Sirt3 protein deacetylates isocitrate dehydrogenase 2 (IDH2) and regulates mitochondrial redox status

Wei Yu, Kristin E. Dittenhafer-Reed, and John M. Denu

Background: NAD⁺⁺-dependent deacetylase SIRT3 is essential for the prevention of age-related hearing loss during caloric restriction.

Results: Oxidative stress resistance by SIRT3 was mediated through IDH2. SIRT3 deacetylates IDH2 at lysine 413 and stimulates activity by as much as 44-fold.

Conclusion: Increased SIRT3 expression protects cells from oxidative stress through IDH2 activation.

Significance: Our results provide the mechanism by which SIRT3 regulates IDH2.

Mitochondria play a central role in oxidative energy metabolism and age-related diseases such as cancer. Accumulation of spurious oxidative damage can cause cellular dysfunction. Antioxidant pathways that rely on NADPH are needed for the reduction of glutathione and maintenance of proper redox status. The mitochondrial matrix protein isocitrate dehydrogenase 2 (IDH2) is a major source of NADPH. Previously, we demonstrated that the NAD⁺⁺-dependent deacetylase SIRT3 was essential for the prevention of age-related hearing loss in mice fed a calorically restricted diet. Here we provide direct biochemical and biological evidence establishing an exquisite regulatory relationship between IDH2 and SIRT3 under acute and chronic caloric restriction. The regulated site of acetylation was mapped to Lys-413, an evolutionarily invariant residue. Site-specific, genetic incorporation of N⁴-acetyllysine into position 413 of IDH2 revealed that acetylated IDH2 displays a dramatic 44-fold loss in activity. Deacetylation by SIRT3 fully restored maximum IDH2 activity. The ability of SIRT3 to protect cells from oxidative stress was dependent on IDH2, and the deacetylated mimic, IDH2⁴⁴¹₃R variant was able to protect mouse embryonic fibroblasts from oxidative stress through increased reduced glutathione levels. Together these results uncover a previously unknown mechanism by which SIRT3 regulates IDH2 under dietary restriction. Recent findings demonstrate that IDH2 activities are a major factor in cancer, and as such, these results implicate SIRT3 as a potential regulator of IDH2-dependent functions in cancer cell metabolism.
the significance and mechanism of regulation remained unknown. Here we provide detailed biochemical and biological evidence that elucidate the exquisite regulatory relationship between SIRT3 and IDH2. Using in vivo and in vitro studies, the site of regulatory (de)acetylation was mapped to Lys-413 of IDH2. In response to calorie and glucose restriction, we demonstrate that SIRT3 (de)acetylation was mapped to Lys-413 of IDH2. In response to acute and chronic caloric restriction. In addition to regulation of mitochondrial redox status, these results implicate SIRT3 as a general regulator of IDH2 functions, particularly in cancer cell metabolism.

**EXPERIMENTAL PROCEDURES**

**In Vivo Site-specific Incorporation of Acetylysine into Proteins**—To generate a homogeneously acetylated IDH2 construct for analysis, we used a three-plasmid system described by Neumann et al. (31, 32). This system allows for the site-specific incorporation of N\textsuperscript{\textalpha}-acetylysine by way of a Methanosarcina Barkeri acetyl-lysyl-tRNA synthetase/tRNA\textsubscript{CUA} pair that recognizes an amide codon. To avoid the isolation of truncated forms of IDH2Ac, wild-type IDH2 was cloned into pTEV-9 (pET-21b backboned with tobacco etch virus cleavage site), producing a C-terminal His\textsubscript{6}-tagged construct. By incorporating an amide codon at Lys-413 (AAG to TAG by site-directed mutagenesis), we produced pTEV-IDH2 that encodes for a homogeneous pool of IDH2Ac. Both proteins were produced and purified as described below.

