERK1/2 affects the protein levels of SIRT2. HEK 293 cells were transfected with Sirt2, the ERK1/2 upstream kinase-MEKEE (constitutively active form) and MEKAA (dominant negative form), and were treated with the MEK inhibitor U0126. The protein levels of SIRT2 were compared by immunoblotting. The exogenous and endogenous protein levels of SIRT2 were markedly increased by increasing amounts of MEKEE (Fig. 1A) and strongly decreased by increasing amounts of MEKAA (Fig. 1B). U0126-mediated inhibition of MEK decreased the protein levels of SIRT2 (Fig. 1C). Increased protein levels of SIRT2 by MEKEE were decreased by U0126 (Fig. 1D). These results suggested that the ERK1/2 pathway regulates the protein levels of the SIRT2.

3.2. ERK1/2 enhances SIRT2 stability

ERK1/2 may regulate the expression of SIRT2 at the level of transcription, translation or protein stability. We confirmed that the protein levels of SIRT2 were increased by the ERK1/2 upstream kinase, MEK (Fig. 1). To identify the molecular mechanism for ERK1/2-induced increase of SIRT2 protein levels, we examined the influence of ERK1/2 on SIRT2 stability using cycloheximide. HEK 293 cells were transfected with Sirt2 with or without the ERK1/2 upstream kinases MEKEE or MEKAA. Transfected cells were treated with 40 μM cycloheximide for the indicated times and harvested. The protein levels of SIRT2 were determined by Western blotting. SIRT2 protein was degraded in the absence of ERK1/2 with a half-life of about 8 h. However, ERK1/2 significantly blocked SIRT2 degradation and prolonged the half-life of SIRT2 protein (Fig. 2). ERK1/2 clearly extended the half-life of the SIRT2 protein and enhanced the accumulation of SIRT2. These results suggest

