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Src regulates the activity of SIRT2

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ABSTRACT

SIRT2 is a mammalian member of the Sirtuin family of NAD$^+$-dependent protein deacetylases. The tyrosine kinase Src is involved in a variety of cellular signaling pathways, leading to the induction of DNA synthesis, cell proliferation, and cytoskeletal reorganization. The function of SIRT2 is modulated by post-translational modifications; however, the precise molecular signaling mechanism of SIRT2 through interactions with c-Src has not yet been established. In this study, we investigated the potential regulation of SIRT2 function by c-Src. We found that the protein levels of SIRT2 were decreased by c-Src, and subsequently rescued by the addition of a Src specific inhibitor, SU6656, or by *siRNA*-mediated knockdown of c-Src. The c-Src interacts with and phosphorylates SIRT2 at Tyr104. c-Src also showed the ability to regulate the deacetylation activity of SIRT2. Investigation on the phosphorylation of SIRT2 suggested that this was the method of c-Src-mediated SIRT2 regulation.

1. Introduction

SIR2 proteins are classified as class III histone deacetylases by their use of 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]. Seven SIR2 homologs have been identified in mammals, SIRT1–7. SIRT proteins affect aging and metabolism, and are often considered as potential targets for pharmacologic intervention due to the importance of their function [3]. Among them, SIRT1 is the best characterized; recent studies have shown that SIRT1 is involved in chromatin remodeling, gene silencing and DNA damage response. SIRT1 can deacetylate non-histone proteins, including various transcription factors involved in growth regulation, stress responses, and endocrine signaling [4]. It can also suppress p53-dependent apoptosis, induced by DNA damage [5]. In contrast to SIRT1, little is known about the precise function of SIRT2.

Human SIRT2 is a type of cytoplasmic protein [6]. It is known to deacetylate lysine40 of α-tubulin, and to co-localize with microtubules and HDAC6. We recently reported that SIRT2 interacts with 14-3-3 β/γ proteins, and presented a novel negative regulatory mechanism for p53, in addition to the well-characterized Mdm2-mediated repression [7]. Acetylation of SIRT2 by p300 attenuates its deacetylase activity [8], and SIRT inhibitors have been shown to induce cell death and p53 acetylation through the targeting of both SIRT1 and SIRT2 [9]. SIRT2 has also been shown to regulate adipocyte differentiation through FoxO1 acetylation/deacetylation [10], and NF-κB dependent gene expression through deacetylation of Lys310 on p65 [11]. Down-regulation in HeLa can induce p53 accumulation via a p38 MAPK activation-dependent decrease in p300, eventually leading to apoptosis [12]. SIRT2 maintains genome integrity, and suppresses tumorigenesis [13]. A dynamic equilibrium between acetylation and deacetylation of proteins, such as transcription factors, can lead to the control of distinct patterns of transcription at different cellular stages. This equilibrium implies that acetyltransferases and deacetylases can act on the same gene regulatory loci, thereby affecting each other.

The Src family of tyrosine kinases is involved in a variety of cellular signaling pathways, leading to the induction of DNA synthesis, cell proliferation, and cytoskeletal reorganization [14]. Src tyrosine kinase activity is tightly regulated, and its deregulation leads to constitutive activation and cellular transformation [15]. Src phosphorylates tyrosine (Y) residues; this phosphorylation process is a critical regulator of numerous cellular processes including cell proliferation, migration, differentiation, survival signaling, and energy metabolism [16].

The regulation of gene expression can be controlled by regulation of transcription factors, which are tightly regulated by post-translational modifications, such as phosphorylation, acetylation, and ubiquitination [17]. The function of SIRT2 is also modulated by post-translational modifications. Cyclin-dependent kinase 1

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phosphorylates SIRT2, leading to mitotic regulation, and Erk1/2 also regulate SIRT2 activity via phosphorylation [18,19]. However, the effects of post-translational modifications on the molecular signaling mechanisms of SIRT2 by c-Src have not yet been characterized.