The three plasmids (TEV-9 with expressed gene, pCDF pYLT-1, and pAcKRS) were transformed into electrocompetent Escherichia coli BL21 DE3 cells (laboratory collection). Overnight cultures were subcultured 1:100 into 2 liters of 2\times YT containing spectinomycin (50 \mu g/ml) and ampicillin (150 \mu g/ml). Cultures were grown at 37 °C with shaking to an A\textsubscript{600} of 0.6, induced with 0.4 \mu M isopropyl-\textalpha-thio-\textbeta-D-galactopyranoside in addition to 2 mM N\textsuperscript{\textalpha}-acetyllysine (Sigma-Aldrich) and 20 \mu M nicotinamide solution (10 \mu M nicotinamide, 20 \mu M NaHCO\textsubscript{3}, 100 \mu M Na\textsubscript{2}CO\textsubscript{3}) and underwent three freeze-thaw cycles to extract NADP\textsuperscript{+} and NADPH. To destroy NADP\textsuperscript{+} in the sample, 90 \mu l of the lysate was incubated in a heating block for 30 min at 60 °C. Twenty-five microliters of each unheated and heated sample was mixed with 225 \mu l of a reaction mixture (100 \mu M Tris, 5 \mu M EDTA, 0.5 \mu M thiazole blue tetrazolium bromide, 2 \mu M phenazine ethosulfate, 1.3 units of glucose-6-phosphate dehydrogenase, pH 8.0) and incubated in a water bath for 5 min at 37 °C. The reaction mixture was transferred to each well of a 96-well plate, and 1 \mu l of 1 mM glucose 6-phosphate was added to initiate the reaction. The absorbance was read at 570 nm every 10 s for 3 min in a microplate reader (Synergy H4, BioTek). All samples were run in duplicate. The reaction rates were calculated, and NADPH levels were determined as the ratio of NADPH (heated sample) to the total of NADP\textsuperscript{+} and NADPH (unheated sample). The GSH:GSSG ratio was determined using the GSH:GSSG-Glo assay kit (Promega).

**Generation of Stable Cell Pools**—HEK293 or immortal Sirt3\textsuperscript{+/+} or Sirt3\textsuperscript{−/−} MEFs were initially cultured in DMEM supplemented with 10% FBS prior to their use in establishing stable transfections. SIRT3 MEFs were kindly provided by Leonard Guarente (34). To establish stable Sirt3-expressing cells, HEK293 cells were transfected with pCDNA3-Sirt3-FLAG by calcium phosphate transfection. A vector control cell line was established by the same method using pCDNA3. To establish stable IDH2-expressing cells, pCDNA3.1-IDH2-FLAG, K413R, and K413Q plasmids were transfected into Sirt3\textsuperscript{+/+} or Sirt3\textsuperscript{−/−} MEF by TurboFect in vitro transfection reagent (Fermentas). After transfection, cells were selected in the medium containing G418 (1.5 mg/ml) for 10 days. The antibiotic-resistant clones were selected, expanded, and further cultured in medium supplemented with adequate amounts of antibiotics. To generate stable IDH2 knockdown cell pools in HEK293 cells stably expressing SIRT3 protein, 30 pmol of siRNA for IDH2 mRNA (Sigma) was transfected into HEK293 by TurboFect reagent. After 36 h of transfection, mitochondria were isolated (2) from 9 \times 10\textsuperscript{6} HEK293 cells. The mitochondrial fraction was used to measure NADPH concentration.

**Cytotoxicity Assay**—The three stable HEK293 cell pools (vector, SIRT3, and SIRT3 with knockdown IDH2 lines) or Sirt3\textsuperscript{+/+} or Sirt3\textsuperscript{−/−} MEF cell pools of stably expressing IDH2\textsuperscript{K413Q} IDH2\textsuperscript{K413R} were first grown on a 96-well plate at a density of 1 × 10\textsuperscript{4} cells/well before oxidant treatment and subsequent assessment of cell viability, using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (35). After overnight culture, 25 \mu M menadione or 1 \mu M hydrogen peroxide was applied to the cells in serum-free DMEM, and cells were incubated for an additional 48 h at 37 °C. After 48 h of oxidant treatment, culture medium was aspirated before 200 \mu l of MTT (1 mg/ml) was added and further incubated for 4 h at 37 °C. The MTT solution was discarded by aspirating, and the resulting formazan product converted by the viable cells was dissolved in 150 \mu l of dimethyl sulfoxide. The absorbance was read in an ELISA plate reader at 595 nm (Synergy H4, BioTek). Cell viability is expressed as a percentage of the absorbance measured in the untreated control cells.
RESULTS

Mapping Functional Acetylation Sites in IDH2—Recent proteomic studies reported that 13 different lysine residues in IDH2 were modified by acetylation (12–14); however, it was unknown which sites played a critical role in regulating IDH2 activity. Because IDH2 is an evolutionarily conserved protein, we speculated that important regulatory sites targeted by acetylation might be conserved. Sequence alignments from diverse species revealed that lysines 256, 263, and 413 are invariant, allowing us to focus our inquiry on those residues (supplemental Fig. S1). We replaced lysine 256, 263, and 413 with either glutamine or arginine (Fig. 1A). As a control, we also mutated Lys-272, which is not conserved but was reported as acetylated. The Lys to Arg mutation retains a positive charge and can act as a surrogate of acetylation (18, 23). The IDH2 variants were overexpressed in HEK293 cells and immunoprecipitated, and activity assays were performed. Wild-type IDH2 activity was set as 100%. Bars and error bars represent mean and S.D. of triplicate assays. B and C, kinetic comparison of wild-type IDH2 and variants. Lys-413 was mutated to glutamine or arginine and bacterially expressed, recombinant proteins were purified, and steady-state kinetic analyses were performed. Comparison of IDH2 (open circles) and variants K413R (open squares) and K413Q (open diamonds) shows that the Lys-413 is important in catalysis.

