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FORUM REVIEW ARTICLE

Mitochondrial Sirtuins and Their Relationships with Metabolic Disease and Cancer

Surinder Kumar[1] and David B. Lombard[1,2]

Abstract

Significance: Maintenance of metabolic homeostasis is critical for cellular and organismal health. Proper regulation of mitochondrial functions represents a crucial element of overall metabolic homeostasis. Mitochondrial sirtuins (SIRT3, SIRT4, and SIRT5) play pivotal roles in promoting this homeostasis by regulating numerous aspects of mitochondrial metabolism in response to environmental stressors. ***Recent Advances:*** New work has illuminated multiple links between mitochondrial sirtuins and cancer. SIRT5 has been shown to regulate the recently described post-translational modifications succinyl-lysine, malonyl-lysine, and glutaryl-lysine. An understanding of these modifications is still in its infancy. Enumeration of SIRT3 and SIRT5 targets *via* advanced proteomic techniques promises to dramatically enhance insight into functions of these proteins. ***Critical Issues:*** In this review, we highlight the roles of mitochondrial sirtuins and their targets in cellular and organismal metabolic homeostasis. Furthermore, we discuss emerging roles for mitochondrial sirtuins in suppressing and/or promoting tumorigenesis, depending on the cellular and molecular context. ***Future Directions:*** Currently, hundreds of potential SIRT3 and SIRT5 molecular targets have been identified in proteomic experiments. Future studies will need to validate the major targets of these enzymes, and elucidate how acetylation and/or acylation modulate their functionality. A great deal of interest exists in targeting sirtuins pharmacologically; this endeavor will require development of sirtuin-specific modulators (activators and inhibitors) as potential treatments for cancer and metabolic disease. *Antioxid. Redox Signal.* 22, 1060–1077.

Introduction

MITOCHONDRIA ARE CYTOPLASMIC organelles that play central roles in diverse intracellular processes such as energy production, metabolism, apoptosis, intracellular signaling, and pathogen responses (158). Mitochondria are responsible for generating the majority of cellular ATP through oxidative metabolism by the Krebs cycle, β-oxidation of fatty acids, and oxidative phosphorylation (OXPHOS). Consequently, mitochondria are the principal source of reactive oxygen species (ROS) within the cell (92, 163). Under normal physiological conditions, low levels of ROS can function as ''redox messengers'' in the regulation of specific signaling pathways (62), whereas excess ROS beyond the cell's detoxification capacity can damage cellular macromolecules and promote cell death *via* the intrinsic apoptotic pathway (28, 146). To neutralize the harmful effects of ROS, cells have evolved numerous antioxidant systems (123). To meet bioenergetic demands of the cell, mitochondrial number, configuration, and/or activity can change in response to a variety of physiological conditions (136). Mitochondrial defects, whether genetic or acquired, are associated with many common diseases, including diabetes and cancer (162, 169). Therefore, normal cellular function requires mechanisms to finely regulate mitochondrial physiology. In recent years, protein post-translational modifications (PTMs) such as ADP-ribosylation and lysine acetylation, succinylation, malonylation, and glutarylation on diverse mitochondrial proteins have emerged as a novel mechanism of mitochondrial regulation. These modifications are directly regulated by members of the sirtuin enzyme family (2, 34, 91, 121, 156).

Sirtuins are mammalian homologues of the yeast silent information regulator 2 (Sir2) protein (42). Sirtuins were initially described as class III histone deacetylases, functionally similar

[1]Department of Pathology, University of Michigan, Ann Arbor, Michigan.
[2]Institute of Gerontology, University of Michigan, Ann Arbor, Michigan.

to other HDACs. The sirtuin-catalyzed deacetylation reaction consumes nicotinamide adenine dinucleotide (NAD^+) as a co-substrate, and generates the sirtuin feedback inhibitor nicotinamide (NAM), 2′-O-acetyl-ADP-ribose, and a deacetylated substrate (66). However, certain sirtuins are now known to possess alternative catalytic functions—for example, deacylase, decrotonylase, desuccinylase, demalonylase, deglutarylase, and ADP-ribosyltransferase activities (Fig. 1)—that play crucial roles in the regulation of diverse cellular processes (2, 10, 32, 34, 36, 48, 65, 72, 121, 156).

Owing to their NAD^+ dependence, fluctuations in NAD^+ levels modulate sirtuin catalytic activities. Calorie restriction (CR), a reduction in calorie intake without malnutrition, is an intervention that promotes extended healthy lifespan. CR promotes expression of nicotinamide phosphoribosyltransferase (NAMPT), the rate-limiting enzyme of NAD^+ biosynthesis, and thus induces increased levels of intracellular NAD^+ (98). Consistently, increased intracellular NAD^+ occur during metabolic stresses such as CR or prolonged fasting (19, 20, 105), and are associated with sirtuin activation in a tissue-specific manner (63, 65). Moreover, NAMPT expression is linked to the circadian clock, thereby regulating NAD^+ levels and sirtuin activity (120). Sirtuins are widely expressed in normal tissues (99) and play diverse biological roles such as regulating oxidative stress, DNA repair, genomic stability, cell survival, apoptosis, metabolism, aging, and longevity (47).

Seven sirtuin members (SIRT1–SIRT7) are encoded in mammalian genomes. These proteins possess a conserved NAD^+-binding and catalytic domain, with distinct flanking N- and C-termini, and differ from one another with regard to catalytic activities (Fig. 1), subcellular localization (Fig. 2), protein targets, and biological functions (42, 47). SIRT6 and SIRT7 are found predominantly in the nucleus, whereas both SIRT1 and SIRT2 can be nuclear and cytosolic. SIRT3, SIRT4, and SIRT5 primarily reside in the mitochondrial matrix (25, 47). Here, we focus on SIRT3, SIRT4, and SIRT5: their catalytic activities, protein substrates, major target pathways, and roles in disease, particularly cancer.

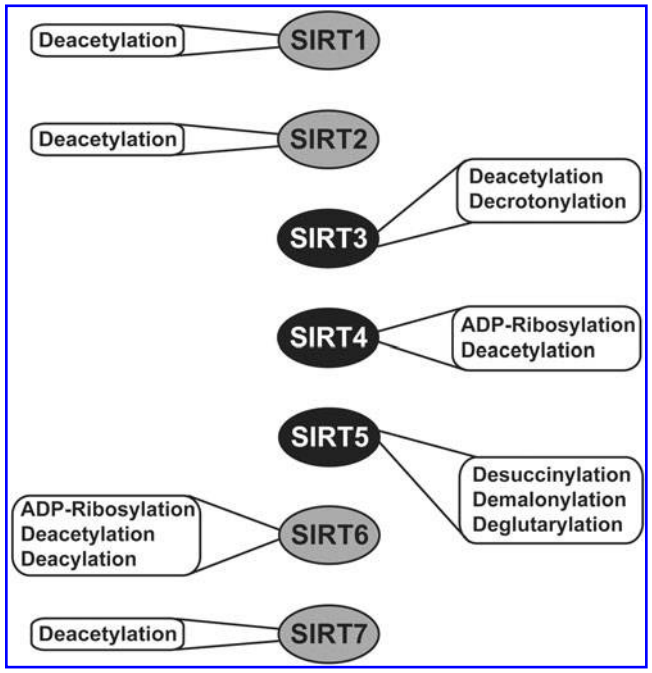

FIG. 1. Catalytic activities of mammalian sirtuins. SIRT1, SIRT2, and SIRT7 function primarily as deacetylases, whereas other mammalian sirtuins catalyze alternative reactions, in addition to or instead of deacetylation. SIRT4 acts as both a deacetylase and an ADP-ribosyltransferase. SIRT5 catalyzes desuccinylation, demalonylation, and deglutarylation. SIRT6 catalyzes ADP-ribosylation and deacylation, in addition to deacetylation. Only activities shown to be biologically significant are depicted.

SIRT3: Activity, Expression, and Metabolic Regulation

SIRT3 activity

Among mitochondrial sirtuins, SIRT3 is, by far, the best characterized, and possesses robust deacetylase activity (93). SIRT3-deficient mice exhibit elevated mitochondrial protein acetylation (91). Sol *et al.* have shown that mouse embryonic fibroblasts (MEFs) derived from *Sirt3* knockout (KO) mice display increased acetylation of more than 100 lysine sites, mostly on mitochondrial proteins (147). Using high-resolution mass spectrometry, two recent studies provided further support for the role of SIRT3 as a major regulator of the mitochondrial acetylome, particularly in response to CR or fasting (57, 128). Interestingly, SIRT3 deacetylase activity exhibits circadian rhythmicity, attributed to clock-driven oscillation in NAD^+ levels in mouse liver (120). Mice with defects in the circadian clock displayed reduced SIRT3 activity, and increased acetylation of multiple mitochondrial enzymes, including well-known targets of SIRT3. NAD^+ supplementation restored SIRT3 activity and increased oxygen consumption in mice with defects in the circadian clock (120).