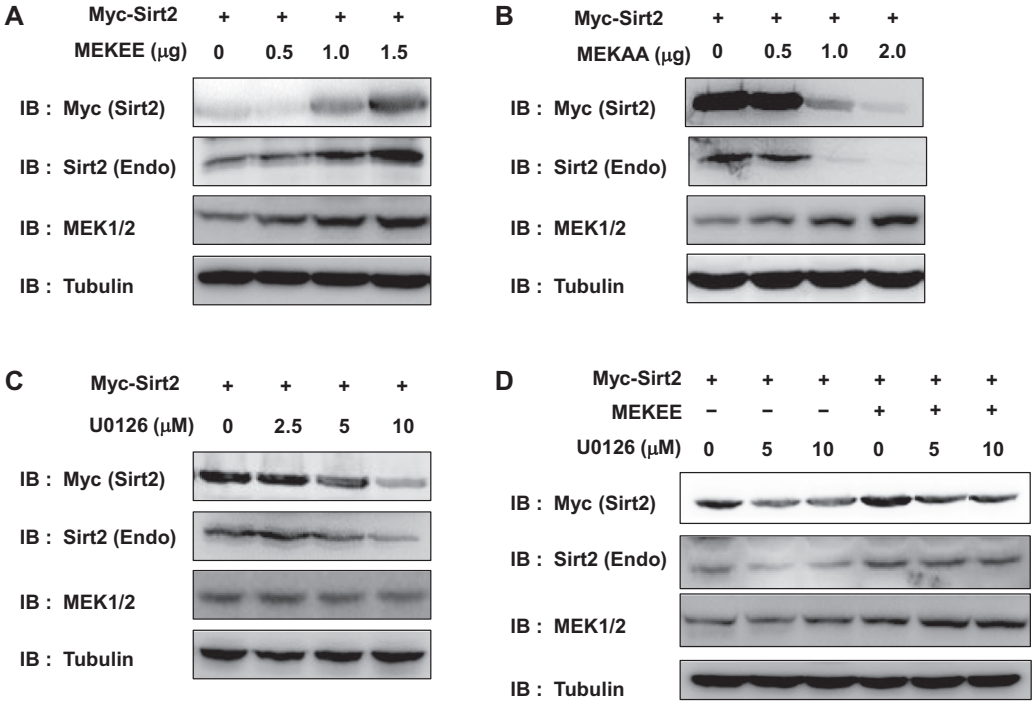

Fig. 1. ERK1/2 increases expression of SIRT2. (A and B) For exogenous and endogenous protein levels, HEK 293 cells were transfected with Myc-tagged Sirt2 (IB: Myc (Sirt2)), the ERK1/2 upstream kinases, MEKEE (active form, IB: MEK1/2), MEKAA (dominant negative form, IB: MEK1/2) expression vectors, or a control vector. Tubulin was used as a loading control (IB: Tubulin). Protein levels were determined by immunoblotting using anti-Myc or SIRT2 antibody. (C) HEK 293 cells were transfected with Myc-tagged Sirt2 (IB: Myc or Sirt2) for 24 h and treated with the MEK inhibitors U0126 (2.5, 5 and 10 μM), or dimethylsulfoxide (DMSO) as a control for 24 h. Tubulin was used as a loading control (IB: Tubulin). Protein levels were determined by immunoblotting using anti-Myc or SIRT2 antibody. (D) HEK 293 cells were transfected with Myc-tagged Sirt2 (IB: Myc or Sirt2) and MEKEE (active form, IB: MEK1/2) for 24 h and treated with MEK inhibitors, U0126 (5 and 10 μM) or DMSO for 24 h. Tubulin was used as a loading control (IB: Tubulin). Protein levels were determined by immunoblotting using anti-Myc or SIRT2 antibody.

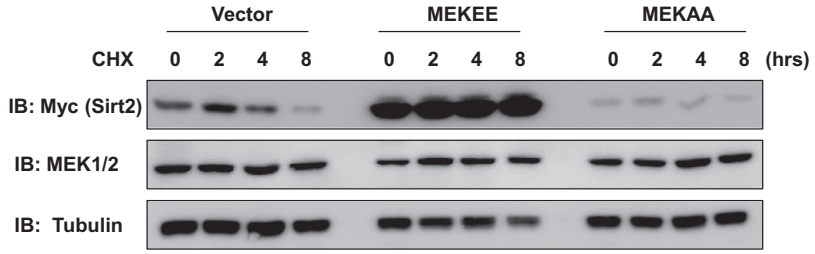

Fig. 2. Protein stability of SIRT2 is markedly increased by ERK1/2. (A) HEK 293 cells were co-transfected with Myc-tagged Sirt2, ERK1/2 upstream kinase active (MEKEE), or dominant negative (MEKAA) and treated with cycloheximide (40 μM). After 24 h, transfected cells were harvested at the indicated times.

that ERK1/2 signaling is critical for the maintenance of SIRT2 stability.

3.3. ERK1/2 interacts with SIRT2

Given the results above, we asked whether SIRT2 might interact with ERK1/2. HEK 293 cells were transfected with GFP-coupled Sirt2, the ERK1/2 upstream kinase MEKEE, or MEKAA, and immunoprecipitation was performed. SIRT2 bound to ERK1/2 (Fig. 3A). Immunoprecipitation was also done to assess the interaction between SIRT2 and endogenous ERK1/2. We determined that SIRT2 bound to endogenous ERK1/2 (Fig. 3B and C). These results indicated that ERK1/2 interacts with SIRT2.

3.4. ERK1/2 regulates SIRT2 deacetylase activity

Acetylation of SIRT2 by p300 attenuates its deacetylase activity [16,22]. SIRT inhibitors induce cell death and p53 acetylation through targeting of both SIRT1 and SIRT2 [18]. SIRT2 regulates adipocyte differentiation through FoxO1 acetylation/deacetylation [11]. We examined whether ERK1/2 could affect the deacetylase activity of SIRT2. The deacetylase activity of SIRT2 was up-regulated by the mediation of the ERK1/2 upstream kinase, MEK (Fig. 4). The deacetylase activity of SIRT2 was up-regulated by the mediation of MEKEE (constitutively active form) (Fig. 4A) and was down-regulated by the mediation of MEKAA (Fig. 4B). Also, the deacetylase activity of SIRT2 was decreased by U0126, a specific inhibitor of MEK (Fig. 4C). HEK 293 cells were transfected with Sirt2, the ERK1/2 upstream kinase MEKEE, and were treated with U0126. MEKEE increased the protein levels and deacetylase activity of SIRT2. But, these increases were blocked by U0126 (Fig. 4D). These results suggested that ERK1/2 increases the deacetylase activity of SIRT2 through regulation of the protein levels of SIRT2.