FIGURE 1. A, maintenance of unacetylated Lys-413 is necessary for IDH2 activity. Wild-type IDH2 and K413R, K413Q, K272Q, K256Q, and K263Q mutants were expressed in HEK293 cells. Proteins were purified by immunoprecipitation (IP), IDH2 levels were normalized for protein, and activity assays were performed. Wild-type IDH2 activity was set as 100%. Bars and error bars represent mean and S.D. of triplicate assays. B and C, kinetic comparison of wild-type IDH2 and variants. Lys-413 was mutated to glutamine or arginine and bacterially expressed, recombinant proteins were purified, and steady-state kinetic analyses were performed. Comparison of IDH2 (open circles) and variants K413R (open squares) and K413Q (open diamonds) shows that the Lys-413 is important in catalysis.
To investigate the mechanism by which Lys-413 acetylation might reduce IDH2 activity, we recombinantly expressed and purified human wild-type IDH2 and the K413R and K413Q mutants from E. coli. Steady-state kinetic analysis of IDH2 and variants (Fig. 1, B and C) indicated that substitution of Lys-413 with arginine did not appreciably alter the $K_m$ values for isocitrate and NADP$^+$; however, the K413Q variant exhibited a dramatic 20-fold decrease in $V_{max}$ compared with WT, whereas the K413R variant was unaffected in $V_{max}$ (supplemental Table S1). Also, the K413Q variant dis-
played an \(~5\)-fold increase in the $K_m$ value for NADP$^+$ but no significant change in the $K_m$ value for isocitrate. These results support an inhibitory effect of Lys-413 acetylation on IDH2 activity.

**Genetic Incorporation of Acetyllysine at Amino Acid Position 413**—Although arginine/glutamine substitutions can provide general insight into possible functional effects of protein acetylation, there is sufficient evidence that such strategies do not reveal the true consequences of acetylation (37). To directly determine the effect of acetylation, we utilized site-specific, genetic incorporation of N-acetyllysine at position 413 of IDH2. The incorporation of N-acetyllysine was genetically encoded through an amber stop codon (TAG) at amino acid position 413. The mutant version of IDH2 was coexpressed in E. coli with an orthogonal N-acetyllysine-tRNA synthetase/tRNA$_{CUA}$ pair in the presence of N-acetyllysine. Only with N-acetyllysine in the growth medium was the full-length protein formed, and incorporated acetyllysine was verified by immunoblotting with an anti-acetyllysine antibody (Fig. 2A).

Acetylation of Lys-413 Decreases Catalysis and SIRT3 Reactivates IDH2 upon Deacetylation—IDH2$^{K413Ac}$ was purified, and catalytic activity was assessed, yielding an enzyme with decreased activity by greater than 10-fold when compared with wild-type enzyme (Fig. 2B). Next, we determined whether SIRT3 could deacetylate Lys-413 and recover IDH2 activity. Using an *in vitro* deacetylation assay, we demonstrated that only when SIRT3 and the co-substrate NAD$^+$ are present does SIRT3 remove the acetyl group from IDH2$^{K413Ac}$ (Fig. 2C). Corresponding IDH2 activity assays revealed that deacetylated IDH2 recovers full catalytic activity when compared with WT (Fig. 2D).

To provide molecular insight into the regulatory mechanism of IDH2 reversible acetylation, a steady-state kinetic analysis was performed with WT and IDH2$^{K413Ac}$. Substrate saturation curves were determined with NADP$^+$ (Fig. 2E) and isocitrate (Fig. 2F) as the varied substrate with either IDH2 or IDH2$^{K413Ac}$. When compared with IDH2, this kinetic analysis revealed that IDH2$^{K413Ac}$ displays a 15-fold (average from supplemental Table S1) decrease in $V_{\text{max}}$ and a 44-fold decrease in the apparent second order rate constant $V_{\text{max}}/K_m$ with NADP$^+$ as substrate. (Fig. 2, E and F). Interestingly, the $K_m$ values for isocitrate were similar between the acetylated and unacetylated enzyme (supplemental Table S1). These results demonstrate for the first time the molecular and functional consequence of Lys-413 acetylation on IDH2 activity.

