SIRT1 Inhibits Transforming Growth Factor β-Induced Apoptosis in Glomerular Mesangial Cells via Smad7 Deacetylation*

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Sir2 (silent information regulator 2) is a class III histone deacetylase with deacetylase activity, depending on intracellular NAD⁺ concentrations (1–3). Sir2 functions, through its deacetylase activity, in a wide array of cellular processes including gene silencing, rDNA recombination, and life span extension under various stress conditions (1–3). Furthermore, mammalian Sir2 homolog (SIRT1) has recently been reported to deacetylate not only the lysine residues of histone proteins but also the lysine residues of some apoptosis-inducible nuclear proteins, such as p53 (4–6) and forkhead family proteins (7, 8). The deacetylation of these nuclear proteins by SIRT1 results in the inhibition of apoptosis (4–8). Thus, it is conceivable that SIRT1 is a key regulator of cell defense and survival of mammalian cells through the interaction with apoptosis-inducible nuclear proteins. However, the target molecules of SIRT1 that are involved in apoptosis have not been fully elucidated.

Transforming growth factor β (TGFβ)² is a multifunctional signaling cytokine that regulates apoptosis, cell cycle, differentiation, and extracellular matrix accumulation (9). Alternation of TGFβ-signaling has been implicated in the progression of kidney diseases (10). In TGFβ-transgenic mice, which exhibit progressive glomerulosclerosis, mesangial cell apoptosis is accelerated in the advanced stage glomerulosclerosis (11). TGFβ signaling from the cell membrane to the nucleus is mediated and regulated by intracellular effector molecules, referred to as Smads (12). Among the Smads, Smad7 was originally recognized as an auto-inhibitory downstream molecule for TGFβ signaling (13). In addition, Smad7 was also reported to modulate TGFβ-induced apoptosis (14–19). However, the role of Smad7 in TGFβ-induced apoptosis depends on the cell type. Smad7 mediates TGFβ-induced apoptosis in podocytes (11) and prostatic carcinoma cells (17), whereas it inhibits TGFβ-induced apoptosis in hematopoietic cells (16, 19) and hepatocytes (14). In renal mesangial cells, TGFβ-induced apoptosis was facilitated by Smad7 overexpression and was inhibited by the antisense oligonucleotide to Smad7 (18). These reports support the notion that Smad7 mediates TGFβ-induced apoptosis in renal mesangial cells. Therefore, a more precise understanding of the regulation of Smad7 expression

The abbreviations used are: TGFβ, transforming growth factor β; E3, ubiquitin-protein isopeptide ligase; PARP, poly(ADP-ribose) polymerase; HA, hemagglutinin; WT, wild type; MMC, murine mesangial cell; GST, glutathione S-transferase; RNAi, RNA interference; DAPI, 4',6-diamidino-2-phenylindole; rDNA, ribosomal DNA.
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in the kidney may lead to the prevention of TGFβ-related pathological changes, including mesangial cell apoptosis and glomerulosclerosis.

It was recently reported that E3 ubiquitin ligase smurf1 (Smad ubiquitination regulatory factor 1)-mediated protein degradation through the ubiquitin-proteasome system regulates the level of Smad7 expression (20, 21). The acetyltransferase (p300)-mediated acetylation of two specific lysine residues (Lys-64 and -70) on Smad7 is one of its post-translational modifications and inhibits its degradation by interfering with the Smurf1-mediated ubiquitination, which mediates the stabilization of Smad7 (22). However, the precise mechanisms for the deacetylation of Smad7 and the correlation between its deacetylation and degradation are yet to be fully understood.

These previous results led us to investigate the possibility that apoptosis-associated nuclear protein Smad7 is a new target protein for SIRT1 and that the SIRT1-mediated deacetylation of Smad7 affects the degradation of Smad7. In the present study, we have investigated the capacity of SIRT1 to interact with Smad7 and inhibit TGFβ-induced apoptosis in renal glomerular mesangial cells. The results suggest that SIRT1 up-regulation is a potentially useful therapeutic target to prevent the progression of kidney disease.

