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Resveratrol is Not a Direct Activator of SIRT1 Enzyme Activity

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Resveratrol is a plant polyphenol capable of exerting beneficial metabolic effects which are thought to be mediated in large by the activation of the NAD$^+$-dependent protein deacetylase SIRT1. Although resveratrol has been claimed to be a bona fide SIRT1 activator using a peptide substrate (Fluor de Lys-SIRT1 peptide substrate), recent reports indicate that this finding might be an experimental artifact and need to be clarified. Here, we show that: (i) the Fluor de Lys-SIRT1 peptide is an artificial SIRT1 substrate because in the absence of the covalently linked fluorophore the peptide itself is not a substrate of the enzyme, (ii) resveratrol does not activate SIRT1 *in vitro* in the presence of either a p53-derived peptide substrate or acetylated PGC-1α isolated from cells, and (iii) although SIRT1 deacetylates PGC-1α in both *in vitro* and cell-based assays, resveratrol did not activate SIRT1 under these conditions. Based on these observations, we conclude that the pharmacological effects of resveratrol in various models are unlikely to be mediated by a direct enhancement of the catalytic activity of the SIRT1 enzyme. In consequence, our data challenge the overall utility of resveratrol as a pharmacological tool to directly activate SIRT1.

Key words: Fluor de Lys, resveratrol, SIRT1

Abbreviations: AMPK, AMP-activated protein kinase; HPLC, high performance liquid chromatography; NAM, nicotinamide; PGC-1α, peroxisome proliferator-activated receptor-γ coactivator-1α; Sir2, silence information regulator 2; SIRTs, sirtuins.

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Sirtuins constitute the unique family of NAD$^+$-dependent protein deacetylases. Silent information regulator 2 (Sir2) is a sirtuin in budding yeast *Saccharomyces cerevisiae* and its activity mediates lifespan extension induced by calorie restriction (1). The mammalian Sir2 ortholog, SIRT1, is also induced by calorie restriction and promotes cell survival (2), triggers lipolysis and loss of fat (3), and controls glucose homeostasis (4). The biological effects of SIRT1 are mediated by its ability to deacetylate several important transcriptional factors such as Peroxisome proliferator-activated receptor-γ coactivator 1alpha (PGC-1α), p53, and FOXO proteins and consequently regulate their activities (5). PGC-1α, which is activated upon deacetylation by SIRT1, plays an important role in the regulation of mitochondrial function and fatty acid oxidation (4). In contrast, p53 is inactivated once deacetylated by SIRT1 (5), suggesting that SIRT1 may protect cells from apoptosis under conditions of nutrient restriction. Taken together, these findings demonstrate that SIRT1 activity stimulates energy metabolism, improves mitochondrial function and promotes cell survival. Therefore, pharmacological activation of SIRT1 *in vivo* may provide a new avenue to maintain metabolic homeostasis.

Molecular screening of SIRT1 activators led to the identification of plant polyphenols as SIRT1 activators, among which is resveratrol (6). The notion that resveratrol is a SIRT1 activator is consistent with two later pharmacological studies, in which resveratrol shifted the physiology of middle-aged mice on high calorie diet, promoted changes associated with longer lifespan, and protected mice against diet-induced obesity and insulin resistance (7,8). One notable finding in both studies is that resveratrol increased PGC-1α activity and improved mitochondrial function (7,8). Despite these findings, which appear to be consistent *in vitro* and *in vivo* results, the notion of resveratrol as a SIRT1 activator has been questioned in recent studies (9,10). The SIRT1 enzyme assay used in the original report employs a fluorescently labeled 4-amino acid peptide substrate (Fluor de Lys substrate) (6), and the enhancement of SIRT1 activity by resveratrol was dependent on the presence of the fluorophore on the substrate (9,10); resveratrol did not activate SIRT1 activity when peptide substrates without fluorescent labeling were used (9,10). Further, in contrast to a previous report (6), resveratrol did not extend the lifespan of several yeast strains (10). These reports raised the important question of whether resveratrol is a bona fide activator of SIRT1. In addition, resveratrol has been reported to inhibit the insulin signaling pathway independently of SIRT1, suggesting that an alternative mechanism for the resveratrol effect on lifespan may exist (11).