**SIRT3-dependent Deacetylation of IDH2 Suppresses Cellular ROS Stress**—Previous studies implicate IDH2-produced NADPH as a protective mechanism against cellular oxidative stress (3). We demonstrated that SIRT3 regulates the ability of cells to resist ROS damage from exogenous and endogenous ROS (2). We proposed that SIRT3 deacetylates IDH2 and stimulates an increase in NADPH, which is utilized by the mitochondrial antioxidant system to combat ROS. To determine how much IDH2 contributes to the steady-state levels of NADPH, we used siRNA to knock down endogenous IDH2 in HEK293 cells (supplemental Fig. 2A) and measured the corresponding change in NADPH levels within mitochondria. The results (Fig. 3B) indicate that IDH2 contributes \(\approx25\%\) of the total NADPH
produced in the mitochondria, which is consistent with previous studies showing IDH2 as one of major mitochondrial sources of NADPH (38).

To test whether the protective effect of SIRT3 against oxidative stress is dependent on IDH2, we used siRNA to knock down endogenous IDH2 in cells stably overexpressing SIRT3. HEK293 cells were treated with H2O2 or menadione, and cell viability was monitored (Fig. 3, C and D). Consistent with our previous observations (2), expression of SIRT3 offers significant protection from ROS insults such as H2O2 and menadione (Fig. 3, C and D). Here we show that when IDH2 levels are knocked down, the protective effects of SIRT3 expression are eliminated (with H2O2, Fig. 3C) or substantially reduced (with menadione, Fig. 3D). These results provide strong biochemical evidence that IDH2 is a major pathway through which SIRT3 provides defense against ROS.

Acetylation of Lys-413 Is Regulated by SIRT3 in Response to Calorie and Glucose Restriction—Next, we investigated in vivo whether Lys-413 is a critical acetylation site that modulates the activity of IDH2 in response to glucose or calorie restriction. To determine the in vivo acetylation status of Lys-413, a rabbit polyclonal anti-acetylated Lys-413 IDH2 antibody was generated as outlined under “Experimental Procedures.” Western blot analysis of IDH2 overexpressed in HEK293 cells revealed an immune-reactive band that displayed increased intensity with cells treated with nicotinamide, a general sirtuin inhibitor (Fig. 4A). The anti-acetylated Lys-413 IDH2 antibody, however, did not recognize the IDH2 K413R mutant, indicating that the antibody is specific for IDH2 K413Ac. These data validate the site-specific antibody and confirm that Lys-413 in IDH2 is indeed acetylated when expressed in HEK293 cells.

Under caloric restriction or fasting, SIRT3 mRNA and protein levels are dramatically increased in mice (2, 39, 40). This led us to inquire whether IDH2 acetylation levels and activity might be directly controlled by glucose availability. We examined the effect of glucose levels on IDH2 acetylation in cultured cells and found that lowering glucose levels led to a decrease in Lys-413 acetylation, as determined by the Lys-413 acetylation-specific antibody (Fig. 4B). Lys-413 acetylation inversely correlated with the measured IDH2 activity (Fig. 4B), in agreement with an inhibitory effect of Lys-413 acetylation on IDH2 activity. Also, consistent with a direct effect by SIRT3, we found that lowered glucose levels increase the protein level of SIRT3 (Fig. 4B). These results suggest an exquisite relationship between nutrient status, SIRT3 levels, and regulated IDH2 activity.

To provide direct evidence that SIRT3 controls IDH2 activity via reversible acetylation of Lys-413, we utilized the site-specific anti-K413Ac antibody and determined acetylation levels of IDH2 from liver mitochondria of WT and Sirt3−/− mice fed control or calorie-restricted diets (Fig. 4C). In CR tissues, the acetylation of Lys-413 is decreased 3-fold when compared with control diet. This change closely matches the 3-fold increase in SIRT3 protein levels under CR (Fig. 4C) (2, 17, 26). In Sirt3−/− mice tissues, hyperacetylation of IDH2 was observed in both control and CR diet conditions, indicating that SIRT3 is required for the CR-induced deacetylation of IDH2 lys-413.

