SIRT FAMILY IN ENDOCRINOLOGY

EDITED BY: Yang Yang and Russel J. Reiter
PUBLISHED IN: Frontiers in Endocrinology and Frontiers in Neuroscience

frontiers Research Topics

ISSN 1664-8714
ISBN 978-2-88963-021-9
DOI 10.3389/978-2-88963-021-9

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SIRT FAMILY IN ENDOCRINOLOGY

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Citation: Yang, Y., Reiter, R. J., eds. (2019). SIRT Family in Endocrinology. Lausanne: Frontiers Media. doi: 10.3389/978-2-88963-021-9

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frontiers in Endocrinology

EDITORIAL
published: 31 May 2019
doi: 10.3389/fendo.2019.00347

Editorial: SIRT Family in Endocrinology

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Keywords: silent information regulator, endocrinology, diabetes, cardiovascular medicine, metabolism

Editorial on the Research Topic

SIRT Family in Endocrinology

Silent information regulator (SIRT), also known as NAD-dependent deacetylase sirtuin, is a member of the class III group of histone deacetylases, collectively called sirtuins. The mammalian sirtuin family consists of 7 members, designated SIRT1 through SIRT7, which are characterized by a conserved 275-amino-acid catalytic core and unique additional N-terminal and C-terminal sequences of variable length. Previous studies have demonstrated that SIRT can deacetylate a range of transcription factors, including forkhead box O (FOXO) transcription factors, p53, nuclear factor-κB (NF-κB), liver X receptor (LXR), peroxisome proliferator-activated receptor γcoactivator-1α(PGC-1α), cAMP-responsive element-binding protein–regulated transcription co-activator 2, and period homolog 2 (1).

It has been reported that SIRT performs a wide variety of functions in human systems, including obesity-associated metabolic diseases, endocrine disease, cancer, and aging. Activated SIRT1 improves the insulin sensitivity of liver, skeletal muscle, and adipose tissues and maintains the homeostasis of function and cell mass among pancreatic β-cells, suggesting that SIRT1 might be a new therapeutic target for the prevention of insulin resistance related disease, e.g., metabolic syndrome and type 2 diabetes mellitus (2). In addition, adipose triglyceride lipase (ATGL)-mediated SIRT1 activation promotes autophagy/lipophagy as a primary mean to control hepatic lipid droplet (LD) catabolism and fatty acid (FA) oxidation (3). In mammals, SIRT1 can deacetylate and thereby deactivate the p53 protein. SIRT1 also stimulates autophagy by preventing acetylation of proteins (via deacetylation) required for autophagy as demonstrated in cultured cells, embryonic, and neonatal tissues, which provides a link between sirtuin expression and the cellular response to limited nutrients due to caloric restriction (4). Furthermore, SIRT1 is shown to deacetylate and affect the activity of both members of the PGC1-alpha/ERR-alpha complex, which are essential to metabolic regulatory transcription factors (5).

The research topic covers the themes of diabetes, thyroid diseases, cardiovascular metabolism, cancer endocrinology, bone metabolism. Liu et al. found that estrogen 17β-estradiol (E2) induces CD34 and downregulates SIRT1 in primary mouse airway smooth muscle cells (ASMCs). Then they showed that loss of CD34 inhibits E2-induced reduction of SIRT1 and its enzymatic activity (measured by p53 deacetylation), demonstrating that E2 downregulates SIRT1 through

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Edited and reviewed by:
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Specialty section:
This article was submitted to Cellular Endocrinology, a section of the journal Frontiers in Endocrinology

Received: *04 May 2019*
Accepted: *14 May 2019*
Published: *31 May 2019*

Citation:
Li T, Peng F, Reiter RJ and Yang Y (2019) Editorial: SIRT Family in Endocrinology. Front. Endocrinol. 10:347. doi: 10.3389/fendo.2019.00347

CD34. Since acetylated p53 is well-established to induce apoptotic cell death, the authors further investigated how the E2/CD38/SIRT1/p53 axis affects hypoxia-induced apoptosis. Hypoxia is shown to stimulate CD38/SIRT1/p53 axis (although there is a discrepancy between mRNA levels and protein levels), and thereby E2 promotes hypoxia-induced apoptosis (17β-estradiol promotes apoptosis in airway smooth muscle cells through CD38/SIRT1/p53 pathway). Zhou et al. found the importance of L-serine in decreasing food intake and ameliorating oxidative stress and inflammation response in the hypothalamus of aging mice. The authors fed the aging-mouse models with different concentrations of L-serine, and found its crucial ability to prevent food intake and age-related body weight gain through regulating the leptin pathway and the orexigenic neuropeptides neuropeptide Y (NPY) and agouti-related neuropeptide (AGRP). In addition, they revealed the anti-oxidative and anti-inflammatory role of L-serine, supported by the decreased levels of reactive oxygen species and pro-inflammatory cytokines (IL-1β and IL-6), which is significantly regulated by the SIRT1 and NFκB pathways (Long-term L-serine administration reduces food intake and improves oxidative stress and SIRT1/NFκB pathway in the hypothalamus in aging mice). Moreover, Xu et al. summarized how SIRT1 in the brain controls systemic metabolic homeostasis and then discussed the role of SIRT1 in regulating mitochondrial functions and promoting neuroprotection in the context of cerebral ischemia and neurodegenerative disorders (Brain SIRT1 mediates metabolic homeostasis and neuroprotection). In addition, Yamamoto and Takahashi demonstrated the role of SIRT1 in the hypothalamic pituitary axis and its pathophysiological significance, showing that SIRT1 is involved in the regulatory mechanism of hypothalamus-pituitary axis with respect to the homeostasis maintenance (The essential role of SIRT1 in hypothalamic-pituitary axis).

In addition, Elibol and Kilic provided an overview regarding the association of the increasing level of SIRT1 protein for regulating some disease related conditions such as obesity, cardiovascular diseases, and neurodegeneration as well as some of the functional partners of SIRT1 (High levels of SIRT1 expression as a protective mechanism against disease-related conditions). Zhong et al. introduced the protective roles of SIRT1 in diabetic kidney disease (DKD). The mechanisms of actions are described, highlighting confirmatory results from mice models with DKD. Several SIRT1 activators are discussed as possible therapeutics to attenuate DKD, including resveratrol, puerarin, a novel specific SIRT1 agonist (BF175), and a bromodomain inhibitor (Sirt1 is a potential drug target for treatment of diabetic kidney disease). The present review article by Sergi et al. provides an overview of a number of molecular mechanisms involved in the etiology of osteosarcoma. They tried to clarify the interaction among insulin/IGF-1R, FOXO, and SIRT1 pathways, further explicating their roles in bone-related diseases. Specifically, the authors focus on the FOXO family of transcription factors (Insulin/IGF-1R, SIRT1, and FOXOs pathways–an intriguing interaction platform for bone and osteosarcoma). In addition, Fujita and Yamashita discussed the roles of the sirtuin family of proteins especially SIRT1 in pathological and physiological conditions of the central nervous system and focused their attention on the potential benefits of activators of SIRT1 in neurodegenerative diseases (Sirtuins in neuroendocrine regulation and neurological diseases). Zhang et al. discussed the predicted and validated roles of SIRT1 in ischemic stroke and potential stroke therapeutics, with a significant focus on Chinese medicines (The role of SIRT1 in ischemic stroke: pathogenesis and therapeutic strategies). Moreover, Liarte et al. aimed to conclude the available presentation on the interplay between SIRT1 and ER/GPER for breast cancer onset and progression. They discussed the ability of SIRT1 to interact with TGFß signaling, a concurrent signaling significantly involved in breast cancer progression, which will benefit for the development of innovative strategies in the assessment of orphan breast cancer subtypes, such as triple negative breast cancer (TNBC) (SIRT1 and estrogen signaling cooperation for breast cancer onset and progression). Frazzi described the role of SIRT1 in cancer with special emphasis on secretory organs' cancer. They sought to summarize the association between SIRT1 overexpression and poor overall survival in patients with liver and lung cancers, and no association with breast cancer, colorectal cancer or gastric carcinoma. The results show that unfavorable overall survival is associated with SIRT1 expression for solid malignancies (SIRT1 in secretory organs' cancer). Artsi et al. reported the role of SIRT1 in marrow adipose tissue (MAT) phenotype, demonstrating its stimulatory effect on a thermogenic gene program in female MAT, showing that SIRT1 activation and blocking sclerostin stimulate a thermogenic gene program in human bone marrow mesenchymal stem cell (BM-MSC) (Sirt1 promotes a thermogenic gene program in bone marrow adipocytes: from mice to (wo)men). Furthermore, Sun et al. confirmed that SIRT1-mediated molecular events and biological processes could be an underlying mechanism for metastasis and SIRT1 is a therapeutic target for inhibiting metastasis (Survival and clinicopathological significance of SIRT1 expression in cancers: A meta-analysis).

In addition to SIRT1, other molecules in the SIRT family also play an important role in endocrine secretion. Zhou et al. presented the recent advances of SIRT1-7 in various insulin resistance-associated physiological processes. In particular, they highlighted the roles of sirtuins in insulin resistance including inflammation, mitochondrial dysfunction, the insulin signaling pathway, glucose and lipid metabolism (Sirtuins and insulin resistance). Song et al. evaluated the roles of sirtuins in diabetes progression and described their involvement in metabolic pathways of skeletal muscle, adipose tissue, and liver. They suggested that SIRT1, SIRT2, SIRT3, and SIRT6 are positive regulators of insulin resistance in most cases while SIRT4 and SIRT7 negatively regulate insulin in secretion and fatty acid oxidation. The identifications of potential pharmacological targets of sirtuins may enhance the understanding on the regulation of glucose metabolism and homeostasis in diabetes (Distinctive roles of Sirtuins on diabetes, protective or detrimental). In addition, Min et al. summarized the current literature on the function of SIRT4, one of the lesser known and studied of the sirtuin family. The review introduces the metabolic functions of SIRT4 in various tissues and its role as

a tumor suppressor (The roles of mitochondrial SIRT4 in cellular metabolism). Wu et al. managed to concisely present the current knowledge on the functions and mechanisms of SIRT7 in cellular regulation and disease (Advances in cellular characterization of the sirtuin isoform, SIRT7).

The research topic highlights some of the recent findings about diabetes, thyroid diseases, cardiovascular metabolism, cancer endocrinology, bone metabolism. I would like to thank all the authors and reviewers for their contribution and discussion to put together this wonderful topic that may inspire further interest in this exciting new field.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

ACKNOWLEDGMENTS

The authors of this editorial would like to thank each and every one of the contributing authors for this Research Topic who worked hard to comply with deadlines and respond to reviewers efficiently.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Endocrinology

REVIEW
published: 27 March 2019
doi: 10.3389/fendo.2019.00187

Sirtuins and Type 2 Diabetes: Role in Inflammation, Oxidative Stress, and Mitochondrial Function

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The rising incidence of type 2 diabetes mellitus (T2DM) is a major public health concern, and novel therapeutic strategies to prevent T2DM are urgently needed worldwide. Aging is recognized as one of the risk factors for metabolic impairments, including insulin resistance and T2DM. Inflammation, oxidative stress, and mitochondrial dysfunction are closely related to both aging and metabolic disease. Calorie restriction (CR) can retard the aging process in organisms ranging from yeast to rodents and delay the onset of numerous age-related disorders, such as insulin resistance and diabetes. Therefore, metabolic CR mimetics may represent new therapeutic targets for insulin resistance and T2DM. Sirtuin 1 (SIRT1), the mammalian homolog of Sir2, was originally identified as a nicotinamide adenine dinucleotide (NAD^+)-dependent histone deacetylase. The activation of SIRT1 is closely associated with longevity under CR, and it is recognized as a CR mimetic. Currently, seven sirtuins have been identified in mammals. Among these sirtuins, SIRT1 and SIRT2 are located in the nucleus and cytoplasm, SIRT3 exists predominantly in mitochondria, and SIRT6 is located in the nucleus. These sirtuins regulate metabolism through their regulation of inflammation, oxidative stress and mitochondrial function via multiple mechanisms, resulting in the improvement of insulin resistance and T2DM. In this review, we describe the current understanding of the biological functions of sirtuins, especially SIRT1, SIRT2, SIRT3, and SIRT6, focusing on oxidative stress, inflammation, and mitochondrial function, which are closely associated with aging.

Keywords: SIRT1, SIRT2, SIRT3, SIRT6, Type 2 diabetes

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Specialty section:
This article was submitted to Cellular Endocrinology, a section of the journal Frontiers in Endocrinology

Received: *22 July 2018*
Accepted: *06 March 2019*
Published: *27 March 2019*

Citation:
Kitada M, Ogura Y, Monno I and Koya D (2019) Sirtuins and Type 2 Diabetes: Role in Inflammation, Oxidative Stress, and Mitochondrial Function. Front. Endocrinol. 10:187. doi: 10.3389/fendo.2019.00187

INTRODUCTION

The rising incidence of type 2 diabetes mellitus (T2DM) has significantly increased worldwide in recent decades, and the development of better treatments for T2DM is urgently needed. Aging is a universal process that affects all organs. Age-related disruptions in cellular homeostasis result in the decline in the responsiveness to physiological stress, including oxidative stress and inflammation, which are implicated in the pathogenesis of metabolic diseases, including insulin resistance and T2DM. Additionally, mitochondria play a central role in energy production and responsiveness to nutrient availability, and they are one of the sources of reactive oxygen species (ROS) (1). Therefore, mitochondrial function decline is also closely related to the impairment of metabolic homeostasis (2) and oxidative stress (3, 4), contributing to the progression of insulin resistance and T2DM, which are associated with aging. Additionally, oxidative stress is closely linked to

TABLE 1 | Seven sirtuins in mammals.

Sirtuin	Catalytic activity	Localization
SIRT1	Deacetylase	Nucleus and cytoplasm
SIRT2	Deacetylase	Cytoplasm and nucleus
SIRT3	Deacetylase	Mitochondria
SIRT4	ADP-ribosyl transferase	Mitochondria
SIRT5	Deacetylase	Mitochondria
SIRT6	Deacetylase and ADP-ribosyl transferase	Nucleus
SIRT7	Deacetylase	Nucleus

inflammation (5, 6); therefore, the suppression of oxidative stress/inflammation and preservation of mitochondrial function should be therapeutic targets for insulin resistance and T2DM, as well as for anti-aging treatments.

Calorie restriction (CR) retards aging or extends the life spans of yeast, worms, flies, and rodents (7). The benefits of CR for the suppression of age-related diseases, including glucose intolerance, cardiovascular disease and cancer, have also been observed in rhesus monkeys or humans (8–10), by improving insulin sensitivity and reducing inflammation and oxidative stress. Sirtuins have received attention for their role in modifying lifespan, especially in relation to the benefits of CR. From the initial studies on aging in yeast, silent information regulator 2 (Sir2), a nicotinamide adenine dinucleotide (NAD^+)-dependent deacetylase, was identified as one of the possible molecules through which CR improves lifespan extension (11). Homologs of Sir2 in higher eukaryotic organisms are known as SIRT1, which may contribute to CR-induced longevity (12–14), and, currently, seven sirtuins, including SIRT1, have been identified in mammals (15, 16) (**Table 1**). Numerous previous reports have shown the multiple physiological roles of sirtuins, including SIRT1, SIRT2, SIRT3 and SIRT6, in cellular function, such as glucose metabolism, mitochondrial function and resistance against cellular stresses, including oxidative stress and inflammation (15–20). Thus, the modulation of sirtuin activity, as a CR mimetic, may be a novel drug target for insulin resistance and T2DM.

In this review, we describe the current understanding of the biological functions of sirtuins, especially SIRT1, SIRT2, SIRT3, and SIRT6, focusing on oxidative stress, inflammation and mitochondrial function, which are closely associated with aging. We also discuss their potential as pharmacological targets to prevent the development of metabolic diseases, such as insulin resistance and T2DM.

INFLAMMATION, OXIDATIVE STRESS, AND MITOCHONDRIAL DYSFUNCTION, WHICH ARE RELATED TO THE PATHOGENESIS OF INSULIN RESISTANCE AND TYPE 2 DIABETES

Chronic inflammation, oxidative stress and impaired mitochondrial function in skeletal muscle, adipose tissue or monocytes/macrophages (21, 22) are closely related to the pathogenesis of insulin resistance and T2DM. Additionally, inflammation and oxidative stress contribute to pancreatic β-cell dysfunction (23, 24), contributing to the progression of T2DM.

The activation of monocytes in the circulation, adipocytes and macrophages residing in adipose tissue leads to the release of various inflammatory mediators, including tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and chemoattractant protein-1 (MCP-1), in insulin-resistant and diabetic states. These cytokines activate inflammatory signaling pathways, such as the inhibitor of IκB kinase (IKK) and c-Jun NH_2-terminal kinase (JNK) pathways, which impair the insulin signaling pathway by modulating phosphoinositide 3-kinase (PI3K) and Akt (25–27), and they play a crucial role in the pathogenesis of insulin resistance in adipose tissue and skeletal muscle. Oxidative stress also impairs insulin signaling, which contributes to insulin resistance in T2DM. In insulin-resistant or diabetic states, in addition to hyperglycemia, other metabolites, including free fatty acids (FFAs) and certain cytokines, such as TNF-α, induce the overproduction of ROS from mitochondria. ROS trigger the activation of serine/threonine kinases, such as p38 mitogen-activated protein kinase (p38 MAPK), JNK, and IKK, which induce the serine phosphorylation of insulin receptor substrate-1 (IRS-1), and then degrade IRS-1 and reduce IRS-1 tyrosine phosphorylation, leading to the suppression of insulin signaling (28–31), as well as inflammation. Inflammatory mediators and oxidative stress are also related to pancreatic β-cell dysfunction, resulting in the impairment of insulin production or excretion from β cells (23, 24).

The impairment of mitochondrial function in skeletal muscle is involved in the pathogenesis of insulin resistance and progression of T2DM, which may be associated with aging. Mitochondria play a central role in energy production and responsiveness to nutrient availability by regulating mitochondrial oxidative phosphorylation (OXPHOS) and fatty acid oxidation. However, previous studies have shown that the rate of mitochondrial OXPHOS is reduced and that intramyocellular lipid accumulation is increased in the skeletal muscle of patients with insulin resistance and T2DM and in elderly individuals (32–35). Aging is closely linked to the impairment of metabolic homeostasis, such as insulin resistance and T2DM, which are closely related to the decline in mitochondria function. Mitochondrial function decline generates excess ROS from mitochondria, and oxidative stress is linked to inflammation. Thus, there is a vicious cycle among oxidative stress, inflammation and mitochondrial dysfunction, and breaking this cycle may be a therapeutic target for the treatment of age-related insulin resistance and T2DM, focusing on SIRT1, SIRT2, SIRT3, and SIRT6 (**Figure 1**).

SIRT1

SIRT1 exists in the nucleus and cytoplasm, and it has NAD^+-dependent deacetylase activity (16). Numerous non-histone proteins, including transcription factors, transcriptional coregulatory proteins and histones, serve as substrates for SIRT1, which is associated with a wide variety of cellular processes (16). SIRT1 may play a crucial role in reducing inflammation and oxidative stress and improving mitochondrial function,

resulting in both the protection of pancreatic β cells and amelioration of insulin resistance in insulin-sensitive tissues such as skeletal muscle and adipose tissue. Therefore, SIRT1 should be a pharmacological therapeutic target to treat insulin resistance and T2DM (17, 36).

Regulation of Inflammation

Accumulated evidence has demonstrated that SIRT1 suppresses inflammatory processes likely through interfering with nuclear factor kappa-B (NF-κB) signaling. Yeung et al. found that SIRT1 deacetylates the p65 subunit of NF-κB at lysine 310 and inhibits its transcriptional activity (37).

In adipocytes and macrophages, SIRT1 reduces inflammatory process through the deacetylation of NF-κB (p65), leading to improved glucose metabolism (38, 39) (**Figure 2A**). Additionally, myeloid cell-specific SIRT1 knockout (KO) mice that were fed a high-fat diet (HFD) exhibited macrophage activation and elevated expression of inflammatory mediators in the liver and adipose tissues, which was associated with the development of systemic insulin resistance (40). Furthermore, we previously reported another mechanism regarding SIRT1 inactivation-induced inflammation (**Figure 2A**): SIRT1 inactivation may enhance the NF-κB signaling pathway by the phosphorylation of NF-κB (p65) via the cellular accumulation of p62/Sqstm1 due to autophagy dysfunction, in THP-1 cells, cultured human monocytes (41). Moreover, SIRT1 inactivation resulted in increased activation of the mammalian target of rapamycin complex 1 (mTORC1) pathway and reduced 5′ AMP-activated kinase (AMPK) activation, possibly contributing to impairment in autophagy (41). In humans, reduction of SIRT1 expression levels in circulating monocytes is correlated with glucose intolerance, insulin resistance and metabolic syndrome in humans (42). Gillum et al. also demonstrated that SIRT1 expression is decreased in adipose tissues from obese males and that the mRNA expression of CD14, a macrophage marker, in adipose tissue is negatively correlated with SIRT1 expression (43).

Interestingly, adipocyte SIRT1 controls systemic glucose homeostasis and insulin sensitivity through cross talk with adipose-resident macrophages (44) (**Figure 2B**). Hui et al. recently reported that adipose-specific SIRT1 KO mice showed a higher susceptibility to HFD-induced insulin resistance, which is associated with an increased number of adipose-resident macrophages and their polarization toward the proinflammatory M1 subtype, overexpressing inflammatory mediators (44). SIRT1 in adipocytes modulates the expression and secretion of several adipokines, including adiponectin, MCP-1, TNF-α, and IL-4, which, in turn, alters the recruitment and polarization of macrophages in adipose tissues. In adipocytes, SIRT1 enhances IL-4 expression through deacetylating the transcription factor nuclear factor of activated T cells, cytoplasmic 1 (NFATc1), leading to polarization of the M2 subtype (44). Thus, SIRT1 may diminish inflammation in adipose tissues and monocytes/macrophages and may improve insulin resistance and T2DM.

In addition, SIRT1 protein expression is reduced in skeletal muscle and primary myotubes derived from T2DM

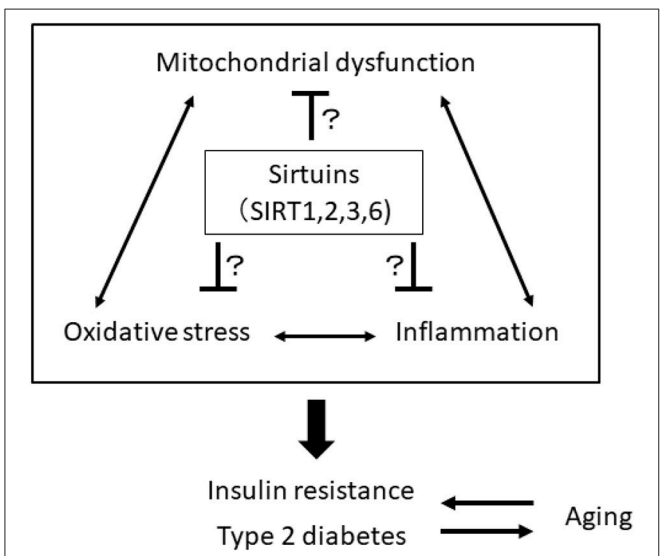

FIGURE 1 | A vicious cycle among oxidative stress, inflammation, and mitochondrial dysfunction is involved in the pathogenesis of insulin resistance, type 2 diabetes, and aging. Sirtuins, including SIRT1, 2, 3, and 6, may be a therapeutic target for the treatment of age-related insulin resistance and T2DM by breaking this vicious cycle.