Although the question whether resveratrol is a SIRT1 activator has not been fully resolved, resveratrol continues to be referred to as a SIRT1 activator in a number of studies and moreover, the Fluor de Lys-SIRT1 substrate is still widely used in *in vitro* SIRT1 assays (12). In contrast, to our knowledge no data have been published to date demonstrating that resveratrol directly activates SIRT1 in cells or cell-free enzyme assays leading to reduced acetylation of a native SIRT1 substrate. To fully understand whether resveratrol activates SIRT1 deacetylase activity, we have used both a peptide substrate lacking the fluorophore and full-length PCG-1α protein in *in vitro* enzyme assays. Further, the acetylation level of PGC-1α was assessed in cells treated with resveratrol. In these experiments, resveratrol did not change the acetylation level of the SIRT1 substrates used. Taken together, our data demonstrate that resveratrol is not a direct SIRT1 activator and the pharmacological profile of this plant polyphenol needs to be accordingly revised.

Materials and Methods

Materials

Full-length His-tagged human SIRT1 enzyme was expressed in an *Escherichia coli* expression system and purified as described previously (13). The Fluor de Lys-SIRT1 substrate is the 4-amino acid acetylpeptide RHKK(Ac) covalently conjugated to a fluorophore (BIOMOL, Plymouth Meeting, PA, USA). Ac-p53 peptide HLKSKKGQSTSRHKK(Ac) LMFK (QCB, Hopkinton, MA, USA) and Fluor de Lys-SIRT1 substrate peptide RHKK(Ac) (Genscript Corp., Piscataway, NJ, USA) lacking the fluorophore were synthesized and purified with greater than 90% purity. Monoclonal and polyclonal antibodies were obtained from the following sources: anti-PGC-1α (rabbit polyclonal H300, Santa Cruz Biotech., Santa Cruz, CA, USA), anti-GCN5 antibody (rabbit polyclonal H-75, Santa Cruz Biotech, Santa Cruz, CA, USA), anti-FLAG M2 (mouse monoclonal, Sigma, St Louis, MO, USA), anti-phospho-AMPK-alpha (Thr172) and anti-AMPK-alpha antibodies (rabbit polyclonal, Cell Signaling, Danvers, MA, USA), anti-Ac-Lys antibody (mouse monoclonal Ac-K-103, Cell Signaling, Danvers, MA, USA), and anti-SIRT1 (rabbit polyclonal Anti-Sir2, Upstate Biotech., Lake Placid, NY, USA).

SIRT1 enzyme assays

High performance liquid chromatography enzyme activity assays using Ac-p53 peptide were performed at room temperature as previously described (13). The HPLC method for quantification of Fluor de Lys-SIRT1 substrate peptide RHKK(Ac) with or without the fluorophore was determined using a Dionex (Sunnyvale, CA, USA) system. A Luna C8 (5 micron, 250 mm $\times$ 4.6 mm; Phenomenx, Torrance, CA, USA) column was used at a flow rate of 1.3 mL/min with buffer A containing 0.1% TFA in water and buffer B containing 0.1% TFA in acetonitrile. A total injection volume of 100 μL of assay reaction was loaded on the column. The separation of the Fluor de Lys-SIRT1 substrate peptide and the de-acetylated Fluor de Lys-SIRT1 substrate peptide was achieved by a binary gradient: 10% B, 0–6 min; 25% B, 6–8 min; 10% B, 8–12 min. The peptides were detected using a UV detector at a wavelength of 215 nm. The assay reactions using the Fluor de Lys-SIRT1 substrate peptide with or without the fluorophore were conducted at room temperature for 30 min. The assay conditions were HDAC buffer (BIOMOL), 50 mM NAD^+, 20 μM Fluor de Lys-SIRT1 substrate peptide, 25 nM or