**IDH2K413R Rescues Oxidant Damage in Sirt3−/− MEFs—**We have shown that substitution of lysine with glutamine mimics an acetylated Lys-413 of IDH2, whereas substitution with an arginine mimics the deacetylated state (Figs. 1, B and C, and 2, E and F). We took advantage of this unique mimicry to investigate, in the absence of SIRT3, whether overexpression of IDH2K413R could protect cells from oxidant stress. For comparison, we separately expressed the IDH2K413Q acetylation mimic. Three stable cell pools expressing IDH2K413R, IDH2K413Q, or a vector control were generated in Sirt3−/− MEFs (34) (Fig. 5A and supplemental Fig. 2B). The levels of GSH:GSSG and cell viability were determined ± H2O2 or ± menadione. In Sirt3−/− MEFs, treatment with H2O2 and menadione caused significant decreases in mitochondrial GSH:GSSG ratios (Fig. 5, A and B). Expression of the IDH2K413R variant in Sirt3−/− MEFs (Fig. 5, B and C) provided maintenance of the GSH:GSSG ratio after H2O2 or menadione treatment. The
The IDH2<sup>K413Q</sup> variant was unable to maintain the GSH:GSSG, yielding similar GSH:GSSG values to those of vector control after ROS treatment. The effects on cell viability are more dramatic and consistent with the trends observed for GSH:GSSG changes (Fig. 5, C and D). Expression of the IDH2<sup>K413R</sup> variant provided complete protection against both oxidant insults (Fig. 5, C and D). In contrast, stable expression of IDH2<sup>K413Q</sup> offered no significant protection against H<sub>2</sub>O<sub>2</sub> and menadione, consistent with the trends on GSH:GSSG levels. These results support a critical role for IDH2 in maintaining the redox balance in mitochondria and a regulatory function of reversible acetylation at Lys-413.

**DISCUSSION**

Here we provide the mechanistic basis for SIRT3-dependent regulation of IDH2 under glucose and caloric restriction. We mapped the regulated site of acetylation to Lys-413 using mutational analysis of evolutionarily invariant lysine residues. To provide unequivocal assessment for the effect of acetylation, we utilized site-specific, genetic incorporation of N<sup>-</sup>/H<sup>9280</sup>-acetyllysine into recombinantly expressed and purified IDH2. When compared with WT enzyme, steady-state kinetic analysis revealed that IDH2<sup>K413Ac</sup> displays an approximate 15- and 44-fold decrease in $V_{\text{max}}$ and $V_{\text{max}}/K_m$ (NADP<sup>+</sup>), respectively, consistent with the kinetics of IDH2<sup>K413Q</sup>. SIRT3 specifically deacetylated IDH2<sup>K413Ac</sup>, resulting in full recovery of IDH2 activity. To our knowledge, this is the first use of site-specific genetic incorporation of acetyllysine to perform a detailed mechanistic analysis on the catalytic effects of acetylation. Previous kinetic analysis of NADP<sup>+</sup>-dependent IDH revealed that catalysis proceeds by a random sequential mechanism where catalysis is more rapid than product release (41). Therefore, the effect of acetylation on $V_{\text{max}}$ likely reflects altered product release. Similarly, the dramatic drop in $V_{\text{max}}/K_m$ for NADP<sup>+</sup> might reflect inefficient formation of the catalytically competent conformation. Structural studies of IDH2 from *Saccharomyces cerevisiae* (57% identity to human IDH2) indicated that the protein undergoes substantial conformational changes during catalysis (42). We hypothesize that acetylation of Lys-413, partially through the ablation of the positively charged binding pocket (supplemental Fig. S3), as well as through potential structural alteration induced by acetylation, prevents the proper binding conformation and positioning of NADP<sup>+</sup> for catalysis.

The ability of SIRT3 to modulate IDH2 activity has wide-ranging implications in metabolism, ROS mitigation, and cancer. Consistent with emerging evidence (2, 15–23, 25, 43), SIRT3 promotes oxidative metabolism and mitochondrial reprogramming under lowered energy input (acute and chronic caloric restriction). Stimulation of oxidative metabolic path-
ways in mitochondria can result in increased production of ROS, which causes damage to cellular components and compromises cell viability (44, 45). The role of IDH2 in the mitochondrial antioxidant pathways suggests a protective role in cancer as it is postulated that decreased cellular damage by ROS slows cancer development (46, 47). We propose that SIRT3 activates the IDH2 pathway as a coordinated program to mitigate increased ROS during periods of increased oxidative metabolism. We demonstrate that SIRT3 can protect cells from mitochondrial oxidative stress and that this effect is almost entirely dependent on IDH2. Cellular knockdown of IDH2 revealed that 25% of mitochondrial NADPH is maintained by IDH2 under unstressed growth, which is consistent with previous reports that IDH2 is one of two major mitochondrial NADPH producers (48). IDH2 knockdown in cells stably over-expressing SIRT3 led to a complete or nearly complete loss of protection from oxidative stress. Importantly, we show that the Lys-413 deacetylation mimic, IDH2K413R, is able to protect Sirt3−/− MEFs from oxidative stress by increasing GSH:GSSG, providing evidence that IDH2 serves as the critical link between SIRT3 and protection from ROS. Recent studies reported that SIRT3 can also activate superoxide dismutase 2 (SOD2) (21) and Mn-SOD (22, 23). SOD catalyzes the conversion of superoxide to H2O2. Hydrogen peroxide, produced by dismutation of superoxide and other cellular redox chemistries, is the major oxidizer of GSH and protein thiols. Oxidized thiols are repaired by the IDH2-dependent antioxidant pathway involving glutathione reductase. The observation that IDH2 is critical for GSH buffering and cell viability against both H2O2 and menadione supports a critical role for IDH2 regardless of ROS origin. Taken together, SIRT3 is a key factor in a coordinate up-regulation of oxidative metabolism and antioxidant pathways in response to acute and chronic caloric restriction.