**EXPERIMENTAL PROCEDURES**

**Reagents and Antibodies**—Dulbecco’s modified Eagle’s medium was purchased from Invitrogen. Anti-SIRT1 antibody was obtained from Upstate Biotechnology (Lake Placid, NY). Anti-cleaved caspase-3 (ASP175), anti-poly(ADP-ribose) polymerase (αPARP), and anti-acetyl-lysine antibody (αAc-K) were obtained from Cell Signaling Technology (Beverly, MA). Protein A/G-agarose and anti-Myc (αMyc) monoclonal and anti-hemagglutinin (αHA) polyclonal antibodies were from Santa Cruz Biotechnology (Santa Cruz, CA). Anti-β actin, anti-FLAG (αFLAG) M2 monoclonal and lactacystin were from Sigma. Lipofectamine reagent was from Invitrogen. Human recombinant TGFβ1 was purchased from R & D Systems (Minneapolis, MN). Anti-Smad7 (αSmad7) antibody was obtained from IMGENEX (San Diego, CA).

**DNA Constructs**—We generated an expression vector for Myc-tagged wild-type (WT) Smad7 by subcloning the PCR products into the pCMV-Myc expression vector (Clontech, Palo Alto, CA) as previously reported (23). Point mutants of Myc-tagged Smad7 (K64R, K70R, and K64R/K70R, respectively) vectors and FLAG-SIRT1(H355A) vector were generated by site-directed mutagenesis (Stratagene, La Jolla, CA). The various fragments of Myc-tagged Smad7 (amino acids 1–812 (ΔCT) and 590–1280 (ΔNT)) vectors were generated by PCR and cloned into the pcDNA-Myc expression vector. FLAG-tagged Smad7 vectors were generated by subcloning into the pFLAG-CMV-6a,b,c expression vector. FLAG-SIRT1 (mouse Sir2α) vector was a kind gift from J. Luo (Columbia University, New York, NY), HA-tagged p300 vector was kindly provided by T. Nakajima (St. Marianna University school of Medicine, Kawasaki, Japan), 6× Myc-tagged Smurf1 vector was a kind gift from K. Miyazono (University of Tokyo, Tokyo, Japan), and His-tagged ubiquitin vector was kindly gifted by D. Bohmann (University of Rochester Medical Center, Rochester, NY).

**Cell Culture**—SV40-transformed murine mesangial cell (MMC) line (MES13 cells) and COS7 cells were purchased from the American Type Culture Collection (Manassas, VA) and cultured at 37 °C in a 5% CO₂ atmosphere in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum, penicillin (100 units/ml), streptomycin (100 g/ml), l-glutamine (2 mM), and glucose (100 mg/dcliter).

**Immunoprecipitation and Immunoblotting**—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 phenylmethylsulfonyl fluoride, 10 mM sodium butyrate, and 1% aprotinin) and cleared by centrifugation at 15,000 revolutions/min for 10 min at 4 °C. For immunoprecipitation analysis using nuclear protein extraction of murine mesangial cells, cells were homogenized and lysed with hypotonic buffer (10 mmol/liter HEPES-KCl, pH 7.9, 1 mmol/liter EDTA, 15 mmol/liter KCl, 2 mmol/liter MgCl₂, 1 mmol/liter dithiothreitol, and protease inhibitor mixture (Roche Molecular Biochemicals, Lewes, UK) with 0.8% Nonidet P-40, and the lysates were centrifuged at 3,000 × g for 10 min. The pellets were resuspended in high salt buffer (hypotonic buffer with 420 mmol/liter NaCl and 25% glycerol), rotated for 30 min at 4 °C, and centrifuged at 17,000 × g for 30 min. The supernatants were used as nuclear extracts. 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 SDS-PAGE, and transferred to nitrocellulose membranes (Millipore, Bedford, MA). The membranes were incubated with the appropriate antibodies, washed, and incubated with horseradish peroxidase-coupled secondary antibodies (Amersham Biosciences). After washing, the blots were visualized by using an enhanced chemiluminescence detection system (PerkinElmer Life Sciences).

**Glutathione S-Transferase (GST) Pulldown Experiments**—GST fusion SIRT1 protein was expressed in Escherichia coli transformed with pGEX-SIRT1 and purified by using MagneticGST protein purification according to the instructions provided by the manufacturer (Promega). GST pulldown assay was performed in vitro translated Myc-tagged Smad7 proteins was performed. Samples from the GST pulldown were resolved by SDS-PAGE and transferred to nitrocellulose membranes. Smad7 was detected by using aMyc antibody.