Tan *et al.* described lysine crotonylation as novel histone PTM, which is specifically enriched at active gene promoters and potential enhancers in mammalian genome (155). A very recent study by Bao *et al.* reported that SIRT3 possesses decrotonylase activity, using a similar catalytic mechanism as for acetyl lysine hydrolysis (10, 36). siRNA-mediated SIRT3

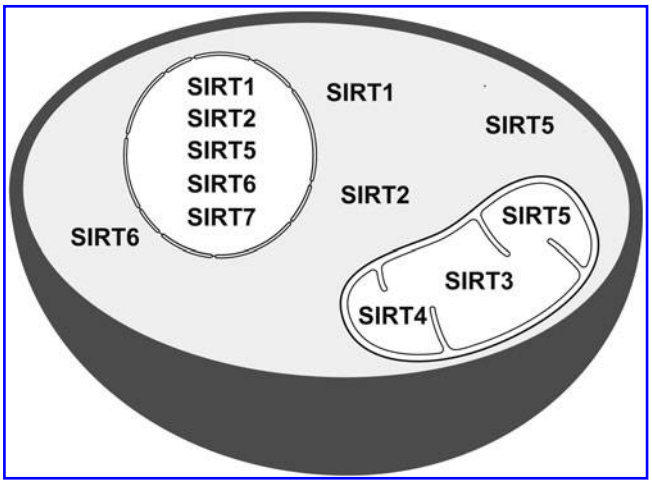

FIG. 2. Subcellular localization of mammalian sirtuins. SIRT7 is present in the nucleus, whereas SIRT1, SIRT2, and SIRT6 are both nuclear and cytosolic. SIRT3, SIRT4, and SIRT5 primarily reside in the mitochondrial matrix; SIRT5 is also found in the cytosol and the nucleus.

knockdown (KD) causes accumulation of global histone crotonylation and enrichment of crotonylation on histone 3 lysine 4 (10). SIRT3 has been reported to interact with chromatin in U2OS cells, resulting in the repression of adjacent genes (68). Interestingly, SIRT3 KD leads to increased lysine crotonylation at five of seven genes analyzed in U2OS cells, along with a significant increase in mRNA levels of three candidate genes (10). These observations suggest that SIRT3 might repress the expression of target genes *via* modulation of histone lysine crotonylation. This implies the surprising conclusion that an active fraction of SIRT3 is present in the nucleus. Indeed, Bao *et al.* report that a substantial amount of full-length (unprocessed) SIRT3 is nuclear. However, unprocessed human SIRT3 has been reported to be catalytically inactive, at least with regard to deacetylation (140). It is conceivable that a fraction of catalytically active SIRT3 is present extra-mitochondrially. Alternatively, SIRT3 deficiency may modulate cellular metabolism *via* activity within mitochondria to promote higher intracellular crotonyl-CoA levels, in turn, leading to increased histone crotonylation. This could occur as a consequence of disordered mitochondrial butyrate or glutaryl metabolism (155). Clearly, more work is needed to fully elucidate the role of SIRT3 in modulating crotonyl-lysine levels, particularly in the extra-mitochondrial compartment.

SIRT3 expression may correlate with human longevity

SIRT3 is expressed abundantly in tissues with high oxidative capacity, such as liver, brain, kidney, skeletal muscle, and brown adipose tissue, and its expression is increased by CR, fasting, or exercise (58, 111, 139, 143). Interestingly, increased expression of SIRT3 may be linked with longevity in humans. The presence of SNP rs11555236 in linkage disequilibrium with the putative functional enhancer region of SIRT3, which is a part of variable number of tandem repeat (VNTR) element located within a SIRT3 intron (4, 12), has been reported to be associated with increased longevity in some human populations (4, 12, 131). SIRT3 expression was higher in peripheral blood mononuclear cells from individuals homozygous for this variant (4). Conversely, SIRT3 expression and activity decline in response to high-fat feeding (9, 59, 77, 111), in insulin resistance (174), and in human subjects with the metabolic syndrome (59). Mice with germline ablation of *Sirt3* have no grossly apparent phenotype under nonstress conditions (91). Similarly, mice with muscle or liver specific *Sirt3* gene deletion are metabolically similar to wild-type (WT) littermates when fed a normal or high-fat diet (HFD), or under conditions of fasting, exercise, or cold challenge (37). However, under stress conditions or with advancing age, SIRT3 plays a crucial role in attenuating the onset of multiple pathologies. In the next section, we will briefly describe the roles of SIRT3 in regulating mitochondrial metabolism and health (summarized in Fig. 3). For more details about SIRT3 biology, readers are referred elsewhere (93).

SIRT3 Regulates Multiple Metabolic Pathways

SIRT3 maintains ATP levels by regulating mitochondrial electron transport chain function

A major role of SIRT3 is to maintain cellular ATP levels by promoting mitochondrial electron transport chain (ETC) activity. Various studies have revealed that SIRT3 deacetylates multiple ETC proteins to enhance energy production and improve ETC efficiency, such as NDUFA9 (complex I), SDHA (complex II), and the ATP synthase subunit β (complex V) (1, 9, 27, 40, 77, 79, 125). Moreover, the activities of complexes III and IV are significantly reduced in *Sirt3*-null mice under high-fat feeding conditions (77). As a consequence, tissues from SIRT3-deficient mice show decreased mitochondrial respiration and reduced ATP levels (1).

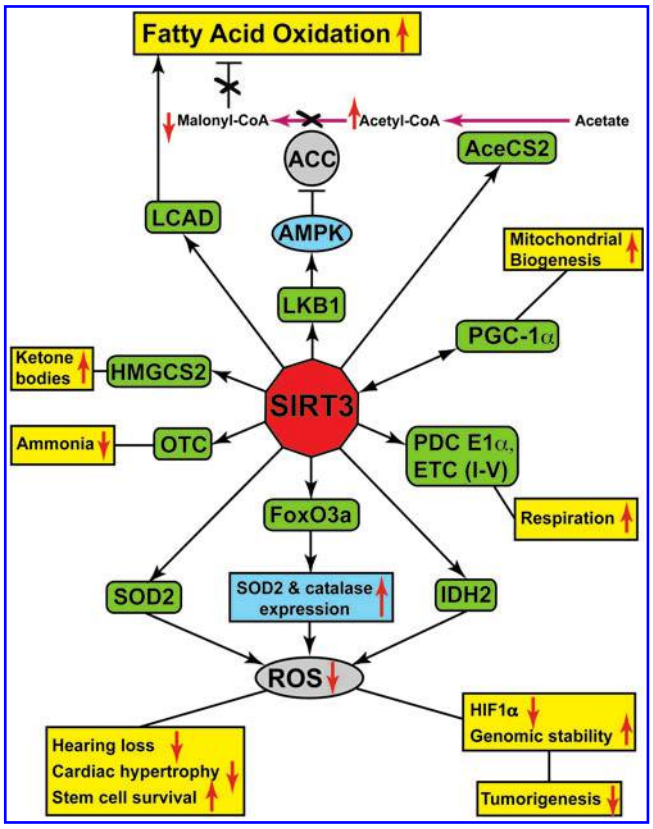

FIG. 3. Schematic representation of SIRT3 targets and downstream functions. SIRT3 deacetylates and activates multiple targets (*green rounded rectangles*), which can either directly regulate key cellular and physiological processes (*yellow*) or alter the activity (*blue ellipse*) or expression levels (*blue rectangle*) of downstream factors. *Upward and downward red arrows* designate promotion or suppression of particular activity or expression. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

Role of SIRT3 in acetate metabolism

Acetyl-CoA synthetase 2 (AceCS2), an enzyme catalyzing the conversion of acetate into acetyl-CoA, was the first SIRT3 substrate identified (53, 138). Under normal feeding conditions, AceCS2-deficient mice display no strong phenotypes; however, under fasting conditions, they show a 75% reduction in acetyl-CoA levels, along with a more than 5-fold increase in plasma acetate levels (134). Indeed, AceCS2 mRNA levels are significantly increased in brown adipose tissue from fasted WT mice (134) and similar to SIRT3, AceCS2 is abundantly expressed in tissues such as heart and skeletal muscle, and its expression is further induced under

ketogenic conditions (43). SIRT3 deacetylates and activates AceCS2 (53, 138), indicating a role for SIRT3 in acetyl-CoA generation under fasting conditions in providing the fasting cells with an alternative source of energy.