Recent studies describe previously unappreciated functions of IDH that suggest a major role in cancer cell metabolism. First, there are known active-site mutations that result in decreased rates of α-ketoglutarate formation, but increased ability to form an oncogenic product, 2-hydroxyglutarate (5−7). Second, IDH2 is critical for the metabolic reprogramming that allows tumor cells to utilize glutamine for macromolecular biosynthesis required for cell growth (8−10). In the latter case, IDH2 catalyzes the reductive carboxylation of α-ketoglutarate to iso-citrate, the reverse direction of the normal IDH2-catalyzed reaction.

Here we have uncovered a previously unknown mechanism by which SIRT3 regulates IDH2 under dietary restriction. In addition to the antioxidant functions described in the current study, these results implicate SIRT3 as a major regulator of IDH2 function and cancer cell metabolism. Several recent studies suggest that SIRT3 acts as a tumor suppressor (34, 49, 50), whereas others report a positive correlation with cancer and SIRT3 expression (51). Given this regulatory connection between these enzymes and their effects in cancer cell metabolism, future investigations will be important to elucidate the role of SIRT3-dependent modulation of IDH2 activity in cancer progression.

Acknowledgments—We thank Leonard Guarente for SIRT3 MEFs, Jason Chin for site-specific incorporation vectors, Tom Prolla and James Vann for supplying MEFs, and members of the Denu laboratory for helpful discussions.

REFERENCES

1. Singh, K. K. (2006) Mitochondria damage checkpoint, aging, and cancer. Ann. N.Y. Acad. Sci. 1067, 182−190
2. Someya, S., Yu, W., Hallows, W. C., Xu, J., Vann, J. M., Leeuwenburgh, C., Tanokura, M., Denu, J. M., and Prolla, T. A. (2010) Sirt3 mediates reduction of oxidative damage and prevention of age-related hearing loss under caloric restriction. Cell 143, 802−812
3. Jo, S. H., Son, M. K., Koh, H. J., Lee, S. M., Song, I. H., Kim, Y. O., Lee, Y. S., Jeong, K. S., Kim, W. B., Park, J. W., Song, B. J., and Huh, T. L. (2001) Control of mitochondrial redox balance and cellular defense against oxidative damage by mitochondrial NADP+-dependent isocitrate dehydrogenase. J. Biol. Chem. 276, 16168−16176
4. Lee, J. H., Yang, E. S., and Park, J. W. (2003) Inactivation of NADP+-dependent isocitrate dehydrogenase by peroxynitrite: implications for cytotoxicity and alcohol-induced liver injury. J. Biol. Chem. 278, 51360−51371
5. Dang, L., White, D. W., Gross, S., Bennett, B. D., Bittinger, M. A., Driggers, E. M., Fantin, V. R., Jang, H. G., Jin, S., Keenan, M. C., Marks, K. M., Prins, R. M., Park, S. P., Zhen, K. E., Liau, L. M., Rabinowitz, J. D., Cantley, L. C., Thompson, C. B., Vander Heiden, M. G., and Su, S. M. (2009) Cancer-associated IDH1 mutations produce 2-hydroxyglutarate. Nature 462, 739−744
6. Ward, P. S., Patel, J., Wise, D. R., Abdel-Wahab, O., Bennett, B. D., Coller, H. A., Cross, J. R., Fantin, V. R., Hedvat, C. V., Perl, A. E., Rabinowitz, J. D., Carroll, M., Su, S. M., Sharp, K. A., Levine, R. L., and Thompson, C. B. (2010) The common feature of leukemia-associated IDH1 and IDH2 mutations is a neomorphic enzyme activity converting α-ketoglutarate to 2-hydroxyglutarate. Cancer Cell 17, 225−234
7. Gross, S., Cairns, R. A., Minden, M. D., Driggers, E. M., Bittinger, M. A., Jang, H. G., Sasaki, M., Jin, S., Schenkein, D. P., Su, S. M., Dang, L., Fantin, V. R., and Mak, T. W. (2010) Cancer-associated metabolite 2-hydroxyxylutarate accumulates in acute myelogenous leukemia with isocitrate dehydrogenase 1 and 2 mutations. J. Exp. Med. 207, 339−344
8. Mullen, A. R., Wheaton, W. W., Jin, S. E., Chen, P. H., Sullivan, L. B., Cheng, T., Yang, Y., Linehan, W. M., Chandel, N. S., and DeBerardinis, R. J. (2012) Reductive carboxylation supports growth in tumor cells with defective mitochondria. Nature 481, 385−388
9. Metallo, C. M., Gameiro, P. A., Bell, E. L., Mattaini, K. R., Yang, J., Hiller, K., Jewell, C. M., Johnson, Z. R., Irvine, D. J., Guarente, L., Kelleher, J. K., Vander Heiden, M. G., Iliopoulos, O., and Stephanopoulos, G. (2012) Reductive glutamine metabolism by IDH1 mediates lipogenesis under hypoxia. Nature 481, 380−384
10. Wise, D. R., Ward, P. S., Shay, J. E., Cross, J. R., Gruber, J. J., Sachdeva, U. M., Platt, J. M., DeMatteo, R. G., Simon, M. C., and Thompson, C. B. (2011) Hypoxia promotes isocitrate dehydrogenase-dependent carboxylation of α-ketoglutarate to citrate to support cell growth and viability. Proc. Natl. Acad. Sci. U.S.A. 108, 19611−19616
11. Lomibard, D. B., Alt, F. W., Cheng, H. L., Bunkencorn, J., Streepser, R. S., Mostoslavsky, R., Kim, J., Yancopoulos, G., Valenzuela, D., Murphy, A., Yang, Y., Chen, Y., Hirschey, M. D., Bronson, R. T., Haigis, M., Guarente, L. P., Farese, R. V., Jr., Weissman, S., Verdin, E., and Schwer, B. (2007) Mammalian Sir2 homolog SIRT3 regulates global mitochondrial lysine acetyltransferase. Mol. Cell. Biol. 27, 8807−8814
12. Kim, S. C., Sprung, R., Chen, Y., Xu, Y., Ball, H., Pei, J., Cheng, T., Kho, Y., Xiao, H., Xiao, L., Grishin, N. V., White, M., Yang, X. J., and Zhao, Y. (2006) Substrate and functional diversity of lysine acetyltransferase revealed by a proteomics survey. Mol. Cell 23, 607−618
13. Choudhary, C., Kumar, C., Gnad, F., Nielsen, M. L., Rehman, M., Walther, T. C., Olsen, J. V., and Mann, M. (2009) Lysine acetylation targets protein complexes and co-regulates major cellular functions. Science 325, 834−840
14. Zhao, S., Xu, W., Jiang, W., Yu, W., Lin, Y., Zhang, T., Yao, J., Zhou, L,
SIRT3 Deacetylates IDH2 and Regulates Redox Status