In this study, we examined whether Src proteins play a role in the regulation of SIRT2 function. We found that SIRT2 is regulated by post-translational modifications. c-Src decreased the protein level and stability of SIRT2. c-Src interacts with and phosphorylates SIRT2. The activity of SIRT2 was decreased by the activation of c-Src. These results provide evidence that the activity of SIRT2 is regulated by c-Src through post-translational modifications.

2. Materials and methods

2.1. Plasmids, antibodies and reagents

Myc-tagged or GFP-tagged SIRT2 wild type and HA-tagged p53 was constructed in a CMV promoter-derived mammalian expression vector (pCS4). GFP-tagged SIRT2 Y104F (phosphorylation site deletion mutant) was generated by PCR-based mutagenesis and confirmed by DNA sequencing. Chicken HA-tagged c-Src WT, HA-tagged-c-Src-KD (a kinase-inactive mutant, K295M) plasmids were generously provided by Dr. N. Kim (Chonnam National University, Korea) [20]. For knockdown of c-Src, oligonucleotides targeting the following sequences were synthesized: sense-(c-Src) 5′-GAT CCC CCA AGA GCA AGC CCA AGG ATT TCA AGA GAA TCC TTG GGC TTG CTC TTG TTT TTG GAA A-3′; and antisense-(c-Src) 5′-AGC TTT TCC AAA AAC AAG AGC AAG CCC AAG GAT TCT CTT GAA ATC CTT GGG CTT GCT CTT GGG G-3′. Antibodies against Myc (9E10, Roche Applied Science), HA (12CA5, Roche Applied Science), GFP (G1544, Santa Cruz Biotechnology), SIRT2 (H-95, Santa Cruz Biotechnology), c-Src (SC-19, Santa Cruz Biotechnology), α-tubulin (B-5-1-2, Sigma–Aldrich), acetylated-tubulin (Sigma–Aldrich), and 4G10 (Upstate Biotech Millipore) were used. SU6656 (Src inhibitor) was purchased from Calbiochem.

2.2. Cell culture and transient transfection

HEK 293 and MCF-7 cell lines were cultured at 37 °C, 5% CO_2, in Dulbecco's Minimal Essential Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 units/ml penicillin, and 100 μg/ml streptomycin. DMEM, FBS and antibiotics were purchased from Invitrogen. Transient transfection was performed using polyethyleneimine (Polysciences, Inc.) or calcium phosphate-mediated method. Total amounts of transfected plasmids in each group were equalized by the addition of an empty vector.

2.3. Protein stability assay

HEK 293 cells were co-transfected with Myc-tagged SIRT2, HA-tagged c-Src WT, HA-tagged-c-Src-KD (a kinase-inactive mutant, K295M) plasmids. Media were refreshed after 24 h. Transfected cells were incubated for the indicated time points, then treated with 40 μM cycloheximide (CHX) and harvested with lysis buffer, as described below. The protein levels were analyzed by immunoblotting using the anti-Myc antibody.

2.4. Immunoblotting (IB) and immunoprecipitation (IP)

For immunoblotting, cells from transfected HEK 293 were harvested after washing with ice-cold PBS, then lysed in 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 then the proteins were transferred to a PVDF membrane. For immunoprecipitation, the supernatants of the cell lysates were incubated with appropriate antibodies and protein A or G-Sepharose beads. The lysate supernatants or immunoprecipitated proteins were resolved by SDS–PAGE and transferred to PVDF membranes. Proteins were visualized using appropriate primary antibodies and horseradish peroxidase-coupled secondary antibodies (Amersham Biosciences) by immunoblotting, and were developed with enhanced chemiluminescence lighting (ECL) solution (Amersham Biosciences). Signals were detected and analyzed by an LAS4000 luminescent image analyzer (Fuji Photo Film Co., Tokyo, Japan).

2.5. Luciferase reporter assay

MCF-7 Cells were seeded on 24-well plates the day before transfection. Cells were transfected with CMV promoter-driven β-galactosidase reporter (pCMV-β-gal), p53 luciferase reporter (p53-Luc) and indicated combinations of expression plasmids. 36 h later, luciferase activities were measured using Luciferase Reporter Assay Kit (Promega, E1501), using a luminometer and normalized with corresponding β-galactosidase activities for transfection efficiency. Experiments were performed in triplicate and repeated at least three times. Average and S.D. of representative experiments are shown.