4. Discussion

SIRT proteins play important roles in the survival and drug resistance of tumor cells, especially during chemotherapy. Sirtuins

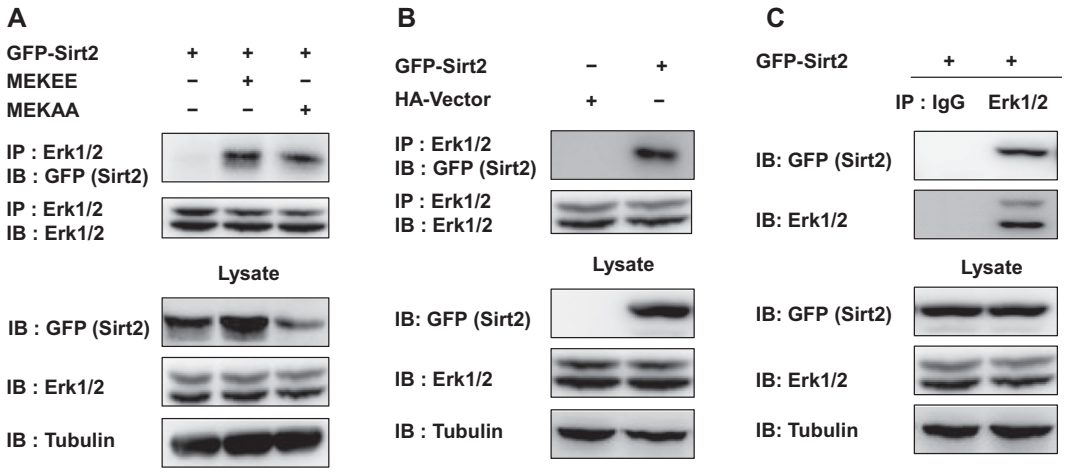

Fig. 3. SIRT2 interacts with ERK1/2. (A) HEK 293 cells were transfected with Myc-tagged Sirt2, ERK1/2 upstream kinase active (MEKEE) and dominant negative (MEKAA). The interaction between SIRT2 and ERK1/2 was determined by anti-ERK1/2 immunoprecipitation [IP: ERK1/2] by Western blot analysis using an anti-GFP antibody [IB: GFP (Sirt2)]. (B) HEK 293 cells were transfected with GFP-tagged Sirt2 and HA-tagged vector. For the endogenous interaction of SIRT2 and ERK1/2, immunoprecipitation was done using Ekr1/2 antibody [IP: ERK1/2] and Western blotting with GFP antibody [IB: GFP (Sirt2)]. (C) GFP-tagged Sirt2 was transfected to examine whether the binding of SIRT2 and ERK1/2 was endogenous. The binding of SIRT2 in HEK 293 cells was performed by immunoprecipitation using IgG or ERK1/2 antibody [IP: IgG and IP: ERK1/2] and Western blotting with GFP antibody [IB: GFP (Sirt2)].