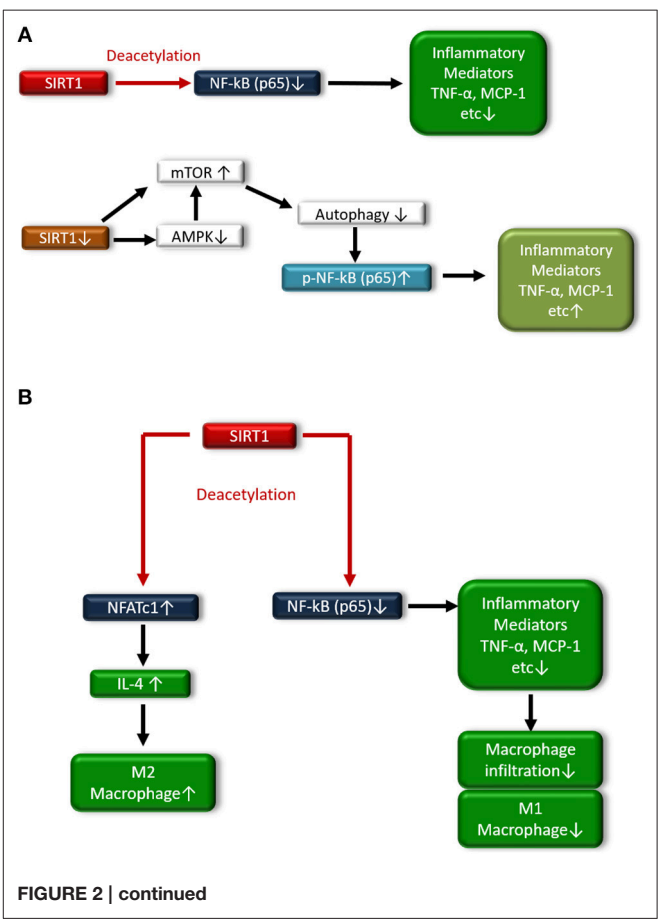

FIGURE 2 | continued

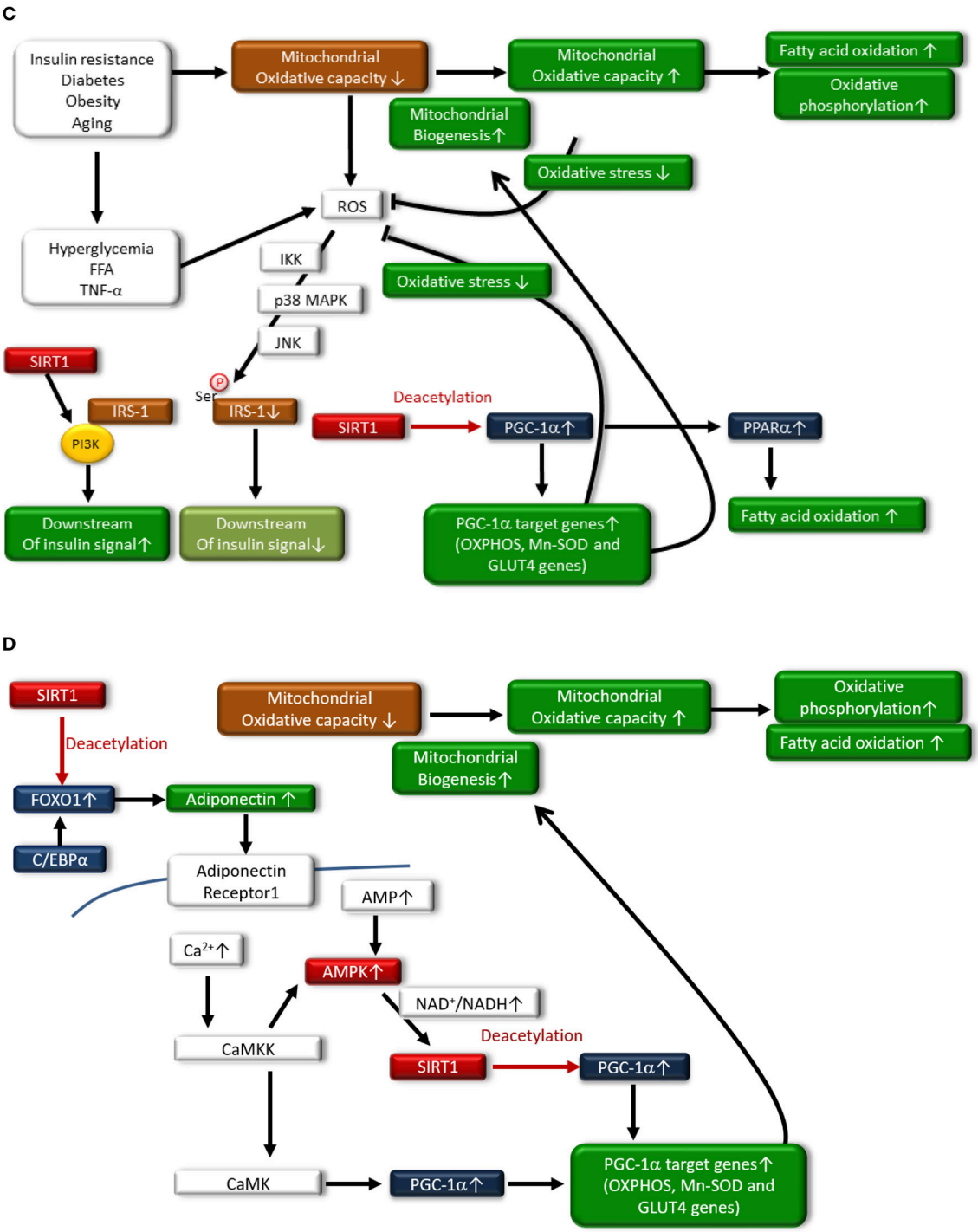

FIGURE 2 | (A) In monocytes/macrophages and adipocytes, SIRT1 deacetylates NF-κB(p65), resulting in reduced expression of inflammatory mediators such as TNF-α and MCP-1. SIRT1 inactivation also induces inflammation through the phosphorylation of the NF-κB pathway via impaired autophagy, which is associated with activation of mammalian target of rapamycin (mTOR) and reduced activation of AMP-activated kinase (AMPK). **(B)** In adipocytes, SIRT1 deacetylates nuclear factor-κB p65 subunit [NF-κB(p65)], resulting in reduced expression of inflammatory mediators such as tumor necrosis factor-α (TNF-α) and chemoattractant protein-1 (MCP-1), and decreased polarization to M1 macrophages and infiltration to adipose tissue. SIRT also induces polarization to M2 macrophages through increased expression of interleukin-4 (IL-4) expression via deacetylation of nuclear factor of activated T-cells 1 (NFATc1). **(C)** In skeletal muscle, SIRT1 increases mitochondrial biogenesis and fatty acid oxidation through acetylation and activation of the peroxisome proliferator-activated receptor (PPAR)-γ coactivator-1α (PGC-1α). Under conditions of insulin resistance, diabetes, obesity, or aging, mitochondrial oxidative capacity is decreased, contributing to the generation of reactive oxygen species

(Continued)

FIGURE 2 | (ROS) in mitochondria. Hyperglycemia, free fatty acids (FFAs) and TNF-α stimulate ROS production from the mitochondria, and increased levels of ROS lead to the serine-phosphorylation of insulin receptor substrate-1 (IRS-1), resulting in reduced insulin signaling. However, SIRT1 interacts with phosphoinositide 3-kinase (PI3K), leading to activation of insulin signaling. Additionally, SIRT1 activates PGC-1α transcriptional activity to induce mitochondrial biogenesis and the induction of antioxidative enzymes, which can inhibit the generation of ROS by mitochondria. Expression of glucose transporter 4 (GLUT4) is enhanced through deacetylation of PGC-1α by SIRT1. Moreover, SIRT1 activates peroxisome proliferator-activated receptor-α (PPAR-α), which induces fatty acid oxidation. **(D)** SIRT1 deacetylates Forkhead box protein O1 (FOXO1) and enhances its interaction with CCAAT/enhancer binding protein α (C/EBPα), resulting in the enhanced transcription of adiponectin in adipocytes. In skeletal muscle, adiponectin is involved in the regulation of Ca^{2+} signaling and PGC-1α expression through calcium/calmodulin-dependent protein kinase kinase (CaMKK) and calcium/calmodulin-dependent protein kinase (CaMK) activation. Adiponectin activates SIRT1 through AMPK activation, thereby deacetylating PGC-1α and resulting in mitochondrial biogenesis, increased fatty acid oxidation, and oxidative phosphorylation.

patients, suggesting that diminished SIRT1 contributes to insulin resistance induced by TNF-α in skeletal muscle (45). SIRT1 interacts with PI3K adaptor subunit p85 in an insulin-independent manner and activates insulin signaling at physiological insulin concentrations in skeletal muscle cells (45) (**Figure 2C**). Furthermore, SIRT1 protects pancreatic β cells against various toxic stresses, including oxidative stress and inflammatory cytokines, through suppressing NF-κB signaling (46).

Regulation of Mitochondrial Function

SIRT1 can be involved in the regulation of metabolism and insulin resistance through the modulation of mitochondrial function. Peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α) maintains mitochondrial biogenesis and OXPHOS proteins, leading to efficient β oxidation of fatty acids in skeletal muscle. However, the levels of PGC-1α in skeletal muscle are decreased in T2DM. SIRT1 regulates mitochondrial function and metabolic homoeostasis, increases the oxygen consumption in skeletal muscle and leads to the expression of OXPHOS genes and mitochondrial biogenesis through the deacetylation of PGC-1α. SIRT1 knockdown largely prevents the upregulation of PGC-1α-induced genes that are involved in mitochondrial fatty acid utilization (47). Furthermore, SIRT1 can regulate peroxisome proliferator-activated receptor-α (PPAR-α) activation by PGC-1α deacetylation, resulting in increased fatty acid oxidation. Thus, the activation of SIRT1 may improve insulin resistance through promoting fatty acid oxidation and mitochondrial biogenesis via deacetylation of PGC-1α and PPAR-α activation in skeletal muscle (**Figure 2C**). Additionally, PGC-1α remarkably increases the expression of glucose transporter 4 (GLUT4) and activation of glucose transport in murine C2C12 myotubes (48). The effects of PGC-1α on GLUT4 gene expression leads to increased transport of glucose in myocytes, suggesting that the activation of PGC-1α by SIRT1 is involved in insulin sensitization (**Figure 2C**). Adiponectin has antidiabetic power (49), and the levels of plasma adiponectin are reduced in insulin resistance and T2DM (50, 51). Treatment with adiponectin can decrease glucose levels and ameliorate insulin resistance in mice (52). Mechanistically, adiponectin enhances insulin sensitivity through increasing fatty acid oxidation via AMPK and PPAR-α activation (49). Additionally, SIRT1 deacetylates Forkhead box protein O1 (FOXO1) and enhances its interaction with CCAAT/enhancer binding protein α (C/EBPα), resulting in enhanced transcription of the adiponectin gene in adipocytes (53) (**Figure 2D**). Iwabu et al. demonstrated that adiponectin signaling plays a crucial role in skeletal muscle cells and is implicated in the regulation of Ca^{2+} signaling and expression/activation of PGC-1α in muscle adiponectin receptor (adipoR) 1 KO mice (54). Adiponectin activates AMPK by binding to adipoR1, thereby activating SIRT1, and deacetylating PGC-1α improves oxidative stress, mitochondrial function, glucose/lipid metabolism, and exercise endurance (54) (**Figure 2D**), resulting in improved insulin resistance and T2DM. Thus, SIRT1-induced PGC-1α deacetylation leads to the improvement of mitochondrial function via mitochondrial biogenesis and induction of GLUT4 and adiponectin, demonstrating the beneficial effects against insulin resistance and T2DM.

Regulation of Oxidative Stress

In addition to mitochondrial function modulation, PGC-1α deacetylation by SIRT1 reduces oxidative stress through the overexpression of antioxidative enzymes, including manganese-superoxide dismutase (Mn-SOD) (55). Additionally, Forkhead box protein O3a (FOXO3a) is deacetylated by SIRT1 and translocates to the nucleus, resulting in the upregulation of other antioxidative enzymes and catalases and protection against oxidative stress (56). Thus, SIRT1 may improve insulin resistance and T2DM possibly through reducing oxidative stress (**Figure 2C**), inducing mitochondrial biogenesis, and increasing mitochondrial function.

SIRT2

SIRT2 is localized in both the cytoplasm and nucleus, and it is widely expressed in various tissues, including the brain, muscle, pancreas, liver, kidney, and adipose tissues. SIRT2 interacts with many histone and non-histone protein substrates, including tubulin and histone H4 (18). SIRT2 is involved in multiple cellular functions, including genomic integrity, cell growth, differentiation, and energy metabolism, and reduced SIRT2 activity has been implicated in cancer, neurodegeneration and metabolic diseases (18). Previous studies have demonstrated that SIRT2 plays an important role in various physiological processes in maintaining metabolic homeostasis, including inflammation, oxidative stress and mitochondrial function, as well as adipocyte differentiation, fatty acid oxidation, gluconeogenesis, and insulin sensitivity (18). A few reports have shown that SIRT2 exerts anti-inflammation and antioxidative stress effects and improves mitochondrial function in metabolic-related tissues, such as skeletal muscle.

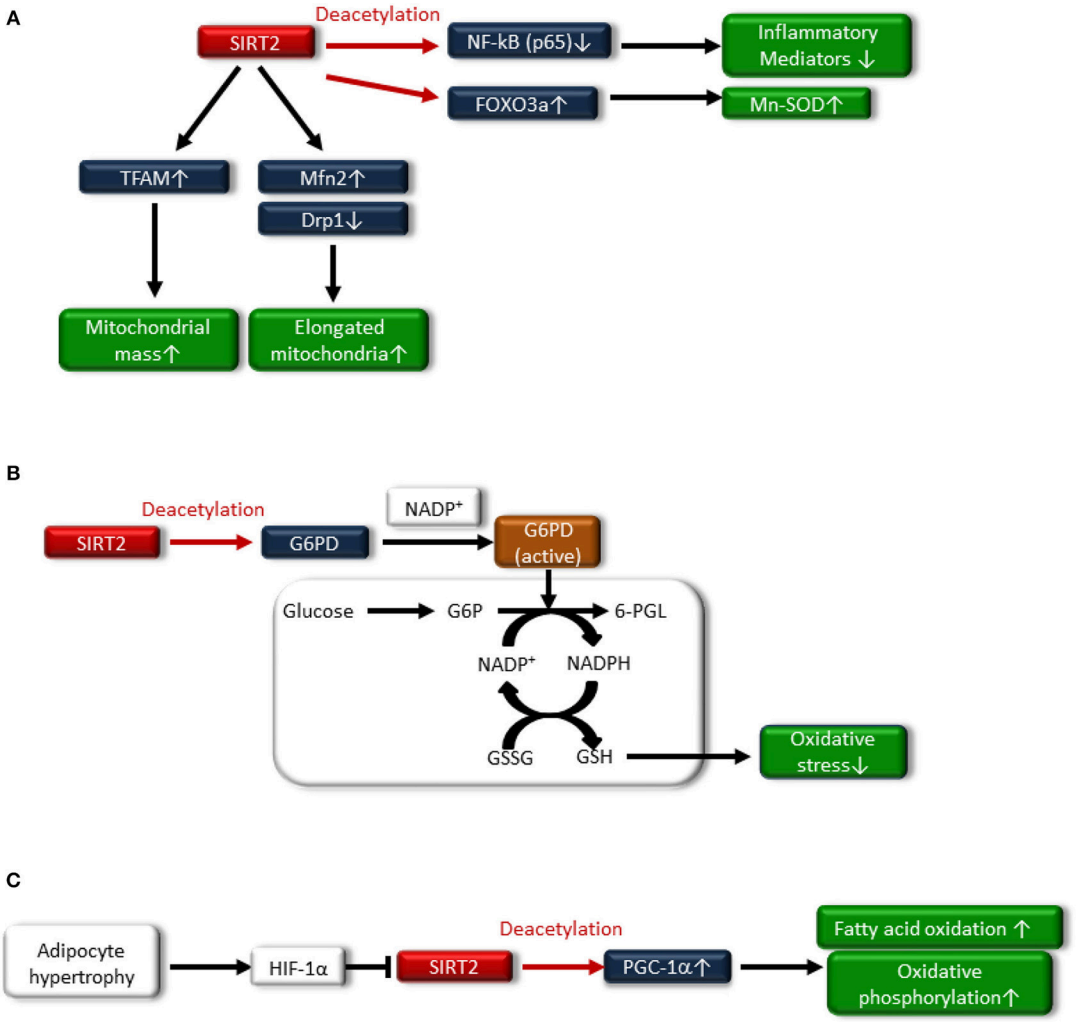

FIGURE 3 | (A) SIRT2 deacetylates nuclear factor-κB p65 subunit [NF-κB (p65)], resulting in decreased expression of inflammatory mediators. Sirt2 also induces Mn-SOD expression by deacetylating Forkhead box protein O3a (FOXO3a). Additionally, SIRT2 increases fusion-related protein mitofusion2 (Mfn2) and decreases mitochondrial-associated dynamin-related protein 1 (Drp1), resulting in an increased number of elongated mitochondria and improved mitochondrial function. SIRT2 also attenuates the downregulation of transcription factor A, mitochondrial (TFAM), a key mitochondrial deoxyribonucleic acid (mtDNA)-associated protein, leading to an increase in mitochondrial mass. **(B)** Glucose-6-phosphate dehydrogenase (G6PD) plays an important role in the oxidative stress response by producing nicotinamide adenine dinucleotide phosphate (NADPH) and the reduced form glutathione (GSH), which is associated with deacetylating G6PD and binding to nicotinamide adenine dinucleotide phosphate ($NADP^+$). **(C)** Hypoxia-inducible factor1α (HIF1α), which is accumulated in the adipocytes of hypertrophy, represses SIRT2 expression, resulting in decreased deacetylation of PGC-1α and the expression of β-oxidation and mitochondrial genes.

Regulation of Inflammation

SIRT2 regulates inflammation by deacetylating the NF-κB p65 subunit (57), similar to SIRT1. Pais et al. demonstrated that SIRT2 plays a crucial role as a major inhibitor of microglia-mediated inflammation and neurotoxicity through the deacetylation of NF-κB (p65) (58) (**Figure 2A**). In other experimental inflammatory disease models, the anti-inflammatory effect of SITR2 has been demonstrated through the suppression of the NF-κB signaling pathway (59, 60). However, further studies are necessary to elucidate whether this anti-inflammatory effect of SIRT2 may be exerted in metabolic diseases, including insulin resistance and T2DM.

Regulation of Oxidative Stress

SIRT2 regulates redox homeostasis in cells. SIRT2-dependent deacetylation of FOXO3a leads to increased expression of Mn-SOD to improve oxidative stress (61) (**Figure 3A**). In addition, glucose-6-phosphate dehydrogenase (G6PD) is a key enzyme in the pentose phosphate pathway (PPP) and plays a crucial role in the oxidative stress response by producing nicotinamide adenine dinucleotide phosphate (NADPH) and reduced form glutathione (GSH), the main intracellular reductant (**Figure 3B**). Wang et al. reported that SIRT2 activates G6PD through deacetylation on lysine 403 in G6PD, which plays an important role in maintaining the cellular redox status and protecting cells from oxidative damage (62).

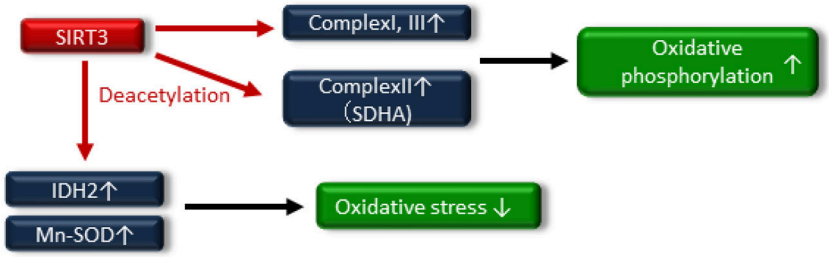

FIGURE 4 | SIRT3 deacetylates the electron transport chain complexes I, II [deacetylate succinate dehydrogenase (SDH) A] and III, leading to increased oxidative phosphorylation. SIRT3 also attenuates oxidative stress by enhancing the glutathione antioxidant defense system via deacetylation and activation of isocitrate dehydrogenase 2 (IDH2) and manganese superoxide dismutase (Mn-SOD).

Regulation of Mitochondrial Function

SIRT2 may be related to the regulation of mitochondrial function. Lemos et al. showed that SIRT2 is downregulated in insulin-resistant hepatocytes and the liver, and is accompanied by increased ROS production, activation of extracellular signal-regulated kinase (ERK1/2), and mitochondrial dysfunction in ob/ob mice (63). SIRT2 overexpression in insulin-resistant hepatocytes improved insulin sensitivity and reduced ROS production. SIRT2 might increase fusion-related protein mitofusion 2 (Mfn2) and decrease mitochondrial-associated dynamin-related protein 1 (Drp1), resulting in an increased number of elongated mitochondria and improving mitochondrial function (**Figure 3A**). SIRT2 also attenuated the downregulation of transcription factor A mitochondrial (TFAM), a key mitochondrial deoxyribonucleic acid (mtDNA)-associated protein, leading to an increase in the mitochondrial mass (63) (**Figure 3A**). Furthermore, SIRT2 expression in peripheral blood mononuclear cells (PBMCs) from human subjects was negatively correlated with obesity, insulin resistance and oxidative stress (63).

SIRT2 is most markedly expressed in adipocytes (61). Nutrient overload-induced adipose expansion enhances intra-adipose hypoxia, promoting the accumulation of adipocyte hypoxia-inducible factor 1α (HIF1α). HIF1α suppresses SIRT2 transcription through interaction at a cross-species conserved hypoxic response element (HRE) on the SIRT2 promoter. HIF1α accumulation in the adipocytes of human obese subjects correlates with low levels of SIRT2 in visceral adipose tissue, and reduced SIRT2 activity directly translates into decreased deacetylation of PGC-1α and expression of β-oxidation and mitochondrial genes. HIF-1α suppresses fatty acid catabolism in mitochondria by negatively regulating the SIRT2-PGC-1α axis (64) (**Figure 3C**).

SIRT3

Preservation of mitochondrial health is crucial to prevent insulin resistance and T2DM during aging. SIRT3 is localized primarily in the mitochondria. SIRT3 is a major mitochondrial deacetylase and plays a major role in deacetylating and modifying the enzymatic activities of several mitochondrial proteins (16). In humans, a polymorphism in the SIRT3 gene has been correlated with reduced enzymatic efficiency and the development of metabolic syndrome (65). SIRT3 is also recognized an anti-aging molecule, and high SIRT3 expression levels are associated with longevity in humans (66, 67). Previous studies have demonstrated that SIRT3 protects organisms against metabolic stress, cancer, the development of cardiac hypertrophy, and oxidative stress (16).

Regulation of Mitochondrial Function and Oxidative Stress

Numerous reports have exhibited that SIRT3 regulates mitochondrial function and maintains redox homeostasis; therefore, the impairment of SIRT3 function is implicated in the pathogenesis of insulin resistance and T2DM. Jing et al. demonstrated that decreased levels of SIRT3 in the skeletal muscle of streptozotocin (STZ)-induced diabetic mice and high-fat diet-induced obese mice were an important component of the pathogenesis of T2DM (68). SIRT3 KO mice exhibited decreased oxygen consumption and increased oxidative stress due to mitochondrial dysfunction via the hyperacetylation of complex I and III in OXPHOS, and these factors led to JNK activation and impaired insulin signaling. In addition, SIRT3 can directly deacetylate succinate dehydrogenase (SDH), a subunit of complex II and that succinate dehydrogenase activity is reduced in SIRT3 KO cells and brown adipose tissue (BAT) from SIRT3 KO mice (69). Thus, SIRT3 may induce mitochondrial oxidative phosphorylation through the deacetylation of complex I, III and SDHA of complex II (**Figure 4**).

A reduction in SIRT3 activity contributes to mitochondrial oxidative stress through decreasing the activation of antioxidative enzymes, such as isocitrate dehydrogenase 2 (IDH2) and Mn-SOD (70–72), by increasing the acetylation of antioxidative enzymes (**Figure 4**). SIRT3 protects pancreatic β cells against lipotoxicity by antagonizing oxidative stress-induced cell damage. Zhou et al. demonstrated that HFD feeding caused elevated oxidative stress accompanied by reduced SIRT3 expression in the pancreatic β cells of wild-type mice (73). Primary pancreatic islets of SIRT3 KO mice and murine pancreatic β-cell line MIN6 cells with downregulated SIRT3 expression showed increased Mn-SOD acetylation and reduced glucose-stimulated insulin secretion and glucose-stimulated ATP generation (73). On the other hand, SIRT3 overexpression,

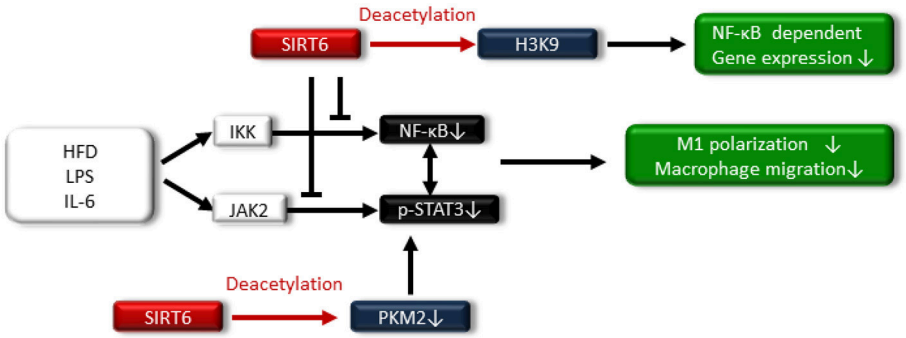

FIGURE 5 | SIRT6 also attenuates NF-κB signaling via histone H3K9 deacetylation at the chromatin level. SIRT6 suppresses the high-fat diet (HFD)-, LPS-, and IL-6-induced I-κ kinase (IKK)-nuclear factor-κB (NF-κB) pathway and Janus activating kinase 2 (JAK2)-signal transducer and activator of transcription 3 (STAT3) pathway, resulting in reduced M1 macrophage polarization and macrophage migration. Additionally, SIRT6 deacetylates pyruvate kinase M2 (PKM2), preventing STAT3 from phosphorylation.

using an adenoviral system, ameliorated palmitate-induced β-cell dysfunction including endoplasmic reticulum (ER) stress in pancreatic β-cell line NIT1 cells (74). In human, reduced expression of SIRT3 in pancreatic β cells from T2DM patients has been linked to impaired β-cell function (75). Thus, novel therapeutic approaches targeting SIRT3 activity may be important in providing new opportunities to treat insulin resistance and T2DM through maintaining mitochondrial health.