Zeng, Y., Li, H., Li, Y., Shi, J., An, W., Hancock, S. M., He, F., Qin, L., Chin, J., Yang, P., Chen, X., Lei, Q., Xiong, Y., and Guan, K. L. (2010) Regulation of cellular metabolism by protein lysine acetylation. Science 327, 1000–1004

15. Ahn, B. H., Kim, H. S., Song, S., Lee, I. H., Liu, J., Vasilopoulos, A., Deng, C. X., and Finkel, T. (2008) A role for the mitochondrial deacetylase Sirt3 in regulating energy homeostasis. Proc. Natl. Acad. Sci. USA. 105, 14447–14452

16. Hallows, W. C., Lee, S., and Denu, J. M. (2006) Sirtuins deacetylate and activate mammalian acetyl-CoA synthetases. Proc. Natl. Acad. Sci. USA. 103, 10230–10235

17. Hirschy, M. D., Shimazu, T., Goetzman, E., Jing, E., Schwer, B., Lombard, D. B., Grueter, C. A., Harris, C., Biddinger, S., Ilkayeva, O. R., Stevens, R. D., Li, Y., Saha, A. K., Ruderman, N. B., Bain, J. R., Newgard, C. B., Farese, R. V., Jr., Alh, F. W., Kahn, C. R., and Verdin, E. (2010) SIRT3 regulates mitochondrial fatty-acid oxidation by reversible enzyme deacetylation. Nature 464, 121–125

18. Schwer, B., Bunkenborg, J., Verdin, R. O., Andersen, J. S., and Verdin, E. (2006) Reversible lysine acetylation controls the activity of the mitochondrial enzyme acetyl-CoA synthetase 2. Proc. Natl. Acad. Sci. USA. 103, 10224–10229

19. Yang, Y., Cimen, H., Han, M. J., Shi, T., Deng, J. H., Koc, H., Palacios, O. M., Montier, L., Bai, Y., Tong, Q., and Koc, E. C. (2010) NAD+-dependent deacetylase SIRT3 regulates mitochondrial protein synthesis by deacetylation of the ribosomal protein MRPL10. J. Biol. Chem. 285, 7417–7429