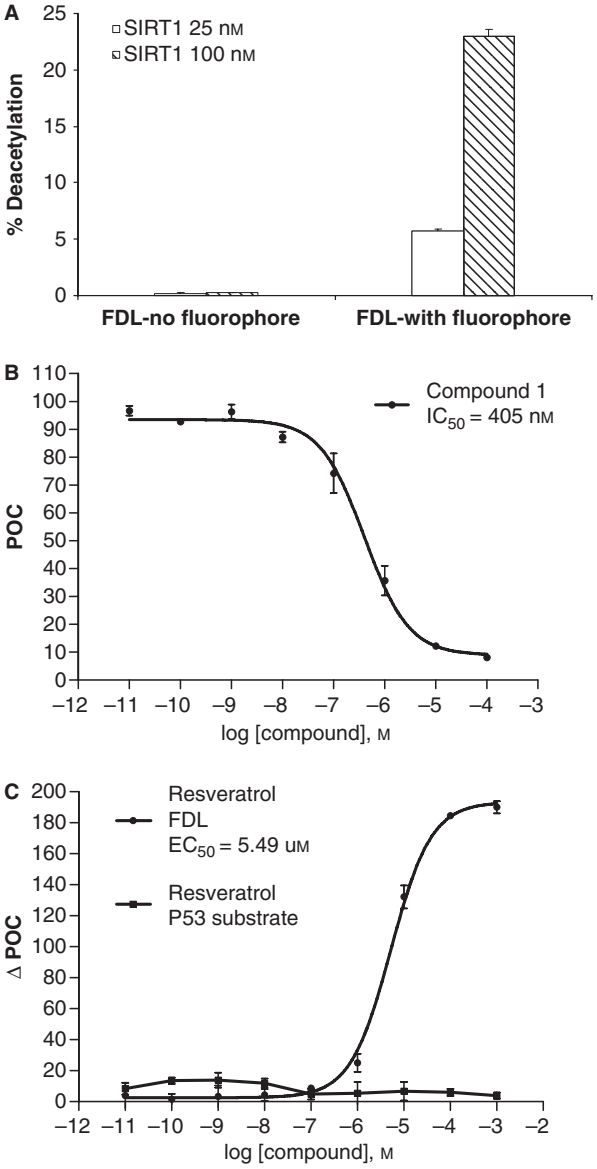

Figure 1: Effect of resveratrol on SIRT1 activity *in vitro* using peptide substrates. (A) HPLC SIRT1 assay using Fluor de Lys-SIRT1 peptide substrate (FDL) with or without the fluorophore. Two enzyme concentrations (25 and 100 nM) were used. (B) HPLC SIRT1 assay using Ac-p53 as a substrate. SIRT1 deacetylase reaction was carried out in the presence of various concentrations of SIRT1 inhibitor compound 1 (6-chloro-2,3,4,9-tetrahydro-1*H*-carbazole-1-carboxamide). Samples were analyzed by HPLC to quantitate deacetylated peptide and dose-dependent inhibition of the enzyme reaction is plotted. (C) Effects of resveratrol on SIRT1 activity using different substrates: FDL with the fluorophore and Ac-p53. The percent of increase in enzyme activity relative to control (without resveratrol treatment) was plotted against resveratrol concentration.

100 nM of recombinant SIRT1 enzyme. The reactions were stopped with 25 μL of cold acetonitrile.

For SIRT1 deacetylation assays using immunoprecipitated PGC-1α from cell lysates, HEK293 cells were transiently transfected with C-terminally FLAG-tagged human PGC-1α (CT-FLAG PGC-1α) and GCN5. On the following day, the cells were treated with 10 mM nicotinamide (NAM) overnight to accumulate acetylated PGC-1α. EZview™ anti-FLAG M2 Affinity Gel (Sigma, St Louis, MO, USA) was initially washed with deionized water and equilibrated with PBS prior to use. The beads (100 μL) were incubated with cell extracts (3 mL) overnight at 4 °C. After three washes in PBS, the bound CT-FLAG PGC-1α was eluted with 0.1 mg/mL FLAG peptide in 50 mM *Tris*-HCl (pH 9.0) containing 150 mM NaCl and 4 nM $MgCl_2$. For *in vitro* deacetylation assay, the eluted CT-FLAG PGC-1α and 50 nM recombinant SIRT1 were incubated in enzyme buffer (50 mM *Tris*-HCl, pH 9.0, containing150 mM NaCl, 4 nM $MgCl_2$, 0.5 mM DTT, and 100 μM NAD^+) for 3 h at 30 °C. The reactions were stopped by addition of SDS sample buffer and analyzed by Western blotting.

Transient transfections with DNA or siRNA knockdown

HEK293 cells were transfected with 10 μg of individual cDNAs using GeneJuice® transfection reagent (Novagen, Gibbstown, NJ, USA) according to the manufacturer's instructions. Similar amounts of empty vector DNA were included when multiple constructs were used to ensure that all transfected cells received the same amount of DNA. For compound treatments, the media were removed 1 day after transfection and the cells were incubated with fresh media containing the compounds and incubated over night.