SIRT6

SIRT6 is located in the nucleus, and it acts as an adenosine diphosphate (ADP)-ribosyl transferase and NAD^+-dependent deacetylase (16). SIRT6 has been associated with longevity regulation. Kanfi et al. reported that the overexpression of SIRT6 extended the lifespan of male mice and was involved in decreased serum levels of insulin-like growth factor (IGF)-1 and increased levels of IGF-binding protein 1 (76). SIRT6 is implicated in DNA repair, telomere maintenance, genomic stability and cell senescence. SIRT6 also attenuates NF-κB signaling via histone H3K9 deacetylation at the chromatin level (77–79) (**Figure 5**).

Regulation of Inflammation, Oxidative Stress, and Mitochondrial Function

SIRT6 is involved in vascular inflammation and oxidative stress. Knockdown of SIRT6 in human umbilical vein endothelial cells (HUVECs) increases the expression of proinflammatory cytokines (80). Balestrieri et al. demonstrated that SIRT6 protein expression in atherosclerotic lesions of T2DM patients was downregulated, which was compared with SIRT6 protein expression in atherosclerotic lesions of non-diabetic patients, and the reduced SIRT6 expression was associated with increased oxidative stress and inflammation (81). Additionally, apolipoprotein E-deficient with SIRT6 knockdown using small hairpin RNA (shRNA) lentivirus-injected mice fed a high-cholesterol diet showed the promotion of atherosclerosis that was associated with increased inflammation in endothelial cells (82).

Regarding the relationship between SIRT6 and metabolic disease, SIRT6 levels increase in rats under the CR condition, and transgenic mice that overexpress SIRT6 are protected against HFD-induced several metabolic impairments, including glucose intolerance (83). By contrast, ablation of neural SIRT6 leads to obesity (84). Lee et al. demonstrated that myeloid-specific SIRT6 KO mice exhibit tissue inflammation and insulin resistance when fed an HFD (85). Myeloid SIRT6 deletion promoted proinflammatory M1 polarization of bone marrow macrophages and enhanced the migration potential of macrophages toward adipose-derived inflammatory mediators. SIRT6 deletion in macrophages facilitated activation of the IKK-NF-κB pathway and endogenous production of IL-6, leading to activation of the Janus activating kinase 2 (JAK2)-signal transducer and activator of transcription 3 (STAT3) pathway and positive feedback circuits for NF-κB stimulation; this cross talk expedited M1 polarization (85) (**Figure 5**). Furthermore, acetylated pyruvate kinase M2 (PKM2) phosphorylates STAT3 in the nucleus (86), PKM2 is deacetylated by SITR6 (87), and the study demonstrated that SIRT6 deacetylates PKM2, preventing STAT3 from being phosphorylated and leading to the suppression of M1 polarization in SIRT6-overexpressed intraperitoneal macrophages treated with LPS (**Figure 5**).

SIRT6 is involved in the regulation of mitochondrial function in skeletal muscle. Cui et al. demonstrated that muscle-specific SIRT6 KO mice impairs glucose homeostasis and insulin sensitivity, attenuates whole-body energy expenditure, and weakens exercise performance. Mechanistically, the deletion of SIRT6 in muscle decreased the expression of genes associated with glucose and lipid uptake, fatty acid oxidation, and mitochondrial OXPHOS in muscle cells caused by the reduced AMPK activity (88).

In pancreatic β cells, SIRT6 regulates insulin secretion in response to glucose stimulation. Xiong et al. demonstrated that the deletion of SIRT6 in pancreatic β-cells in mice leads to the impairment of glucose-stimulated insulin secretion (89, 90) and they further found that SIRT6 regulates insulin secretion by maintaining mitochondrial function and modulating

intracellular Ca^{2+} dynamics (89, 90). Additionally, SIRT6 plays an important role in the protection of pancreatic β cells from lipotoxicity (palmitic acid, PA)-induced cellular dysfunction or even cell death (90). Oxidative stress generated by fatty acid oxidation is involved in the pathogenesis of PA-induced β-cell dysfunction and apoptosis (91). SIRT6 may exert the effect of antioxidative stress by coactivating NF-E2-related factor 2 (NRF2) (92). However, it is unclear whether SIRT6 plays a role in antioxidative stress in pancreatic β cells.

Thus, SIRT6 may have beneficial effects on glucose metabolism, including insulin resistance and T2DM, by reducing inflammation and improving mitochondrial function. Additionally, SIRT6 protects pancreatic β cells from lipotoxicity-induced cellular damage, through maintaining mitochondrial function and possibly antioxidative stress.

CONCLUDING REMARKS

The knowledge of sirtuins has expanded from the original description of a NAD^+-dependent deacetylase responsible for longevity in yeast, which is associated with CR. As described above, sirtuin family members, such as SIRT1, 2, 3, and 6, may induce beneficial effects in glucose metabolism, partially through improving inflammation, oxidative stress, and maintaining mitochondrial function. Therefore, pharmacological modulation of sirtuins may represent a novel therapeutic tool to improve insulin resistance and T2DM. Among the sirtuins, several SIRT1 activators, such as resveratrol and synthesized activators, have been evaluated for their antidiabetic effects in animal models (93). In humans, several small trials have shown that SIRT1 activators exert beneficial effects on glucose metabolism and insulin resistance, which resemble the effect of CR (94). However, there is still insufficient clinical data regarding the effect of SIRT1 activators on insulin resistance and T2DM. In addition, SIRT2, SIRT3, and SIRT6, which are induced by CR, play crucial roles in regulating cellular processes, including metabolism, inflammation, oxidative stress and mitochondrial function. However, further investigation into the targets and functions of SIRT1, SIRT2, SIRT3, and SIRT6 will aid in the development of new strategies to treat insulin resistance and T2DM. In addition to SIRT1, SIRT2, SIRT3, and SIRT6, other sirtuins, such as SIRT4, SIRT5, and SIRT7, play crucial roles in cellular homeostasis and functions, including redox homeostasis, anti-inflammation, cell survival, and mitochondrial quality control (95–100), which may be involved in the pathogenesis of insulin resistance and T2DM. However, further basic studies are necessary to elucidate the detailed molecular mechanisms.

AUTHOR CONTRIBUTIONS

MK and DK designed the manuscript, wrote and edited the manuscript. YO, IM, and DK contributed to the discussion. DK is the guarantor of this work.

FUNDING

This work was supported by a Grant-in-Aid for Scientific Research (C) (17K09718) to MK, a Grant-in-Aid for Young Scientists (B) (17K16104) to YO, and a Grant-in-Aid for Young Scientists (B) (18K16013) to IM. Boehringer Ingelheim, Mitsubishi Tanabe Pharma, Kyowa Hakko Kirin, Taisho Toyama Pharmaceutical Co., and Ono Pharmaceutical Co. contributed to establishing the Division of Anticipatory Molecular Food Science and Technology.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Endocrinology

SYSTEMATIC REVIEW
published: 13 March 2019
doi: 10.3389/fendo.2019.00121

Survival and Clinicopathological Significance of SIRT1 Expression in Cancers: A Meta-Analysis

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OPEN ACCESS

Edited by:
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Specialty section:
This article was submitted to Cellular Endocrinology, a section of the journal Frontiers in Endocrinology

Received: 10 December 2018
Accepted: 11 February 2019
Published: 13 March 2019

Citation:
Sun M, Du M, Zhang W, Xiong S, Gong X, Lei P, Zha J, Zhu H, Li H, Huang D and Gu X (2019) Survival and Clinicopathological Significance of SIRT1 Expression in Cancers: A Meta-Analysis. Front. Endocrinol. 10:121. doi: 10.3389/fendo.2019.00121

Background: Silent information regulator 2 homolog 1 (SIRT1) is an evolutionarily conserved enzymes with nicotinamide adenine dinucleotide $(NAD)^+$-dependent deacetylase activity. SIRT1 is involved in a large variety of cellular processes, such as genomic stability, energy metabolism, senescence, gene transcription, and oxidative stress. SIRT1 has long been recognized as both a tumor promoter and tumor suppressor. Its prognostic role in cancers remains controversial.

Methods: A meta-analysis of 13,138 subjects in 63 articles from PubMed, EMBASE, and Cochrane Library was performed to evaluate survival and clinicopathological significance of SIRT1 expression in various cancers.

Results: The pooled results of meta-analysis showed that elevated expression of SIRT1 implies a poor overall survival (OS) of cancer patients [Hazard Ratio (HR) = 1.566, 95% CI: 1.293–1.895, $P < 0.0001$], disease free survival (DFS) (HR = 1.631, 95% CI: 1.250–2.130, $P = 0.0003$), event free survival (EFS) (HR = 2.534, 95% CI: 1.602–4.009, $P = 0.0001$), and progress-free survival (PFS) (HR = 3.325 95% CI: 2.762–4.003, $P < 0.0001$). Elevated SIRT1 level was associated with tumor stage [Relative Risk (RR) = 1.299, 95% CI: 1.114–1.514, $P = 0.0008$], lymph node metastasis (RR = 1.172, 95% CI: 1.010–1.360, $P = 0.0363$), and distant metastasis (RR = 1.562, 95% CI: 1.022–2.387, $P = 0.0392$). Meta-regression and subgroup analysis revealed that ethnic background has influence on the role of SIRT1 expression in predicting survival and clinicopathological characteristics of cancers. Overexpression of SIRT1 predicted a worse OS and higher TNM stage and lymphatic metastasis in Asian population especially in China.

Conclusion: Our data suggested that elevated expression of SIRT1 predicted a poor OS, DFS, EFS, PFS, but not for recurrence-free survival (RFS) and cancer-specific survival (CCS). SIRT1 overexpression was associated with higher tumor stage, lymph node metastasis, and distant metastasis. SIRT1-mediated molecular events and biological processes could be an underlying mechanism for metastasis and SIRT1 is a therapeutic target for inhibiting metastasis, leading to good prognosis.

Keywords: SIRT1, cancer, prognosis, clinicopathological significance, meta-analysis

INTRODUCTION

Silent information regulator 2 homolog 1 (SIRT1) is an evolutionarily conserved enzymes with nicotinamide adenine dinucleotide $(NAD)^+$-dependent deacetylase activity and a member of the mammalian sirtuin family. It is expressed in almost all human tissues and localized in both nuclei and cytoplasm (1). Its substrates include histones and non-histone proteins such as transcription factors (2–4). SIRT1 is involved in a large variety of cellular processes, such as genomic stability, energy metabolism, senescence, gene transcription, and oxidative stress (5). It has been shown to be involved in a spectrum of diseases, including cancer, diabetes, obesity, and neurodegenerative diseases (6–8). SIRT1 plays an important role in regulating glucose and lipid metabolism and regulates malignancy in tumors (9).

SIRT1 has long been recognized as both a tumor promoter and tumor suppressor (10–12). This is also shown in recent studies. SIRT1 promotes proliferation, migration, and invasion of breast cancer cell line MCF-7 (13). SIRT1 promotes proliferation and paclitaxel-resistance of human cervical cancer cells (14). Yang et al. found that SIRT1 levels are lower in non-small cell lung cancer (NSCLC) than the normal control group (15), but Gharabaghi et al. found that SIRT1 are over expressed in NSCLC (16). The role of SIRT1 in prognosis of cancer was also investigated in several studies. Over expression of SIRT1 suggests poor prognosis in luminal breast cancer (17) and serous epithelial ovarian cancer (EOC) (18), gastric cancer (19), high pathological stage and worse overall survival in the lung adenocarcinoma patients (20), decreased survival and increased relapse in breast cancer patients (3, 21), colorectal carcinoma patients (22), lymphangiogenesis, lymphovascular invasion, and prognosis in pN0 esophageal squamous cell carcinoma (23), soft tissue sarcomas (24), both operable triple-negative and non-triple-negative breast cancer (25), hepatocellular carcinoma (26), gastric carcinoma (27), diffuse large B-cell lymphoma (28). On the other hand, SIRT1 expression is found to be associated with good prognosis for head and neck squamous cell carcinoma patients (29), and colorectal cancer (30). Therefore, the prognostic and clinicopathological significance of SIRT1 abnormal expression in cancers remain to be elucidated.

Prognostic value and clinicopathological association of SIRT1 with cancers have been analyzed in previous meta-analysis (31–36). However, the studies included in these meta-analysis were limited to mostly Asian population, single or several cancer types, or they were published several years ago (31–36). In the present study, we conducted an updated and more comprehensive meta-analysis and subgroup analysis to reveal the prognostic value and clinicopathological association of SIRT1 abnormal expression in cancers.

Abbreviations: OS, overall survival; SIRT1, silent information regulator 1, sirtuin-1; HR, hazard ratio; RR, relative risk; CI, confidence interval; DFS, disease free survival; EFS, event free survival; PFS, progress-free survival; RFS, recurrence-free survival; CCS, cancer-specific survival; NAD, nicotinamide adenine dinucleotide; q-PCR, quantitative real-time polymerase chain reaction; IHC, immunohistochemistry; ISH, *in situ* hybridization; NA, not available; NOS, newcastle-ottawa scale; NSCLC, non-small cell lung cancer; EOC, epithelial ovarian cancer; TNM, tumor, node, metastasis; MOOSE, meta-analysis of observational Studies in Epidemiology.

METHODS

Search Strategy

We retrieved literature published in between 1966 and April 1st, 2018 by searching PubMed, EMBASE, and Cochrane Library with the keywords (1) "SIRT1" OR "sirtuin 1" OR "SIR2" OR "SIR2L1" OR "SIR2alpha" OR "silent mating type information regulation 2 homolog-1" AND (2) "tumor OR cancer OR carcinoma OR neoplasm" and using the search strategies as illustrated in **Supplementary Tables 1A–C**. We selected and evaluated all relevant studies and review articles about SIRT1 and inquired the authors for unpublished raw data. The search and selection of articles for the study were separately conducted based on a common set of criteria. The divergence in opinion were settled through discussion among ourselves.

Inclusion and Exclusion Criteria

This meta-analysis was conducted according to Meta-analysis of Observational Studies in Epidemiology (MOOSE) Checklist. Studies enrolled in this analysis satisfied the following requirements: (i) patients must be diagnosed with cancer via pathology; (ii) The expression of SIRT1 must be determined by quantitative real-time polymerase chain reaction (q-PCR), immunohistochemistry (IHC), or *in situ* hybridization (ISH); (iii) The correlation between SIRT1 expression and prognosis or clinicopathological features was investigated; (iv) The Hazard Ratio (HR) and its 95% confidence interval (CI) for survival indicator on the basis of SIRT1 expression level were readily available or could be calculated indirectly; (v) The most representative and most accurate study was adopted when a single sample source was used in multiple studies to avoid unnecessary cohort overlapping. Studies that have satisfied the abovementioned inclusion requirements were further ruled out if they had any of the following flaws: (i) duplicated articles or data; (ii) not human studies; (iii) review articles or letters; (iv) lack of sufficient data or information to get HR; (v) articles not written in English.

Quality Assessment of Included Studies

We used the Newcastle-Ottawa Scale (NOS) to assess the quality of each included study. Scores ≥ 7 were considered high quality. We used a "star system" for case-control studies (**Supplementary Table 2**).

Data Extraction

We extracted the following data from the full texts of eligible studies: (i) the first author; (ii) publication year; (iii) characteristics of the studies, which comprised of the patients' nationality, sample size, tumor type, and clinicopathological characteristics; (iv) the assay method and cut-off value of SIRT1; (v) HRs of SIRT1 expression for OS, disease-free survival (DFS), event-free survival (EFS), recurrence-free survival (RFS), cancer-specific survival (CCS), progression-free survival (PFS); (vi) if the HR for OS, DFS, EFS, RFS, CCS and PFS were

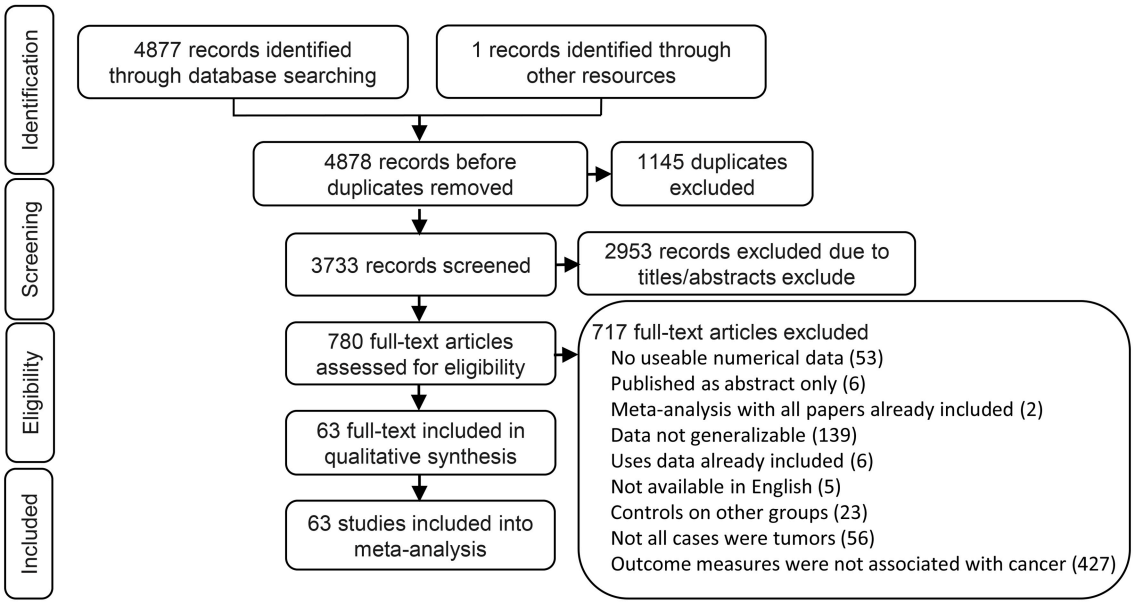

FIGURE 1 | Flow chart of the identification process for eligible studies.

calculated by both univariate and multivariate analyses, the latter was our first choice, given that these results were adjusted for confounding factors. If a study did not report the HR, we estimated HR and their corresponding 95% CI using the method described by Parmar et al. (37) and Tierney et al. (38). We recovered the data of Kaplan-Meier curves via the Engauge Digitizer version 9.8 (http://markummitchell.github.io/engauge-digitizer) and calculated the HR and its 95% CI. We repeated this process three times to reduce variability. Any divergence regarding the extraction and interpretation of all data was resolved by discussion among ourselves until consensus was reached.

Statistical Analysis

All the HRs and their 95% CIs were combined to evaluate the effect of SIRT1 high expression on prognosis. If the pooled HR < 1 and their 95% CI did not overlap the invalid line in the forest plot, the high expression of SIRT1 predicted a good OS. If the 95% CI overlapped the invalid line, the combined HR was considered insignificant. Otherwise, the combined HR predicted a poor OS. The heterogeneity of the pooled results was examined via Cochrane's Q test and Higgins' I-squared, and $P < 0.1$ or $I^2 \geq 25\%$ was considered high heterogeneity. If $P > 0.1$ and $I^2 < 25\%$, we ignored the influence of heterogeneity and pooled the overall result using a fixed effects model, otherwise employing the random effects model. The potential publication bias was assessed by a funnel plot, and Egger's test (39). $P < 0.05$ was considered significant. Statistical analysis was carried out using the "metafor" and "meta" packages of the R/BioConductor (version 3.5.1).

RESULTS

Search Results

We found 2,397 articles in PubMed, 2,460 articles in EMBASE, 20 articles in Cochrane library, and one articles through the references. We had a total of 3,733 articles after removing 1,145 duplicated articles. We then ruled out 2,953 articles which were review, letters, laboratory studies, or articles irrelevant to present research. We further excluded 717 full-text articles according to the inclusion and exclusion criteria of this study. The remaining 63 articles were finally eligible and included in this meta-analysis (**Figure 1**).

Study Demographics

The 63 eligible articles were published in between 2008 and 2017 with 63 studies that included a total of 13,138 participants from 9 countries who represented 16 cancer types and Asian and Caucasian ethnic groups (**Table 1**). The mean and median value were selected as the cut-off value in most articles. All studies measured SIRT1 expression in tumor tissue or serum via q-PCR, IHC, or ISH.

Correlation Between SIRT1 Expression and Prognosis

We performed meta-analysis of correlation between SIRT1 expression and OS, DFS, EFS, RFS, CCS, and PFS. The results and analysis of publication bias are presented in **Table 2**. The results showed that higher SIRT1 expression indicated an unfavorable OS ($n = 48$, HR: 1.566, 95% CI: [1.293, 1.895], $P < 0.0001$, $I^2 = 81.3\%$) (**Figure 2**), poor patient DFS ($n = 14$, HR: 1.631, 95% CI: [1.250–2.130], $P = 0.0003$, $I^2 = 72.6\%$, **Figure 3A**), poor

TABLE 1 | Main characteristics of 63 included studies.