20. Hallows, W. C., Yu, W., Smith, B. C., Devries, M. K., Ellinger, J. J., Someya, S., Shortreed, M. R., Pro, T., Markley, J. L., Smith, L. M., Zhao, S., Guan, K. L., and Denu, J. M. (2011) Sirt3 promotes the urea cycle and fatty acid oxidation during dietary restriction. Mol. Cell 41, 139–149

21. Qi, X., Brown, K., Hirschy, M. D., Verdin, E., and Chen, D. (2010) Calorie restriction reduces oxidative stress by SIRT3-mediated SOD2 activation. Cell Metab. 12, 662–667

22. Chen, Y., Zhang, J., Lin, Y., Lei, Q., Guan, K. L., Zhao, S., and Xiong, Y. (2011) Tumor suppressor SIRT3 deacetylates and activates manganese superoxide dismutase to scavenge ROS. EMBO Rep. 12, 534–541

23. Tao, R., Coleman, M. C., Pennington, J. D., Oxzen, O., Park, S. H., Jiang, H., Kim, H. S., Flynn, C. R., Hill, S., Hayes McDonald, W., Olivier, A. K., Spitz, D. R., and Gius, D. (2010) Sirt3-mediated deacetylation of evolutionarily conserved lysine 122 regulates Mn-SOD activity in response to stress. J. Biol. Chem. 285, 103, 14447–14452

24. Schlicker, C., Gertz, M., Papatheodorou, P., Kachholz, B., Becker, C. F., Kim, H. S., Flynn, C. R., Hill, S., Hayes McDonald, W., Olivier, A. K., Spitz, D. R., and Gius, D. (2010) Sirt3-mediated deacetylation of evolutionarily conserved lysine 122 regulates Mn-SOD activity in response to stress. J. Biol. Chem. 285, 103, 14447–14452

25. Guan, K. L., and Denu, J. M. (2011) Sirt3 promotes the urea cycle and fatty acid oxidation during dietary restriction. Mol. Cell 41, 139–149

26. Shi, T., Wang, F., Stieren, E., and Tong, Q. (2005) SIRT3, a mitochondrial deacetylase of evolutionarily conserved lysine 122 regulates Mn-SOD activity in response to stress. Mol. Cell 40, 893–904

27. Chen, Y., Zhang, J., Lin, Y., Lei, Q., Guan, K. L., Zhao, S., and Xiong, Y. (2011) Tumor suppressor SIRT3 deacetylates and activates manganese superoxide dismutase to scavenge ROS. EMBO Rep. 12, 534–541

28. Schlicker, C., Gertz, M., Papatheodorou, P., Kachholz, B., Becker, C. F., and Steegborn, C. (2008) Substrates and regulation mechanisms for the human mitochondrial sirtuins Sirt3 and Sirt5. J. Mol. Biol. 382, 790–801

29. Guan, K. L., and Xiong, Y. (2011) Regulation of intermediary metabolism by protein acetylation. Trends Biochem. Sci. 36, 108–116

30. Shi, T., Wang, F., Stieren, E., and Tong, Q. (2005) SIRT3, a mitochondrial sirtuin deacetylase, regulates mitochondrial function and thermogenesis in brown adipocytes. J. Biol. Chem. 280, 13560–13567

31. Weindruch, R., and Walford, R. L. (1988) The Retardation of Aging and Disease by Dietary Restriction, C.C. Thomas, Springfield, IL

32. Sohal, R. S., and Weindruch, R. (1996) Oxidative stress, caloric restriction, and aging. Science 273, 59–63

33. Lee, C. K., Klop, R. G., Weindruch, R., and Prolla, T. A. (1999) Gene expression profile of aging and its retardation by caloric restriction. Science 285, 1390–1393

34. Colman, R. J., Anderson, R. M., Johnson, S. C., Kastman, E. K., Kosmatka, K. I., Beasley, T. M., Allison, D. B., Cruzen, C., Simmons, H. A., Kempf, J. W., and Weindruch, R. (2009) Caloric restriction delays disease onset and mortality in rhesus monkeys. Science 325, 201–204

35. Neumann, H., Hancock, S. M., Buning, R., Routh, A., Chapman, L., Somers, J., Owen-Hughes, T., van Noort, J., Rhodes, D., and Chin, J. W. (2009) A method for genetically installing site-specific acetylation in recombinant histones defines the effects of H3 K56 acetylation. Mol. Cell 36, 153–163

36. Neumann, H., Peak-Chew, S. Y., and Chin, J. W. (2008) Genetically encoding N-acetylsine in recombinant proteins. Nat. Chem. Biol. 4, 232–234