2.6. RT-PCR analysis

Total cellular RNA was prepared using TRIzol reagent, (Life Technologies) according to the manufacturer's instructions. Random hexamer-primed cDNA was synthesized from 1 μg of total RNA using the SuperScript III First-Strand Synthesis System (Life Technologies). The following conditions were used for amplification by PCR: initial denaturation at 94 °C for 1 min, followed by 25–28 cycles including denaturation at 94 °C for 1 min, annealing at a temperature optimized for each primer pair for 1 min, and extension at 72 °C for 1 min, with a final extension at 72 °C for 7 min. The following PCR primers were used: *p21* Forward 5′-AGA CAT GCC TAG ACA TGC CTA GAC ATG CCT AGA CAT GCC T-3′ and Reverse 5′-AGG CAT GTC TAG GCA TGT CTA GGC ATG TCT AGG CAT GTC T-3′; *BAX* Forward 5′-TTT GCT TCA GGG TTT CAT CC-3′ and Reverse 5′-CAG TTC AAG TTG CCG TCA GA-3′; *NOXA* Forward 5′-CTG GAA GTC GAG TGT GCT ACT-3′ and Reverse 5′-TCA GGT TCC TGA GCA GAA GAG-3′; *PUMA* Forward 5′-GCC CAG ACT GTG AAT CCT GT-3′ and Reverse 5′-TCC TCC CTC TTC CGA GAT TT-3′; *GAPDH* Forward 5′-ACC ACA GTC CAT GCC ATC AC-3′ and Reverse 5′-TCC ACC ACC CTG TTG CTG TA-3′.

2.7. In vitro tubulin deacetylation assay

For the tubulin deacetylation assay, HEK 293 cells transfected with Myc-SIRT2 and HA-tagged c-Src (WT) were harvested after washing with ice-cold PBS, then lysed in 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]. Supernatants containing 30 μg of total protein were subjected to SDS–PAGE after centrifugation. The proteins were transferred to a PVDF membrane, and acetylated tubulin was visualized by Western blotting using antisera specific for acetylated tubulin [21].

2.8. Statistical analysis

All experiments were performed with three independent replicate samples, and were repeated at least twice to give qualitatively

identical results. Results are expressed as mean ± standard error of the mean. Data were analyzed using the Student's *t*-test, and $p < 0.05$ was considered significant.

3. Results

3.1. c-Src affects the protein levels and stability of SIRT2

In order to understand the mechanism of SIRT2, we first examined whether c-Src had an effect on the protein levels of SIRT2. HEK 293 cells were transfected with SIRT2, c-Src WT or *siRNA c-Src* (*si-Src*), and the protein levels of SIRT2 were determined by Western blotting. The exogenous and endogenous protein levels of SIRT2 decreased with increasing amounts of c-Src WT (Fig. 1A), but expression was increased with increasing amounts of c-Src KD (a kinase-inactive mutant, K295M) (Fig. 1B). We next examined whether knockdown of c-Src by the specific *siRNA* (*si-Src*) also affected the protein levels of SIRT2. Src levels were decreased by *siRNA* (*si-Src*). The protein levels of SIRT2 that had been decreased by the transformed c-Src WT were restored by the addition of *siRNA c-Src* (*si-Src*) (Fig. 1C). In addition, protein levels of SIRT2 were also increased by the Src inhibitor, SU6656 (Fig. 1D). Since c-Src may regulate the expression of SIRT2 at the level of transcription, translation, or protein stability, the influence of c-Src on the stability of SIRT2 was examined using a translation inhibitor, cycloheximide (CHX), to identify the mechanism of regulation. Cells were incubated for the indicated time periods, In the absence of c-Scr WT, SIRT2 protein degradation showed a half-life of about 4 h. However, c-Src WT significantly blocked the SIRT2 stability, shortening the SIRT2 half-life, as shown in Fig. 1E. These results suggest that c-Src regulates the stability of SIRT2.