References	Study design	Country	Case (N)	Type of Cancer	Disease stage	Follow-up (Mo.) median(range)	Race	Sample type	Survival end points	Adjusted variables	NOS score
Stenzinger et al. (40)	Retrospective	Germany	129	Pancreatic ductal adenocarcinoma	I–IV	54	Caucasian	Tissue	OS	Stage, grade	7
Li et al. (41)	Retrospective	China	91	Pancreatic ductal adenocarcinoma	I–IV	NA	Asian	Tissue	NA	NA	8
Teramae et al. (42)	Retrospective	Japan	62	Uterine cervical cancer	III	NA	Asian	Tissue	OS	NA	7
Asaka et al. (43)	Retrospective	Japan	108	Endometrial carcinoma	I–IV	NA	Asian	Tissue	OS, DFS	NA	8
Jang et al. (44)	Retrospective	Korea	64	Ovarian cancer	I–IV	29 (1–137)	Asian	Tissue	OS	Age, P53	6
Shuang et al. (18)	Retrospective	China	63	Ovarian cancer	I–IV	NA	Asian	Serum	OS	Age, grade, FIGO stage, lymphatic metastasis	6
Mvunta et al. (45)	Retrospective	Japan	68	Ovarian cancer	I–IV	74	Asian	Tissue	OS	Stage, lymphovascular space invasion	7
Zhang et al. (46)	Retrospective	China	22	Osteosarcoma	NA	NA	Asian	Tissue	OS	NA	7
Feng et al. (47)	Retrospective	China	89	Osteosarcoma	I–III	11 (3–83)	Asian	Tissue	OS	Stage, neoadjuvant chemotherapy	8
Kim et al. (24)	Retrospective	Korea	104	Soft tissue sarcoma	I–IV	NA	Asian	Tissue	OS, EFS	P53, β-catenin, stage, depth of tumor, tumor necrosis, distant metastasis	8
Noguchi et al. (29)	Retrospective	Japan	437	Head and neck squamous cell carcinoma	I–IV	46.6 (1–174)	Asian	Tissue	RFS, CSS	Age, gender, tumor site, tumor status, lymph node status, distant metastasis, pathologic grade	8
Yu et al. (48)	Retrospective	China	120	Laryngeal and hypopharyngeal carcinomas	I–IV	NA	Asian	Tissue	OS	NA	7
Batra et al. (49)	Retrospective	India	94	Retinoblastoma	I–IV	59	Asian	Tissue	NA	NA	7
Chen et al. (50)	Retrospective	China	206	Esophageal squamous cell carcinoma	I–II	55.9 (5–86)	Asian	Tissue	OS, DFS	Differentiation, T status, stage, VEGF-C, peritumoral lymphatic microvessel density	7
He et al. (51)	Retrospective	China	86	Esophageal squamous cell carcinoma	I–III	NA	Asian	Tissue	OS	Gender, clinical stage, histological grade, lymph node metastasis	7
Feng et al. (52)	Retrospective	China	34	Pelvis chondrosarcoma	I–III	37.7 vs. 53.8*	Asian	Tissue	OS	NA	8
Noh et al. (53)	Retrospective	Korea	200	Renal cell carcinoma	I–III	NA	Asian	Tissue	OS, RFS, CSS	NA	6
Jeh et al. (54)	Retrospective	Korea	102	Renal cell carcinoma	I–IV	73	Asian	Tissue	CSS	Age, T stage, grade, metastasis, karnofsky performance status	7
Jang et al. (28)	Retrospective	Korea	104	Diffuse large B cell lymphoma	I–IV	17.3 (1–135)	Asian	Tissue	OS, EFS	International prognostic index	8

(Continued)

TABLE 1 | Continued

References	Study design	Country	Case (N)	Type of Cancer	Disease stage	Follow-up (Mo.) median(range)	Race	Sample type	Survival end points	Adjusted variables	NOS score
Ren et al. (55)	Retrospective	China	45	Angioimmunoblastic T cell lymphoma	I–IV	NA	Asian	Tissue	PFS	P53, LDH, hemoglobin, γ-Globulin, sex, age, international prognostic index score, stage	8
Nosho et al. (56)	Retrospective	USA	485	Colorectal cancer	I–IV	NA	Caucasian	Tissue	OS, CSS	Age, year of diagnosis, sex, body Mass index, tumor location, stage, grade, microsatellite instability, the CpG island methylator phenotype	6
Jang et al. (2)	Retrospective	Korea	497	Colorectal cancer	I–IV	70.8	Asian	Tissue	OS, DFS	Histological grade, AJCC stage	8
Jung et al. (30)	Retrospective	Korea	349	Colorectal cancer	I–IV	55.3	Asian	Tissue	OS	Age, location, TNM stage, Histologic grade, β-catenin	8
Benard et al. (57)	Retrospective	Netherlands	254	Colorectal cancer	I–III	103.2	Caucasian	Tissue	OS	NA	7
Chen et al. (22)	Retrospective	China	102	Colorectal cancer	II–IV	NA	Asian	Tissue	OS	Gender, age, metastasis, TNM stage	8
Lv et al. (58)	Retrospective	China	120	Colorectal cancer	I–IV	53.3 (1–78)	Asian	Tissue	OS	NA	6
Lee et al. (59)	Retrospective	China	351	Colorectal cancer	I–IV	NA	Asian	Tissue	DFS, CSS	NA	7
Cheng et al. (60)	Retrospective	China	90	Colorectal cancer	I–III	NA	Asian	Tissue	OS	NA	8
Chen et al. (61)	Retrospective	China	172	Hepatocellular Carcinoma	I–III	125 (45–236)	Asian	Tissue	OS	NA	6
Jang et al. (62)	Retrospective	Korea	154	Hepatocellular Carcinoma	I–IV	NA	Asian	Tissue	OS, DFS	Stage, albumin, AFP, c-Myc, P53	8
Hao et al. (63)	Retrospective	China	99	Hepatocellular Carcinoma	I–IV	NA	Asian	Tissue	OS	NA	7
Cheng et al. (64)	Retrospective	China	148	Hepatocellular Carcinoma	I–III	NA	Asian	Tissue	OS	NA	8
Li et al. (65)	Retrospective	China	72	Hepatocellular Carcinoma	I–III	NA	Asian	Tissue	OS, DFS	NA	8
Liu et al. (66)	Retrospective	China	148	Hepatocellular Carcinoma	I–III	NA	Asian	Tissue	NA	NA	7
Cha et al. (27)	Retrospective	Korea	177	Gastric Cancer	I–IV	NA	Asian	Tissue	OS, RFS	TNM stage	7
Feng et al. (67)	Retrospective	China	176	Gastric Cancer	I–IV	NA	Asian	Tissue	OS	NA	6
Kang et al. (68)	Retrospective	Korea	452	Gastric Cancer	I–IV	53.3 (3–83)	Asian	Tissue	OS	Lymph node metastasis, depth of invasion, lymphatic invasion, histologic grade, DBC1, cytoplasmic β-catenin	7
Noguchi et al. (19)	Retrospective	Japan	557	Gastric Cancer	I–IV	69 (6–142)	Asian	Tissue	CSS	NA	7

(Continued)

TABLE 1 | Continued

References	Study design	Country	Case (N)	Type of Cancer	Disease stage	Follow-up (Mo.) median(range)	Race	Sample type	Survival end points	Adjusted variables	NOS score
Qiu et al. (69)	Retrospective	China	96	Gastric Cancer	I–IV	31.6 (6–78)	Asian	Tissue	OS, RFS	Lymph node metastasis, Beclin1 expression	8
Szász et al. (70)	Retrospective	Hungary	1065	Gastric Cancer	I–IV	NA	Caucasian	Tissue	OS	NA	8
Zhang et al. (71)	Retrospective	China	128	Gastric Cancer	I–IV	NA	Asian	Tissue	OS	NA	8
Zhang et al. (72)	Retrospective	China	176	Gastric Cancer	I–IV	NA	Asian	Tissue	OS	NA	6
Ren et al. (73)	Retrospective	USA	348	Colorectal cancer	I–IV	NA	Caucasian	Tissue	OS	NA	8
Shin et al. (74)	Retrospective	Korea	45	Ovarian cancer	NA	NA	Asian	Tissue	OS	NA	6
Zhang et al. (75)	Retrospective	China	50	Colorectal cancer	NA	NA	Asian	Tissue	NA	NA	7
Cao et al. (76)	Retrospective	China	150	Breast carcinoma	I–IV	161	Asian	Tissue	OS, DFS	Lymph node metastasis, TNM stage, ER status, PR status, Snail expression	8
Jin et al. (77)	Retrospective	Korea	319	Breast carcinoma	I–III	NA	Asian	Tissue	OS, DFS	AJCC stage, lymphatic invasion, DCIS	6
Kim et al. (78)	Retrospective	Korea	278	Breast carcinoma	NA	63.78 (2–74)	Asian	Tissue	DFS	T stage, caspase3, lymphovascular invasion	7
Lee et al. (21)	Retrospective	USA	142	Breast carcinoma	I–IV	NA	Caucasian	Tissue	OS, EFS	Stage, HER2 status, P53 expression	8
Chung et al. (79)	Retrospective	Korea	344	Breast carcinoma	I–III	NA	Asian	Tissue	OS, DFS	T stage, lymphatic invasion, DCIS	6
Derr et al. (3)	Retrospective	Netherlands	822	Breast carcinoma	I–III	11.8 (0.16–27.55)	Caucasian	Tissue	OS, DFS	NA	6
Wu et al. (25)	Retrospective	China	134	Breast carcinoma	I–III	154	Asian	Tissue	OS, DFS	Stages, P53, Lymph nodes status	8
Lee et al. (80)	Retrospective	Korea	688	Breast carcinoma	I–IV	190.8	Asian	Tissue	NA	NA	8
Chung et al. (81)	Retrospective	Korea	427	Breast carcinoma	I–III	NA	Asian	Tissue	DFS	NA	6
Zhang et al. (82)	Retrospective	China	149	Breast carcinoma	NA	101.03 vs. 88.38*	Asian	Tissue	OS	NA	6
Sung et al. (83)	Retrospective	Korea	28	Breast carcinoma	I-IV	NA	Asian	Tissue	NA	NA	6
Gharabaghi et al. (16)	Retrospective	Iran	40	NSCLC	NA	NA	Caucasian	Tissue	OS	Gender, age, histologic grade, T stage, lymph node metastasis, BIRC6 expression	6
Li et al. (20)	Retrospective	China	75	NSCLC	I–IV	NA	Asian	Tissue	OS	Age, TNM stage	7
Lin et al. (84)	Retrospective	China	260	NSCLC	NA	37.1 (0–128)	Asian	Tissue	OS	NA	8
Noh et al. (85)	Retrospective	Korea	144	NSCLC	NA	NA	Asian	Tissue	NA	NA	7
Zhang et al. (86)	Retrospective	China	295	NSCLC	III–IV	NA	Asian	Tissue	OS	Tumor stage, tumor differentiation	6
Chen et al. (23)	Retrospective	China	125	NSCLC	I–IV	NA	Asian	Tissue	NA	NA	8
Grbesa et al. (87)	Retrospective	Spain	179	NSCLC	I–IV	45	Asian	Tissue	OS, RFS	Stage	6

Mo., month; NSCLC, non-small cell lung cancer; NA, not available; NOS, newcastle-ottawa scale. FIGO, the international federation of gynecology and obstetrics; OS, overall survival; EFS, event-free survival; DFS, disease-free survival; CSS, cancer-specific survival; RFS, recurrence-free survival; LDH, lactate dehydrogenase; DBC1, deleted in breast cancer 1; ER, estrogen receptor; PR, progesterone receptor; DCIS, ductal carcinoma in situ; HER2, human epidermal growth factor receptor 2; BIRC6, Baculoviral IAP repeat-containing 6.

**The median survival time of high expression group vs. low expression group.*

TABLE 2 | Survival effects of SIRT1 overexpression and the prognosis of patients.

Outcome	No. of trials (patients)	HR (95%CI) Fixed-effect estimate	*P*-value of Fixed-effect Model	HR (95%CI) Random-effect estimate	*P* value of Random-effect Model	Heterogeneity I^2(%), *P*-value	*P*-value of Egger's test, Begg's test
OS	48 (9573)	1.259 (1.170–1.355)	<0.0001	***1.566 (1.293–1.895)***	***<0.0001***	81.3%, <0.0001	0.0043, 0.1884
DFS	14 (3982)	1.482 (1.308–1.679)	<0.0001	***1.631 (1.250–2.130)***	***0.0003***	72.6%, <0.0001	0.2234, 0.2503
EFS	3 (350)	***2.534 (1.602–4.009)***	***0.0001***	2.534 (1.602–4.009)	0.0001	0.0%, 0.8557	0.1174, 0.1172
RFS	5 (1089)	1.253 (0.996–1.575)	0.0542	1.936 (0.903–4.151)	0.0898	88.90%, <0.0001	0.0037, 0.3272
CCS	6 (2132)	1.097 (0.900–1.338)	0.3591	1.229 (0.757–1.994)	0.4037	77.3%, 0.0005	0.6331, 0.3476
PFS	2 (340)	***3.325 (2.762–4.003)***	***<0.0001***	3.325 (2.762–4.003)	<0.0001	0.0%, 0.9089	NA, NA

HR, hazard ratio; CI, confidence interval; OS, overall survival; DFS, disease free survival; EFS, event free survival; RFS, recurrence-free survival; CCS, cancer-specific survival; PFS, progress-free survival; NA, not available. I^2, index for assessing heterogeneity; value ≥25% indicates a moderate to high heterogeneity; Egger's test, P-value of Egger's regression for asymmetry assessment; Begg's test, P-value of Begg and Mazumdar rank correlation test for asymmetry assessment. Bold italics indicate statistically significant values (P < 0.05).

EFS ($n = 3$, HR: 2.534, 95% CI: [1.602, 4.009], $P = 0.0001$, $I^2 = 0\%$, **Figure 3B**), and poor PFS ($n = 2$, HR: 3.325, 95% CI: [2.762, 4.003], $P < 0.0001$, $I^2 = 0\%$, **Figure 3C**), but not correlated with RFS of the Asian or tissue ($n = 5$, HR: 1.936, 95% CI: [0.903 - 4.151], $P = 0.0898$, $I^2 = 88.9\%$) (**Figure 3D**) or CCS ($n = 6$, HR: 1.229, 95% CI: [0.757–1.994], $P = 0.4037$, $I^2 = 77.3\%$) (**Figure 3E**).

Correlation Between SIRT1 Expression and Prognosis of Cancer Types

Cancer type subgroup analysis showed that SIRT1 overexpression was associated with a worse OS in osteosarcoma ($n = 2$, HR: 1.661, 95% CI: [1.162, 2.372], $P = 0.0053$, $I^2 = 0\%$), esophageal squamous cell carcinoma ($n = 2$, HR: 1.781, 95% CI: [1.197, 2.652], $P = 0.0044$, $I^2 = 0\%$), hepatocellular carcinoma ($n = 5$, HR: 1.969, 95% CI: [1.539, 2.520], $P < 0.0001$, $I^2 = 0\%$), breast carcinoma ($n = 7$, HR: 1.744, 95% CI: [1.022, 2.978], $P < 0.0416$, $I^2 = 70.18\%$), NSCLC ($n = 5$, HR: 1.929, 95% CI: [1.259, 2.957], $P < 0.0025$, $I^2 = 59.40\%$), whereas SIRT1 overexpression was not correlated with the OS in ovarian cancer ($n = 4$, HR: 1.971, 95% CI: [0.899, 4.323], $P = 0.0903$, $I^2 = 55.18\%$), colorectal carcinoma ($n = 8$, HR: 0.932, 95% CI: [0.636, 1.366], $P = 0.7198$, $I^2 = 82.96\%$), gastric carcinoma ($n = 7$, HR: 1.535, 95% CI: [0.864, 2.726], $P = 0.1436$, $I^2 = 87.90\%$) (**Supplementary Figure 1A**).

SIRT1 overexpression was associated with a worse DFS in colorectal cancer ($n = 3$, HR: 1.544, 95% CI: [1.061, 2.247], $P = 0.0233$, $I^2 = 68.69\%$), and breast carcinoma ($n = 7$, HR: 1.819, 95% CI: [1.026, 3.223], $P = 0.0404$, $I^2 = 84.59\%$), whereas it was not correlated with the DFS in hepatocellular carcinoma ($n = 2$, HR: 1.357, 95% CI: [0.872, 2.113], $P = 0.1758$, $I^2 = 9.41\%$) (**Supplementary Figure 2A**).

SIRT1 overexpression was correlated with RFS of Gastric Cancer ($n = 2$, HR: 2.734, 95% CI: [1.694, 4.413], $P < 0.0001$, $I^2 = 0\%$), Renal cell carcinoma ($n = 1$, HR: 2.233, 95% CI: [1.088, 4.583]), and NSCLC (HR: 2.698, 95% CI: [1.457, 4.996]), whereas SIRT1 overexpression was negatively correlated with RFS of head and neck squamous cell carcinoma (HR: 0.655, 95% CI: [0.478, 0.897], $P = 0.0084$) (**Supplementary Table 3**).

SIRT1 overexpression was correlated with CCS in head and neck squamous cell carcinoma ($n = 1$, HR: 0.640, 95% CI: [0.453, 0.905], $P = 0.0116$), gastric cancer ($n = 1$, HR: 1.450, 95% CI: [1.018, 2.066], $P = 0.0396$), and renal cell carcinoma ($n = 2$, HR: 1.478, 95% CI: [0.124, 17.621]), but not with CCS in colorectal cancer (HR: 1.344, 95% CI: [0.716, 2.521], $P = 0.3577$) (**Supplementary Table 3**).

Correlation Between SIRT1 Expression and Prognosis of Cancer in Different Countries

Analysis of country subgroups showed that high expression of SIRT1 was correlated with poor OS in China ($n = 24$, HR: 1.661, 95% CI: [1.339, 2.060], $P < 0.0001$, $I^2 = 63.03\%$), Korea ($n = 12$, HR: 1.902, 95% CI: [1.187, 3.047], $P = 0.0075$, $I^2 = 80.65\%$), Japan ($n = 3$, HR: 1.940, 95% CI: [1.029, 3.655], $P = 0.0405$, $I^2 = 0\%$), but not in USA ($n = 3$, HR: 1.043, 95% CI: [0.465, 2.338], $P = 0.9193$, $I^2 = 84.36\%$), or Netherlands ($n = 2$, HR: 1.003, 95% CI: [0.671, 1.498], $P = 0.9893$, $I^2 = 73.79\%$) (**Supplementary Figure 1B**).

SIRT1 overexpression was also correlated with poor DFS in China ($n = 6$, HR: 2.021, 95% CI: [1.612, 2.534], $P < 0.0001$, $I^2 = 0\%$), but not in Korea ($n = 6$, HR: 1.321, 95% CI: [0.773, 2.259]) (**Supplementary Figure 2B**).

SIRT1 overexpression was correlated with poor EFS in Korea ($n = 2$, HR: 2.714, 95% CI: [1.506, 4.894], $P = 0.0009$, $I^2 = 0\%$) and USA ($n = 1$, HR: 2.280, 95% CI: [1.098, 4.734]).

Correlation Between SIRT1 Expression and Prognosis of Cancer in Asian and Caucasian

Elevated SIRT1 expression predicted a significantly worse OS in Asian population with cancers (HR: 1.708, 95% CI: [1.406, 2.076], $P < 0.0001$, $I^2 = 69.59\%$) rather than in Caucasian population (HR: 1.04, 95% CI: [0.75, 1.45], $P < 0.01$, $I^2 = 81\%$) (**Supplementary Figure 1C**).

SIRT1 expression predicted a significantly worse DFS in Asian population with cancers ($n = 13$, HR: 1.683, 95% CI: [1.235, 2.294], $P < 0.0010$, $I^2 = 74.27\%$), whereas one article suggested that increased expression of SIRT1 is correlated with Caucasian patient DFS (HR: 1.344, 95% CI: [1.040; 1.736], $P = 0.0237$) (**Supplementary Figure 2C**).

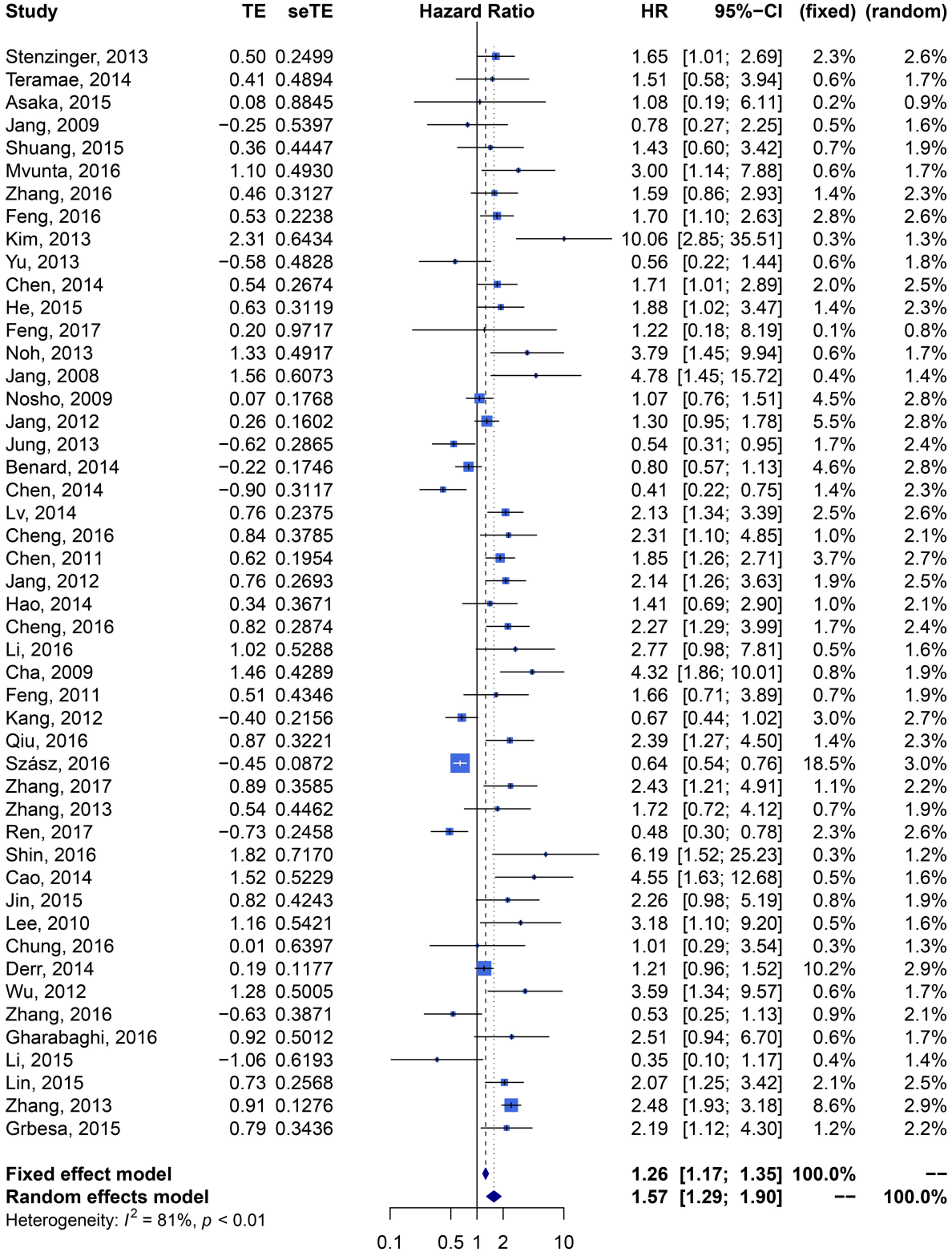

FIGURE 2 | Forest plot of SIRT1 expression and overall survival in various cancers.

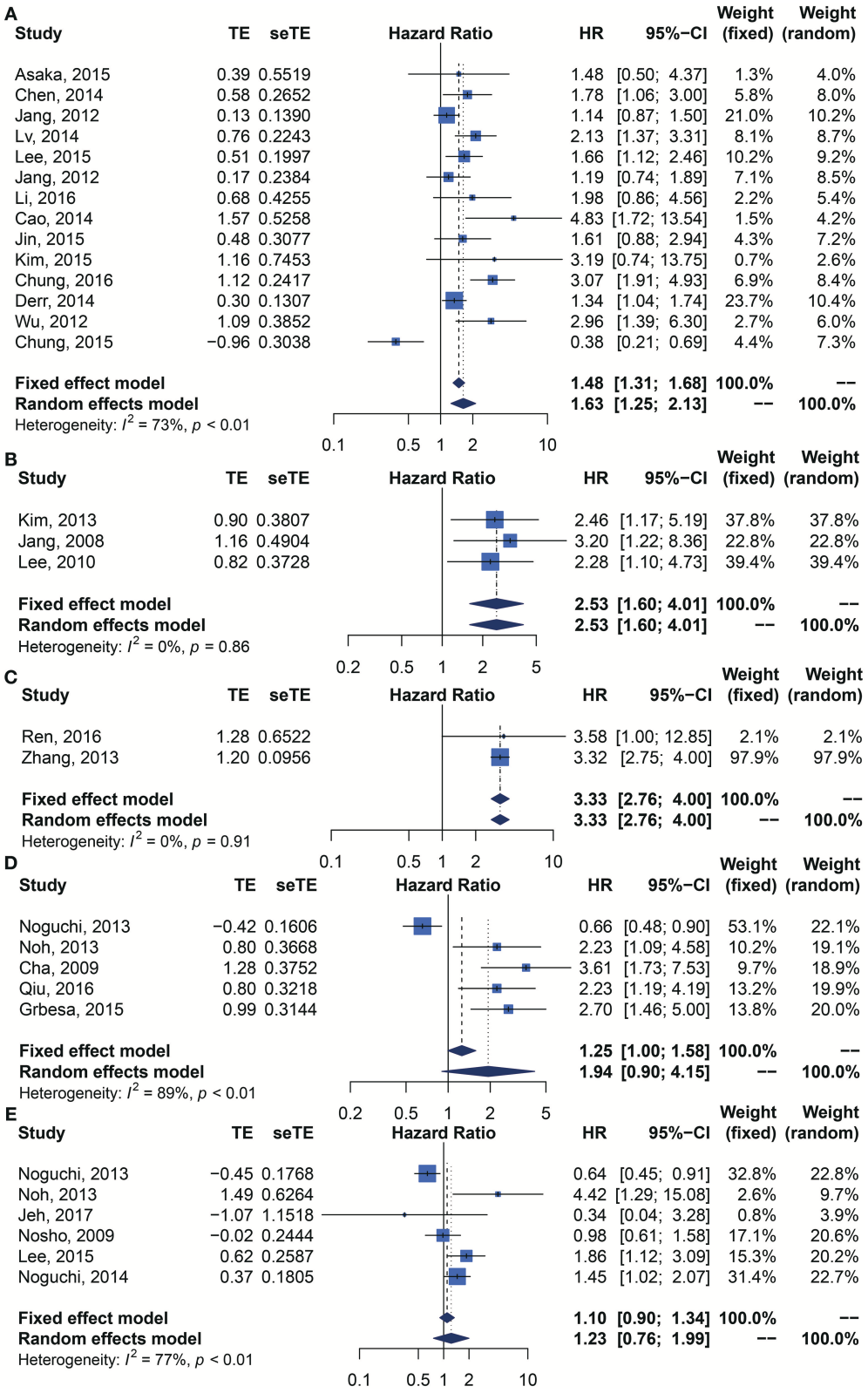

FIGURE 3 | Forest plots of main survival outcomes compared SIRT1 overexpression with underexpression. **(A)** DFS. **(B)** EFS. **(C)** PFS. **(D)** RFS. **(E)** CCS.

TABLE 3 | The associations of SIRT1 overexpression with the clinicopathological characteristics of the study patients.