SIRT3 promotes fatty acid oxidation

SIRT3 plays a pivotal role in regulating fatty acid β-oxidation by deacetylation and activation of long-chain-specific acyl-CoA dehydrogenase (LCAD) (58). Under fasting conditions, SIRT3-deficient mice show decreased fatty acid β-oxidation, resulting in elevated serum long-chain fatty acid levels (54, 58). Inefficient fatty acid oxidation may contribute to the reduced ATP levels of SIRT3-deficient mice under fasting conditions (1).

As previously mentioned, SIRT3 expression and activity decline in response to high-fat feeding (9, 59, 77, 111), and mice lacking SIRT3 show accelerated obesity, insulin resistance, hyperlipidemia, and hepatic steatosis when fed an HFD over a prolonged period (59). In this context, SIRT3-deficient mice fed a HFD expressed elevated levels of the lipogenic enzyme stearoyl-CoA desaturase 1 (SCD1) (59). Increased SCD1 levels are associated with obesity and type 2 diabetes (64). Another study demonstrated that SIRT3 suppresses hepatic lipid accumulation, *via* AMP-activated kinase (AMPK)-dependent phosphorylation and inhibition of acetyl-CoA carboxylase (ACC) (142). ACC is a biotin-dependent enzyme that plays a crucial role in fatty acid synthesis by catalyzing the production of malonyl-CoA by irreversible carboxylation of acetyl-CoA (133).

SIRT3 deacetylates PDC E1α to promote glucose oxidation

The pyruvate dehydrogenase complex (PDC) catalyzes oxidative decarboxylation of pyruvate into acetyl-CoA, which is subsequently used in Krebs cycle to generate ATP (110, 118). PDC plays a key role in glucose metabolism, linking glycolysis with the Krebs cycle. Roughly half of all caloric intake passes through PDC (117). Phosphorylation of the PDC E1α subunit by pyruvate dehydrogenase kinases (PDK1–4) inhibits PDC activity, whereas dephosphorylation by pyruvate dehydrogenase phosphatases (PDP1 and 2) restores its activity (110). Reduced PDC activity and increased glycolysis are common in cancer cells, as a part of an overall metabolic reprograming associated with malignancy (179). Fan *et al.* found that increased lysine acetylation of PDC E1α and PDP1 is common in diverse types of human cancer cells (35). Putative mitochondrial acetyltransferases are poorly characterized. Acetyl-CoA acetyltransferase 1 (ACAT1) is a mitochondrial enzyme involved in the final step of isoleucine catabolism, and it converts 2-methyl-acetoacetyl-CoA into propionyl-CoA and acetyl-CoA. Fan *et al.* found that recombinant ACAT1 directly acetylates PDC E1α and PDP1 *in vitro* (35). Further, treatment of PDC E1α and PDP1 with cell lysates from ACAT1 KD H1299 cells results in their decreased lysine acetylation and increased activity (35). Conversely, incubation of recombinant PDC E1α with cell lysates from SIRT3 KD H1299 leads to their increased acetylation and decreased activity (35). In EGF-treated cancer cells, phosphorylation of PDP1 results in dissociation of SIRT3 and recruitment of ACAT1, which acetylates PDC E1α and PDP1. This, in turn, induces dissociation of PDP1 from PDC E1α and recruitment of PDK1, leading to reduced PDC activity. (35). Indeed, *Sirt3* deletion in skeletal muscle and myoblasts results in increased PDC E1α acetylation, increasing its phosphorylation and decreasing its activity (74). Decreased PDC activity promotes a switch of skeletal muscle metabolism from glucose oxidation toward lactate production and fatty acid utilization (74). Recently, a study by Ozden *et al.* confirmed the role of SIRT3 in directly deacetylating PDC E1α (110). A PDC E1α mutant mimicking the deacetylated state showed increased activity as compared with an acetylation mimic. Cells expressing an acetylation mimic showed increased proliferation, colony formation, and survival on treatment with ionizing radiation, all of which are characteristics of the transformed state (110).

SIRT3 facilitates ketone body production

SIRT3 also regulates the activity of 3-hydroxy-3-methylglutaryl CoA synthase 2 (HMGCS2) (144), the enzyme that performs the initial step in the conversion of acetyl-CoA into ketone bodies (acetoacetate and β-hydroxybutyrate) in the liver. Ketone bodies, in turn, serve as an important source of energy for tissues such as heart, skeletal muscle, and brain under fasting conditions. SIRT3-mediated HMGCS2 deacetylation promotes its activity and thus facilitates ketone body formation (144). Consequently, *Sirt3* KO mice display reduced serum β-hydroxybutyrate levels during prolonged fasting (144).

SIRT3 regulates the urea cycle via *ornithine transcarbamoylase activation*

Using a high-throughput approach combining acetyl-peptide arrays with metabolomics analysis, Hallows *et al.* identified ornithine transcarbamoylase (OTC) as a SIRT3 substrate (54). OTC is an enzyme that catalyzes the second step of the urea cycle, the key process in the detoxification of ammonia generated by amino-acid catabolism. SIRT3 directly deacetylates OTC at lysine 88 and stimulates its activity, thus promoting urea cycle function (54). As a consequence, SIRT3-deficient mice display an inability to deacetylate and activate mitochondrial OTC, and show urea cycle functional deficiency in response to CR along with elevated urinary orotic acid levels (54), a well-known clinical marker of human OTC deficiency (38).

SIRT3 Promotes Diverse Aspects of Healthy Aging

SIRT3 prevents hearing loss during CR

Age-related hearing loss (AHL), characterized by a decline of auditory function with increasing age, is associated with attrition of spiral ganglion neurons and sensory hair cells in the cochlea of the inner ear (90, 148). Studies have shown that CR delays the onset of AHL and reduces cochlear pathology (141, 150) by reducing oxidative stress (150), which plays a major role in AHL (73, 149). Notably, SIRT3 is required for the beneficial effect of CR in preserving auditory function. Hearing function is normal in young SIRT3-deficient mice, but with age they lose cochlear cells, manifest hearing loss, even under CR conditions, and display hearing loss (151). SIRT3 maintains hearing by suppressing oxidative stress under CR, by deacetylation and activation of isocitrate dehydrogenase 2 (IDH2). Activation of IDH2, in turn, increases

NADPH levels, used to regenerate reduced glutathione, a key component of cellular ROS defense (151, 176).

SIRT3 promotes hematopoietic stem cell survival by reducing oxidative stress

Brown *et al.* identified a role for SIRT3 in preserving the functions of hematopoietic stem cells (HSCs) during aging by lowering ROS levels (15). SIRT3 is dispensable for HSC function at a young age; however, SIRT3 deficiency results in a reduced HSC pool size in aged mice. Moreover, in a serial transplantation experiment, *Sirt3* KO results in impaired HSC self-renewal and reconstitution (15). Indeed, HSCs from WT aged mice show reduced levels of SIRT3 expression and activity, contributing to an increase in ROS levels occurring in aged HSCs. SIRT3 overexpression suppresses ROS levels *via* deacetylation and activation of the mitochondrial antioxidant enzyme, manganese superoxide dismutase (SOD2), and increases the colony-forming ability of aged HSCs (15). Moreover, in competitive transplantation assay, SIRT3 overexpression results in increased functional reconstitution by HSCs (15). Thus, SIRT3 upregulation rejuvenates aged HSCs by partially rescuing their functional defects.

SIRT3 suppresses cardiac hypertrophy

SIRT3 has also emerged as a crucial factor in maintaining cardiac health (132). Under stress conditions, increased SIRT3 levels in cardiomyocytes are protective against genotoxic and oxidative stress-mediated cell death (154). Furthermore, SIRT3-deficient mice show cardiac hypertrophy basally, and this effect becomes more pronounced in response to hypertrophic stimuli (50, 153). Conversely, SIRT3-overexpressing mice are protected from hypertrophy induction (153). Mechanistically, the cardioprotective role of SIRT3 has been ascribed to multiple functions of this sirtuin. According to one proposed mechanism, SIRT3 reduces cellular ROS levels by deacetylating and activating forkhead box O3a (FoxO3a) (153), a transcription factor that promotes expression of antioxidant-encoding genes such as SOD2 and catalase (86, 157). Another study identified a role for SIRT3 in deacetylating cyclophilin D, a modulator of the mitochondrial permeability transition pore (mPTP), to suppress mPTP opening, thereby inhibiting induction of cell death in cardiomyocytes and potentially in other cell types (50, 145). In addition, SIRT3 catalyzes the deacetylation and activation of LKB1, a serine/threonine kinase that phosphorylates and activates AMPK. Activated AMPK blocks hypertrophy by a number of mechanisms, including promotion of catabolic pathways to upregulate ATP production (122).