3.2. c-Src interacts with SIRT2

c-Src can modulate the function of target proteins through direct modifications, or through the modulation of upstream effectors. As shown above, the protein levels and stability of SIRT2 are negatively regulated by c-Src (Fig. 1). It is possible that this regulation is caused by the interaction of c-Src with SIRT2, so this possibility was investigated with immunoprecipitation assays of the expressed proteins in HEK 293 cells. Results showed that endogenous SIRT2 was bound to overexpressed c-Src WT (Fig. 2A, top panel). Immunoprecipitation performed to assess the interaction between exogenous SIRT2, overexpressed c-Src-WT, and c-Src KD. We revealed that c-Src bound to and regulated the expression levels of SIRT2 (Fig. 2B, top panel). These results indicated that c-Src interacts with SIRT2.

3.3. c-Src Tyr104 is responsible for the phosphorylation of SIRT2

We suggested that a protein–protein interaction between SIRT2 and c-Src is important for regulating the stability of SIRT2 (Fig. 2). Src phosphorylates tyrosine (Y) residues of target proteins. This phosphorylation process is a critical regulator of numerous cellular processes, including cell proliferation, migration, differentiation, survival signaling, and energy metabolism [16]. We next hypothe-

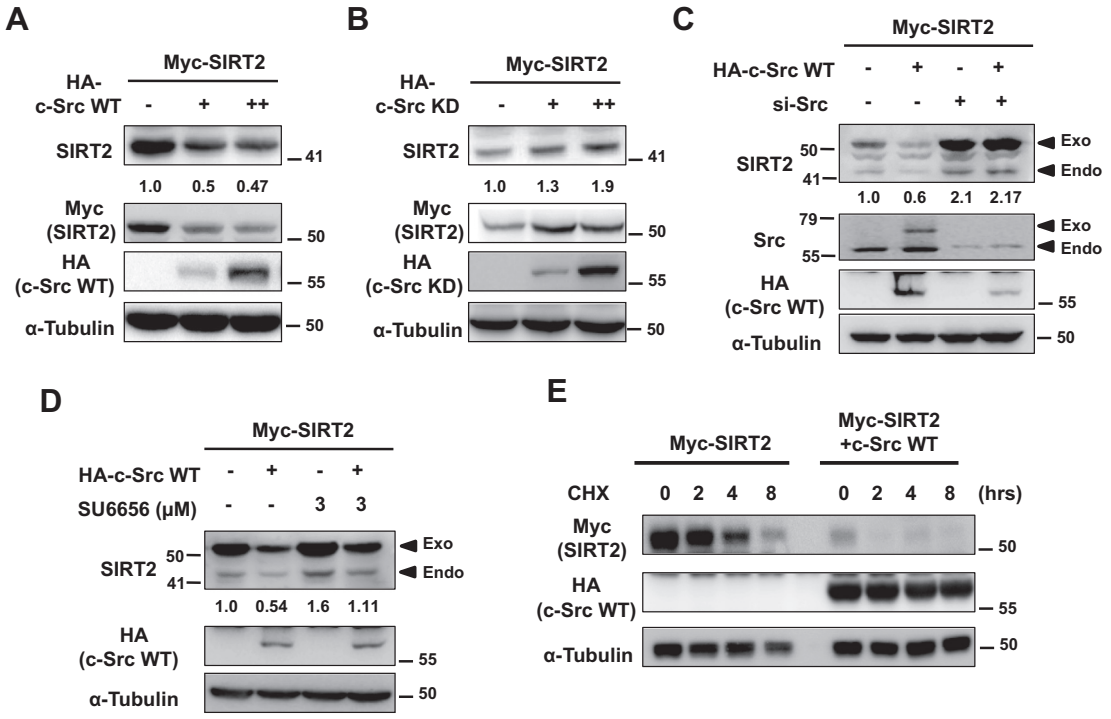

Fig. 1. c-Src reduces the expression levels and stability of SIRT2. (A) For protein levels of SIRT2 by overexpressed c-Src WT (wild type), HEK 293 cells were transfected with indicated Myc-tagged SIRT2, HA-tagged c-Src WT, or a control vector. Protein levels of the SIRT2 were determined by immunoblotting (IB) using anti-SIRT2 and anti-Myc antibody [SIRT2 or Myc (SIRT2)]. Tubulin was used as a loading control. (B) For protein levels of SIRT2 by overexpressed c-Src KD (a kinase-inactive mutant of c-Src, K295M), HEK 293 cells were transfected with indicated Myc-tagged