**Retroviral Infection**—The pBABE, pBABE-SIRT1, pSUPER-retro, and pSUPERetro-SIRT1 RNA interference (RNAi) vectors were previously generated (24). Retroviral infection was performed as previously described (24). Human embryonic kidney 293T cells were transfected with pBABE, pBABE-SIRT1, pSUPERetro, or pSUPERetro-SIRT1 RNAi by using Lipofectamine reagent. At 48 h after transfection, the media containing retroviruses were collected, centrifuged, and transferred to MMCS treated by polybrene (1 μg/ml). The infected cells were selected by treatment with puromycin (2.5 μg/ml) for several days.

**In Vitro Translation**—[35S]Methionine-labeled, in vitro translated FLAG-tagged SIRT1 and each Myc-tagged Smad7 (WT, ΔCT, or ΔNT) were generated with the rabbit reticulocyte lysate in vitro translation system (Promega), and these pro-
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RESULTS

SIRT1 Interacts with Smad7 Both in Vivo and in Vitro—To investigate the general interaction between SIRT1 and Smad7 in vivo, we performed co-immunoprecipitation analysis in transiently transfected COS7 cells using either Myc-tagged Smad7 vector, FLAG-tagged SIRT1 vector, or both of these vectors (Fig. 1, A and B). Myc-tagged Smad7 was detected in the immunoprecipitates with αMyc of co-transfection (Fig. 1A), and FLAG-tagged SIRT1 was detected in the immunoprecipitates with αMyc of co-transfection (Fig. 1B). We next performed immunoprecipitation experiments in vitro using either in vitro translated FLAG-tagged SIRT1 proteins, Myc-tagged Smad7 proteins, or both of these proteins (Fig. 1C), which provided additional evidence for an interaction between SIRT1 and Smad7 (Fig. 1C). In further immunoprecipitation analysis using the deletion

teins were used to perform immunoprecipitation analysis as described previously (25). Immunoprecipitation results were detected by autoradiography after 16 h at −80 °C.

Pulse-Chase Analysis—Myc-tagged Smad7 vector was transfected in the MMCs infected with either pBabe or pBabe-SIRT1 retroviral vector. Twenty-four hours after transfection, these cells were incubated for 1 h with 35S-labeled cysteine and methionine (Amersham Biosciences) in cysteine- and methionine-free Dulbecco’s modified Eagle’s medium. The cells were washed and incubated in Dulbecco’s modified Eagle’s medium supplemented with 50 μg/ml cysteine and methionine for the indicated time periods (0, 30, 60, and 120 min) followed by immunoprecipitation using αMyc. Immunoprecipitated samples were separated by SDS-PAGE, and the gels were dried and analyzed by autoradiography after 16 h at −80 °C. The amount of 35S-labeled Myc-Smad7 at each point was plotted as the percent of the amount at the start of the chase.

RNA Interference—The small interference RNA to mouse Smad7 (5’-CAUCAAGGCUUUGACUAGAGAAA-3’) was designed by and bought from iGENE Therapeutics (Tsukuba, Japan). MMCs on a 6-well plate were transfected with Smad7 expressed as the mean ± S.E. Analysis of variance with subsequent Scheffe’s test was used to determine the significance of differences in multiple comparisons. p < 0.05 was considered statistically significant.

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FIGURE 1. SIRT1 interacts with Smad7 in vivo and in vitro. Immunoprecipitation analysis using Myc-tagged Smad7 vector (lanes 1 and 2) and FLAG-tagged SIRT1 vector (lanes 2 and 3) in COS7 cells. Immunoprecipitation (IP) was performed using αFLAG (A) or αMyc (B). Whole cell lysates (input) and immunoprecipitates were estimated by immunoblotting (IB) with αFLAG or αMyc. Immunoprecipitation analysis in vitro. Either 35S-labeled FLAG-tagged SIRT1 (lanes 1, 4, and 7), Myc-tagged Smad7 (lanes 2, 5, and 8), or both proteins (lanes 3, 6, and 9) were produced in the rabbit reticulocyte lysate translation system. Immunoprecipitation was performed with αFLAG (lanes 4–6) or αmhc (lanes 7–9). In vitro translated proteins (input) and the immunoprecipitates were resolved by SDS-PAGE and estimated by autoradiography. D, immunoprecipitation analysis using deletion mutant vectors of Myc-tagged Smad7 (WT, lanes 1 and 2; ΔCT, lanes 3; ΔNT, lane 4) and FLAG-tagged SIRT1 vector (lanes 2–5) in COS7 cells. Immunoprecipitation was performed using αMyc. E, 35S-labeled deletion mutant proteins of Myc-tagged Smad7 (WT, lanes 2 and 6; ΔCT, lanes 3 and 7; ΔNT, lanes 4 and 8) and FLAG-tagged SIRT1 protein (lanes 1–8) were generated in vitro. Immunoprecipitation was performed using αMyc (lanes 3–8). F, GST pulldown assay. Direct interaction of Smad7 with GST-SIRT1 was determined by GST pull-down assay with in vitro translated Myc-tagged Smad7.
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**A)**