SIRT3 regulates mitochondrial fusion

Mitochondrial quality control is crucial for overall cellular health. In this regard, mitochondria continuously undergo fusion and fission; a balance between these processes is necessary to maintain mitochondrial morphology and function (21). Optic atrophy protein 1 (OPA1), a dynamin-related GTPase, mediates the fusion of inner mitochondrial membranes (126). A recent study found that activity of OPA1 is regulated by SIRT3-mediated deacetylation (135). Stress conditions induce OPA1 hyperacetylation, leading to a reduction in its GTPase activity. SIRT3 binds to and deacetylates OPA1 to restore its activity. Consistently, OPA1 isolated from *Sirt3* KO cells displayed reduced GTPase activity, and mitochondria isolated from *Sirt3* KO hearts showed disorganized mitochondrial morphology (135), suggesting a role for SIRT3 in maintaining mitochondrial integrity *via* OPA1.

SIRT3 plays a role in mitochondrial biogenesis

Maintenance of mitochondrial function and integrity require the selective degradation of defective mitochondria and the generation of new mitochondria. While defective mitochondria are targeted for degradation by the lysosome for hydrolytic digestion by a process known as mitophagy, the process of mitochondrial biogenesis induces mitochondrial DNA (mtDNA) replication and synthesis of mitochondrial proteins, resulting in increased mitochondrial number and mass (14, 106). A major regulator of mitochondrial biogenesis is PGC-1α, which, by co-activating NRF-1 and NRF-2, induces the expression of nuclear encoded mitochondrial transcription factor A (TFAM) (14, 173). TFAM is an essential protein that binds to mtDNA, regulates mitochondrial transcription initiation, and participates in mitochondrial genome replication (17). A study by Kong *et al.* reported that PGC-1α promotes expression of the *Sirt3* gene in muscle cells and hepatocytes, mediated by an estrogen-related receptor-binding element (ERRE) in the *Sirt3* promoter (82). Interestingly, activation of *Sirt3* gene expression *via* ERRE is required for PGC-1α-mediated mitochondrial biogenesis. Overexpression of PGC-1α results in increased mtDNA copy number and induces the transcription of cytochrome c oxidase subunits I, II, and VIIa. However, SIRT3 KD impairs PGC-1α-induced mitochondrial biogenesis and blocks PGC-1α-induced mitochondria-related gene expression (82). Another recent study by Dai *et al.* showed that treatment of rat cortical neurons with H_2O_2 causes oxidative stress-induced injury and significantly decreases mtDNA content (31). However, SIRT3 overexpression inhibits H_2O_2-induced neuronal damage and increases expression of PGC-1α, NRF-1, and TFAM, resulting in increased mtDNA content (31). These findings highlight roles of SIRT3 in regulating mitochondrial biogenesis.

SIRT3 regulates the mitochondrial unfolded protein response

Maintenance of protein homeostasis is essential for cell function and survival. Accumulation of misfolded and aggregated proteins in the mitochondria induces cellular proteotoxic stress and initiates the mitochondrial unfolded protein response (UPR^{mt}) (75). The UPR^{mt} activates expression of nuclear encoded protective genes to reduce proteotoxic stress and to re-establish mitochondrial homeostasis (56, 75). A recent study by Papa and Germain described a novel role for SIRT3 in the UPR^{mt} to coordinate both the antioxidant defenses and mitophagy (112). Proteotoxic stress leads to increased levels of FoxO3a, SOD2, catalase, and the autophagy marker LC3B-II (112). Interestingly, proteotoxic stress also induces SIRT3 expression. siRNA-mediated SIRT3 inhibition expression significantly attenuates LC3B-II induction (112). Moreover, SIRT3 inhibition prevents upregulation of SOD2 and FoxO3a and results in a significant

increase in mitochondrial O_2^- levels after proteotoxic stress. In addition, loss of SIRT3 leads to a decrease in the mitochondrial membrane potential, increases aggregation of mitochondrial proteins, and reduces the viability of cells undergoing proteotoxic stress (112). These observations indicate that SIRT3 acts as a major coordinator of UPR^{mt} induced by mitochondrial proteotoxic stress.

Roles for SIRT3 in Cancer

SIRT3 functions as a tumor suppressor

The tumor suppressor role of SIRT3 was first identified with the observations that SIRT3-deficient cells are more easily transformed than WT controls, and SIRT3-deficient mice develop mammary tumors with a long latency (51, 79). SIRT3 expression is reduced in human breast cancer, colon carcinoma, osteosarcoma, and hepatocellular carcinoma (11, 39, 51, 79, 177, 178). Moreover, deletion of the *SIRT3* locus is present in about 20% of all human cancers, and 40% of human breast and ovarian cancers, further supporting a tumor-suppressor role for this protein (39). SIRT3 KD in human cancer cells resulted in increased tumor size and reduced latency in xenografts, whereas SIRT3 overexpression decreased xenograft tumorigenicity, indicating that SIRT3 continues to retard tumor growth in the context of preformed cancer cells (11).

As previously noted, SIRT3 functions as a tumor suppressor, in part by suppressing the production of ROS *via* deacetylation and activation of antioxidant enzyme SOD2 (124, 159), IDH2 (151), and FoxO3a (153). Increased ROS levels promote nuclear and mitochondrial genome instability, and stabilize hypoxia-inducible factor (HIF) 1-alpha, a part of a protein complex that promotes a shift toward glycolysis, whose upregulation is associated with tumor development (11, 39, 79). SIRT3 also plays other functions that are relevant for tumor suppression. SIRT3 overexpression, by reducing ROS levels, decreases the expression of the transferrin receptor, TfR1, by inhibiting iron regulatory protein 1 (IRP1), thereby suppressing the proliferation of pancreatic cancer cells (70). IRP1, which serves as an ROS sensor (100), displays reduced binding to the iron response element (IRE) in SIRT3 overexpressing cells (70). IRE is found in the 5′ untranslated regions of mRNAs whose protein products are associated with iron metabolism. Furthermore, SIRT3 may also be involved in tumor suppression by modulating the activity of extramitochondrial factors. In this regard, Inuzuka *et al.* found that SIRT3 deacetylates the proto-oncoprotein S-phase kinase-associated protein 2 (Skp2) (67), which is overexpressed in multiple types of cancer and functions as an E3 ubiquitin ligase to target numerous tumor suppressors for proteasome-mediated degradation (41). SIRT3-mediated deacetylation leads to Skp2 nuclear import, thereby preventing its targeting of E-cadherin (67). Reduced E-cadherin expression occurs in many cancer types, and is a characteristic of epithelial-mesenchymal transition and cancer metastasis (160). Overall, these studies indicate that SIRT3 functions as a tumor suppressor by increasing mitochondrial respiration, repressing ROS production, promoting nuclear and mtDNA integrity, destabilizing HIF1-alpha, decreasing TfR1 expression, and promoting nuclear import of Skp2.

Potential role of SIRT3 in tumor promotion

As with other sirtuins (179), there are reports of tumor-promoting roles for SIRT3. SIRT3 is overexpressed in many oral squamous cell carcinomas (OSCCs) relative to normal oral mucosa, and SIRT3 depletion in these cells impairs their growth and proliferation, and sensitizes them to genotoxic therapy (5). However, another study reported that despite increased expression of SIRT3 in OSCC, its catalytic activity is significantly reduced (22). Further, it has been found that 23.8% of OSCC patients analyzed carried a germline point mutation in *SIRT3*, resulting in substitution of a valine residue with isoleucine at position 208 in the SIRT3 catalytic domain (22). Recombinant SIRT3 with this V208I mutation displayed reduced catalytic efficiency (22). Consistent with the notion that SIRT3 can play an oncogenic function, a recent study reported the presence of an extra copy of the *SIRT3* locus in a family with Li-Fraumeni Syndrome (8), an inherited condition characterized by an increased risk of developing diverse cancer types. Consistently, Ashraf *et al.* reported that increased SIRT3 expression was associated with human lymph node-positive breast cancer (7). In context of these observations, ectopic expression of SIRT3 rescued p53-induced growth arrest in human bladder tumor-derived Ej-p53 cells (87). These opposing roles of SIRT3, as both a tumor suppressor and an oncogene, are context- and cell-type specific.

SIRT4-Regulated Processes: Targets and Physiological Implications

Although SIRT4 possesses a conserved sirtuin deacetylase domain (42), initial reports did not identify any deacetylase activity of this sirtuin (2, 52, 107). Recently, however, SIRT4 has been reported to possess specific deacetylase activity toward at least one particular substrate (see subsequent section on SIRT4 and fatty acid metabolism) (84). SIRT4 also exhibits strong ADP-ribosyltransferase activity (2, 52). In the next sections, we describe the effect of SIRT4-mediated regulation on various target substrates (Fig. 4) and their physiological functions.