Clinicopathological parameters	No. of trials (patients)	RR (95%CI) Fixed-effect estimate	*P*-value of Fixed-effect Model	RR (95%CI) Random-effect estimate	*P*-value of Random-effect Model	Heterogeneity I^2(%), *P*-value	*P*-value of Egger's test, Begg's test
Tumor stage	33 (5857)	1.133 (1.062–1.209)	0.0002	***1.299 (1.114–1.514)***	***0.0008***	77.4%, <0.0001	0.0070, 0.1827
Lymphatic metastasis	29 (6354)	1.046 (0.995–1.100)	0.0763	***1.172 (1.010–1.360)***	***0.0363***	86.3%, <0.0001	0.0637, 0.4308
Distant metastasis	14 (2632)	1.607 (1.312–1.968)	<0.0001	***1.562 (1.022–2.387)***	***0.0392***	71.0%, <0.0001	0.6780, 0.3520
Tumor size	21 (2469)	1.143 (1.050–1.245)	0.0021	1.101 (0.984–1.232)	0.0924	41.7%, 0.0241	0.1660, 0.2047
Depth of tumor invasion	19 (4689)	1.036 (0.982–1.093)	0.1912	1.113 (0.985–1.258)	0.0852	81.70%, <0.0001	0.0903, 0.1955
Differentiation	28 (5740)	1.010 (0.940–1.085)	0.7841	1.055 (0.931–1.196)	0.3996	63.10%, <0.0001	0.1170, 0.3847
Age	38 (7223)	1.052 (1.004–1.102)	0.0345	1.043 (0.973–1.118)	0.2373	43.50%, 0.0027	0.5651, 0.8308
Gender	34 (6129)	1.003 (0.967–1.040)	0.8739	0.991 (0.950–1.035)	0.6858	35.00%, 0.0247	0.1727, 0.3353

RR, relative risk; CI, confidence interval; I^2, index for assessing heterogeneity; value ≥25% indicates a moderate to high heterogeneity; Egger's test, P-value of Egger's regression for asymmetry assessment; Begg's test: P-value of Begg and Mazumdar rank correlation test for asymmetry assessment. Bold italics indicate statistically significant values (P < 0.05).

Correlation Between SIRT1 Expression and Clinicopathological Characteristics

We performed an analysis of the association of SIRT1 expression with clinicopathological characteristics (**Table 3**). The results showed that overexpression of SIRT1 was significantly correlated with TNM stage. Higher SIRT1 expression indicated high TNM stage for various malignancies ($n = 33$, RR: 1.299; 95% CI: [1.114, 1.514], $P = 0.0008$, $I^2 = 77.4\%$, **Figure 4**). SIRT1 expression was significantly correlated with lymphatic metastasis ($n = 29$, RR: 1.172, 95% CI: [1.010, 1.360], $P = 0.0363$, $I^2 = 86.3\%$, **Figure 5**), distant metastasis ($n = 14$, RR: 1.562, 95% CI: [1.022, 2.387], $P = 0.0392$, $I^2 = 71.0\%$, **Figure 6**), but not correlated with tumor size (RR:1.101, 95% CI [0.984-1.232], $I^2 = 41.7\%$), depth of tumor invasion (RR: 1.113, 95% CI [0.985–1.258], $I^2 = 81.7\%$), differentiation (RR: 1.055, 95% CI [0.931–1.196], $I^2 = 63.1\%$), gender (RR: 0.991, 95% CI [0.950–1.035], $I^2 = 35.0\%$), or age (RR: 1.043, 95% CI [0.973–1.118], $I^2 = 43.5\%$) (**Table 3**, **Supplementary Figure 3**).

Correlation Between SIRT1 Expression and Clinicopathological Characteristics of Cancers Types

We performed analysis of correlation between SIRT1 expression and clinicopathological characteristics of cancers types (**Supplementary Table 4**). The results showed that SIRT1 overexpression was associated with a higher TNM stage in hepatocellular carcinoma ($n = 6$, RR: 1.611, 95% CI: [1.185, 2.188], $P = 0.0023$, $I^2 = 55.30\%$), but not correlated with the TNM stage in pancreatic ductal adenocarcinoma ($n = 2$, RR: 2.275, 95% CI: [0.928, 5.579], $P = 0.0725$, $I^2 = 0\%$), ovarian cancer ($n = 2$, RR: 0.820, 95% CI: [0.561, 1.201], $P = 0.3082$, $I^2 = 3.18\%$), colorectal cancer ($n = 4$, RR: 1.146, 95% CI: [0.817, 1.608], $P = 0.4290$, $I^2 = 70.90\%$), gastric cancer ($n = 6$, RR: 1.264, 95% CI: [0.823, 1.942], $P = 0.2842$, $I^2 = 92.48\%$), breast carcinoma ($n = 5$, RR: 1.411, 95% CI: [0.846, 2.356], $P = 0.1873$, $I^2 = 65.10\%$), or NSCLC (n = 2, RR: 1.389, 95% CI: [0.661, 2.917], $P = 0.3853$, $I^2 = 16.51\%$) (**Supplementary Figure 4A**).

SIRT1 overexpression was associated with distant metastasis in pancreatic ductal adenocarcinoma ($n = 2$, RR: 2.046, 95% CI: [1.153, 3.631], $P = 0.0144$, $I^2 = 0\%$) and breast carcinoma ($n = 2$, RR: 3.257, 95% CI: [1.777, 5.970], $P = 0.0001$, $I^2 = 0\%$), but not in colorectal cancer ($n = 3$, RR: 1.140, 95% CI: [0.444, 2.923], $P = 0.7857$, $I^2 = 80.57\%$) or gastric cancer ($n = 2$, RR: 1.316, 95% CI: [0.679, 2.551], $P = 0.4160$, $I^2 = 0\%$) (**Supplementary Figure 5A**).

Correlation Between SIRT1 Expression and Clinicopathological Characteristics of Cancers in Different Countries

We performed analysis of correlation between SIRT1 expression and clinicopathological characteristics of cancers in different countries (**Supplementary Table 4**). The results showed that SIRT1 overexpression was associated with a higher TNM stage ($n = 17$, RR: 1.638, 95% CI: [1.404, 1.911], $P < 0.0001$, $I^2 = 41.16\%$) (**Supplementary Figure 4B**) and lymphatic metastasis in China ($n = 11$, RR: 1.411, 95% CI: [1.155, 1.724], $P = 0.0007$, $I^2 = 68.48\%$), and not with lymphatic metastasis in Japan ($n = 3$, RR: 0.964, 95% CI: [0.657, 1.415]), or Korea ($n = 12$, RR: 1.166, 95% CI: [0.898, 1.516]) (**Supplementary Figure 6A**).

Correlation Between SIRT1 Expression and Clinicopathological Characteristics of Cancers in Asian and Caucasian

We performed analysis of correlation between SIRT1 expression and clinicopathological characteristics of cancers in Asian and Caucasian (**Supplementary Table 4**). The results showed that SIRT1 overexpression predicted a significantly higher TNM stage in Asian population with cancers ($n = 30$, RR: 1.323, 95% CI: [1.124, 1.559], $P = 0.0008$, $I^2 = 78.76\%$) rather than that in Caucasian population ($n = 3$, RR: 0.919, 95% CI: [0.744, 1.136], $P = 0.4352$, $I^2 = 0\%$) (**Supplementary Figure 4C**). However, publication bias was suspected based on the Egger's test ($P = 0.0070$) rather than Begg's test ($P = 0.1827$).

Elevated SIRT1 expression predicted a significantly distant metastasis in Caucasian population with cancers ($n = 2$, RR:

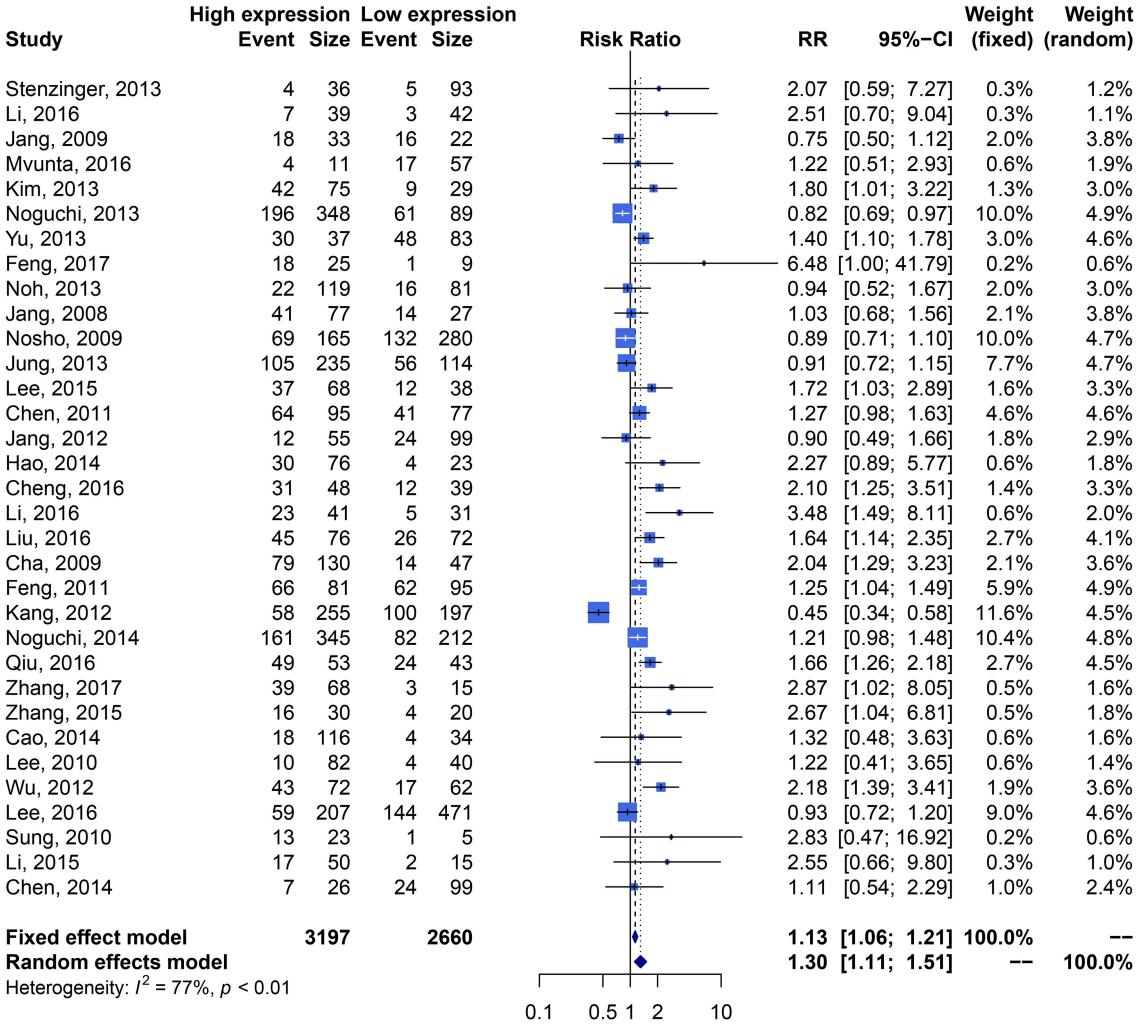

FIGURE 4 | Forest plots of tumor stage compared SIRT1 overexpression with underexpression.

3.830, 95% CI: [1.445, 10.154], $P = 0.0069$, $I^2 = 0\%$), but not in Asian population ($n = 12$, RR: 1.422, 95% CI: [0.913, 2.217], $P = 0.1198$, $I^2 = 72.85\%$) (**Supplementary Figure 5B**).

Elevated SIRT1 expression predicted a significantly higher lymphatic metastasis in Asian population with cancers ($n = 29$, RR: 1.239, 95% CI: [1.056, 1.453], $P = 0.0086$, $I^2 = 86.81\%$), but not correlated with lymphatic metastasis in Caucasian population ($n = 3$, RR: 0.777, 95% CI: [0.526, 1.147], $P = 0.2040$, $I^2 = 76.11\%$) (**Supplementary Figure 6B**).

Meta-Regression Analysis of Heterogeneity for Overall Survival and Publication Bias

We performed a meta-regression to explore the source of high heterogeneity for OS (**Table 4**). All potential factors could not significantly explain heterogeneity in the meta-analyses of the SIRT1 expression with survival outcomes in the *post-hoc* analysis, with the exception of ethnicity (**Supplementary Table 5**). Meta-regression analysis demonstrated a statistically significant correlation between ethnicity and OS ($P = 0.022$). From the meta-regression result, we conducted a subgroup analysis with groups of patients Asian or Caucasian (**Supplementary Figure 1C**). This subgroup analysis demonstrated a significantly lower heterogeneity value in Asian group ($n = 40$, RR: 1.708, 95% CI: [1.406, 2.076], $I^2 = 69.59\%$), which suggests that the high SIRT1 expression has stronger efficacy in the Asian population than the Caucasian population.

Meta-regression also used to explore the source of high heterogeneity for clinicopathological outcomes (Further details are provided in **Supplementary Table 6**). As to tumor stage, meta-regression analysis demonstrated a statistically significant correlation between tumor stage and country ($P < 0.05$), published year ($P = 0.0169$), and sample size ($P = 0.0004$). This subgroup analysis demonstrated a significantly lower heterogeneity value in China ($n = 17$, RR: 1.638, 95% CI: [1.404, 1.911], $I^2 = 41.16\%$), which suggests that the high SIRT1

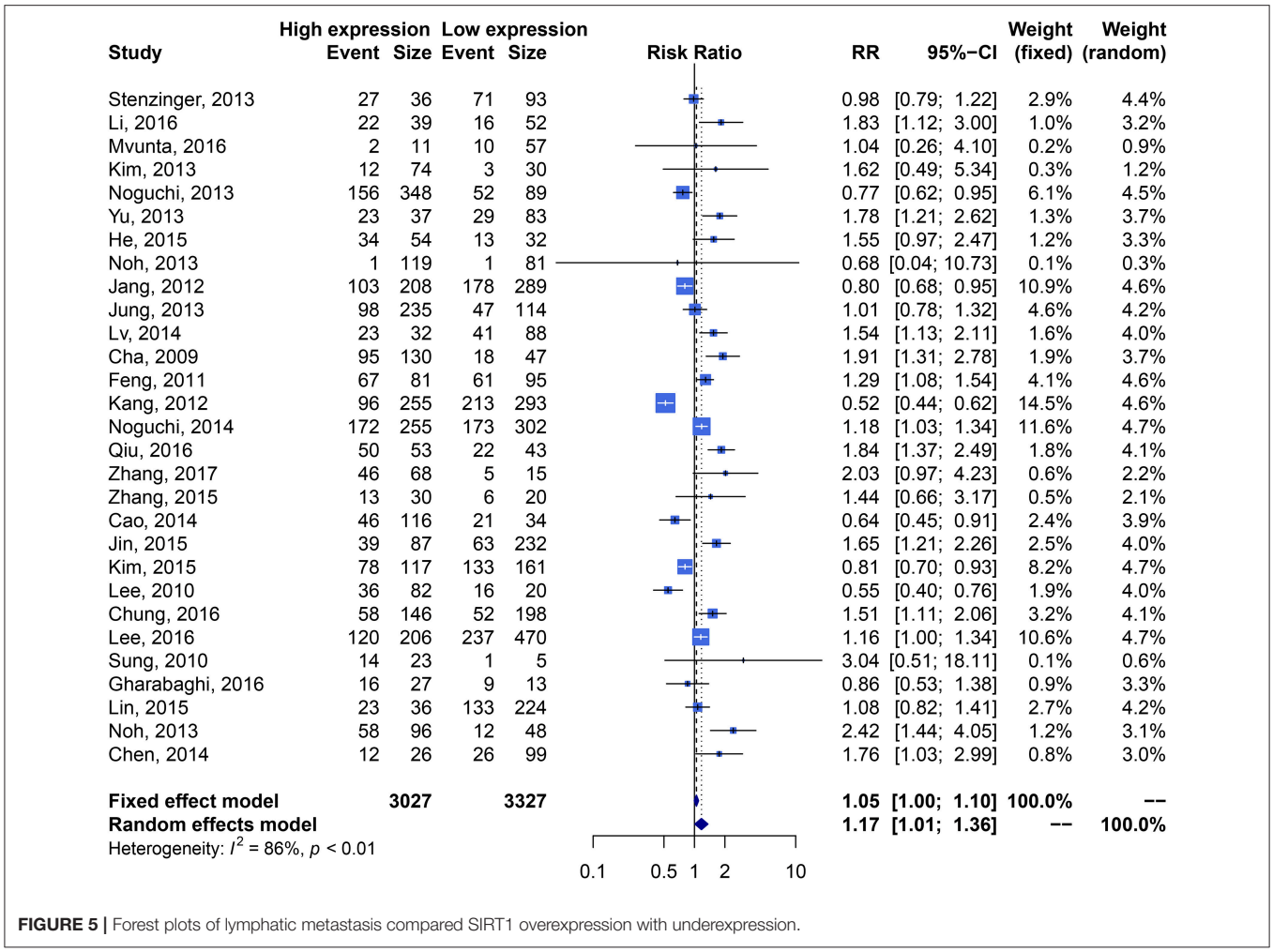

FIGURE 5 | Forest plots of lymphatic metastasis compared SIRT1 overexpression with underexpression.

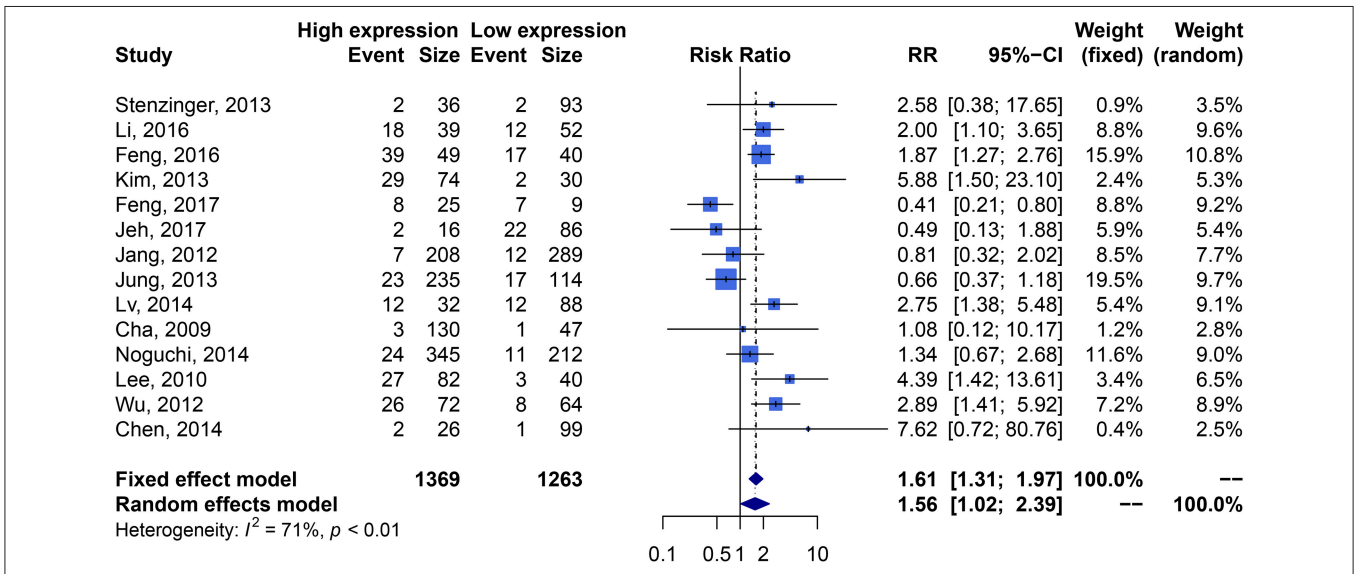

FIGURE 6 | Forest plots of distant metastasis compared SIRT1 overexpression with underexpression.

TABLE 4 | Meta-regression analysis of heterogeneity for overall survival.

Moderators	Variables of regression	$HR_{interaction}$ (95% CI)	*P*-value of regression	I^2	Cochrane Q (*P*-value)
Year	Year	1.001(0.990–1.012)	0.840	81.69%	<0.001
Sample size	Sample size	2.578(0.674–9.860)	0.166	43.18%	0.152
Follow up	Follow up	0.741(0.076–7.236)	0.796	72.89%	<0.001
Country	Intercept	1.648(1.291–2.104)	<0.001	71.56%	<0.001
	Germany	1.001(0.334–2.997)	0.999	71.56%	<0.001
	Hungary	0.388(0.143–1.051)	0.063	71.56%	<0.001
	Iran	1.523(0.380–6.105)	0.553	71.56%	<0.001
	Japan	1.134(0.463–2.775)	0.783	71.56%	<0.001
	Korea	1.079(0.695–1.675)	0.736	71.56%	<0.001
	Netherlands	0.600(0.285–1.263)	0.178	71.56%	<0.001
	Spain	1.330(0.405–4.374)	0.638	71.56%	<0.001
	USA	0.605(0.302–1.215)	0.158	71.56%	<0.001
Tumor type	Intercept	1.716(1.055–2.792)	0.030	75.22%	<0.001
	Colorectal cancer	0.543(0.293–1.008)	0.053	75.22%	<0.001
	Diffuse large B cell lymphoma	2.786(0.559–13.892)	0.211	75.22%	<0.001
	Endometrial carcinoma	0.629(0.082–4.849)	0.657	75.22%	<0.001
	Esophageal squamous cell carcinoma	1.044(0.412–2.640)	0.928	75.22%	<0.001
	Gastric Cancer	0.851(0.442–1.638)	0.630	75.22%	<0.001
	Hepatocellular Carcinoma	1.168(0.574–2.373)	0.668	75.22%	<0.001
	Laryngeal and hypopharyngeal carcinomas	0.326(0.078–1.370)	0.126	75.22%	<0.001
	NSCLC	1.071(0.521–2.204)	0.852	75.22%	<0.001
	Osteosarcoma	0.960(0.384–2.399)	0.930	75.22%	<0.001
	Ovarian cancer	1.135(0.478–2.692)	0.774	75.22%	<0.001
	Pancreatic ductal adenocarcinoma	0.961(0.294–3.144)	0.948	75.22%	<0.001
	Pelvis chondrosarcoma	0.711(0.080–6.345)	0.760	75.22%	<0.001
	Renal cell carcinoma	2.208(0.520–9.383)	0.283	75.22%	<0.001
	Soft tissue sarcoma	***5.863(1.115–30.823)***	***0.037***	75.22%	<0.001
	Uterine cervical cancer	0.880(0.208–3.727)	0.862	75.22%	<0.001
Race	Intercept	1.705(1.414–2.056)	<0.001	73.32%	<0.001
	Caucasian	***0.619(0.411–0.932)***	***0.022***	73.32%	<0.001
Sample type	Intercept	1.430(0.353–5.799)	0.617	81.72%	<0.001
	Tissue	1.097(0.267–4.510)	0.898	81.72%	<0.001

$HR_{interaction}$, interaction effect calculated by meta-regression; Positive direction indicates that possible moderators might strengthen OS in the SIRT1 overexpression relative to underexpression. Bold italics indicate statistically significant values (P < 0.05).

expression has stronger efficacy in the China population than the other countries. As to tumor size, meta-regression analysis demonstrated a statistically significant correlation between tumor size and published year ($P = 0.0260$). As to depth of tumor invasion, meta-regression analysis demonstrated a statistically significant correlation between depth of tumor invasion and sample size ($P = 0.0044$).

We used funnel plots and Egger's regression models to assess potential publication bias (**Tables 2**, **3**). The association between HRs (**Supplementary Figure 7**) or RRs (**Supplementary Figure 8**) and standard error for the SIRT1 expression was demonstrated in funnel plots, with each plot point representing a study. In regards to the OS, RFS, and TNM stage, we found that Egger's regression yielded potential publication bias (**Figure 7**).

DISCUSSION

In the current study, we conducted a meta-analysis of 13,138 subjects in 63 articles from PubMed, EMBASE and Cochrane library to evaluate prognostic and clinicopathological significance of SIRT1 expression in cancers. We found that elevated expression of SIRT1 was correlated with a poor OS of cancer patients, DFS, EFS, and PFS, but couldn't predict RFS or CCS. Elevated SIRT1 expression was associated with TNM stage, lymph node metastasis, and distant metastasis, but not with tumor size, depth of tumor invasion, differentiation, gender, or age. Our findings provide a clue to understanding prognostic and clinicopathological significance of SIRT1 expression in cancers.