**FIGURE 2. SIRT1 deacylates lysine residues of Smad7.** A, In vivo acetylation assay. Each Myc-tagged empty (lanes 1 and 2), Smad7(WT) (lanes 3 and 4), Smad7(K64R) (lanes 5 and 6), Smad7(K70R) (lanes 7 and 8), or Smad7(K64R/K70R) (lanes 9 and 10) was expressed in COS7 cells in the absence or presence of HA-tagged p300 vector. Each Myc-tagged Smad7 was immunoprecipitated (IP) using αMyc followed by immunoblotting (IB) with anti-acetyl lysine antibody (αAcK). Whole cell lysates (input) were estimated by immunoblotting using αHA and αMyc. B, In vivo acetylation assay. Myc-tagged Smad7 (lanes 1–5), HA-tagged SIRT1 (lanes 3–5), and FLAG-tagged SIRT1(H355A) (lanes 4 and 5) were expressed in COS7 cells in the presence (+) or absence (−) of HA-tagged p300. Myc-tagged Smad7s were immunoprecipitated using αMyc followed by immunoblotting with αAcK.

mutants of Myc-tagged Smad7 vectors, SIRT1 was detected in the immunoprecipitates of both Smad7(WT) and Smad7(ΔCT) but not Smad7(ΔNT), indicating that SIRT1 interacts with the N terminus of Smad7 (Fig. 1, D and E). We confirmed a direct interaction between SIRT1 and Smad7 by using GST pulldown assay (Fig. 1F). These findings show that SIRT1 directly interacts with Smad7.

**SIRT1 Deacylates the Lysine Residues of Smad7**—To confirm that two specific lysine residues (Lys64 and -70) of Smad7 are acetylated by acetyltransferase p300 (22), we generated two single-mutated and a double-mutated Smad7 vector in which these lysine residues were mutated to arginine residues (K64R, K70R and K64R/K70R, respectively). In vivo acetylation assays revealed that wild-type Smad7 was acetylated by p300 (Fig. 2A). However, the acetylation of the lysine residues was partially decreased in the single-mutated Smad7(K64R and K70R), and it was completely inhibited in the double-mutated Smad7(K64R/K70R) (Fig. 2A). These results indicate that two specific lysine residues (Lys64 and -70) of Smad7 were acetylated by p300 as previously reported (22). We next investigated whether SIRT1 could deacetylate the lysine residues of Smad7. The p300-mediated acetylation of the lysine residues of Smad7 was decreased in SIRT1-overexpressing cells (Fig. 2B, lane 3). SIRT1(H355A) was reported to function as a dominant negative mutant and to inhibit the endogenous SIRT1-mediated deacetylation (4). SIRT1(H355A) inhibited SIRT1-mediated decreases in the p300-induced acetylation of the lysine residues of Smad7 in a dose-dependent manner (Fig. 2B, lanes 4 and 5). These results indicate that SIRT1 functionally interacts with Smad7, resulting in the deacetylation of the lysine residues of Smad7.