SIRT4 suppresses glutamate dehydrogenase activity

The first target of SIRT4 identified was glutamate dehydrogenase (GDH), a mitochondrial enzyme that catalyzes the second step in glutamine catabolism. Glutamine is initially hydrolyzed by glutamate synthase (GLS) to glutamate, which is then subsequently converted to a Krebs cycle intermediate, α-ketoglutarate, by the action of GDH (95). SIRT4 interacts with GDH in pancreatic β-cells and ADP-ribosylates GDH to repress its activity (52). Pancreatic islets isolated from *Sirt4* KO mice show increased GDH activity compared with controls. The SIRT4-mediated decrease in GDH activity results in the repression of amino-acid-stimulated insulin secretion (AASIS) in pancreatic β-cells and, thus, *Sirt4* KO mice show elevated circulating insulin levels (52). These observations suggest an inhibitory role of SIRT4 in glutamine metabolism by repressing the activity of GDH.

SIRT4 inhibits fatty acid metabolism

SIRT4 deacetylates and inhibits malonyl-CoA decarboxylase. Malonyl-CoA decarboxylase (MCD) is an enzyme that catalyzes the generation of acetyl-CoA from malonyl-

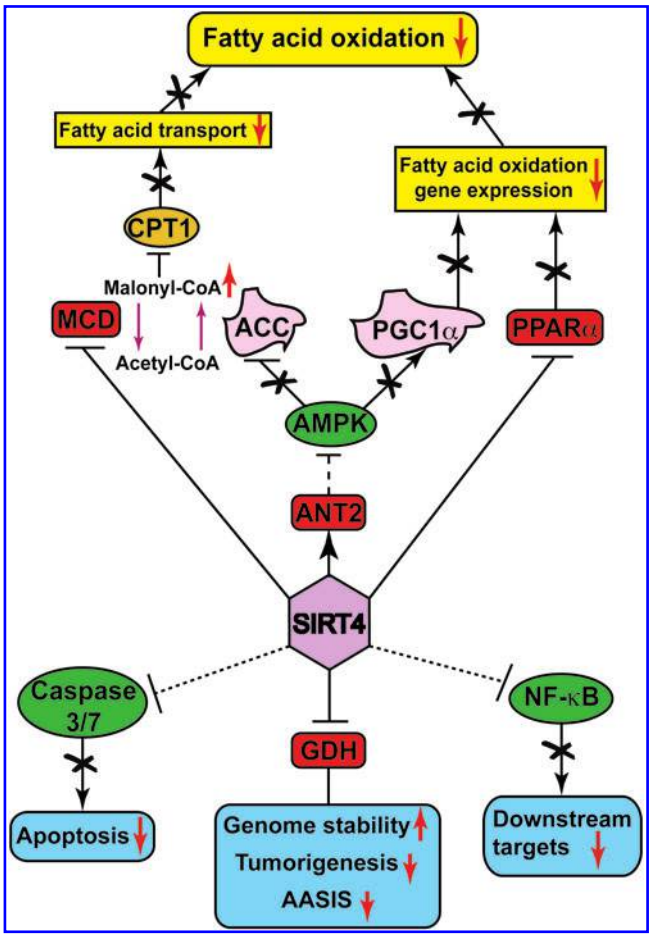

FIG. 4. Overview of SIRT4 target substrates and cellular functions. SIRT4 directly (*red rounded rectangles*) or indirectly (*green ellipses*) modulates the activity of various target substrates, which either regulate aspects of fatty acid metabolism (*yellow rectangles*) or play crucial roles in other cellular processes (*blue rounded rectangles*). *Upward and downward red arrows* indicate the promotion or suppression of a particular activity, gene expression, or physiological activity. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

CoA. Malonyl-CoA, in turn, allosterically inhibits the activity of carnitine palmitoyltransferase 1 (CPT1), the enzyme that catalyzes mitochondrial uptake of fatty acids for β-oxidation (133). Under nutrient-rich conditions, SIRT4 deacetylates MCD, thereby repressing its activity (84). SIRT4 overexpression in myocytes and adipocytes resulted in reduced MCD activity, whereas muscles and white adipose tissues from *Sirt4* KO mice showed elevated MCD activity and a reduction in malonyl-CoA levels (84). As a consequence, *Sirt4* KO mice showed increased fatty acid oxidation, associated with increased exercise capacity and resistance to diet-induced obesity (84). However, SIRT4-deficient mice did not show improved insulin sensitivity commensurate with their relative leanness.

SIRT4 inhibits β-oxidation gene expression by repressing PPARα activity. Peroxisome-activated receptor α (PPARα) is a ligand-activated transcription factor that promotes the expression of genes involved in fatty acid catabolism (78, 85). Nasrin *et al.* reported that SIRT4 KD in mouse primary hepatocytes and myotubes resulted in increased expression of genes encoding fatty acid metabolism enzymes, along with elevated SIRT1 expression (104). Consequently, a significant increase in fatty acid oxidation was observed upon SIRT4 KD in primary hepatocytes, which was found to be SIRT1 dependent, as SIRT1/SIRT4 double KD hepatocytes showed a blunted increase in fatty acid oxidation (104). Recently, SIRT4 overexpression has been shown to repress PPARα transcriptional activity, resulting in suppression of fatty acid catabolism gene expression, and, hence, reduced fatty acid oxidation (83). Conversely, PPARα target gene expression levels were remarkably elevated in *Sirt4* KO livers (83). *Sirt4* deletion resulted in increased NAD^+ levels, leading to increased activity of SIRT1. Interestingly, SIRT1 has been shown to activate PPARα, and this activation was repressed by SIRT4 overexpression (83). Collectively, both studies identify SIRT4 as a suppressor of fatty acid oxidation by inhibiting SIRT1-mediated activation of PPARα.

SIRT4 negatively regulates AMPK activity to suppress fatty acid oxidation. AMPK plays a key role in promoting fatty acid oxidation, by phosphorylating and inhibiting ACC. As previously mentioned, ACC catalyzes the production of malonyl-CoA from acetyl-CoA. Thus, AMPK-driven ACC phosphorylation reduces malonyl-CoA levels, resulting in increased CPT1-mediated mitochondrial fatty acid uptake (3, 133, 172). A recent study showed that during fasting, increased SIRT4 levels inhibit AMPK activity and suppress fatty acid oxidation (61). AMPK also activates PGC-1α, a transcriptional co-activator of fatty acid oxidation genes (164). Consistent with these reports, livers from *Sirt4* KO mice showed elevated levels of active AMPK, resulting in increased phosphorylated ACC and PGC1-α induction (61). ANT2 is a mitochondrial protein associated with the inner mitochondrial membrane that catalyzes the exchange of ATP generated in the mitochondria with cytosolic ADP (119). ANT2 KD in SIRT4-overexpressing cells rescues decreased AMPK activity, indicating that ANT2 plays a crucial role in SIRT4-dependent AMPK regulation (61). Altogether, SIRT4 suppresses fatty acid oxidation by modulating the activity of MCD, PPARα, and AMPK.

Other targets of SIRT4

Caspases are a family of cysteine proteases that play key roles in apoptosis. SIRT4 overexpression decreases the activities of caspases 3 and 7 under hypoxic conditions, and reduces induction of caspases 3 and 9 by hypoxia (89). Consequently, SIRT4 protects H9c2 cardiomyoblast cells against hypoxia-induced apoptosis (89). More recently, SIRT4 has been shown to inhibit the activity of NF-κB by inhibiting IkBα degradation (23). NF-κB is a transcription factor that plays a key role in inflammatory responses, and transcriptionally regulates the expression of surface adhesion molecules, such as VCAM-1 and E-selectin. Treatment of human pulmonary microvascular endothelial cells (HPMECs) with cigarette smoke extract (CSE) strongly induces expression of VCAM-1 and E-selectin (23). However, overexpression of SIRT4 in HPMECs inhibits the CSE-induced expression of these surface adhesion molecules and mononuclear cell adhesion (23). Tissues and cells

from *Sirt4* KO mice show reduced ATP levels, whereas overexpression of SIRT4 increases ATP content (61). The interaction of SIRT4 with ANT2 is essential for maintaining ATP homeostasis (61). However, there are no reports showing that SIRT4 biochemically modifies ANT2.