Co-transfections of plasmid DNAs and siRNAs were performed in HEK293 using Lipofectamine™ 2000 according to the manufacturer's instructions (Invitrogen, Carlsbad, CA, USA). The following siRNAs were used and sequences listed are for the target sequences [sense strand (5′–3′)] for the siRNA duplex: C-luciferase siRNA control: ACGTACGCGGAATACTTCG; a pool of three SIRT1 siRNAs which were empirically determined to yield the most efficient knockdown of SIRT1 protein expression: GCAACAGCATCTTGCCTGATTTGTA ('Stealth'-modified siRNA from Invitrogen), CCTGATTTGTAAATACAAA, and CCTGTAAAGCTTTCAGAAA. The final concentrations of individual SIRT1 siRNAs were 20 nM and C-luciferase siRNA 60 nM. Ten micrograms of plasmid DNA was used. Media were changed the next day and after 68 h total incubation time, protein expression was analyzed by Western blotting.

Immunoprecipitations and Western blot analyses

Cells were scraped in PBS and collected by centrifugation for 5 min at 6000× *g*. The cell pellets were solubilized in 500 μL of 1% Igepal CA-630, 0.5% Triton X-100, 150 mM NaCl, 50 mM Tris (pH 7.5), 2 mM EDTA, and 1× EDTA-free protease inhibitor cocktail (Roche Applied Science, Indianapolis, IN, USA). Insoluble debris was removed by centrifugation at 4 °C for 10 min at 20 000× *g* after incubation for 15 min on ice with repeated vortexing. The supernatants were used for subsequent studies.

Equal volumes of cell extracts were incubated with anti-Ac-Lys or anti-FLAG antibodies followed by the addition of protein G beads and incubation overnight at 4 °C. Bound proteins were eluted in SDS sample buffer by boiling for 5 min after three washes with PBS. Equal amounts of protein were separated by SDS-polyacrylamide gel electrophoresis and transferred to nitrocellulose membranes. Probing of the membranes was carried out with various antibodies as indicated in the figure legends. Individual polypeptides were visualized by infrared imaging using Alexa Fluor-conjugated goat anti-rabbit $F(ab')_2$ fragments (Molecular Probes, Carlsbad, CA, USA) and a LI-COR® Odyssey infrared imager (LI-COR Biosciences Inc., Lincoln, NE, USA).

HepG2 cells were grown in DMEM with 1 g/L D-glucose and supplemented with 10% FBS. Cells were serum started overnight before treatment with resveratrol for 24 h. Phospho-AMPK-alpha and total AMPK-alpha were analyzed by Western blotting using LI-COR Odyssey infrared imager.

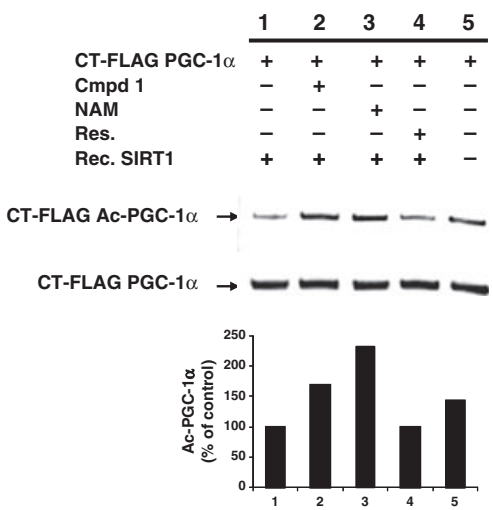

Figure 2: Effect of resveratrol on SIRT1 deacetylase activity using purified PGC-1α as a substrate. Acetylated CT-FLAG PGC-1α was isolated by immunoprecipitation from lysates of cells transfected with PGC-1α and GCN5 and treated with 10 mM nicotinamide (NAM) overnight to accumulate acetylated PGC-1α. Equal aliquots (20 μL) were incubated in enzyme buffer in the presence of 50 nM recombinant SIRT1 and various compounds (DMSO vehicle control, 10 μM SIRT1 inhibitor compound 1, 5 mM NAM or 10 μM resveratrol) for 4.5 h at 30 °C. The reactions were stopped by addition of SDS-sample buffer and acetylated PGC-1α (CT-FLAG Ac-PGC-1α) was detected by Western blotting with an anti-Ac-Lys monoclonal antibody. To confirm equal substrate loading, the blot was stripped and re-probed with the anti-PGC-1α antibodies. The density of the acetylated PGC-1α band was scanned and normalized by that of the total PGC-1α in each lane. All data are expressed as % of the lane 1 value (control).