**SIRT1 Accelerates Degradation of Smad7 in Mesangial Cells**—We confirmed that endogenous SIRT1 interacted with endogenous Smad7 in the nuclei of MMCs (Fig. 3A). To examine the physiological effects of SIRT1 on Smad7 in MMCs, SIRT1 expression in MMCs was modified through retroviral infection with either pBABE-SIRT1 or pSUPER-SIRT1 RNAi for overexpression (10-fold) or knockdown (7-fold), respectively, of the SIRT1 gene (Fig. 3B). The acetylated lysine residues of Smad7 have been reported to protect it against Smurf1-mediated ubiquitination and degradation (22). We therefore investigated the role of SIRT1 in the regulation of Smad7 degradation. The levels of Smad7 expression were significantly decreased in the SIRT1-overexpressing MMCs and were increased in the SIRT1 knockdown MMCs (Fig. 3B). However, SIRT1(H355A) significantly attenuated the decreased Smad7 expression in the SIRT1-overexpressing MMCs in a dose-dependent manner (Fig. 3, C and D). To confirm that the decreased amount of Smad7 expression in the SIRT1-overexpressing MMCs was caused by the stimulation of degradation, we performed degradation assays using methionine pulse-chase analysis. This analysis revealed that the degradation of Smad7 was significantly accelerated in the SIRT1-overexpressing MMCs (Fig. 4A). To test the effect of SIRT1 on the ubiquitin-proteasome system in MMCs, we performed ubiquitination assays in the SIRT1-overexpressing and knockdown MMCs. Ubiquitination assays revealed that the ubiquitination of Smad7 was notably increased in the SIRT1-overexpressing MMCs and was decreased in the SIRT1 knockdown MMCs (Fig. 4B). SIRT1(H355A) inhibited the acceleration of Smad7 ubiquitination in the SIRT1-overexpressing MMCs (Fig. 4C). Furthermore, the decreased amount of Smad7 expression in the SIRT1-overexpressing MMCs was significantly reversed by lactacystin, known as a proteasome inhibitor (Fig. 4, D and E). These findings suggest that SIRT1 enhances the smurf1-mediated ubiquitination and degradation of Smad7 in MMCs.

**SIRT1 Inhibits Smad7-induced Mesangial Cell Apoptosis**—Overexpression of Smad7 has been reported to induce mesangial cell apoptosis (18). Therefore, we investigated whether SIRT1 could modulate Smad7-induced mesangial cell apoptosis. The number of apoptotic cells detected by DAPI staining in the Smad7-overexpressing MMCs was increased 36 h after transfection in a vector dose-dependent manner (Fig. 5A). Similarly, cleavages of caspase-3 and PARP were increased in the Smad7-overexpressing cells (Fig. 5B). Compared with MMCs infected with the pBABE control retroviral vector, Smad7-induced apoptotic cells and cleavage of caspase-3 and PARP were decreased in the SIRT1-overexpressing MMCs (Fig. 5, C and D). In contrast, the increased amount of these apoptotic markers by Smad7 overexpression were significantly increased in the SIRT1 knockdown MMCs compared with the MMCs infected with pSUPER control retroviral vector (Fig. 5, C and D). Interestingly, SIRT1 failed to inhibit double-mutated Smad7(K64R/K70R)-induced mesangial cell apoptosis, although SIRT1 could inhibit Smad7(WT)-induced apoptosis (Fig. 5, E and F). These results suggest that SIRT1 acts a negative regulator of Smad7.

**NOTE**

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Induced apoptosis in MMCs through interaction with the specific lysine residues of Smad7.

SIRT1 Inhibits TGFβ-induced Mesangial Cell Apoptosis—Smad7 has been reported to accelerate TGFβ-induced mesangial cell apoptosis (18). We examined the effects of SIRT1 on TGFβ-induced apoptosis in MMCs. The number of apoptotic cells was significantly increased by TGFβ-stimulation after 48 h of incubation in a dose-dependent manner (Fig. 6, A and C). Similarly, the amounts of cleaved caspase-3 and cleaved PARP were also markedly increased by TGFβ stimulation in MMCs (Fig. 6, B and D). In the Smad7 knockdown MMCs using Smad7 RNAi, TGFβ failed to increase the amount of cleaved caspase-3 and cleaved PARP (Fig. 6E). These findings suggest that Smad7 is required for TGFβ-induced apoptosis in MMCs. We finally examined the effects of SIRT1 on TGFβ-induced mesangial cell apoptosis using the SIRT1-overexpressing and knockdown MMCs. TGFβ-induced apoptotic cells, cleavage of caspase-3, and PARP were decreased in the SIRT1-overexpressing MMCs, whereas these apoptotic markers were additionally increased in the SIRT1-knockdown MMCs (Fig. 6, F and G). In the similar way to Smad7-induced apoptosis, SIRT1 also acts as a negative regulator of TGFβ-induced apoptosis in MMCs.