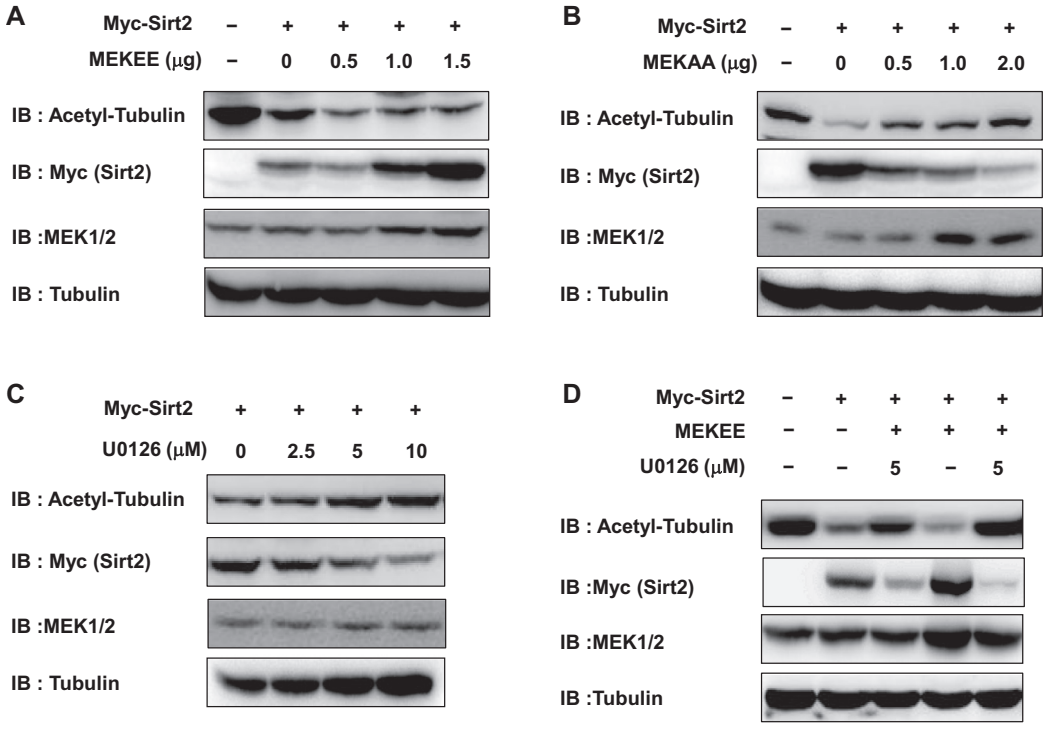

Fig. 4. ERK1/2 increases SIRT2 deacetylase activity. For the tubulin deacetylation assay, cell lysates were harvested after washing with ice-cold PBS and lysed in an ice-cold lysis buffer. After centrifugation, supernatants containing 30 μg of total protein were subjected to SDS–PAGE. Proteins were transferred to a PVDF membrane. (A and B) HEK293 cells were transfected with indicated combination of Myc-tagged Sirt2 [IB: Myc (Sirt2)], ERK1/2 upstream kinase active form, (MEKEE) and dominant negative (MEKAA) (IB: MEK1/2) or a control vector. Acetylated tubulin was visualized by Western blotting using specific antisera for acetylated tubulin (IB: Acetyl-Tubulin) [6]. The levels of α-tubulin were used as a loading control (IB: Tubulin). (C) HEK 293 cells were transfected with Myc-tagged Sirt2 [IB: Myc (Sirt2)] for 24 h and treated U0126 (2.5, 5 and 10 μM) or DMSO for 24 h. Acetylated tubulin was visualized by Western blotting using specific antisera for acetylated tubulin (IB: Acetyl-Tubulin) [6]. The levels of α-tubulin were used as a loading control (IB: Tubulin). (D) HEK 293 cells were co-transfected with Myc-tagged Sirt2 [IB: Myc (Sirt2)], ERK1/2 upstream kinase active form, (MEKEE) (IB: MEK1/2) and treated with U0126 (5 μM) or DMSO for 24 h. Acetylated tubulin was visualized by Western blotting using specific antisera for acetylated tubulin (IB: Acetyl-Tubulin) [6]. The levels of α-tubulin were used as a loading control (IB: Tubulin).

are NAD-positive–dependent class III HDACs that share extensive homologies with the yeast HDAC Silent Information Regulator 2 (Sir2). SIRT2 down-regulation in HeLa cells induced p53 accumulation via p38 MAPK activation-dependent p300 decrease, eventually leading to apoptosis [17]. p53 rescue through HDM2 antagonism suppresses melanoma growth and potentiates MEK inhibition [20]. The Raf/MEK/ERK pathway regulates the subcellular localization of p53 and the relative contribution of transcription-dependent and transcription-independent pathways in p53-mediated apoptosis. We thus surmised that SIRT2-mediated deacetylation

of p53 is regulated by the signaling of ERK1/2. Our results suggest correlations between SIRT2 and ERK1/2 signaling. ERK1/2 signaling is important in regulating the histone deacetylase activity of SIRT2. ERK1/2 interacts with SIRT2 exogenously and endogenously, and the resulting ERK1/2 activation increases protein levels and stability of SIRT2. The deacetylase activity of SIRT2 is up-regulated by ERK1/2 signaling. So, we confirmed that ERK1/2 regulates SIRT2 by increasing the protein level, stability, and deacetylase activity of SIRT2.