SIRT4 Acts as a Tumor Suppressor *via* Repression of Glutamine Metabolism

SIRT4 mRNA levels are reduced in several human cancers (13, 26, 45, 71, 170), and reduced *SIRT4* mRNA levels correlate with inferior survival in patients with lung tumors (71). Recent studies confirmed that SIRT4 indeed acts as a tumor suppressor, by repressing glutamine metabolism and promoting genomic stability (30, 69, 71). Glutamine is a key amino acid required for diverse intracellular processes such as macromolecular synthesis, redox homeostasis, oxidative metabolism, and many others (95). Although most mammalian cells can synthesize glutamine, under conditions of rapid cell proliferation, such as cancer, a steady extracellular source of glutamine becomes essential. Glutamine serves as an anaplerotic substrate by replenishing the Krebs cycle *via* α-ketoglutarate, a product of glutamine catabolism. Consistently, many cancer cells are ''glutamine addicted,'' and require exogenous glutamine to support survival and proliferation (95). For example, cell cycle progression in HeLa cells is absolutely dependent on glutamine (29). Jeong *et al.* showed that genotoxic stress, which arrests cell cycle progression to allow DNA damage repair, induces SIRT4 expression, which, in turn, represses mitochondrial glutamine metabolism (71). *Sirt4* KO MEFs show increased entry of glutamine-derived metabolites into the Krebs cycle, and are unable to repress cellular glutamine uptake in response to DNA damage (71). Moreover, these cells display an increased proliferation rate, a phenotype abrogated by inhibitors of glutamine metabolism, highlighting the glutamine-dependent proliferation of these cells (71). Consistently, HeLa cells, which use glutamine as a major energy source, show growth inhibition in response to SIRT4 overexpression (71). Moreover, SIRT4 deficiency is associated with larger tumor formation in a nude mice allograft model, and two independently derived strains of *Sirt4* KO mice displayed increased incidence of spontaneous lung tumors (71).

The tumor-suppressor activity of SIRT4 was further evaluated in the context of Myc-driven human Burkitt lymphoma cells (69). c-Myc is a transcription factor that upregulates glutaminase expression, by reducing the expression of inhibitory microRNAs targeting this mRNA (95). Myc-driven cancers typically show marked glutamine dependence (44, 168). Overexpression of SIRT4 reduces glutamine utilization in Burkitt lymphoma cells, inhibits their proliferation, and sensitizes them to glucose depletion (69). Moreover, loss of SIRT4 in a mouse Burkitt lymphoma model increases lymphomagenesis and mortality. Malignant B cells derived from these mice display increased glutamine uptake and GDH activity (69).

Csibi *et al.* showed that a mechanistic target of rapamycin complex 1 (mTORC1) negatively regulates SIRT4 expression by promoting proteasome-mediated degradation of the *SIRT4* transcriptional regulator CREB2 (30). Tuberous sclerosis 2 (TSC2) is a negative regulator of mTORC1; thus, *Tsc2* KO MEFs show increased mTORC1 activation. Inhibition of mTORC1 activity by rapamycin results in increased SIRT4 expression and reduced GDH activity in *Tsc2* KO cells (30). Consistently, SIRT4 overexpression inhibits transformation and proliferation of *Tsc2* KO MEFs *in vitro*, and delays tumor development in xenograft models (30).

SIRT5 Regulates Newly Described PTMs

SIRT5 is phylogenetically most closely related to prokaryotic (so-called class III) sirtuins (42). SIRT5 is broadly expressed with the highest expression levels in brain, heart, liver, kidney, muscles, and testis (99, 102). SIRT5 is predominantly mitochondrial (33, 99, 102, 137); however, several reports have revealed the existence of functional extra-mitochondrial SIRT5 (46, 96, 116). In this regard, Park *et al.* reported that a significant amount of SIRT5 is present in the cytosol in mouse hepatocytes and human 293T cells, and that a number of cytosolic and nuclear proteins, in addition to many mitochondrial proteins, were hypersuccinylated in the absence of SIRT5 (116). To date, no strong phenotype or major metabolic abnormality has been described in *Sirt5* KO mice (91, 101, 175). Thus, SIRT5 seems to be largely dispensable for gross metabolic homeostasis under basal, unstressed conditions, which is true of most sirtuins except SIRT1 and SIRT6. SIRT5 overexpression was reported to enhance ATP synthesis and oxygen consumption in HepG2 cells, whereas SIRT5 KD had no effect in this context (16). Conversely, SIRT5 KD human cells and mitochondria isolated from SIRT5-deficient mouse livers showed increased respiration in the presence of succinate and pyruvate, indicating that SIRT5 inhibits mitochondrial respiration under some conditions (116).

SIRT5 and Protein Deacylation

Based on homology to other sirtuins, SIRT5 was originally annotated as a deacetylase (42). However, recently SIRT5 has been shown to preferentially deacylate negatively charged modifications: malonylation, succinylation, and glutarylation (34, 116, 121, 127, 156). Du *et al.* showed that SIRT5 possesses minimal deacetylase activity compared with SIRT1 and SIRT3 (34); however, the catalytic efficiency of SIRT5 for demalonylation and desuccinylation was much higher than for deacetylation (34, 121). Protein lysates from SIRT5-deficient livers showed increased lysine malonylation and lysine succinylation, with little impact on lysine acetylation (121). Du *et al.* described the presence of an arginine residue (Arg^{105}) and tyrosine residue (Tyr^{102}) in the acyl-binding pocket of SIRT5, which are conserved in most class III sirtuins (34). The presence of arginine and tyrosine residues in the catalytic pocket of SIRT5 is likely responsible for their preference for negatively charged acyl groups.

Via mass spectrometry approaches, two independent studies have identified multiple SIRT5 succinylated targets in mouse liver mitochondria (127), and globally in MEFs and liver tissues (116). Among the quantifiable sites identified by Park *et al*., more than 90% showed hypersuccinylation in *Sirt5* KO cells, strongly suggesting that SIRT5 is a major regulator of lysine succinylation in mammals (116). Similarly, Rardin *et al.* reported that SIRT5 deficiency resulted in hypersuccinylation of 32% sites in 56% of mitochondrial proteins overall (127). The preference of SIRT5 for negatively charged acyl groups was further corroborated by

another recent study (156). Tan *et al.* identified and validated lysine glutarylation as an evolutionary conserved post-translational modification; similar to lysine succinylation, this modification was also regulated by SIRT5 (156). Proteomic screening of liver extracts from *Sirt5* KO mice revealed hyperglutarylation of 683 lysine sites on 191 proteins; more than three quarters of these proteins were found to be mitochondrial (156). These studies emphasize the major cellular role of SIRT5 in removing negatively charged lysine modifications, primarily within the mitochondrial matrix.

Although many metabolic enzymes have been identified as malonylated or succinylated, the significance of these PTMs is still unclear. It has been proposed that protein acylation results from the nonenzymatic lysine modification (167), due to accumulation of intrinsically reactive carbon metabolites, which can negatively impact protein function and, hence, disrupt cellular homeostasis (166). Therefore, by removing these lysine PTMs, sirtuins may contribute to maintaining the quality of the proteome, especially in mitochondria.

Metabolic Targets of SIRT5

Similar to other sirtuins, SIRT5 targets a number of protein substrates (Fig. 5) implicated in diverse metabolic pathways. Cytochrome c, a mitochondrial protein with central roles in oxidative metabolism and apoptosis, was the first reported target of SIRT5 (137). However, no *in vivo* evidence indicates a role for SIRT5 in regulating functions of this protein. Carbamoyl phosphate synthetase 1 (CPS1) is the enzyme catalyzing the initial step of the urea cycle for ammonia detoxification and disposal (55, 97). Deacetylation (102), desuccinylation (34), and deglutarylation (156) of CPS1 by SIRT5 result in its increased enzymatic activity. *Sirt5*-null mice display lower CPS1 activity (34, 102) and have reduced capacity to detoxify ammonia. During conditions of high amino-acid catabolism (fasting, CR, or a high protein diet), SIRT5-deficient mice showed elevated blood ammonia levels (102). These findings were further complemented by another study, which revealed increased expression of *Sirt5* mRNA in the livers of WT mice during fasting, and, in addition, increased CPS1 activity in the livers of SIRT5-overexpressing transgenic mice (108). Analysis of mitochondrial proteins in the livers of SIRT5-overexpressing transgenic mice identified urate oxidase as another target of SIRT5 (103). Urate oxidase catalyzes the conversion of urate to allantoin, the last step of purine catabolism in most mammals (but not humans) (6). In the livers of SIRT5-overexpressing transgenic mice, urate oxidase showed decreased acetylation and increased activity (103).