**DISCUSSION**

In this study, we have presented the first evidence that SIRT1 can modulate TGFβ-induced apoptosis in renal mesangial cells through direct interaction with Smad7. To date, SIRT1 has been reported to interact with some nuclear proteins, such as p53 (4–6) or forkhead family proteins (7, 8), which could modulate the functions of these proteins through the deacetylation. Here, we show (a) that Smad7 is a new substrate for SIRT1, (b) that the SIRT1-mediated deacetylation of Smad7 stimulates the smurf1-mediated degradation of Smad7 through the ubiquitin-proteasome system, and (c) that SIRT1 inhibits TGFβ- and Smad7-induced apoptosis in MMCs.

The deacetylation/degradation of lysine residues on nuclear proteins is thought to affect multiple protein functions, such as transcriptional activity, DNA binding, protein binding, protein stability, and translation. Recently, SIRT1-mediated degradation of apoptosis-inducible nuclear proteins, such as p53 (4–6), FOXO (7, 8), and Ku70 (26), has been reported to promote cell survival. Consistent with this evidence, our results indicating that the SIRT1-mediated degradation of two lysine residues (Lys-64 and -70) on Smad7 suggest that SIRT1 plays an important role in renal mesangial cell survival under stimulation of TGFβ. This evidence strongly supports the contribution of the SIRT1-mediated deacetylation of the nuclear proteins to cell survival.

The present study has demonstrated that SIRT1 directly interacts with Smad7, resulting in the deacetylation of Smad7. Furthermore, our study has revealed that SIRT1 accelerates the Smurf1-mediated ubiquitination and degradation of Smad7, which causes the instability of Smad7. A previous report shows that both acetylated Smad7 and mutated Smad7(K64R/K70R) fails to bind to ubiquitin ligase and smurf-1, and subsequently both expressions were protected from the ubiquitin-proteasome degradation, resulting in the increased stability of Smad7 expression (22). In addition to these results, our results indicating that SIRT1 accelerates the ubiquitin-proteasome-mediated degradation of Smad7 through the deacetylation of Smad7 provided further evidence that unacetylated/deacetylated lysine residues (Lys-64 and -70) of Smad7 were required for the binding to smurf1. With respect to the regulation of Smad7 acetylation, it has been recently reported that some class I and II histone deacetylase interact with Smad7 (27). This interaction results in the deacetylation of Smad7 with subsequent enhancement of degradation by ubiquitination; however, the direct interaction between SIRT1 and Smad7 was not fully examined (27). Our results clearly show that class III histone deacetylase and SIRT1 directly interact with Smad7, resulting in the acceleration of Smad7 degradation. Several nuclear proteins, including p53 (4–6,28) and NFκB (RelA/p65) (29), are also deacetylated both by class I histone deacetylase and SIRT1, which modulate the functional effects of these nuclear proteins. At present, the differential roles between class I histone deacetylase- and SIRT1-mediated deacetylation of these nuclear proteins remain unclear.
Our results provided the possibility that SIRT1 inhibits p300-mediated acetylation of lysine residues of Smad7 through direct and functional interaction. A recent report suggests that SIRT1 deacetylates lysine residues of p300, resulting in the inhibition of acetyltransferase activity of p300 (30). Therefore, it is possible that SIRT1 may decrease acetylation of lysine residues of Smad7 through both direct interaction and the inhibition of transferase activity of p300, although this study did not reveal the precise mechanism by which the acetylation of Smad7 was decreased. However, we did show that SIRT1 directly interacts with Smad7 and inhibits the p300-mediated acetylation of the lysine residues of Smad7. These results suggest that Smad7 is a new target for SIRT1 deacetylase activity.