Sirtuins are involved in many physiological and pathological processes, and their activity has been associated with different human diseases, including cancer. SIRT1 and SIRT2 may have roles in the development of cancer. SIRT1 can activate stress defense and DNA repair mechanisms, allowing the preservation of the genomic integrity [23]. Conversely, it has also been shown that SIRT1 can enhance tumor growth and promote cell survival in response to stress and drug resistance. Nevertheless, SIRT1 is up-regulated in a spectrum of cancers, including lymphomas, leukemia and soft tissue sarcomas, prostate cancer, and lung and colon carcinomas [24,25]. Tumors with high levels of SIRT2 are refractory to chemotherapy, especially microtubule poisons [26]. The dual role of SIRTs in modulating the acetylation of tumor suppressor proteins and chromatin makes them attractive therapeutic targets for anticancer drug development [23]. Especially two Sirtuin members, SIRT1 and SIRT2, antagonize p53-dependent transcriptional activation and apoptosis in response to DNA damage by catalyzing p53 deacetylation. The findings that SIRT1 levels are increased in a number of tumors highlights the oncogenic role of Sirtuins, in particular, in the down-modulation of p53 tumor suppressor activity. Cancers carrying wild-type p53 protein deregulate activity by other mechanisms.

Inhibition of SIRT1 and SIRT2, aimed at restoring wild type p53 transcriptional activity in tumors that retain the ability to express normal p53, might represent a valid therapeutic cancer approach specially when combined with standard therapies.

Acknowledgment

This work was supported by a Grant (2011-0010844) from the National Research Foundation of Korea to Y.H. Jin.