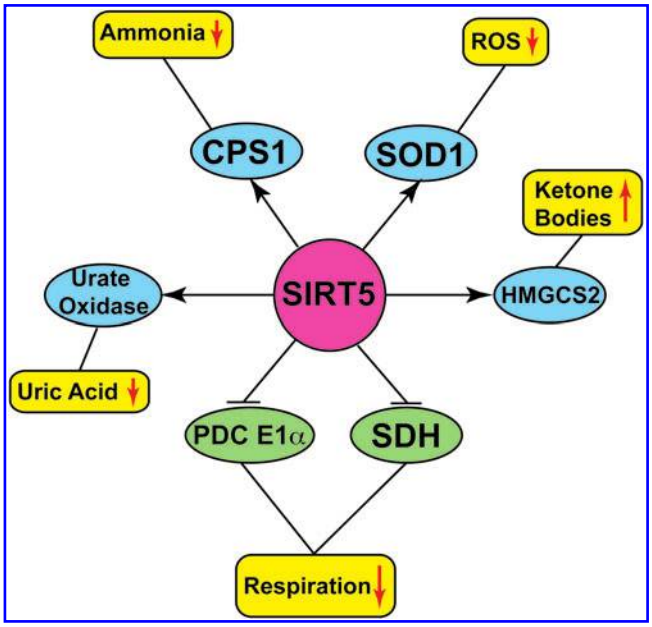

FIG. 5. Major targets regulated by SIRT5. SIRT5 deacetylates, desuccinylates, demalonylates, and/or deglutarylates multiple metabolic enzymes to activate (*blue ellipses*) or inhibit (*green ellipses*), either increasing (*yellow rounded rectangle* with *upward red arrow*) or decreasing (*yellow rounded rectangles* with *downward red arrow*) the levels/activity of particular compound/cellular activity. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

In the context of ROS management, it was recently shown that SIRT5 binds to, desuccinylates, and activates Cu/Zn superoxide dismutase (SOD1). SOD1 is a key cellular antioxidant enzyme, and SOD1-mediated ROS detoxification is significantly increased when SOD1 is co-overexpressed with SIRT5 (88). A number of observations potentially implicate SOD1 in tumorigenesis (114). For example, increased SOD1 expression was found in a panel of breast cancer cell lines (113); overexpression of SOD1 promotes growth of lung cancer cells (152); and inhibition of SOD1 induces cell death in the lung carcinoma cell line A549 (49). Mutation of the SOD1 succinylation site inhibited the growth of lung cancer cells (88), suggesting a role for SIRT5-mediated SOD1 desuccinylation and activation in promoting tumorigenesis.

In their global analysis of lysine succinylation, Park *et al.* reported widespread succinylation in diverse mitochondrial metabolic enzymes (116). Among a large number of putative SIRT5 targets, they further analyzed the role of SIRT5 in regulating PDC E1α and succinate dehydrogenase (SDH), an enzyme that catalyzes the oxidation of succinate to fumarate. SIRT5 robustly desuccinylated PDC E1α *in vitro* and repressed its activity. Consequently, SIRT5 KD resulted in elevated PDC E1α activity, along with a substantial increase in SDH activity and elevated cellular respiration (116). Rardin *et al.* showed hypersuccinylation of HMGCS2 in the absence of SIRT5 (127). HMGCS2 is the rate-limiting enzyme of ketone body synthesis, and hypersuccinylation decreases its activity. Consequently, there is a mild defect in ketone body formation during fasting in SIRT5-deficient animals (127). Loss of SIRT5 also leads to hypersuccinylation of proteins involved in fatty acid β-oxidation; liver and skeletal muscle from *Sirt5* KO mice show modest accumulation of medium- and long-chain acylcarnitines (127).

Overall, SIRT5 target a number of protein substrates involved in glucose oxidation, ketone body formation, fatty acid oxidation, ammonia detoxification, and ROS management. One way to rationalize the functions of SIRT5 may be that this protein suppresses glucose oxidation while facilitating use of alternative energy sources, such as fatty acids, ketone bodies, and amino acids. These conditions occur during fasting and CR. However, no role for SIRT5 in CR has been directly identified as yet.

SIRT5 and Cancer

Until recently, no reports assessed roles for SIRT5 in any type of malignancy. A recent analysis of human high-grade serous ovarian carcinomas found that the region encompassing the *SIRT5* locus was amplified in 30% of these tumors (18). Altered activities of SIRT5 targets, PDC and SDH, are implicated in neoplasia and cancer cell metabolic reprogramming. As previously noted, PDC activity is frequently suppressed in tumor cells, in part *via* reversible phosphorylation (60, 76, 80, 81, 109, 115, 129, 130). It is possible that SIRT5 contributes to metabolic reprogramming in cancer cells, potentially by participating in PDC inhibition. Very recently, Lu *et al.* showed that SIRT5 is overexpressed in advanced non-small cell lung cancer (NSCLC), and that SIRT5 KD repressed the growth rate of NSCLC cell lines (94). SIRT5 KD in these cells increased their susceptibility to genotoxic drugs (94). NRF2 is a transcription factor that regulates development and drug resistance of human NSCLC (24). NSCLCs show NRF2 upregulation, and SIRT5 KD resulted in reduced NRF2 levels, and expression of downstream targets of NRF2 (94). These observations highlighted the potential role of SIRT5 in promoting lung cancer growth and drug resistance, by promoting the expression of NRF2 and its downstream targets (94). Another study proposed that SIRT5-mediated SOD1 desuccinylation and activation might be relevant in lung tumor cell growth (88). SOD1 KD lung tumor cells expressing succinylation-resistant SOD1 mutant displayed reduced proliferation (88), potentially indicating that regulation of SOD1 *via* succinylation is critical for lung tumor cell growth. Clearly, further studies are warranted to explore the possible involvement of SIRT5 in tumorigenesis and reprograming of cancer cell metabolism.

An Interplay Between Mitochondrial Sirtuins Contributes to Metabolic Homeostasis

Maintenance of metabolic homeostasis is crucial for proper cellular function, and it is achieved by tightly regulated interactions among different metabolic pathways under diverse physiological conditions. Mitochondrial sirtuins are critical regulators of multiple metabolic processes, and they influence numerous aspects of metabolic homeostasis in a carefully orchestrated manner (Fig. 6). For instance, under chow feeding conditions, SIRT4 represses AASIS by inhibiting the activity of GDH (52), which catalyzes the conversion of glutamate into the Krebs cycle intermediate α-ketoglutarate (95), thereby promoting cellular glucose metabolism. However, during CR, SIRT4 activity is suppressed, and thus GDH is released from SIRT4-mediated inhibition (52, 165). Moreover, it has been reported that acetylation of GDH also reduces its activity, whereas SIRT3-mediated deacetylation activates it (91, 137). Therefore, it is possible that during CR, increased SIRT3 activity induces hepatic glucose production from amino acids by activating GDH-mediated conversion of glutamate into α-ketoglutarate (91, 137), which fuels the Krebs cycle. Another example where coordination between SIRT3 and SIRT4 has been observed is the regulation of FAO. As noted, during fasting, SIRT3 promotes FAO through LCAD and AMPK (58, 142). Conversely, SIRT4 suppresses FAO by negatively regulating the activities of MCD, PPARα, and AMPK (83, 84). Further, SIRT4 KD increases the expression of SIRT1, which activates PPARα and increases FAO gene expression (83, 104). Collectively, these results suggest that under conditions of glucose deprivation, crosstalk between SIRT3 and SIRT4 may stimulate utilization of alternative energy sources such as amino acids and fatty acids.