SIRT2, HA-tagged c-Src KD, or a control vector. Protein levels of the SIRT2 were determined by immunoblotting (IB) using anti-SIRT2 and anti-Myc antibody [SIRT2 or Myc (SIRT2)]. Tubulin was used as a loading control. (C) For protein levels of SIRT2 by knockdown of c-Src by the specific *siRNA* (*si-Src*), HEK 293 cells were transfected with indicated Myc-tagged SIRT2, knockdown of Src (*si-Src*), or a control vector. Protein levels of the SIRT2 were determined by immunoblotting (IB) using anti-SIRT2 antibody. Tubulin was used as a loading control. (D) HEK 293 cells were transfected with Myc-tagged SIRT2, HA-tagged c-Src WT, and treated with c-Src inhibitors, SU6656 or vehicle as a control (DMSO) for 15 h. Protein levels of SIRT2 were determined by immunoblotting (IB) anti-SIRT2 antibody. Tubulin was used as a loading control. (E) HEK 293 cells were co-transfected with Myc-tagged SIRT2, HA-tagged c-Src WT, and treated with cycloheximide (40 μM). After 24 h, transfected cells were harvested at the indicated times point. Protein levels of SIRT2 were determined by immunoblotting (IB) using anti-Myc antibody [Myc (SIRT2)]. Tubulin was used as a loading control.

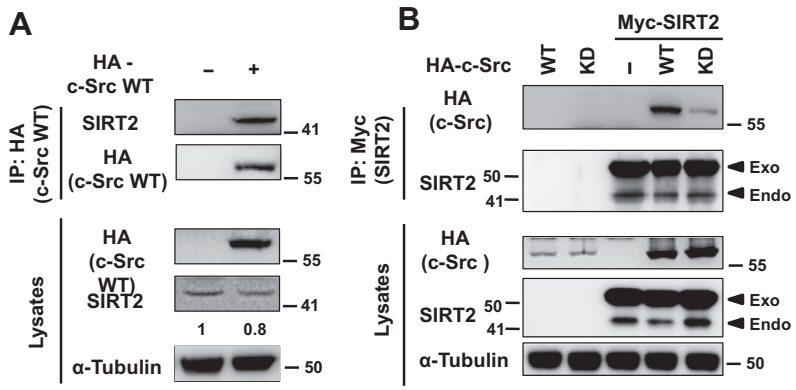

Fig. 2. c-Src interacts with SIRT2. (A) HEK 293 cells were transfected for 36 h with the combinations of indicated plasmids. The interaction between endogenous SIRT2 and overexpressed c-Src WT was analyzed by immunoprecipitation (IP) for c-Src using anti-HA antibody [IP: HA (c-Src WT)], followed by IB for SIRT2 (top panels). The levels of SIRT2 and c-Src WT in cell lysates were also compared (bottom panels). Tubulin was used as a loading control. (B) HEK 293 cells were transfected with Myc-tagged-SIRT2, HA-tagged-c-Src WT, and HA-tagged-c-Src KD. The interaction between exogenous SIRT2 and overexpressed c-Src was analyzed by IP for SIRT2 [IP: Myc (SIRT2)], followed by IB for c-Src [HA (c-Src)] (top panels).