Our current study indicates that overexpression of SIRT1 is correlated with poor OS, DFS, EFS, and PFS, but not with RFS or

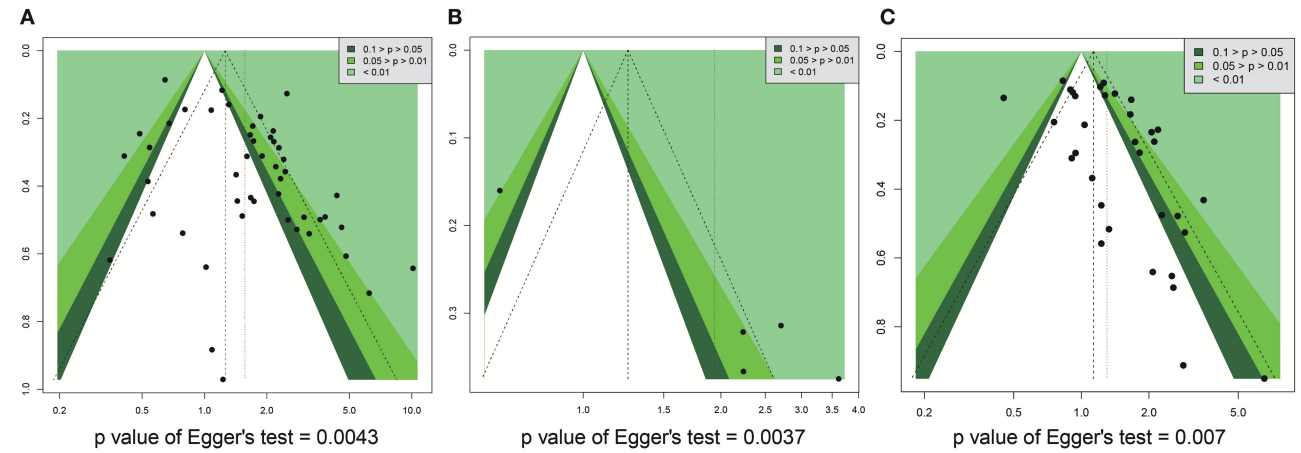

FIGURE 7 | Funnel plot for publication bias in overall survival, recurrence free survival, and TNM stage. **(A)** OS. **(B)** RFS. **(C)** TNM stage.

CCS, suggesting that SIRT1 expression is significantly correlated with poor prognosis as a global factor but not a restricted factor to tumor itself. It has been shown that SIRT1 is not a protein only found to a specific tissues or organs, instead, its expression can be found in almost all human tissues (1) and involved in a large variety of cellular processes, such as genomic stability, energy metabolism, senescence, gene transcription, and oxidative stress (5) by acting on a wide spectrum of proteins, including histones and transcription factors (2–4). This render SIRT1 plays multiple roles to regulate biological process in multi-systems. Melatonin is a pleiotropic molecule synthesized by pineal gland and many other organs and has important cytoprotective effects in many tissues including aging, neurodegenerative diseases, immunomodulation, and cancer and modulates DNA damage response (88, 89). Melatonin counteracts tumor metastases by modulating cell-cell and cell-matrix interaction, extracellular matrix remodeling, cytoskeleton reorganization, epithelial-mesenchymal transition, and angiogenesis (90). Recent studies showed that the upregulated SIRT1 signaling pathway is involved in protective effects of melatonin on vascular endothelium against aging-, oxidative stress-, lipopolysaccharide-, and ischemia-induced damage (91) and delays ovarian aging (92). SIRT1 is induced in normal cells and inhibited in tumor cells by melatonin (88, 89). SIRT1 may mediate the pleiotropic function of melatonin in cancer progression and metastasis. SIRT1 is an endocrine regulator of thyroid and parathyroid hormone function (93–95), and steroid hormone receptor activity (96, 97). SIRT1 is a regulator in immunity and autoimmunity, such as dendritic cell activation, T-regulatory cells (98–102). SIRT1 is also a regulator of lipid and carbohydrate metabolism (9, 103–105). In addition, SIRT1 regulates nervous system by inhibiting neuronal apoptosis and damage as well module nerve regeneration (103, 106, 107). Therefore, SIRT1 is a global factor for endocrine, immunity, metabolism, and nervous system, and affect poor OS, DFS, EFS, and PFS, but not with RFS or CCS in cancer patients.

In the current study, we found that SIRT1 overexpression was associated with TNM stage, lymph node metastasis, and distant metastasis, but not with tumor size, depth of tumor invasion, differentiation, gender, or age, suggesting that SIRT1 promotes metastasis but not growth, proliferation, and invasion of cancer tissues. Tumor is locally initiated and proliferated and may invade near tissues. Tumor size, depth of tumor invasion, and differentiation are terms used to characterize tumors which are locally confined in the early stage of malignancy (108, 109). Metastasis is the characteristics of advanced malignancy of cancer progression (110–113). Our data indicate that SIRT1 overexpression is associated with metastasis but not with tumor characteristics of early stage, suggesting that SIRT1 expression can predict advanced malignancy and is a potential therapeutic target for inhibiting metastasis of advanced cancer.

We performed subgroup analysis because of high heterogeneity in included studies. Correlation analysis between SIRT1 expression and prognosis of cancer types showed that SIRT1 overexpression predicted worse OS of osteosarcoma, esophageal squamous cell carcinoma, OS but not DFS of hepatocellular carcinoma, OS and DFS of breast carcinoma, OS and RFS of NSCLC, DFS but not OS or CCS in colorectal cancer, RFS and CCS but not OS of gastric cancer, RFS and CCS of renal cell carcinoma, CCS but not RFS in head and neck squamous cell carcinoma. SIRT1 overexpression cannot predict OS in ovarian cancer. Correlation analysis between SIRT1 expression and clinicopathological characteristics of cancers types showed that SIRT1 overexpression was associated with a higher TNM stage in hepatocellular carcinoma, but not in pancreatic ductal adenocarcinoma, ovarian cancer, colorectal cancer, gastric cancer, breast carcinoma, or NSCLC. SIRT1 overexpression was associated with distant metastasis in pancreatic ductal adenocarcinoma and breast carcinoma, but not in colorectal cancer or gastric cancer. From these results, we are unable to draw a unanimous conclusion, probably because there is a deficiency of studies that employ all prognostic indexes OS, DFS,

EFS, RFS, CCS, and PFS or a full range of clinicopathological characteristics to study the role of SIRT1 expression in survival of patients with a specific cancer type. More thorough studies are warranted.

Our subgroup correlation analysis between SIRT1 expression and prognosis of cancer in different countries and ethnic groups showed that high expression of SIRT1 predicted poor OS and DFS in China, poor OS and EFS but not DFS in Korea, poor OS in Japan, EFS in USA, but not OS in USA or Netherlands. Elevated SIRT1 expression predicted worse OS and DFS in Asian population with cancers, poor DFS but not OS in Caucasian population. Our subgroup analysis between SIRT1 expression and clinicopathological characteristics of cancers in different countries and ethnic groups showed that SIRT1 overexpression was associated with a higher TNM stage and lymphatic metastasis in China and Asian population except lymphatic metastasis in Japan or Korea, and not higher TNM stage and lymphatic metastasis in Caucasian population, We also found that SIRT1 overexpression predicted distant metastasis in Caucasian population, but not in Asians. These results indicate that ethnic background has influence on the role of SIRT1 expression in predicting the OS and clinicopathological characteristics of cancers. This is consistent with recent studies that showed SIRT1 expression is lower in NSCLC than the normal control group in a group of Chinese patients (15), and overexpressed in NSCLC in an Iran population (16). Our study showed that overexpression of SIRT1 predicted a worse OS in the Asian but not in the Caucasian, a higher TNM stage and lymphatic metastasis in Asian population especially in China but not in the Caucasian. This is consistent with the results of our meta-regression analysis. The effects of ethnic background on the role of SIRT1 expression in predicting the OS and clinicopathological characteristics of cancers need further collaborative investigation.

It has been established that there are significant differences between Asian and Caucasian populations in genetic and epigenetic background, dietary, environmental factors (114, 115). These factors are essential for not only initiation and progression, but also metastasis of cancers (116, 117). Mutations and extensive polymorphisms of SIRT1 were found in Chinese and Japanese (118–121) and 41 cancer lines (122). Although the data on mutations and polymorphisms of SIRT1 are very limited, we speculate that difference in SIRT1 mutations and polymorphisms may be one of accounts for difference in predicting OS and TNM stage and lymphatic metastasis of cancer by SIRT1 expression. This deserves further investigation (123).

It is known that metastasis is an independent predictor for poor prognosis of many cancer types (124–126). We find that elevated expression of SIRT1 was correlated with OS, DFS, EFS, and PFS. SIRT1 overexpression is also correlated with TNM stage, lymph node metastasis, and distant metastasis, but not with tumor size, depth of tumor invasion, differentiation, gender, or age. Overexpression of SIRT1 predicted a worse OS and higher TNM stage and lymphatic metastasis in Asian population especially in China. Therefore, overexpression of SIRT1 may promote lymphatic metastasis of cancers that lead to poor OS, DFS, EFS, and PFS. It is likely that SIRT1-mediated molecular events and biological processes could be an underlying mechanism for metastasis.

Our study is consistent with the most recent study by Wang et al. in that SIRT1 overexpression was significantly correlated with the OS in solid cancers, especially in liver cancer and lung cancer based on 7,369 cases from 37 studies and most of them are Asians (34). Consistently, the study by Hong et al showed that high SIRT1 expression correlated with vascular invasion and was not significantly correlated with overall survival rates in colon cancer (36). Study with 3024 patients by Wu et al showed that high SIRT1 expression predicts poor survival in non-colorectal gastrointestinal cancer, but not in colorectal cancer (35). SIRT1 expression was correlated with depth of invasion, lymph node metastasis and TNM stage and predicted a poor OS in colorectal cancer patients based on an analysis with seven studies (33). In an analysis of 1,650 patients in seven studies, high SIRT1 expression predicts a poor prognosis of gastric cancer patients and linked with patients' age, T stage, N stage, and tumor differentiation (32). Analysis by Cao et al. based on six studies involving 604 patients showed that SIRT1 expression was correlated with poor DFS and OS and high TNM stage and lymph node metastasis (31). However, we have performed study on survival and clinicopathological significance of SIRT1 expression in cancers more comprehensively. First, we included 63 eligible articles and a total of 13,138 participants in our study. These patients represented 9 countries and 16 cancer types as well as Asian and Caucasian ethnic groups. Second, we investigated both clinicopathological and prognostic significance of SIRT1 expression based on comprehensive clinical data and performed a series of subgroup analysis based on prognostic types, clinicopathological characteristics, cancer types, ethnic groups, countries. These stratifications provide more vehicles in understanding the survival and clinicopathological significance of SIRT1 expression in cancers.

There are also limitations in our study. Firstly, we found that heterogeneity existed in the meta-analysis as indicated by the I^2 values. It is predictable because of presence of inter-study differences in study design (prospective and retrospective), enrolled populations, treatment regimen, duration of follow-up, outcome measures, and other study and clinical characteristics (127). The heterogeneity among the studies remained, despite the usage of a random-effects model and subgroup analyses (128). Secondly, there is publication bias for SIRT1 expression and prognosis or clinicopathological characteristics as indicated by asymmetry of funnel plots for OS, DFS, EFS, RFS, CCS, PFS, and clinicopathological characteristics. Thirdly, we barely explored the correlation between SIRT1 overexpression and patient survival in terms of clinical parameters. Other elements that may contribute to the heterogeneity, such as therapeutic regimen, pathological grade, body mass index, and mean age, were not analyzed due to the lack of sufficient data (129). Fourthly, we performed a quantitative meta-analysis based mostly on secondary data, which could lead to inaccurate results because of a shortage of original individual patient data (130). Finally, we conducted our study based on the mRNA expression of SIRT1 or the protein levels, although the changes in the mRNA and protein levels of SIRT1 are consistent in several cancer types

(15, 16, 131, 132). The study by Hong et al who determined SIRT1 expression using immunohistochemistry showed similar results to ours study in relation with vascular invasion and overall survival rates in colon cancer (36). We should extensively investigate the prognostic and clinicopathological significance of SIRT1 expression at protein level in the future.

In conclusion, we have found that elevated expression of SIRT1 can predict poor OS, DFS, EFS, and PFS, but not with RFS or CCS, TNM stage, lymph node metastasis, and distant metastasis, but not tumor size, depth of tumor invasion, differentiation of cancers. Ethnic background has influence on the role of SIRT1 expression in predicting survival and clinicopathological characteristics of cancers. Overexpression of SIRT1 predicted a worse OS and higher TNM stage and lymphatic metastasis in Asian population especially in China. SIRT1-mediated molecular events and biological processes could be an underlying mechanism for metastasis and SIRT1 is a potential therapeutic target for inhibiting cancer metastasis. More studies that employ all prognostic indexes OS, DFS, EFS, RFS, CCS, and PFS or a full range of clinicopathological characteristics to study the role of SIRT1 expression in survival of patients with a specific cancer type, and mutations and polymorphisms of SIRT1 in cancers of different ethnic groups need to be further investigated in the future.

DATA AVAILABILITY

All datasets generated for this study are included in the manuscript and the supplementary files.

AUTHOR CONTRIBUTIONS

MS, DH, XGu, and HZ participated in research design. MS, WZ, MD, SX, XGo, PL, HL, and JZ performed data analysis. MS and XGu wrote or contributed to the writing of the manuscript.

FUNDING

This research was supported by the Natural Science Foundation of Hubei Provincial Department of Education (Q20182105), Chen Xiao-ping Foundation for the development of science and technology of Hubei Provincial (CXPJJH11800001-2018333), Natural Science Foundation of Hubei Province of China (2016CFB530) and Faculty Development Foundation of Hubei University of Medicine (2014QDJZR01), and National Students' platform for innovation and entrepreneurship training program (201810929005, 201810929009, 201810929068, and 201813249010).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2019.00121/full#supplementary-material

Supplementary Table 1 | Databases searching terms. **(a)** Search criterion of Medline (via PubMed, from inception to April 1st, 2018) ($n = 2397$). **(b)** Search criterion of Embase (from 1966 to July 24, 2018) ($n = 2460$). **(c)** Search criterion of Cochrane Library (April 1st, 2018) ($n = 20$).

Supplementary Table 2 | Results of quality assessment using the Newcastle–Ottawa Scale for the included studies.

Supplementary Table 3 | Subgroup analysis for SIRT1 overexpression and the prognosis in cancers.

Supplementary Table 4 | Subgroup analysis for SIRT1 overexpression and clinicopathological parameters in cancers.

Supplementary Table 5 | Meta-regression analysis of heterogeneity for survival outcomes.

Supplementary Table 6 | Meta-regression analysis of heterogeneity for clinicopathological parameters in cancers.

Supplementary Figure 1 | Forest plot of subgroup analysis for SIRT1 overexpression and OS in cancers. **(A)** Cancer subgroup, **(B)** Country subgroup, **(C)** Ethnicity subgroup.

Supplementary Figure 2 | Forest plot of subgroup analysis for SIRT1 overexpression and DFS in cancers. **(A)** Cancer subgroup, **(B)** Country subgroup, **(C)** Ethnicity subgroup.

Supplementary Figure 3 | Forest plots of non-essential clinicopathological outcomes compared SIRT1 overexpression with underexpression. **(A)** Tumor size, **(B)** Depth of tumor invasion, **(C)** Differentiation, **(D)** Gender, **(E)** Age.

Supplementary Figure 4 | Forest plot of subgroup analysis for SIRT1 overexpression and TNM stage in cancers. **(A)** Cancer subgroup, **(B)** Country subgroup, **(C)** Ethnicity subgroup.

Supplementary Figure 5 | Forest plot of subgroup analysis for SIRT1 overexpression and distant metastasis in cancers. **(A)** Cancer subgroup, **(B)** Ethnicity subgroup.

Supplementary Figure 6 | Forest plot of subgroup analysis for SIRT1 overexpression and lymphatic metastasis in cancers. **(A)** Country subgroup, **(B)** Ethnicity subgroup.

Supplementary Figure 7 | Funnel plot for publication bias for SIRT1 expression and prognosis. **(A)** OS, **(B)** DFS, **(C)** EFS, **(D)** RFS, **(E)** CCS.

Supplementary Figure 8 | Funnel plot for publication bias for SIRT1 expression and clinicopathological characteristics. **(A)** Age, **(B)** Gender, **(C)** Tumor stage, **(D)** Distant metastasis, **(E)** Lymphatic invasion, **(F)** Tumor size, **(G)** Depth of tumor invasion, **(H)** Differentiation.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Endocrinology

PERSPECTIVE
published: 01 March 2019
doi: 10.3389/fendo.2019.00093

Insulin/IGF-1R, SIRT1, and FOXOs Pathways—An Intriguing Interaction Platform for Bone and Osteosarcoma

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Specialty section:
This article was submitted to Cellular Endocrinology, a section of the journal Frontiers in Endocrinology

Received: 15 October 2018
Accepted: 01 February 2019
Published: 01 March 2019

Citation:
Sergi C, Shen F and Liu S-M (2019) Insulin/IGF-1R, SIRT1, and FOXOs Pathways—An Intriguing Interaction Platform for Bone and Osteosarcoma. Front. Endocrinol. 10:93. doi: 10.3389/fendo.2019.00093

Aging is a substantial risk factor for the development of osteoarthritis (OA) and, probably, an essential substrate for the development of neoplastic disease of the bone, such as osteosarcoma, which is the most common malignant mesenchymal primary bone tumor. Genetic studies have established that the insulin/insulin-like growth factor 1 (IGF-1)/phosphatidylinositol-3 kinase (PI3K)/AKT (Protein Kinase B) signal transduction pathway is involved across species, including nematodes, fruit flies, and mammals. SIRT1, a phylogenetically-conserved family of deacetylases, seems to play pleiotropic effects in epithelial malignancies of the liver and interact with the IGF-1/PI3K/AKT signal transduction pathway. Some of the most critical processes in degenerative conditions may indeed include the insulin/IGF1R and SIRT1 signaling pathways as well as some specific transcription factors. The Forkhead box O (FOXO) transcription factors (FOXOs) control diverse cellular functions, such as metabolism, longevity, and cell death. FOXOs play a critical role in the IGF-1/PI3K/AKT signal transduction pathway. FOXOs can indeed be modulated to reduce age-related diseases. FOXOs have advantageous inhibitory effects on fibroblast and myofibroblast activation, which are accompanied by a subsequent excessive production of extracellular matrix. FOXOs can block or decrease the fibrosis levels in numerous organs. Previously, we observed a correlation between nuclear FOXO3 and high caspase-8 expression, which induces cellular apoptosis in response to harmful external stimuli. In this perspective, we emphasize the current advances and interactions involving the insulin/IGF1R, SIRT1, and FOXOs pathways in the bone and osteosarcoma for a better understanding of the mechanisms potentially underpinning tissue degeneration and tumorigenesis.

Keywords: IGF1, FOXO, SIRT1, signaling pathways, bone, osteosarcoma

INTRODUCTION

Aging is a substantial risk factor for the development of inflammatory conditions, such as osteoarthritis (OA) and, probably, other degenerative, and neoplastic diseases of the bone, such as Paget disease of the bone and osteosarcoma (1, 2). Some growth factors linked to cartilage repair following damage in animal models have been considered to increase the risk of neoplasia (3–7). In this paper, we review the insulin/IGF1R, SIRT1, and FOXOs signaling pathways and emphasize the interaction involving these crucial factors in the bone physiology and oncogenesis, with

regard to osteosarcoma, which is considered the most common mesenchymal malignant primary tumor of the skeletal system.

INSULIN/IGF1R SIGNALING PATHWAY

The insulin/IGF-1 signaling system (IIS) is the route that regulates not only the organism's metabolism, but also the growth, development, and longevity concerning the availability status of nutrients. It is an ancient system. In fact, it is highly conserved across species. In invertebrates, e.g., in the worm *C. elegans*, the IIS system begins with the secretion of numerous, insulin-like peptides in reply to food or, ultimately, to the sensory perception of food. These insulin-like ligands can connect to a single (common) receptor, called the insulin/IGF-1 like tyrosine kinase receptor or *dauer* formation 2 (DAF-2). In the 1990s, two DAF genes, DAF-2, and DAF-16, were discovered after isolating *dauer-constitutive* (DAF-c) mutants and *dauer-defective* mutants (DAF-d). The worm *C. elegans*, under conditions of high population density and low food, forms an alternative 3rd larval stage, called the *dauer* stage. This stage is resistant to dehydration and harsh environments (8). The *C. elegans* genome encodes AGE-1 adaptor protein (AAP-1), a single PI3K adaptor subunit, and a putative IRS homolog, i.e., the adaptor protein or insulin receptor substrate (IST-1) homolog (9). After the ligand binds, the signal is progressively transduced from the activated receptor to AGE-1, which is a phosphatidylinositol 3-kinase either directly or using the adaptor protein called IST-1 (9). The phosphatidylinositol 3-kinase AGE-1 changes the phospholipid PIP2 into the second messenger PIP3. Subsequently, the increased level of PIP3 initiates the 3-phosphoinositide-dependent protein kinase 1 (PDK1) and the protein kinases B1 and B2 (PKB1 and PKB2). Ultimately, it leads to the phosphorylation of the DAF-16 molecule, which causes its extrusion from the nucleus to the cytoplasm (10). DAF-18, a homolog of the mammalian phosphatase and tensin homolog (PTEN), can dephosphorylate PIP3 to PIP2. Gene mutations in daf-2 and kinase components of the IIS pathway harboring reduction of functional significance can extend the life span of the worm. Conversely, mutations harboring the same meaning but in daf-18 abolish the life-span extension of daf-2 and age-1 mutants. The downstream targets of DAF-16 include metabolic genes, cellular stress response genes, and genes encoding antimicrobial peptides (11, 12). The fruit fly (*Drosophila melanogaster*) shows powerful similarities to *C. elegans* about the IIS pathway. In the fruit fly, multiple extracellular ligands are binding to a single tyrosine kinase receptor, which is a transmembrane protein, the insulin/IGF-1 common receptor. The binding of the ligands to the common receptor promotes some intracellular phosphorylation events that end in the phosphorylation and nuclear extrusion of dFOXO. In the fruit fly, several indirect losses of function gene mutations have been linked to an enhancement of the life span, such as the insulin receptor and its substrate. These events are particularly pronounced in the female fruit fly. In mammals, the core of the insulin/IGF-1 signaling path is preserved, but there is an increase in complexity moving from invertebrates to vertebrates. Specifically, there are three different ligand molecules of insulin/IFG-1 receptor in mammals. They include insulin, IGF-1, and IGF-2. Also, there are three diverse mammalian insulin/IGF tyrosine kinase receptors, including insulin receptor (IR), IGF-1 receptor (IGF-1R), and the so-called orphan IR related receptor (IRR). An orphan receptor is a protein that harbors a structure similar to other identified receptors but whose endogenous ligand has not yet been discovered. Following the ligand binding, the activated IGF-1 or insulin receptor starts the phosphorylation of numerous intracellular substrates. The phosphorylated substrates give precise docking sites for intracellular effectors. These sites include the growth-factor-receptor-bound protein-2 (Grb2) and the p85 regulatory subunit of PI-3K. Eventually, it leads to the activation of two major signaling pathways, which are the Ras-MAPK pathway and the PI-3K-PKB/AKT pathway. The former path (PI-3K-PKB/AKT) has been shown to regulate most of the metabolic effects of insulin/IGF-1 signaling (13). The latter pathway (Ras-MAPK) gave evidence of the regulation of most of the effects (mitogenic) of insulin/IGF-1 signaling. Also, most of the crucial components of the insulin/IGF-1 signaling cascade show some further complexity in mammals, because different forms have been revealed that are encoded by several genes and/or isoforms determined by a single gene.