Results and Discussion

Effect of resveratrol on SIRT1 activity using peptide substrates

To directly monitor SIRT1 deacetylase activity without potential chemical or biological interference, we developed an analytical method using HLPC to directly quantify products from SIRT1 enzymatic reaction by their retention times (13). We first assessed the deacetylation of Fluor de Lys-SIRT1 peptide substrate that has been widely used in the literature to assess SIRT1 deacetylase activity (6,12). The Fluor de Lys-SIRT1 substrate is the acetylpeptide RHKK(Ac) covalently conjugated to a fluorophore. It was previously demonstrated that covalent attachment of a fluorophore to other SIRT1 peptide substrates produced resveratrol-mediated SIRT1 activation (9,10). When the fluorophore was removed from the labeled peptide substrates, resveratrol failed to activate SIRT1 (9,10). These data suggest that the resveratrol-mediated activation of SIRT1 is dependent on the presence of the fluorophore on the substrate. We asked the question if the fluorophore on the Fluor de Lys-SIRT1 substrate plays a similar role in causing SIRT1 activation by resveratrol. To our surprise, when the fluorophore was removed from the Fluor de Lys-SIRT1 peptide substrate, it could not even function as a SIRT1 substrate (Figure 1A). In contrast, the Fluor de Lys-SIRT1 peptide substrate (with the fluorophore) was a substrate for SIRT1 in the same assay (Figure 1A). These data suggest that the fluorophore on the Fluor de Lys-SIRT1 peptide is not only a fluorescent label for detection but more importantly converts the Fluor de Lys-SIRT1 peptide into an artificial SIRT1 substrate. Therefore, the Fluor de Lys-SIRT1 peptide is not an appropriate SIRT1 substrate.

Since p53 is a known SIRT1 substrate *in vivo* (14), we synthesized a p53-derived peptide in our assay to assess the effect of resveratrol. Incubation of recombinant SIRT1 with the acetylated form of the p53 peptide resulted in the appearance of deacetylated form as analyzed by HPLC (data not shown). The amount of accumulated deacetylated peptide was inhibited by a known SIRT1 inhibitor (13) in a dose-dependent manner (Figure 1B), suggesting that the assay is a selective assessment of SIRT1 activity. However, resveratrol did not have any effect on SIRT1 activity when the acetylated p53 peptide was used as a substrate (Figure 1C). In contrast, resveratrol only activated SIRT1 dose-dependently when the Fluor de Lys-SIRT1 peptide was used (Figure 1C). These data are consistent with previous findings that SIRT1 activation by resveratrol is dependent on the fluorophore on the Fluor de Lys-SIRT1 peptide substrate (9,10), and it is the fluorophore that helps produce enzymatic activation by resveratrol (9). Our data, along with those in the literature, suggest that SIRT1 activation by resveratrol could be an artifact caused by the Fluor de Lys-SIRT1 peptide substrate. However, since all the above assays were conducted with synthetic acetylpeptide substrates, further studies with a physiologically relevant protein substrate are warranted to support this conclusion.

Effect of resveratrol on SIRT1 deacetylase activity using purified PGC-1α

PGC-1α has been implicated as a SIRT1 substrate and mediates SIRT1 effects on mitochondrial function and energy metabolism (4). To further assess whether resveratrol activates SIRT1 deacetylase activity, we carried out a deacetylase assay using immunoprecipitated PGC-1α. We found that when PGC-1α was expressed in cells