sized that c-Src regulates the activity of SIRT2 via phosphorylation. HEK 293 cells were transfected with c-Src-WT, c-Src-KD expressing plasmid or control plasmid. SIRT2 was immunoprecipitated and immunoblotted using anti-phosphotyrosine, a 4G10 antibody. c-Src WT phosphorylated SIRT2, while the c-Src-KD showed abolishment of the tyrosine phosphorylation of SIRT2 (Fig. 3A, top panel). The widespread nature of protein phosphorylation/dephosphorylation underscores its key role in cell signaling metabolism, growth and differentiation. SIRT2 has one potential c-Src phosphorylation target site, at Tyr104 (http://scansite.mit.edu). We generated a tyrosine-to-phenylalanine substitution mutant. In the results shown above, the protein levels of SIRT2 were decreased by a c-Src-dependent mechanism (Fig. 1). However, expression levels of SIRT2 were not affected by transfection with c-Src-WT, or c-Src-KD in the SIRT2 Y104F substitution mutant (Fig. 3B, top panel). Phosphorylation by c-Src was greatly blocked by the mutation in SIRT2 Y104F, compared to that of the wild-type SIRT2 (Fig. 3C, top panel). These results indicate that c-Src-dependent regulation of SIRT2 protein level is dependent on the phosphorylation of Tyr104.

3.4. c-Src regulate the activity of SIRT2-induced the down-regulation of p53

In previous reports, SIRT inhibitors were shown to induce cell death and p53 acetylation by targeting SIRT2 [9], and acetylation of SIRT2 by p300 has been shown to attenuate SIRT2's deacetylase activity [8]. Therefore, we examined whether c-Src could also affect the activity of SIRT2. The activity of SIRT2 was attenuated by c-Src mediation (Fig. 4A, top panel). We decided to examine whether c-Src could affect p53 transcriptional activity via regulation of SIRT2. SIRT2 activity-mediated down-regulation of p53 transcriptional activity was attenuated by c-Src, likely because of the decrease in endogenous SIRT2 activity (Fig. 4B). Sirtuins are involved in gene silencing, cell cycle control, apoptosis and even energy homeostasis [22]. SIRT2 is important in cell cycle regulation, causing various effects dependent on its concentration. For example, SIRT2 protein levels increase during the mitotic phase and its over-expression delays mitosis [23]. Specifically, it is required for an efficient mitotic cell death caused by the spindle checkpoint and induced by microtubule inhibitors [24]. SIRT2 down-regulation induces apoptosis,