In the kidney, Smad7 is considered to play an important role in the regulation of TGFβ-induced apoptosis besides the inhibition of TGFβ signaling (11, 15, 18). A recent report shows that overexpression of Smad7, but not regulatory Smads (Smad2/3), enhances apoptosis in mesangial cells (18). In addition, antisense oligonucleotides to Smad7 prevent TGFβ-induced apoptosis in mesangial cells (18). Our results also confirmed the role of Smad7 on TGFβ-induced mesangial cell apoptosis. Furthermore, we showed that SIRT1 could inhibit Smad7- and TGFβ-induced mesangial cell apoptosis through the enhancement of Smad7 degradation and inhibition of caspase-3 and PARP activation. Other roles of Smad7, except for the inhibition of TGFβ-signaling, may be involved in TGFβ-induced apoptosis. Interactions between Smad7 and some pro-apoptotic molecules, such as the activation of p38 (31, 32) or NF-κB (11, 31), have been reported to be important for TGFβ-induced apoptosis. The relationship between these pro-apoptotic molecules, Smad7 and SIRT1, has not been clarified in our study, although we have demonstrated that SIRT1 inhibits TGFβ-induced mesangial cell apoptosis through Smad7 degradation. To clarify further the role of SIRT1 and Smad7 in TGFβ-induced apoptosis in all cell types including mesangial cells, more investigation is required.

In conclusion, our study has provided new information regarding the functional significance of the interaction between SIRT1 and Smad7 in TGFβ-induced mesangial cell apoptosis. We suggest that up-regulation of SIRT1 is a potentially useful
FIGURE 5. SIRT1 inhibits Smad7-induced apoptosis in MMCs. (A and B) Smad7-induced apoptosis in MCCs. MMCs were transfected with Myc-tagged Smad7-overexpressing vector at the various concentrations. A, the number of apoptotic cells detected by DAPI staining was quantified (apoptotic nuclei/Myc-positive cells). Quantitative results of five independent experiments are shown. Data are mean ± S.E.; *, p < 0.05 versus Smad7 (−). B, immunoblotting revealed that cleaved caspase-3 and PARP, biochemical apoptotic markers, were detected as a 17-kDa band and an 89-kDa band, respectively. C and D, the effects of SIRT1-overexpressing (BABE-SIRT1) and knockdown (SUPER-SIRT1) on Smad7-induced mesangial cell apoptosis were assessed by DAPI staining (C) and immunoblotting (D). Quantitative results of five independent experiments are shown. Data are mean ± S.E.; *, p < 0.05 versus BABE; Smad7 (−); †, p < 0.05 versus BABE-SIRT1, Smad7 (−). E and F, the effect of Smad7 overexpression on the double mutated Smad7(K64R/K70R)-induced mesangial cell apoptosis assessed by DAPI staining (E) and immunoblotting (F). Quantitative results of five independent experiments are shown. Data are mean ± S.E.; *, p < 0.05 versus BABE, Smad7(WT) (−), Smad7(K64R/K70R) (−); †, p < 0.05 versus BABE, Smad7(WT) (−); ‡, p < 0.05 versus BABE-SIRT1, Smad7(WT) (−).

FIGURE 6. SIRT1 inhibits TGF-β-induced apoptosis in MMCs. A and B, TGF-β-induced apoptosis in MMCs. MMCs were exposed to TGF-β (5.0 ng/ml) for the indicated time periods. Apoptosis was evaluated by DAPI staining (A) and immunoblotting (B). Quantitative results of three independent experiments are shown. Data are mean ± S.E.; *, p < 0.05 versus TGF-β (0 h). C and D, MMCs were exposed to various concentrations of TGF-β for 48 h. Quantitative results of three independent experiments are shown. Data are mean ± S.E.; *, p < 0.05 versus TGF-β (0 ng/ml). E, the effect of Smad7 knockdown using Smad7 RNAi on TGF-β-induced mesangial apoptosis. The levels of Smad7 expression in the Smad7 knockdown MMCs were detected by immunoblotting using anti-Smad7 antibody. The Smad7 knockdown MMCs were exposed to TGF-β (5 ng/ml) for 48 h. Cleaved caspase-3 and cleaved PARP were detected by immunoblotting. F and G, the effects of SIRT1-overexpressing (BABE-SIRT1) and knockdown (SUPER-SIRT1) on TGF-β-induced mesangial cell apoptosis were determined by DAPI staining (F) and immunoblotting (G). Quantitative results of five independent experiments are shown. Data are mean ± S.E.; *, p < 0.05 versus BABE, TGF-β (−); †, p < 0.05 versus BABE, TGF-β (+); **, p < 0.05 versus SUPER, TGF-β (−); ‡, p < 0.05 versus SUPER, TGF-β (+).

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therapeutic strategy for prevention of glomerular diseases through its effect on cell survival.

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