SIRT1 SIGNALING PATHWAY

In mammals, Sirtuins constitute a family of NAD+-dependent deacetylases (14–19). Seven members (SIRT1–SIRT7) are included in this family. All of them share the conserved Sirtuin domain conferring NAD+-dependent deacetylase activity (20). However, they also have different amino- and carboxy-terminal extensions. Also, they display distinct subcellular localization and biological functions. Although SIRT1 is predominantly located in the nucleus, it transfers between the nucleus and the cytoplasm during the ontogenesis and in response to physiological stress and pathological conditions. If SIRT1 is located in the nucleus, different is the situation for SIRT2, SIRT3, SIRT4, and SIRT5. The proteins SIRT3 through SIRT5 are located in the mitochondria, while SIRT2 is mostly identified in the cytoplasm. Like SIRT1, SIRT6, and SIRT7 are localized in the nucleus. The former is a chromatin-associated protein, while the latter is found in the nucleolus. In mammals, deacetylase activity was reported as conserved, but the acyl group preference is different according to the Sirtuins with SIRT4-7 harboring a weak or, probably, undetectable deacetylase activity *in vitro* (21–23). An efficient demyristoylase activity is found in SIRT2, while SIRT5 has a demalonylase and lysine desuccinylase activities associated with an adequate and efficient NAD+-dependent protein. SIRT4 and SIRT6 harbor ADP-ribosyltransferase activity. In mammals, Sirtuins are crucial in regulating a broad variety of cellular processes, including metabolism, oxidative/anti-oxidative balance, mitochondrial homeostasis, autophagy, and apoptosis as well as pathological conditions, such as inflammation. SIRT1 has been proved to repress inflammation in multiple cells and tissues (24–26). Moreover, there is an important contribution of Sirtuins in aging and aging-related diseases, such as obesity,

type 2 diabetes mellitus (T2DM), cardiovascular disease, neurodegenerative diseases, and cancer (16, 17). Most probably, SIRT1 (and SIRT6 for certain aspects) is the most extensively characterized proteins of this class. In several species and across species, the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) remains a chief transcriptional factor in cellular physiology. NF-κB is a complex that panels the transcription of DNA, production of several cytokines, and, ultimately, cell survival. In the cell, NF-κB is tangled in responses to stimuli such as stress, free radicals, cytokines, but also bacterial or viral antigens as well as heavy metals, ultraviolet irradiation, oxidized lipoproteins. NF-κB mediates the expression of multiple inflammatory factors, including IL-1β, IL-6, and TNF-α. The nuclear translocation and activation of NF-κB rely on its acetylation. SIRT1 deacetylates NF-κB (p65 subunit) at lysine 310 (K310). It inhibits the transcriptional activity of NF-κB. A co-repressive action has also been identified with transducing-like enhancer of split 1 (TLE1). SIRT1 interacts with TLE1 to inhibit NF-κB-mediated transcription and suppress inflammation (27, 28). Other two actions of the SIRT1 are well-known and include (1) the deacetylation of activator protein-1 (AP-1) able to decrease the expression of cyclooxygenase 2 (COX-2) in macrophages and (2) the deacetylation of TP53 to repress macrophage activation. Sirtuins significantly contribute to their functions on insulin resistance. The activation of SIRT1 leads to the repression of c-Jun N-terminal kinases (JNKs) and inhibitor of NF-κB kinase subunit beta (IKK-β) inflammatory pathways substantially. JNKs were initially discovered as kinases that bind and phosphorylate c-Jun on Ser-63 and Ser-73 (two serine amino acid residues) within its transcriptional activation domain (29, 30). They are a member of the mitogen-activated protein kinase (MAPK) family and are responsive to stress stimuli. IKK- β is a protein that in humans is encoded by the gene labeled inhibitor of kappa light polypeptide and gene enhancer in B-cells, kinase beta (IKBKB). SIRT1 improves glucose tolerance, reduced hyperinsulinemia, and enhanced systemic insulin sensitivity and Sirt1 controls specifically the inflammatory status of macrophages and T lymphocytes regulating the metabolism and that of adipose tissues in obese mice (31, 32). Besides, SIRT1 promotes mitochondrial biogenesis by deacetylating the peroxisome proliferator-activated receptor and gamma coactivator 1-alpha (PGC-1α), which is a transcriptional coactivator that regulates mitochondrial biogenesis and respiration. SIRT1 activator resveratrol induces PGC-1α increasing the number of mitochondria, and this may have a beneficial effect in T2DM patients, who have fewer mitochondria in the muscle than insulin-sensitive individuals. SIRT1 activator resveratrol inducing PGC-1α activity protects mice against diet-induced obesity and insulin resistance (33–35). Mitochondria biogenesis is found in a balance with the clearance of damaged mitochondria (mitophagy or mitochondria autophagy). SIRT1 binds to and deacetylates autophagy (ATG) regulators (e.g., ATG5, ATG7, and ATG8) to promote mitophagy (29, 36–39). SIRT1 also deacetylates Forkhead box protein O1 (FoxO1) and Forkhead box protein O3a (FoxO3a). Both deacetylations induce the expression of elements of the autophagy machinery. The deacetylation and activation of FoxO3a upregulate the expression of MnSOD and catalase, which is an enzyme detected in almost all living organisms exposed to O_2. Catalase catalyzes the decomposition of hydrogen peroxide (H_2O_2) to water and O_2. Calatase plays a major role in protecting the cell from oxidative damage by reactive oxygen species (ROS). Finally, SIRT1 promotes the transcriptional activity of Nuclear factor (erythroid-derived 2)-like 2 (NRF2). SIRT1 acts in this way by deacetylating it. SIRT1 upregulates the expression of NRF2 target antioxidant genes, including mitochondrial antioxidant manganese superoxide dismutase (MnSOD), catalase, heme oxygenase-1 (HO-1), and glutathione (29, 40, 41). Overall, Sirtuins are histone deacetylases that are crucial in regulating organismal lifespan as well as oxidative stress and DNA damage.

FOXOS SIGNALING PATHWAY—GENERAL REMARKS

An impressive class of transcription factors for cancer therapeutic modulation is represented by the forkhead box transcription factors (FOXO) family. FOXOs or FOXO proteins are growth factors and stress-related factors. These transcription factors naturally reside in the nucleus of cells and function as regulators of gene transcription. FOXO proteins may be transferred to the cytoplasm and go through degradation of the ubiquitin-proteasome pathway (UPP), which is the chief mechanism for protein catabolism in the mammalian cell. Following translation of the mRNA into proteins, and no cellular survival initiative of growth factors, FOXOs translocate to the nucleus upregulating a series of target genes of the cell cycle, stress resistance, and longevity. FOXOs regulate numerous cellular functions, and these functions include cellular differentiation, cellular proliferation, DNA damage, apoptosis, DNA repair, and oxidative stress modulation (42–45). These very delicate functions indicate how dysregulation of the FOXOs may implicate abnormal cellular and tissue physiology, tumorigenesis, and neoplastic progression. Different from most common transcription regulators, such as extracellular signal-regulated kinase (ERK), which are located in the cytoplasm where kinases are phosphorylated and translocated into the nucleus, FOXOs are transcription factors with a nuclear location. Growth factor pathways endorse the nuclear exclusion and translocation of phosphorylated FOXO to the cytoplasm. In the cytosol, the phosphorylated FOXO is subjected to degradation via the UPP. The founding member of the FOX family is the forkhead transcription factor of the forkhead box (FOX) family in the fruit fly *Drosophila melanogaster*, of which a mutation in Foxo genes results in defective head shrinkage. The forkhead box is a conserved domain, which has been described due to a butterfly-like appearance on nuclear magnetic resonance and X-ray crystallography. This domain consists of three α-helices and three β-sheets that are accompanied with two loops usually referred to like the wings. In invertebrates, there is only one FOXO gene, termed dFOXO in the fruit fly and daf-16 in the worm. Conversely, there are four FOXO genes, FOXO1, 3, 4, and 6 in mammals. In the mammals, forkhead transcription

factors of the O class, i.e., FOXO1, FOXO3, FOXO4, and FOXO6 proteins, play a paramount role in the usual functional complexity of the cell, its proliferation, differentiation, and death as well as in the progression of several diseases. FOXO1 is a transcription factor that plays crucial roles in the regulation of gluconeogenesis and glycogenolysis by insulin signaling and is also vital to the decision for a preadipocyte to commit to adipogenesis. In mammals, FOXO localization at the subcellular level is exquisitely controlled by post-translational modifications, including ubiquitination, phosphorylation, and acetylation (29, 44–52). During development and tumorigenesis, the protein kinase B (AKT) path plays a significant role in cell growth and survival. AKT is activated by AKT kinase PDK1/2. AKT kinase PDK1/2 is considered to be a downstream target kinase of phospholipid kinase phosphatidylinositol 3-kinase (PI3K). In mammals, there are three isoforms which are determined by distinct loci. FOXOs are proteins active in growth factor signaling. In the pathway, they are positioned downstream of AKT. In addition to AKT, there are negative regulators of FOXOs, including the casein kinase 1 (CK1), dual-specificity tyrosine-phosphorylation-regulated kinase 1A (DYRK1A), serum and glucocorticoid-regulated kinase (SGK), IκB kinase (IKK), and the ubiquitous ERK. Other kinases, which control FOXOs are c-Jun N-terminal kinase (JNK) and mammalian ste20-like kinase (MST1) which act under circumstances of elevated oxidative stress. In the last few years, it has been suggested how FOXO regulation may contribute to fibrosis of several organs (42). In particular, FOXO1/3 have been demonstrated to have promising inhibitory effects on fibroblast activation and extracellular matrix production improving the degree of fibrosis levels in several organs, including the heart, kidney, liver, and lung (42). FOXOs have been cataloged to be tumor suppressors due to their antiproliferative and pro-apoptotic actions, despite some data revealed unpredicted functions of FOXOs in the advancement of cancer and in modifying responses to cancer treatment (49). A complex array of posttranslational modifications regulates FOXO transcriptional activity. These posttranslational modifications can be either activating or inactivating. These modifications alter modify the DNA binding affinity, nuclear import and export, and alter the pattern of transcriptional activity for specific target (53). FOXO factors play a crucial role in cell fate decision.

FOXO AND BONE

In a concerted fashion, bone is continuously degraded and replaced by the action of operating cells toward growth and operating cells toward remodeling. The builder cells are the osteoblasts, while the refining cells are the osteoclasts. This process of regeneration is active throughout the entire life of a vertebrate organism and is also present during tumorigenesis. Neoplasm is intrinsically linked to inflammation, and this aspect is not only relevant to the liver, where parasitic cholangitis can evolve to cancer, but it is a phenomenon across several organs and structures, including the skeletal system (54). Osteoclasts, which are short-lived giant multinucleated cells, arise from the fusion of myeloid lineage progenitor cells. They are under the influence of macrophage colony-stimulating factor (M-CSF) and the receptor activator of nuclear κ-B ligand (RANKL) and their respective receptors (55). The action of proteolytic enzymes and hydrogen ions (H^+) on the mineralized bone matrix evolves along all life of an individual. The "podosome belt" that tightly adheres to the bone area targeted for removal relies on a polarized secretion of proteolytic enzymes and H^+ ions (56–58). In the skeleton of an adult individual, bone mass is maintained, but the process is more complicated than thought initially. The maintenance of the bone mass requires the activity of both the re-absorption process and the re-apposition process. Thus, the resorption process is regulated by the osteoclast, while the deposition of new bone relies on osteoblasts. Human pathology and aging determine an imbalance between bone deposition and resorption. Conversely, osteoblasts are short-lived mesenchymal cells derived from bone progenitor cells that express the osteoblast identifying transcriptions factors RUNX2 and OSTERIX1 (59–62). FOXOs repress proliferation of OSTERIX1+ committed osteoblast precursors by inhibiting the canonical Wnt/β-catenin signaling (59). The osteoblasts are responsible for the deposition of osseous matrix (osteoid) in the bony tissue spaces emptied by the osteoclasts. One of the critical aspects of bone remodeling is played by the Wnt signaling (63). The WNT proteins present in the extracellular space bind to receptor frizzled and coreceptor lipoprotein-like receptor protein (LRP) 5/6 present on the cell surface of mesenchymal progenitors. The Wnt-frizzled and Wnt-LRP 5/6 binding triggers an intracellular set of events, which culminates in the release of β-catenin from a proteasomal degradation complex, its translocation into the nucleus, and subsequent binding T-cell factor/lymphoid enhancer factor (TCF/LEF) transcription factors. This bond β-catenin—TCF/LEF can activate or suppress the expression of Wnt target genes (64). Although most of the osteoblasts once finished their function die by apoptosis, a subgroup of them will incorporate in the bone matrix becoming long-lived dendritic cells called osteocytes. The osteocytes are a strong pillar in bone remodeling by producing RANKL and sclerostin, which are essential for both bone resorption and deposition, respectively (65).

The FOXO1, 3, and 4 proteins are critical for bone development, and the control of bone mass in both physiology and pathology (**Figure 1**). FOXOs are essential regulators of osteoclast differentiation and bone resorption by decreasing the ROS (66). The loss of all FOXO1, 3, and 4 in osteoclast progenitors does increase proliferation, osteoclast formation, and bone resorption, which lead to reduced trabecular and cortical bone mass. Contrarywise, gain-of-function of FOXO3 inhibits bone resorption by osteoclast differentiation. This aspect results by an increase of the expression of catalase, as an example of antioxidant enzymes able to impede H_2O_2 (66). The accumulation of ROS is due to RANKL, which decrease the levels and activity of FOXO1, 3, and 4 via AKT-mediated phosphorylation and proteasomal degradation (66–69). FOXOs also excite heme oxygenase-1 (HO-1) to be expressed in osteoclast progenitors. Furthermore, macrophages are the site where HO-1 catabolizes heme and attenuates oxidative phosphorylation and ATP production in mitochondria

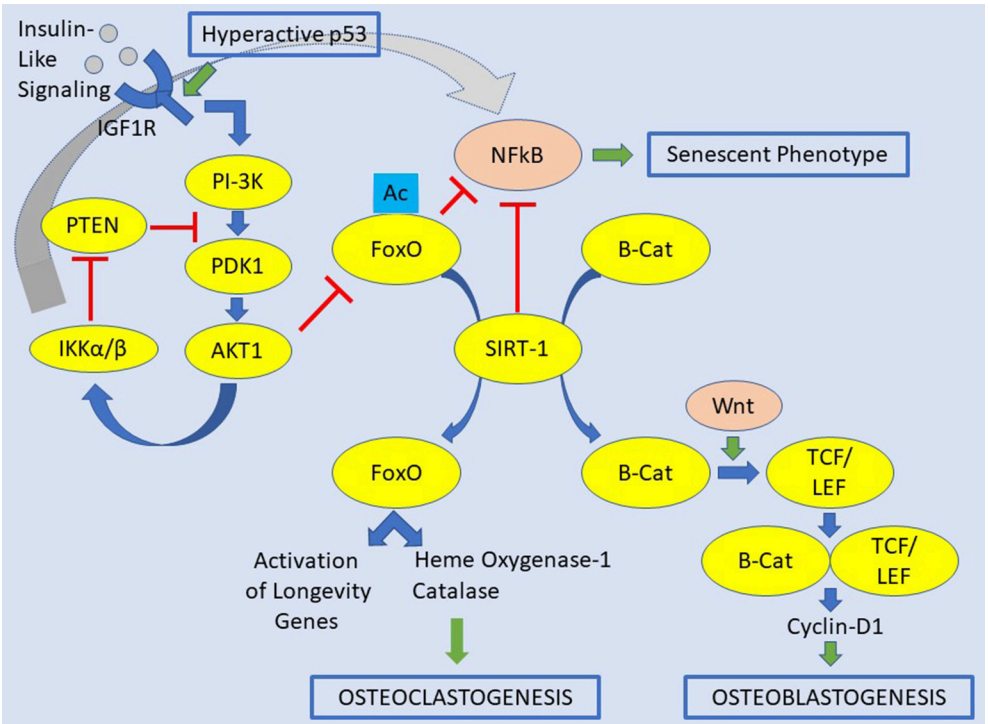

FIGURE 1 | Signaling pathways contemplating insulin-like signaling/IGFR1, FOXOs, and SIRT1 for the osteoclastogenesis and osteoblastogenesis. In this original picture is also depicted the occurrence of a hyperactive p53. A hyperactive p53 would be a condition leading to osteosarcoma-genesis. The abbreviations of this figure are shown in the text and please see the text for details.

(70). Overall, HO-1 contributes to the anti-osteoclastogenic effects of FOXOs. Further, FOXOs act directly to reduce the osteoblastogenesis by restraining Wnt signaling in which FOXOs bind to and sidetrack β-catenin away from TCF/LEF-mediated transcription. Consequently, there is a decrease in cyclin D1 and cell proliferation, and finally bone mass (59). However, FOXOs seems also to promote survival of osteoblasts by increasing the expression of catalase and superoxide dismutase, two major antioxidant enzymes, preventing oxidative cellular stress (71, 72). Also, FOXO1 promotes the accumulation of glutathione, a peptide that reduces ROS due to its redox-active sulfhydryl moieties. There is also a paracrine mechanism, but details of this process are still missing. The anti-osteoclastogenic actions of FOXOs are probably due to stimulation of osteoprotegerin (OPG), which is the decoy receptor for RANKL (59, 73).

MOLECULAR INTERACTION BETWEEN IGF-1, SIRT1, AND FOXOS

The PI3K-PKB/AKT pathway is the canonical pathway regulating the transcriptional activity of FOXOs. While FOXOs and SIRT1 add conceptually to the longevity of the bone through an equilibrated balance of bone formation and bone remodeling, the action related to IGF1 and IGF-R1 may act in the opposite direction. SIRT1 mediates posttranslational modifications of FOXOs and seems to prevent bone resorption and stimulate bone formation. Multiple kinases can modulate FOXOs through phosphorylation, and post-translational changes may work influencing FOXO activity. These modifications include methylation, ubiquitination, acetylation, PARylation, glycosylation, and hydroxylation. Poly ADP-ribosylation (PARylation) is a highly dynamic post-translation protein modification at DNA lesions, which is catalyzed by poly (ADP-ribose) polymerases (74). The accumulation of FOXOs in the nucleus determines its binding to various transcription-cofactors regulating the transcription of genes involved in the cell cycle, apoptosis, metabolism, redox homeostasis, angiogenesis, and GFR signaling. As seen that insulin-like signaling leads to PI(3)K activation, this tie induces AKT to inhibit FOXO by phosphorylation. Also, the human tumor suppressor PTEN inhibits AKT activity, conceivably by phosphorylation of PIP3. In the setting of oxidative stress, cells with high AMP/ATP ratios determine an increase of JNK and AMPK. Both kinases activate FOXOs by phosphorylation, and the active form of FOXOs relocates to the nucleus. Once in the nucleus, it promotes the expression of genes that promote longevity (75). Oxidative stress activates JNK with the consequence to increase the FOXOs activity. Subsequently, FOXOs reduces both WNT signaling and insulin-like signaling (ILS). The decreased Wnt signaling leads to protein aggregation, which involves the early cell degeneration with the formation of abnormal exocellular bodies,

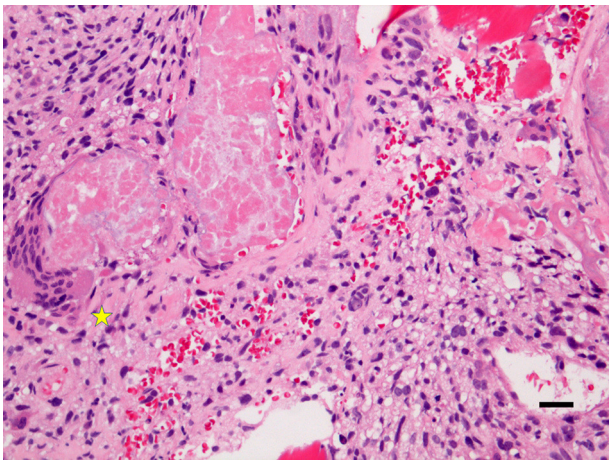

FIGURE 2 | This is an original microphotograph (X200, Hematoxylin & Eosin staining) of osteosarcoma showing osteoclastogenesis (left, asterisk) and osteoblastogenesis (right). The diagnosis of osteosarcoma relies on the identification of anaplastic cells with the osteoid formation (Hematoxylin and Eosin staining, X100 original magnification, bar: 20 μm).

e.g., β-amyloid plaques. The decrease of ILS heads to insulin resistance, hyperglycemia, and development of T2DM (30, 76).

OSTEOSARCOMA: THE MOST FREQUENT MALIGNANT MESENCHYMAL TUMOR OF BONE

The osteogenic sarcoma of the skeletal system or bone osteosarcoma is a malignant mesenchymal tumor of bone. It is the most common primary bony malignancy with both active osteoclastogenesis and osteoblastogenesis (**Figure 2**). The osteosarcoma derives from primitive bone-forming mesenchymal cells. The incidence rates of osteosarcoma per year per million of persons are about 4.0% for the range of 0–14 years and 5.0% for children aged 0–19 years, being eighth (2.4%) in the pediatric cancer incidence after leukemia (30%), brain tumors (22.3%), neuroblastoma (7.3%), nephroblastoma (5.6%), lymphoma of non-Hodgkin type (4.5%), rhabdomyosarcoma (3.1%), and retinoblastoma (2.8%) (1). Similar to other neoplasms, osteosarcoma has a bimodal age distribution, having the first peak during adolescence and the second peak in the elderly. Since the first peak is in the 10-14-year-old age group, there is an essential endocrinologic coincidence to be considered. This time corresponds to the pubertal growth spurt and may indicate a close relationship between the endocrinologic changes occurring during the adolescent growth spurt and the endocrinologic platforms of the bone metabolism. In the elderly osteosarcoma, there is a strong link with Paget disease of the bone. That there is a connection with the pubertal growth spurt, it may also be supported from the most frequent site of osteosarcoma. Indeed, it occurs near the metaphyseal growth plates of the long bones of the extremities (1). Although death rates for osteosarcoma have been declining by about 1% per year, the global 5 year survival rate for this tumor is 68%, without substantial gender difference. It has been suggested that osteosarcoma is more often identified in patients with abnormal glucose metabolism, although clear epidemiology data are lacking (77–80). The abnormal glucose metabolism may also be relevant locally other than generally. Interestingly, a teenager was described harboring a premature aging syndrome with diabetes mellitus, osteoporosis, and osteosarcoma (81). By studying animal models, spontaneous osteosarcoma was found in about 7% diabetic (non-obese) mice (79, 80). In osteosarcoma, genetics changes include point-mutations, aneuploidy, and chromothripsis, in which there are numerous rearrangements of the genome. It leads to oscillations of the copy number states, which has been labeled as a dramatic cellular catastrophe (82, 83). There is chromosomal instability in osteogenic osteosarcoma. This instability leads to the breakdown of the cell-cycle checkpoints and DNA-repair mechanisms. Moreover, there are numerous aneuploidy losses or gains at multiple chromosomal sites. Chromosomes 9, 10, 13, and 17 may be lost. The chromosomes 3, 6, 9, 10, 13, 17, and 18 may have a deletion of some parts of the chromosome. Amplification of chromosomal parts can also be detected. DNA sequence copy number upsurges have been distinguished on the regions 1q21, 3q26, 6p, 8q, 12q12-13, 14q24-qter, 17p11-12 of the autosomes and on the regions Xp11.2-21, and Xq12 of the allosomes. In most of the regions harboring chromosomal changes, there are sites of tumor suppressors and oncogenes. Apart of tumor suppressor and oncogenic sites, there are regions involved in the transcription procedure of the genetic information, including c-MYC and c-FOS that seem to play substantial roles in the etiology and/or pathogenesis (84, 85). In listing the other oncogenes connected with amplifications in osteosarcoma, we need to mention Cell Division Cycle five-Like (*CDC5L*), Mitogen-Activated Protein Kinase 7 (*MAPK7*), Mesenchymal to Epithelial Transition (*MET*), *PIM1*, peripheral myelin protein 22 (*PMP22*), DNA Primase Subunit 1 (*PRIM1*) other than Runt Related Transcription Factor 2 (*RUNX2*), and Vascular endothelial growth factor A (*VEGFA*) (86). Of these genes, *MAPK7*, *MET*, *PIM1*, *PMP22*, *RUNX2*, and *VEGFA* have been described to be associated with diabetes (87–91). Finally, epigenetic changes have also been demonstrated to play a role in osteosarcoma. Epigenetic modifications are specific changes in gene expression that are not due to direct changes in the DNA sequence. In osteosarcoma, there are epigenetic changes, which include methylation of DNA and modification of histones, nucleosome remodeling, and RNA-facilitated events (84, 92–94). Among others, p16 is a tumor-suppressor protein that plays a central role in osteosarcoma. The tumor suppressor p16 is a cell-cycle regulation factor, which acts by decelerating cells progression. The methylation of the cytosine residue within a gene can alter its expression. This event occurs in cytosine-phosphate-guanine (CpG) islands, i.e., DNA sites of 200 bp or more, GC rate >50%, and a detected-to-expected CpG ratio >60%. Gene silencing is the consequence of the methylation of CpG islands in promoter regions. The hypermethylation, when it occurs, reduces the genetic expression at the p16INK4 locus (84). Moreover, lysine-specific demethylase 1 (LSD1), a histone demethylase of the cell,

is overexpressed in osteosarcoma. Cell lines treated with the inhibitor of LSD1 exhibit reduced cell growth (84, 95). Likewise, demethylation of the promoter regions of TSSC3, which is a proapoptotic gene, caused in overexpression of the gene with the consequence of suppression of the osteosarcoma cells (96). The demethylation of tumor-suppressor genes in osteosarcoma seems to alter the metastatic capability of the tumor (97, 98). Some microRNAs have also been identified and suggested to be markers of prognosis influencing the genetic expression of osteosarcoma (99). Transcription factors, which enable the process of transcribing coding information from the DNA to single-stranded RNA by binding to promoter sequences on the genome, are carefully supervised in cells at different levels. Osteosarcoma cells show that such regulatory mechanism is disturbed. The study of both FOS and JUN has evidenced that their oncogenes are upregulated in osteosarcoma. Both FOS and JUN are components of the activator protein one complex, which is a transcription factor that controls cell proliferation, differentiation, and metabolism in the bone. The activator protein one complex has also been concerned in the tendency of these tumors to invade and metastasize (100–102). Intranuclear MYC, which is a transcription factor and endorses cellular growth and proliferation, is also overexpressed in osteosarcoma. It is also linked with struggle to conventional chemotherapy protocols (85, 103, 104). That transcription factors play an important role in osteosarcoma may be underlined by the discovery that niclosamide, a traditional anti-helminthic drug, is successful in some osteosarcomas by inducing apoptosis and inhibiting cell-cycle progression in osteosarcoma cells. Niclosamide inhibits the transcription factors E2F1, AP1, and c-MYC-responsive reporters strongly (105, 106). Melatonin, which is a hormone, produced mainly by the pineal gland, regulates wakefulness, and protects mesenchymal stem cells of the bone marrow against iron overload-induced aberrant differentiation and cellular senescence, has also been seen influencing the progression of osteosarcoma *in vitro*. Melatonin weakens osteosarcoma cell invasion by inhibition of the c-Jun N-terminal kinase pathway (107, 108). Melatonin increased and decreased the activation of ERK 1/2 and JNK 1/2, respectively, in a dose-dependent manner in U2OS and HOS osteosarcoma cells (108). This occurred while exerting no apparent influence on the level and activation of P38, AKT, PTK2 protein tyrosine kinase 2 (PTK2), also known as focal adhesion kinase or FAK, steroid receptor coactivator, or Rapidly accelerated fibrosarcoma (RAF).