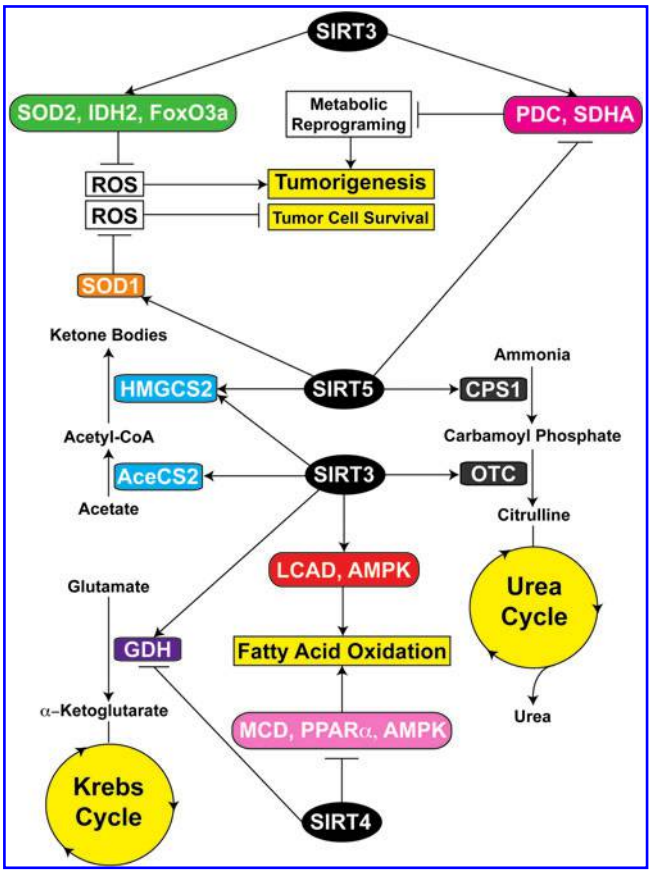

FIG. 6. Overview of interplay between mitochondrial sirtuins. Enzymes involved in ketogenesis (*blue boxes*) and urea cycle (*gray boxes*) are activated by both SIRT3 and SIRT5. While SIRT5 inhibits the enzymatic activities of glucose metabolism (*magenta box*), SIRT3 activates them to suppress metabolic reprogramming and tumorigenesis. SIRT3 also suppresses tumorigenesis by decreasing ROS levels through activation of antioxidant machinery (*green box*). SIRT5 activates SOD1 (*orange box*) to maintain ROS below toxic levels to support tumor cell survival. SIRT3 also shares common target/pathways with SIRT4. SIRT3 promotes FAO by activating LCAD and AMPK (*red box*), whereas SIRT4 represses FAO through inhibition of MCD, PPARα, and AMPK (*pink box*). Similarly, SIRT3 activates GDH (*violet box*), which is inhibited by SIRT4. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

Recent studies illustrate potential crosstalk between SIRT3 and SIRT5, both of which activate the enzymes involved in ketogenesis and the urea cycle. Ketone bodies, an important source of energy for extrahepatic tissues under fasting conditions, are produced in mitochondria from acetyl-CoA. SIRT3-mediated activation of AceCS2 results in enhanced acetyl-CoA production (53, 138). Acetyl-CoA is then converted into 3-hydroxy-3-methylglutaryl-CoA by HMGCS2, which is finally converted into ketone bodies.

HMGCS2 is activated by both SIRT3-mediated deacetylation (144) and SIRT5-mediated desuccinylation (127). In addition, under the conditions of fasting, amino acids are catabolized as a carbon source for gluconeogenesis. Toxic ammonia is generated in this process, requiring conversion into urea for proper disposal *via* the urea cycle. During CR, SIRT3 and SIRT5 play pivotal roles in the urea cycle by activating OTC (54) and CPS1 (34, 102, 156), respectively. CPS1 catalyzes the initial rate-limiting step in the urea cycle and converts ammonia into carbamoyl phosphate (171). OTC is the second enzyme involved in the mitochondrial urea cycle, and catalyzes the conversion of carbamoyl phosphate into citrulline (171). Although modulation of enzymatic activities in ketogenesis and urea cycle by SIRT3 and SIRT5 highlights their important roles in the adaptive response to fasting, mechanistic understanding of this coordination is incomplete.

We have already discussed roles of SIRT3 and SIRT5 in ROS regulation, generated as a by-product of OXPHOS, and also in the modulation of activities of enzymes involved in glucose metabolism. While ROS are produced as a product of normal cellular functioning, increased ROS levels often result in oxidative stress and adversely affect genomic stability, promoting tumorigenesis (161). Moreover, increased ROS levels stabilize HIF1α, which promotes a shift toward glycolysis, providing advantages to rapidly dividing tumor cells (11, 39, 79). As described earlier, SIRT3 enhances the ability of mitochondria to detoxify ROS by activating IDH2 (151) and SOD2 (124, 159) and potentially by increasing the expression of antioxidants through an interaction with FoxO3a (153). Therefore, by reducing the ROS levels, SIRT3 functions as a tumor suppressor by maintaining genomic stability and destabilizing HIF1α. As previously noted, elevated ROS promote carcinogenesis; however, excessive ROS levels beyond a toxic threshold may overwhelm cellular antioxidant capacity and trigger cell cycle arrest and apoptosis (161). Therefore, reduced SIRT3 expression in cancer cells is likely to impose increased demand on the antioxidant machinery to protect cells from the deleterious effects of elevated ROS levels. Studies from several groups indicate an important role of SOD1 in tumor initiation and progression (114). SOD1 is overexpressed in many types of cancer cells (113, 152), and its activity may be essential for limiting ROS levels to a level that is consistent with robust cellular proliferation. In this regard, a recent study by Lin *et al.* found that succinylation of SOD1 leads to decreases in its activity (88). SIRT5 binds to, desuccinylates, and activates SOD1. Expression of SIRT5 potentiates SOD1-mediated ROS scavenging (88). These results highlight a potential role of SIRT5 in the defense mechanisms of cancer cells against ROS-induced apoptosis, by promoting SOD1 activity. In this context, Lu *et al.* showed that SIRT5 is overexpressed in NSCLC, and SIRT5 KD results in the repression of NSCLC cells growth (94). In addition, SIRT5 represses the activities of PDC and SDH (116), both of which have a critical role in cancer cell metabolic reprogramming, and PDC activity is suppressed in many types of tumor cells. This represents an example of mutual antagonism between SIRT3 and SIRT5, as SIRT3 increases the activities of PDC (35) and SDHA (27, 40).

Concluding Remarks

Recent findings in sirtuin biology have highlighted the importance of mitochondrial sirtuins in regulating multiple metabolic pathways. Owing to their important roles in metabolic regulation, mitochondrial sirtuins may represent attractive candidates for development of therapeutic interventions against cancer and other diseases. However, many protein targets are common among mitochondrial sirtuins, and the degree to which these proteins functionally interact with each other has yet to be addressed. Additional work is also required to better understand potential redundancy in their functions, and the ways they respond to different environmental stimuli. Finally, the identification of novel lysine acyl modifications regulated by mitochondrial sirtuins and the diverse array of their putative targets suggest that our understanding of these important regulators is truly still in its infancy.

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Address correspondence to:
Dr. David B. Lombard
Department of Pathology
University of Michigan
3015 BSRB
109 Zina Pitcher Place
Ann Arbor, MI 48109

E-mail: davidlom@umich.edu

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Abbreviations Used

AASIS = amino acid stimulated insulin secretion
ACAT1 = acetyl-CoA acetyltransferase 1
ACC = acetyl-CoA carboxylase
AceCS2 = acetyl-CoA synthetase 2
AHL = age-related hearing loss
AMPK = AMP-activated kinase
ANT2 = ATP/ADP translocase 2
CPS1 = carbamoyl phosphate synthetase 1
CPT1 = carnitine palmitoyltransferase 1
CR = calorie restriction
CREB2 = cAMP response element binding protein 2
CSE = cigarette smoke extract
ERRE = estrogen-related receptor-binding element
ETC = electron transport chain
FoxO3a = forkhead box protein O3a
GDH = glutamate dehydrogenase
GLS = glutamate synthase
HFD = high-fat diet
HIF1α = hypoxia-inducible factor 1-alpha
HMGCS2 = 3-hydroxy-3-methylglutaryl CoA synthase 2
HPMECs = human pulmonary microvascular endothelial cells
HSCs = hematopoietic stem cells
IDH2 = isocitrate dehydrogenase 2
IRE = iron response element
IRP1 = iron regulatory protein 1
KD = knockdown
KO = knockout
LCAD = long-chain-specific acyl-CoA dehydrogenase
MCD = malonyl-CoA decarboxylase
MEFs = mouse embryonic fibroblasts
mPTP = mitochondrial permeability transition pore
mtDNA = mitochondrial DNA
mTORC1 = mechanistic target of rapamycin complex 1
NAD^+ = nicotinamide adenine dinucleotide
NAM = nicotinamide
NAMPT = nicotinamide phosphoribosyltransferase
NDUFA9 = NADH dehydrogenase (ubiquinone) 1 alpha subcomplex 9
NSCLC = non-small cell lung cancer
OPA1 = optic atrophy protein 1
OSCC = oral squamous cell carcinoma
OTC = ornithine transcarbamoylase
PDC = pyruvate dehydrogenase complex
PDK = pyruvate dehydrogenase kinase
PDP1 = pyruvate dehydrogenase phosphatase 1
PGC-1α = peroxisome proliferator-activated receptor gamma coactivator 1-alpha
PPARα = peroxisome-activated receptor α
PTM = post-translational modification
ROS = reactive oxygen species
SCD1 = stearoyl-CoA desaturase 1
SDH = succinate dehydrogenase
SDHA = succinate dehydrogenase subunit A
Skp2 = S-phase kinase-associated protein 2
SOD1 = Cu/Zn superoxide dismutase
SOD2 = Mn superoxide dismutase
TFAM = mitochondrial transcription factor A
TSC2 = tuberous sclerosis 2
UPR^{mt} = mitochondrial unfolded protein response
WT = wild type

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