References

[1] G. Blander, L. Guarente, The Sir2 family of protein deacetylases, Annu. Rev. Biochem. 73 (2004) 417–435.
[2] M. Hernick, C.A. Fierke, Zinc hydrolases: the mechanisms of zinc-dependent deacetylases, Arch. Biochem. Biophys. 433 (2005) 71–84.
[3] R. Marmorstein, Structure and chemistry of the Sir2 family of NAD+-dependent histone/protein deactylases, Biochem. Soc. Trans. 32 (2004) 904–909.
[4] A. Vaquero, M. Scher, D. Lee, H. Erdjument-Bromage, P. Tempst, D. Reinberg, Human SirT1 interacts with histone H1 and promotes formation of facultative heterochromatin, Mol. Cell 16 (2004) 93–105.
[5] G. Afshar, J.P. Murnane, Characterization of a human gene with sequence homology to *Saccharomyces cerevisiae* SIR2, Gene 234 (1999) 161–168.
[6] B.J. North, B.L. Marshall, M.T. Borra, J.M. Denu, E. Verdin, The human Sir2 ortholog, SIRT2, is an NAD+-dependent tubulin deacetylase, Mol. Cell 11 (2003) 437–444.
[7] C.M. Southwood, M. Peppi, S. Dryden, M.A. Tainsky, A. Gow, Microtubule deacetylases, SirT2 and HDAC6, in the nervous system, Neurochem. Res. 32 (2007) 187–195.
[8] Q. Zuo, W. Wu, X. Li, L. Zhao, W. Chen, HDAC6 and SIRT2 promote bladder cancer cell migration and invasion by targeting cortactin, Oncol. Rep. 27 (2012) 819–824.
[9] F. Nahhas, S.C. Dryden, J. Abrams, M.A. Tainsky, Mutations in SIRT2 deacetylase which regulate enzymatic activity but not its interaction with HDAC6 and tubulin, Mol. Cell. Biochem. 303 (2007) 221–230.
[10] F. Wang, C.H. Chan, K. Chen, X. Guan, H.K. Lin, Q. Tong, Deacetylation of FOXO3 by SIRT1 or SIRT2 leads to Skp2-mediated FOXO3 ubiquitination and degradation, Oncogene 31 (2012) 1546–1557.
[11] E. Jing, S. Gesta, C.R. Kahn, SIRT2 regulates adipocyte differentiation through FoxO1 acetylation/deacetylation, Cell Metab. 6 (2007) 105–114.
[12] K.M. Rothgiesser, S. Erener, S. Waibel, B. Luscher, M.O. Hottiger, SIRT2 regulates NF-kappaB dependent gene expression through deacetylation of p65 Lys310, J. Cell Sci. 123 (2010) 4251–4258.
[13] K. Harting, B. Knoll, SIRT2-mediated protein deacetylation: an emerging key regulator in brain physiology and pathology, Eur. J. Cell Biol. 89 (2010) 262–269.
[14] V. Todorovic, B.V. Desai, M.J. Patterson, E.V. Amargo, A.D. Dubash, T. Yin, J.C. Jones, K.J. Green, Plakoglobin regulates cell motility through Rho- and fibronectin-dependent Src signaling, J. Cell Sci. 123 (2010) 3576–3586.
[15] Y.H. Jin, Y.J. Kim, D.W. Kim, K.H. Baek, B.Y. Kang, C.Y. Yeo, K.Y. Lee, Sirt2 interacts with 14–3-3 beta/gamma and down-regulates the activity of p53, Biochem. Biophys. Res. Commun. 368 (2008) 690–695.
[16] Y. Han, Y.H. Jin, Y.J. Kim, B.Y. Kang, H.J. Choi, D.W. Kim, C.Y. Yeo, K.Y. Lee, Acetylation of Sirt2 by p300 attenuates its deacetylase activity, Biochem. Biophys. Res. Commun. 375 (2008) 576–580.
[17] Y. Li, H. Matsumori, Y. Nakayama, M. Osaki, H. Kojima, A. Kurimasa, H. Ito, S. Mori, M. Katoh, M. Oshimura, T. Inoue, SIRT2 down-regulation in HeLa can induce p53 accumulation via p38 MAPK activation-dependent p300 decrease, eventually leading to apoptosis, Genes Cells 16 (2011) 34–45.
[18] B. Peck, C.Y. Chen, K.K. Ho, P. Di Fruscia, S.S. Myatt, R.C. Coombes, M.J. Fuchter, C.D. Hsiao, E.W. Lam, SIRT inhibitors induce cell death and p53 acetylation through targeting both SIRT1 and SIRT2, Mol. Cancer Ther. 9 (2010) 844–855.
[19] S.Y. Jang, S.Y. Kim, Y.S. Bae, P53 deacetylation by SIRT1 decreases during protein kinase CKII downregulation-mediated cellular senescence, FEBS Lett. 585 (2011) 3360–3366.
[20] Z. Ji, C.N. Njauw, M. Taylor, V. Neel, K.T. Flaherty, H. Tsao, P53 rescue through HDM2 antagonism suppresses melanoma growth and potentiates MEK inhibition, J. Invest. Dermatol. 132 (2012) 356–364.
[21] K. Kojima, M. Konopleva, I.J. Samudio, V. Ruvolo, M. Andreeff, Mitogen-activated protein kinase kinase inhibition enhances nuclear proapoptotic function of p53 in acute myelogenous leukemia cells, Cancer Res. 67 (2007) 3210–3219.
[22] J. Shin, D. Zhang, D. Chen, Reversible acetylation of metabolic enzymes celebration: SIRT2 and p300 join the party, Mol. Cell 43 (2011) 3–5.
[23] F.J. Alcain, J.M. Villalba, Sirtuin inhibitors, Expert Opin. Ther. Pat. 19 (2009) 283–294.
[24] M.F. Fraga, M. Esteller, Epigenetics and aging: the targets and the marks, Trends Genet. 23 (2007) 413–418.
[25] C.S. Lim, Human SIRT1: a potential biomarker for tumorigenesis?, Cell Biol Int. 31 (2007) 636–637.
[26] N. Matsushita, Y. Takami, M. Kimura, S. Tachiiri, M. Ishiai, T. Nakayama, M. Takata, Role of NAD-dependent deacetylases SIRT1 and SIRT2 in radiation and cisplatin-induced cell death in vertebrate cells, Genes Cells 10 (2005) 321–332.