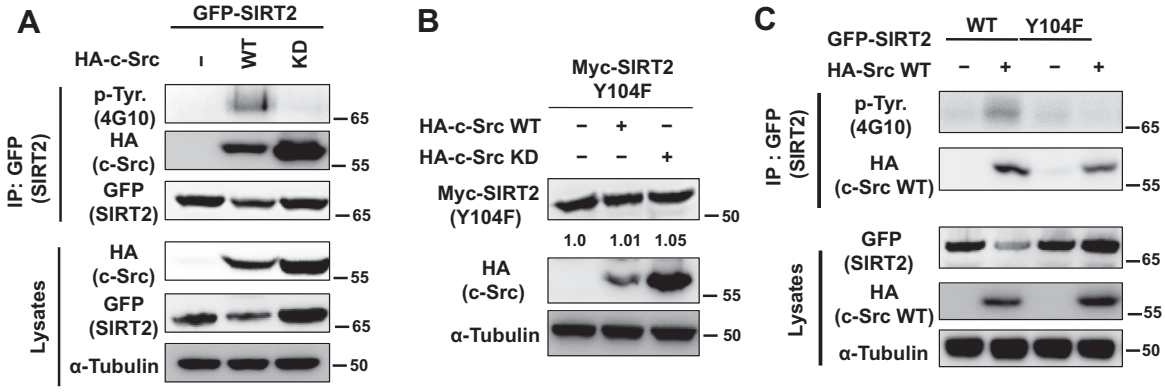

Fig. 3. c-Src phosphorylates SIRT2 at Tyr104. (A) HEK 293 cells were transfected for 36 h with the combinations of indicated plasmids.GFP-tagged-SIRT2, HA-tagged-c-Src WT, and HA-tagged-c-Src KD were transfected to examine the phosphorylation of SIRT2. Phosphorylation of SIRT2 was analyzed by IP for SIRT2 [IP: GFP (SIRT2)] followed by IB using anti-phosphotyrosine antibody 4G10 [p-Try. (4G10)] (top panels). The levels of SIRT2, c-Src WT, and c-Src KD in cell lysates were also compared (bottom panels). Tubulin was used as a loading control. (B) HEK 293 cells were transfected with the indicated combinations of Myc-tagged-SIRT2 Y104F (a tyrosine-to-phenylalanine substitution mutant), HA-c-Src WT and HA-c-Src KD or the control vector. Protein levels were determined by immunoblotting (IB) using anti-Myc antibody [Myc (SIRT2 Y104F)]. (C) HEK 293 cells were transfected with the indicated combinations of Myc-tagged SIRT2 (wild-type or Y104F mutant) and HA-tagged c-Src WT, or the control vector plasmid. The phosphorylation between SIRT2 Y104F and c-Src WT was determined by anti-GFP immunoprecipitation [IP: GFP (SIRT2)] followed by anti-phosphotyrosine antibody 4G10 immunoblotting [p-Tyr. (4G10)].

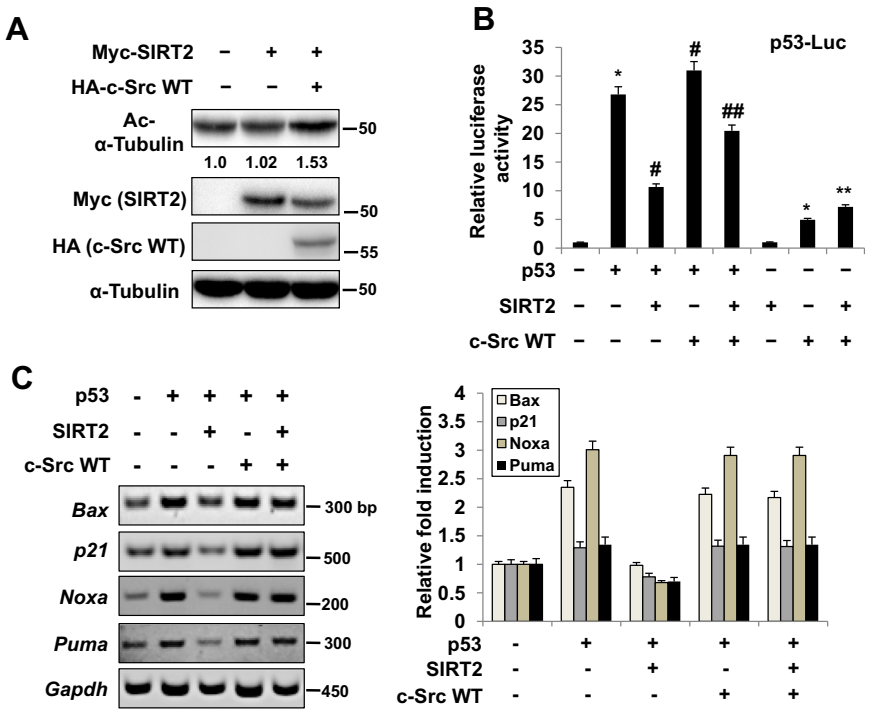

Fig. 4. SIRT2-mediated down-regulation of p53 is attenuated by c-Src. (A) HEK 293 cells were transfected with the indicated combinations of Myc-tagged SIRT2, HA-tagged c-Src, or a control vector. Acetylated tubulin was visualized by Western blotting using antisera specific for acetylated tubulin (Ac-α-tubulin). The levels of α-tubulin were used as loading controls. (B) MCF-7 cells were transfected with p53-luc (0.3 μg), pCMV-β-gal (0.05 μg) and indicated combinations of p53 (0.3 μg), SIRT2 (0.3 μg), and c-Src WT (0.5 μg). Luciferase activities were measured, and the average and S.D. of triplicate are shown. * and ** indicate a comparison to control or SIRT2 transfection alone ($p < 0.05$, by *t*-test). # and ## indicate a comparison to p53 alone, or co-transfection of p53 and Sirt2 ($p < 0.05$, by *t*-test). (C) MCF-7 cells were transfected for 24 h with the indicated combinations of HA-tagged-p53, Myc-tagged-SIRT2 and HA-tagged-c-Src WT. The expression levels of apoptosis marker genes (*Puma*, *Noxa,* and *Bax*), or *p21*-induced cell cycle arrest were compared by RT-PCR. *Gapdh* was used as a loading control. The expression levels of apoptosis marker genes in panel C were determined by densitometry, and then plotted.