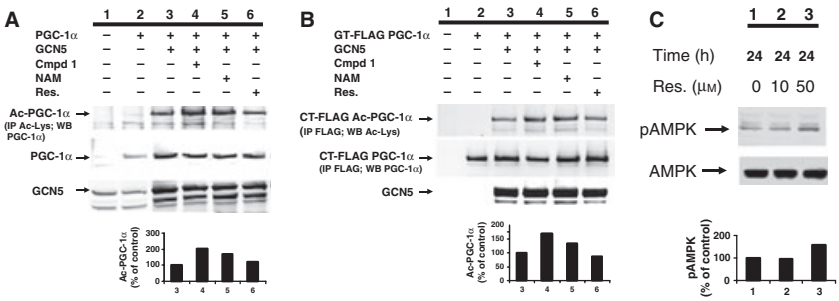

Figure 3: Effect of resveratrol on PGC-1α acetylation in HEK293 cells. (A) HEK293 cells were transiently transfected with various cDNA constructs and on the following day, treated with compounds (50 μM SIRT1 inhibitor compound 1, 10 mM NAM, 10 μM resveratrol (Res) or vehicle DMSO overnight. Total PGC-1α and GCN5 were detected by Western blotting of 50 μg cell lysate protein. Acetylated PGC-1α (Ac-PGC-1α) was detected by immunoprecipitation of cell lysates using an anti-Ac-Lys mouse monoclonal antibody followed by Western blotting with anti-PGC-1α polyclonal antibodies. The density of the acetylated PGC-1α band was scanned and normalized by that of the total PGC-1α in each lane. All data are expressed as % of the lane 3 value (control). The relative densities are not shown for lanes 1 and 2 since there is no total PGC-1α in lane 1 and there is very little acetylated PGC-1α without combined PGC-1α and GCN5 overexpression in lanes 1 and 2. (B) Transfection, compound treatments and GCN5 detection were carried out as described in (A). Total CT-FLAG PGC-1α and acetylated CT-FLAG PGC-1α were detected by Western blotting of immunoprecipitated CT-FLAG PGC-1α with anti- PGC-1α antibodies and anti-Ac-Lys mouse monoclonal antibody, respectively. The density of the acetylated PGC-1α band was scanned and normalized by that of the total PGC-1α in each lane. All data are expressed as % of the lane 3 value (control). The relative densities are not shown for lanes 1 and 2 since there is no total PGC-1α in lane 1 and there is very little acetylated PGC-1α without combined PGC-1α and GCN5 overexpression in lanes 1 and 2. (C) To confirm the activity of resveratrol in cells, HepG2 cells were treated with resveratrol for 24 h and phosphorylated AMPK was measured in cell lysates. The density of phosphorylated AMPK was normalized by the total AMPK in the same lane. All data are expressed as % of the lane 1 value (control).

alone, there was very little acetylation (see below). We co-transfected PGC-1α with its acetylase GCN5 to boost its acetylation level (15). The PGC-1α expressed in transfected cells was purified by immunoprecipitation. When PGC-1α was incubated with SIRT1, there was a significant decrease in its acetylation level (Figure 2, lanes 1 and 5). The addition of SIRT1 inhibitors, compound 1 (6-chloro-2,3,4,9-tetrahydro-1*H*-carbazole-1-carboxamide 1) or NAM (13), suppressed the SIRT1-dependent deacetylation of PGC-1α, resulting in the increased PGC-1α acetylation level compared with control (Figure 2, lanes 2 and 3). However, resveratrol had no effect on the acetylation level of PGC-1α in the same assay (Figure 2). These data suggest that PGC-1α acetylated by GCN5 in cells is a SIRT1 substrate and resveratrol does not activate SIRT1 activity.

Evaluation of the effect of resveratrol on SIRT1 activity and PGC-1α acetylation in cells

To further demonstrate any potential effects of resveratrol on SIRT1 activity, we carried out co-transfection experiments to assess the acetylation level of PGC-1α in cells treated with SIRT1 inhibitors or resveratrol. The acetylation level of PGC-1α is low when it is expressed alone in cells (Figure 3A), probably because of either high level of endogenous deacetylase activity or low level of endogenous acetylase activity. When PGC-1α was co-transfected with its acetylase GCN5 (15), there was a significant increase in the acetylation level compared with PGC-1α alone (Figure 3A). When the cells were treated with SIRT1 inhibitor compound 1 or NAM, further increases in PGC-1α acetylation were observed (Figure 3A), suggesting that the endogenous SIRT1 acts as a PGC-1α deacetylase in the cells. When the cells were treated with resveratrol, there was no change in the PGC-1α acetylation level (Figure 3A), suggesting that resveratrol did not activate the endogenous SIRT1. To confirm these findings, we took a different approach in the immunoprecipitation experiment. Instead of immunoprecipitating acetylated proteins and blotting with anti-PGC-1α antibodies, we immunoprecipitated FLAG-tagged PGC-1α and blotted with an anti-acetylated lysine antibody (Figure 3B). Consistent with the observations in Figure 3A, GCN5 increased PGC-1α acetylation in cells and SIRT1 inhibitor further increased the acetylation level (Figure 3B). Resveratrol had no effect on the acetylation level of PGC-1α (Figure 3B). These data demonstrate that resveratrol does not activate SIRT1 in cells. To confirm the activity of resveratrol in cells, we measured AMPK activation by resveratrol in HepG2 cells (Figure 3C). Our data are consistent with the report by Bauer *et al.* that resveratrol activates AMPK at 50 μM but does not at 12.5 μM in cells (7). It has been reported that AMPK enhances SIRT1 activity indirectly by increasing cellular NAD^+ levels (16). Since our goal is to assess if resveratrol directly activates SIRT1 in cells, it is important that our cell-based experiment is carried out at a resveratrol concentration that does not activate AMPK to avoid false positive result. Bauer *et al.* reported that resveratrol can activate AMPK at 25 μM but not at 12.5 μM (7). Using 10 μM resveratrol in our cell-based assay is therefore appropriate because it does not activate AMPK based on our data and those by Bauer *et al.* (7).

To further demonstrate that the endogenous SIRT1 is a major PGC-1α deacetylase, siRNA was used to knock down SIRT1 protein level and complement the pharmacological studies with SIRT1 inhibitors. SIRT1 siRNA significantly reduced the endogenous SIRT1 protein level and there was a concomitant increase in PGC-1α acetylation level (Figure 4). These data demonstrate that SIRT1 is an endogenous PGC-1α deacetylase.

In conclusion, resveratrol activates SIRT1 only when the Fluor de Lys-SIRT1 peptide substrate was used. We demonstrated that it acts as a SIRT1 substrate exclusively because of the fluorophore on the peptide and therefore, it is not an appropriate SIRT1 substrate. Resveratrol did not activate SIRT1 in *in vitro* assays using a p53-derived peptide substrate that is not covalently labeled with a fluorophore. Further, resveratrol did not activate SIRT1 activity when purified PGC-1α was used as a substrate. Finally, in cells that express both PGC-1α and GCN5, resveratrol had no effect on PGC-1α acetylation. The claim of resveratrol being a SIRT1 activator is likely to be an experimental artifact of the SIRT1 assay that employs the Fluor de Lys-SIRT1 peptide as a substrate. However, the beneficial metabolic effects of resveratrol have been clearly demonstrated in diabetic animal models (7,8). Our data do not support the notion that these metabolic effects are mediated by direct SIRT1 activation. Rather, they could be mediated by other mecha-

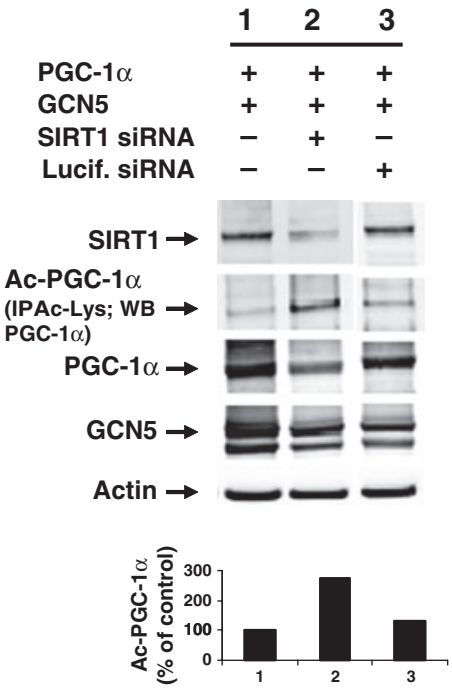

Figure 4: SIRT1 siRNA knockdown increases PGC-1α acetylation in cells. HEK293 cells were co-transfected with cDNA constructs and SIRT1 pool or C-luciferase control siRNAs as indicated. After 68 h incubation, cells were lysed and SIRT1, total and acetylated PGC-1α, GCN5 and actin were detected by Western blotting using 50 μg cell lysate protein. The density of the acetylated PGC-1α band was scanned and normalized by that of the total PGC-1α in each lane. All data are expressed as % of the lane 1 value (control). The white line between lanes 2 and 3 indicates that lanes from the same blot have been spliced.

nisms such as AMPK activation (17–19), which could occur independently of SIRT1, although recent work has shown that SIRT1 activation can indirectly activate AMPK (20). In the meantime, AMPK enhances SIRT1 activity by increasing intracellular NAD^+ levels (16). These findings suggest that resveratrol could indirectly activate SIRT1 *in vivo*. How resveratrol activates AMPK is unknown but it may be mediated by targets other than SIRT1. Future work will be required to test whether resveratrol exerts its pharmacological action via AMPK activation in a SIRT1-independent manner.

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