caused by the accumulation of p53 [12]. Therefore, we next examined whether c-Src was able to regulate apoptosis by the down-regulation of SIRT2 in MCF-7 cells. An apoptosis response is mediated by *Bax*, *Puma* and *Noxa* as key actors of apoptosis, or by a *p21*-induced cell cycle arrest. Our experiments showed that increased cell death (apoptosis) by p53 accumulation was attenuated by SIRT2 activity, but was augmented by c-Src-induced SIRT2 down-regulation (Fig. 4C). These results suggested that c-Src affects SIRT2 activity and has an effect on the p53 transcriptional activity, through the regulation of SIRT2 protein levels.

4. Discussion

Sirtuin (silent mating type information regulation 2 homolog) 2 is NAD-dependent protein deacetylase, involved in the control of mitotic exit in the cell cycle, probably via its role in the regulation of the cytoskeleton.

In the present study, we found evidence that a non-receptor tyrosine kinase, c-Src play roles in the regulation of SIRT2 function. c-Src may affect the protein stability and transcriptional activity of SIRT2 through post-translational modifications, such as phosphorylation. Our results indicated that c-Src activation negatively regulates the protein levels and stability of SIRT2 through the phosphorylation of Try104 (Figs. 1 and 3). Moreover, the deacetylase activity of SIRT2 was also shown to be attenuated by the action of c-Src. Cell death, cell cycle, and p53 acetylation are especially regulated by SIRT2 [9,12,25]. The transcriptional activity and cell death (apoptosis) by p53 accumulation was attenuated by SIRT2, but augmented by c-Src-induced SIRT2 down-regulation (Fig. 4). Our results suggest that c-Src acts as a negative regulator of SIRT2. The studies about c-Src will enhance our understanding about the regulatory mechanisms of SIRT2.

Sirtuins are involved in many physiological and pathological processes, and their activity has been associated with different human diseases, including cancer. SIRT2 levels are increased in a number of tumors. In particular, involvement in the down-modulation of p53 tumor suppressor activity highlights the oncogenic role of Sirtuins. p53 is specifically acetylated by p300/CBP or PCAF (p300/CBP-associated factor). Acetylation has been shown to augment p53 DNA binding, and to stimulate p53-mediated transactivation of its downstream target genes through the recruitment of co-activators. p53 can also be deacetylated by HDAC1 and SIRT1 [26]. HDAC6 and SIRT2 regulate the acetylation state, thereby participating in the regulation of microtubule dynamics and possibly cell cycle progression [27,28]. Acetylation of SIRT2 by p300 has been shown to attenuate its deacetylase activity [8]. This acetylation can have additional consequences, due to its ability to regulate the function of target proteins. Acetylation of SIRT2 may affect its interaction with other signaling modulators. Acetylation of p53 can be regulated by the signaling mechanism of SIRT2 through the modulation of the activity of c-Src. GSK3 beta binding to p53 also promotes its acetylation [29], and the phosphorylation of Src and activation of GSK-3 in prostate cancer cells inhibits prostate cancer progression *in vitro* and in vivo [30]. Suppression of Src/ERK and GSK-3/beta-catenin signaling by pinosylvin inhibits the growth of human colorectal cancer cells [31]. The protein levels and activity of SIRT2-downregulated p53 activity can be modulated by the signaling of Src and GSK-3. Our studies on this may provide a valid therapeutic cancer approach, especially when combined with standard therapies.

In conclusion, our results indicate that non-receptor tyrosine kinase, c-Src has an important role in SIRT2 signaling mechanism through the down-regulation of stability and activity.

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