IGF-1, SIRT1, AND FOXO1 IN OSTEOSARCOMA

In osteosarcoma, increased levels of IGF-1 and IGF-1R have been found that seem to lead to tumor progression through transformation, proliferation, decreased susceptibility to apoptosis, and a phenotype prone to metastasis, including increase cell motility, invasion, and angiogenesis (109–111). Also, an over-expression of IGF-1/IGF-1R signaling also contributes to tumor cell survival, metastasis, and resistance to chemotherapy protocols. Some worrisome data for patients harboring osteosarcoma regard the interaction of IGF-1 signaling and osteosarcoma suggesting that supplementation of osteosarcoma cell lines with IGF-1 increases their growth (112, 113). IGF-1 stimulates cell growth, and proliferation exceeds cell death. In fact, the IGF-1 signaling pathway is activated in osteosarcoma promoting proliferation and chemotherapy resistance by activating the AKT signaling pathway. Jentzsch et al.'s study suggest that local IGF-1 expression is associated with more aggressive tumor types. Although relatively rare in human, the osteosarcoma is 27 times more frequent in dogs (114). In the canine osteosarcoma, IGF-1R mRNA and protein expression are strictly associated with surgical stage and distant metastasis (115). MacEwen et al. have indeed demonstrated that IGF-1R expression is correlated with a poor prognosis of both human and canine osteosarcoma (116).

The role of SIRT1 in cancer is controversially discussed. SIRT1 promotes osteosarcoma metastasis by regulating the expression of genes that are associated with a metastatic phenotype. Zhang et al. found that SIRT1 was upregulated in most primary osteosarcoma tumors when compared with normal tissues (117). In this investigation, Zhang et al. hypothesize that SIRT1 might be coupled with a metastatic phenotype in human osteosarcoma. Cell migration and wound-healing assays supported the invasive activity of osteosarcoma cells and downregulating SIRT1 with shRNA inhibition determined that the migration ability of osteosarcoma cells *in vitro* decreased. It blocked tumor lung metastasis in mice (117). Other groups have challenged this data. In other investigations, SIRT1 inhibits tumor progression, deacetylates β-catenin, drives cell proliferation, and inhibits carcinogenesis in patients harboring colon cancer (118, 119). These incongruencies have been explained considering the different features of various tumors.

FOXO1 is generally low or absent in osteosarcoma, and the FOXO1 locus has been associated with copy number variation (CNV) and loss of heterozygosity (LOH) in osteosarcoma confirming that chromosomal aberrations may be (at least partially) responsible for the low FOXO1 expression in some cases of osteosarcoma (120). In osteosarcoma cell lines, the activation of FOXO1 promotes the pathway leading to cell cycle arrest and apoptosis that has been associated with repressed Wnt/β-catenin signaling. The inhibition of FOXO1 induced cell proliferation and decreased the osteogenic differentiation of osteosarcoma cells. By rebuilding the FOXO1 activity, there was impaired proliferation and apoptosis. Both the retinoblastoma-1 gene (RB1) and FOXO1 are situated in 13q14. This locus is often associated with recurrent losses in osteosarcoma (121–123). Interestingly, patients that harbor a loss of 13q14 show lower event-free survival. Other tumors, including cellular angiofibroma, spindle cell lipoma, as well as mammary and vaginal myofibroblastomas, may share a monoallelic loss of RB1 and FOXO1, although none of them is a malignant tumor (124). Guan et al. (120) identified no point mutations in the coding sequence or DNA hypermethylation in the promoter region of FOXO1, which is similar to what has been published in previous studies, i.e., FOXO1 might be involved in the genomic loss, but rarely engaged in mutation or DNA hypermethylation in tumorigenesis. The same authors found

five gains, six losses, and 15 cases of LOH of the FOXO1 locus in 34 cases of osteosarcoma by analysis of whole-genome sequencing (120). Guan et al. found that FOXO1 represses the survival of osteosarcoma cells by inhibition of the Wnt/β-catenin signaling, showing that the inhibition of Wnt/β-catenin signaling by FOXOs is conserved during the development of bone and osseous tumorigenesis. FOXO1 does not seem to have any significant influence on the subcellular localization of β-catenin, but FOXO1 inhibits expression of β-catenin. FOXO1 activation induces cell cycle arrest and its inhibition by impairing osteogenic differentiation of osteosarcoma cell lines considering that FOXO1 is a positive promoter of osteoblastogenesis *in vitro* (125). FOXO1 loss might contribute to the disturbed terminal differentiation observed in osteosarcoma. The rebuilding of FOXO1 activity could be a potential therapeutic strategy for therapy of osteosarcoma.

CONCLUSIONS

There is an intricate relationship that does occur in the interaction of IGF1, SIRT1, and FOXOs in the skeletal system. This relationship is particularly essential not only for altered bone metabolism, i.e., deposition and absorption of osteoid but also putatively for the inflammatory status that may trigger osteosarcoma to develop. A research platform in metabolomics of bone tumors is growing, and the mole of data will shape the treatment of osteosarcoma of the 21st century.

AUTHOR CONTRIBUTIONS

CS conceived the study and wrote the manuscript. FS critically reviewed the signaling pathways. S-ML critically reviewed the manuscript. All authors approved the final manuscript.

FUNDING

This research has been funded by the generosity of the Stollery Children's Hospital Foundation and supporters of the Lois Hole Hospital for Women through the Women and Children's Health Research Institute (WCHRI Grant Application ID #: 2096), Austrian Tyrolean Cancer Research Institute (Tiroler Krebsforschungsinstitut, Innsbruck, Austria), Austrian Research Fund (Fonds zur Förderung der wissenschaftlichen Forschung, FWF), and the Saudi Cultural Bureau, Ottawa, Canada.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Endocrinology

ORIGINAL RESEARCH
published: 28 February 2019
doi: 10.3389/fendo.2019.00126

Sirt1 Promotes a Thermogenic Gene Program in Bone Marrow Adipocytes: From Mice to (Wo)Men

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OPEN ACCESS

Edited by:
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Université Paris-Sorbonne, France

Reviewed by:
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Specialty section:
This article was submitted to Cellular Endocrinology, a section of the journal Frontiers in Endocrinology

Received: 31 July 2018
Accepted: 11 February 2019
Published: 28 February 2019

Citation:
Artsi H, Gurt I, El-Haj M, Müller R, Kuhn GA, Ben Shalom G, Cohen-Kfir E, Abramowitz E, Kandel L, Safran O and Dresner-Pollak R (2019) Sirt1 Promotes a Thermogenic Gene Program in Bone Marrow Adipocytes: From Mice to (Wo)Men. Front. Endocrinol. 10:126. doi: 10.3389/fendo.2019.00126

Bone marrow adipose tissue (MAT) is influenced by nutritional cues, and participates in whole body energy metabolism. To investigate the role of Sirtuin1 (Sirt1), a key player in metabolism, in MAT, marrow adiposity was evaluated in inbred 5-month-old 129/Sv *Sirt1* haplo-insufficient (*Sirt1*$^{\Delta/+}$) and wild type (WT) mice. Decreased expression of the thermogenic genes: *Prdm16, Pgc1α, Foxc2, Dio2,* and *β3AR* was detected in whole tibiae derived from *Sirt1*$^{\Delta/+}$ compared to WT female mice. Similarly, decreased expression of *Prdm16* and *Pgc1α* was observed in primary bone marrow mesenchymal stem cell (BM-MSC) cultures obtained from *Sirt1*$^{\Delta/+}$ compared to WT female mice, suggesting a cell autonomous effect of Sirt1 in BM-MSCs. *In vitro*, Sirt1 over-expression in the mesenchymal embryonic fibroblast stem cell line C3HT101/2 increased Pgc1α and Prdm16 protein level. Similarly, pharmacologic activation of Sirt1 by SRT3025 increased *Foxc2, Pgc1α, Dio2, Tfam,* and *Cyc1* expression while inhibition of Sirt1 by EX527 down-regulated *UCP1* in C3HT101/2 cells. Importantly, in human femoral BM-MSCs obtained from female patients undergoing hip operations for fracture or osteoarthritis, Sirt1 activation by SRT3025 increased *PGC1α* mRNA and protein level. Blocking sclerostin, an inhibitor of the WNT pathway and a Sirt1 target, by the monoclonal humanized antibody (Sc-AbII), stimulated *β3AR, PRDM16,* and *UCP1* gene expression, and increased PGC1α protein level. These results show that Sirt1 stimulates a thermogenic gene program in marrow adipocytes in mice and humans via PGC1α activation and sclerostin inhibition. The implications of these findings to bone health, hematopoiesis and whole body energy metabolism remain to be investigated.

Keywords: sirtuin1, marrow adipose tissue, PGC1-alpha, bone marrow mesenchymal stem cells, thermogenic genes

INTRODUCTION

Adipose tissue consists of three main fat depots: visceral, subcutaneous, and marrow. Marrow adipose tissue (MAT) was initially thought to be metabolically inert and a filler only. Recent studies however have found that MAT responds to nutritional cues and exercise, and participates in whole body fat metabolism (1–4). Studies in mice and humans undergoing bone

marrow transplantation have demonstrated trafficking of bone marrow (BM)-derived circulating progenitor cells to adipose tissue, their differentiation into subcutaneous adipocytes and increased representation in obesity (5, 6). Furthermore, MAT was shown to contribute to circulating adiponectin in mice subjected to calorie restriction and in humans undergoing anti-cancer therapy (1). Interestingly, MAT response to nutritional cues can be distinct than visceral and subcutaneous fat depots. Calorie restriction, anti-cancer therapy, type 1 diabetes mellitus and anorexia nervosa all lead to peripheral fat loss but are surprisingly associated with increased MAT volume (7). In addition, MAT volume is inversely related to bone mass and strength in postmenopausal osteoporosis, aging and glucocorticoid excess (8–11). Finally, marrow adipocytes negatively regulate hematopoiesis and support bone homing cancers (12, 13). Thus, elucidating the mechanisms that regulate MAT may reveal novel pathways that influence bone turnover, hematopoiesis and whole body energy metabolism.

Three types of adipocytes reside in fat depots: white, brown, and beige. Lipid rich white adipocytes expand with energy intake and store triglycerides. Mitochondria rich brown adipocyte arise from a muscle-like cell lineage (*Myf5*$^{+}$) and dissipate energy as heat, while beige adipocytes (brite) emerge in white fat depots, bear characteristics of brown adipocytes, but do not originate from the *Myf5*$^{+}$ lineage (14).

The origin of bone marrow adipocytes is still unknown, and tools to genetically manipulate it are limited. The prevailing model suggests that a self-renewing bone marrow (BM) mesenchymal stem cell (BM-MSC) exists within the bone marrow that gives rise to osteoblasts, adipocytes, chondrocytes and marrow stromal cells (15). This BM-MSC was identified as the bone marrow stromal stem cell (BMSC) that surrounds bone marrow sinusoids (16), and has *in vivo* osteogenic and adipogenic potential. The regulatory factors that are involved in BMSC commitment to the adipocyte lineage are starting to unravel. BMSCs that express the leptin receptor (LepR) have the capacity to differentiate into both adipocytes and osteoblasts, while LepR is not expressed by neither mature osteoblasts nor marrow adipocytes, suggesting that LepR in BMSCs influences lineage allocation (17). Consistently, Leptin signaling via the LepR induced by high-fat-diet failed to promote marrow adipogenesis in mice with LepR deletion in BMSCs but not in osteoblasts, confirming that the effect is restricted to BMSCs (18). Another hormonal pathway affecting the BMSC fate is the parathyroid hormone/parathyroid hormone related peptide (PTH/PTHrP) receptor signaling pathway. Genetic loss PTH/PTHrP receptor (PTH1R) in mesenchymal stem cells using the paired related homeobox transcription factor 1 (*Prx1*)-*Cre* driver was reported to induce marrow adipogenesis, while PTH administration reduced marrow fat in mice and male patients with idiopathic osteoporosis, suggesting that PTH inhibits the differentiation of adipocyte progenitors to the adipocyte lineage (19). On another level of complexity, region-specific variation in MAT development, regulation and phenotype was reported in mice, rats and humans (20).

Sirtuin1 (Sirt1), a member of the sirtuin family of NAD^{+}-dependent protein deacetylases, is a key cellular energy sensor and a mediator of the beneficial effects of calorie restriction in some animal models (21). Sirt1 regulates glucose and fat metabolism (22, 23). *In vitro*, Sirt1 inhibited the generation of white adipocytes in 3T3L-1 pre-adipocytes by down-regulating Pparγ, a master gene in white adipocytes differentiation (24). *In vivo*, adipose selective over-expression of a dominant negative Sirt1 resulted in dyslipidemia and ectopic lipid deposition (25). Targeted Sirt1 deficiency in mature adipocytes accelerated the onset of obesity-induced insulin resistance and glucose intolerance (26). On the other hand, Sirt1 gain-of-function induced a brown adipocyte-like phenotype in white adipocytes by deacetylating Pparγ and modulating its transcriptional activity (27).

Others and we have previously reported that Sirt1 directly regulates bone osteoblasts, osteoclasts and osteocytes (28–33). However, the role of Sirt1 in MAT is still largely unknown. *In vitro*, Sirt1 and its pharmacologic activation decreased adipogenesis of bone marrow MSCs (34–36). MSC-specific S*irt1* knock-out mice using the *Prx1-Cre* driver (MSCKO mice) exhibited reduced subcutaneous fat with aging, but no significant change in marrow adipocyte size compared to young mice (37).

Marrow adipogenesis is influenced by the WNT signaling pathway (38, 39). We have previously reported that Sirt1 is a negative regulator of sclerostin, an inhibitor of the canonical WNT pathway in bone (28). Our findings were recently confirmed (40). Moreover, we have shown that the administration of the Sirt1 activator, SRT3025 reduced sclerostin in bone in mice *in vivo* (29), and in human femoral BM-MSCs *in vitro* (41). In the current study we investigated the role of Sirt1 in MAT, and discovered that it induces a thermogenic gene program, characteristic of brown adipocytes, in mouse and human BM-MSCs via PGC1α stimulation and sclerostin inhibition.

METHODS

Animals

S*irt1* haplo-insufficient mice (*Sirt1*$^{\Delta/+}$) lacking exon 4 of the *Sirt1* gene and their wild type (WT) littermates of 129/Sv background were a generous gift (see Acknowledgments), and were used for this study (42). Adult 5–7-month-old inbred *Sirt1*$^{\Delta/+}$ and WT female and male mice were studied. Animals were housed under specific pathogen free (SPF) conditions with free access to water and chow #2018 (Teklad Diets, Madison WI), containing 6.2% fat and energy density of 3.1 kCal/gr. Daily food intake was determined for each mouse for 3 weeks between age 5 and 6 months. Fasting (overnight) glucose was determined in blood collected from the tail vein by an automatic glucometer (Accuchek; Roche Diagnostics GmbH, Mannheim, Germany). For Glucose Challenge Test (GCT) mice were fasted overnight. Glucose 2 g/kg was injected intra-peritoneal, and blood was collected in 15 min intervals for 2 h. Mice were sacrificed using isoflurane inhalation (Minrad INC, USA). Whole tibia with marrow and L3–L5 were removed, immediately frozen in liquid nitrogen and stored in −80°C until analyzed. For bone fat volume determination tibiae were kept in 10% formalin for 48 h and then transferred to phosphate buffered saline (PBS) until

analyzed. For bone histology tibiae were dehydrated in 50% and then in 70% EtOH and kept at 4°C. All experiments were performed with the approval of the Animal Study Committee of the Hebrew University-Hadassah Medical School (MD-12-13154-3).

Determination of Bone Marrow Fat

To determine tibiae bone marrow fat volume osmium tetroxide staining followed by micro-computed tomography (μCT) analysis was performed in WT and *Sirt1*$^{\Delta/+}$ female mice, as previously described (43). Briefly, tibiae were fixed in 10% formalin, decalcified in 0.5 M EDTA, soaked in a 1:1 solution of equal volumes of 2% aqueous osmium tetroxide (O_sO_4) and 5% potassium dichromate. The intact bones were scanned at 6 μm resolution using micro-focus conebeam X-ray computed tomography (μCT40 Scanco Medical AG Brüttisellen, Switzerland). The scanner was operated at 55 KVp, 144 μA, collecting 2,000 projections per rotation at 300 ms integration time. Total tibiae marrow fat volume as well as marrow fat volumes in the proximal and distal fourths were measured separately for each mouse. Histologic analysis was performed on 4 μm thick decalcified tibial sections from the proximal tibiae and stained with hematoxylin. Adipocytes were identified as empty oval structures and were manually counted, as previously described (44). Bone marrow fat area was determined as the fraction of adipocyte area per total area. Images were obtained with a DS-Fi camera attached to an Eclipse 80i microscope (Nikon, USA), and analyzed with ImageJ (National Institutes of Health, USA, https://imagej.nih.gov/ij/).

Primary Bone Marrow Cell Cultures

Bone marrow cells were harvested from femurs and tibiae of 6 month-old *Sirt1*$^{\Delta/+}$ and WT female mice. The femurs and tibiae were removed and cleaned of connective tissue, the ends were cut, and the marrow was flushed with α-MEM/15% fetal bovine serum. Single-cell suspensions were prepared in α-MEM by drawing the cells several times through graded needles. Cells collected from each mouse were plated in 100 mm plate. Non-adherent cells were removed after 3 days and the medium was changed every 3 days. Ten days later cells were harvested and plated in a density of 2x10^5 cells/well in six-well-plates. Adipogenesis was induced by 10 μg/ml insulin/50 μM dexamethasone/100 μM indomethacin/500 μM 3-isobutyl-1-methylxanthine administered on day 14 post plating at 70% confluence. RNA was isolated on day 3 post adipogenic induction.

Experiments in the Murine Mesenchymal Stem-Cell Line C3H10T1/2

The C3H10T1/2 (ATCC CCL-226) murine mesenchymal stem cell line is an established cell line model to investigate bone marrow adipocytes (45, 46). Sirt1 over-expressing C3H10T1/2 cells (*Sirt1-OE*) were previously generated and reported by us through stable retroviral infection with pBABE-*Sirt1* (28). Adipogenesis was induced in C3H10T1/2 and in *Sirt1-OE* cells with 10 μg/ml insulin/50 μM dexamethasone/100 μM indomethacin/500 μM 3-isobutyl-1-methylxanthine administered for 4 days followed by 10 μg/ml insulin/50 μM dexamethasone/5 μM rosiglitazone administration with medium changes twice a week (47). Protein was purified on day 7 post adipogenic induction. Adipogenesis was determined by oil-red-o staining on day 8–10 and was normalized to cell number determined by crystal violet staining (28, 48). In another set of experiments the Sirt1 activating compound SRT3025 (29, 49), kindly provided by SIRTRIS/GSK, was dissolved in dimethyl sulfoxide (DMSO) according to the manufacturer's instructions and was co-administered at a final concentration of 10 μM with the adipogenic medium to C3H10T1/2 cells. RNA was isolated on day 1. Oil-red-o staining and protein purification were conducted as described above.

The Sirt1 inhibiting compound Ex527 (6-Chloro-2,3,4,9-tetrahydro-1H-Carbazole-1-carboxamide; E7034, Sigma-Aldrich, Ukraine) (29, 50, 51) was dissolved in dimethyl sulfoxide (DMSO) according to the manufacturer's instructions and was co-administered at a final concentration of 10 μM with the adipogenic medium to C3H10T1/2 cells. RNA purification was conducted as described above.

Experiments in Human Bone Marrow Mesenchymal Stromal Cells

Human bone marrow mesenchymal stromal cells (hBM-MSCs) have the capacity to spontaneously differentiate into adipocytes in *ex vivo* cell cultures without the addition of an adipogenic medium (52). Fresh femoral bone marrow was harvested during femoral canal preparation from three female patients (age 68 ± 9.3 years) undergoing hip replacement for hip osteoarthritis or fractured head of femur (n = 4, age 81 ± 8.1), as part of an ongoing research project which was previously reported by us (41). None of the patients had diabetes or was treated with medications known to affect glucose, lipid or bone metabolism. The study was approved by the Hadassah-Hebrew University Medical Center ethics committee (HMO-0369-10), and informed consent was obtained from each patient prior to surgery. The bone marrow aspirate was collected in growing medium (GM) containing DMEM/5 mM glucose/10%FBS/100 Units/ml penicillin/100 mg/ml streptomycin sulfate/0.25 mg/ml amphotericin B, treated with Lymphoprep #1114544 (Ficoll, Axis-Shield PoC AS, Oslo, Norway), and centrifuged at 900 g for 30 min. Cells at the intermediate interface were collected and centrifuged again at 900 g for 10 min. The resulting mononuclear pellet was re-suspended in GM, plated at a density of 5 × 10^5 cells/35 mm dish and cultured in GM with a medium change twice a week (53). The Sirt1 activator SRT3025 at concentration of 5 μM or a vehicle was added upon confluence and with every medium change. RNA was collected 3 days following treatment initiation, while protein collection was carried out on day 10.

Gene Expression Analysis

Whole tibiae and vertebrae with marrow, primary cultures of BMSCs, C3H10T1/2 cells and human BM-MSCs were homogenized in TRIzol (Invitrogen, Carlsbad CA). Total RNA was extracted, converted to cDNA using the qScript kit (Quanta BioSciences, Inc. Gaithersburg, MD, USA). Gene expression

analysis was performed using SYBR Green-based real-time-PCR (Kapa Syber, Kapa Biosystems (Pty) Ltd, Cape Town, South Africa). **Supplementary Table 1** provides all of the primer sequences used in this study. Relative gene expression was determined by the comparative CT method with *βActin* and *Polr2a* as controls in murine and cell line experiments (geometric mean). *βActin* was used as control for analysis of experiments conducted in human BM-MSCs**.** For experiments conducted in C3H10T1/2 cells gene expression was further normalized to the expression of *Adipoq*. For experiments conducted in human BM-MSCs data was further normalized to *PPARγ* expression.

Protein Analysis

Protein was extracted in Laemmli buffer (2% SDS/10% glycerol/5% 2-mercaptoethanol/ 0.01% bromphenol blue/60 mM Tris HCl). Antibodies for immunoblotting: Prdm16 (AbCam, ab106410), Pgc1α (Cell Signaling, #2178). α-Tubulin (AbCam, ab106375). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH, Abcam, ab8245).

Statistical Analysis

Results are presented as Mean ± SEM. Data was analyzed by unpaired Student's *t*-test to compare group means. 1-way ANOVA followed by Holm-Sidak's analysis was used to compare three groups. Analysis was performed using GraphPad (San Diego, CA, USA) Prism version 6.01. Each experiment was conducted in triplicates and was repeated at least 3 times. $P < 0.05$ was considered statistically significant.

RESULTS

Reduced Expression of Thermogenic Genes in MAT of Sirt1$^{\Delta/+}$ Female Mice

Basal metabolic parameters including body weight, daily food intake, fasting glucose and the response to glucose load did not differ between WT and *Sirt1*$^{\Delta/+}$ mice of both genders (**Supplementary Figures 1A–H**). Previous work by others and us has demonstrated a sexual dimorphism with regard to the effects of Sirt1 deficiency in bone, showing a bone phenotype in female but not in male mice (28, 30, 31). We therefore conducted most of our studies in female mice. Whole tibiae bone marrow fat volume determined by osmium staining was not different between WT and *Sirt1*$^{\Delta/+}$ female mice (**Figure 1A**). Proximal and distal tibial marrow fat volume also did not differ between WT and *Sirt1*$^{\Delta/+}$ mice. In agreement with these results, tibial marrow adipocyte number and area was

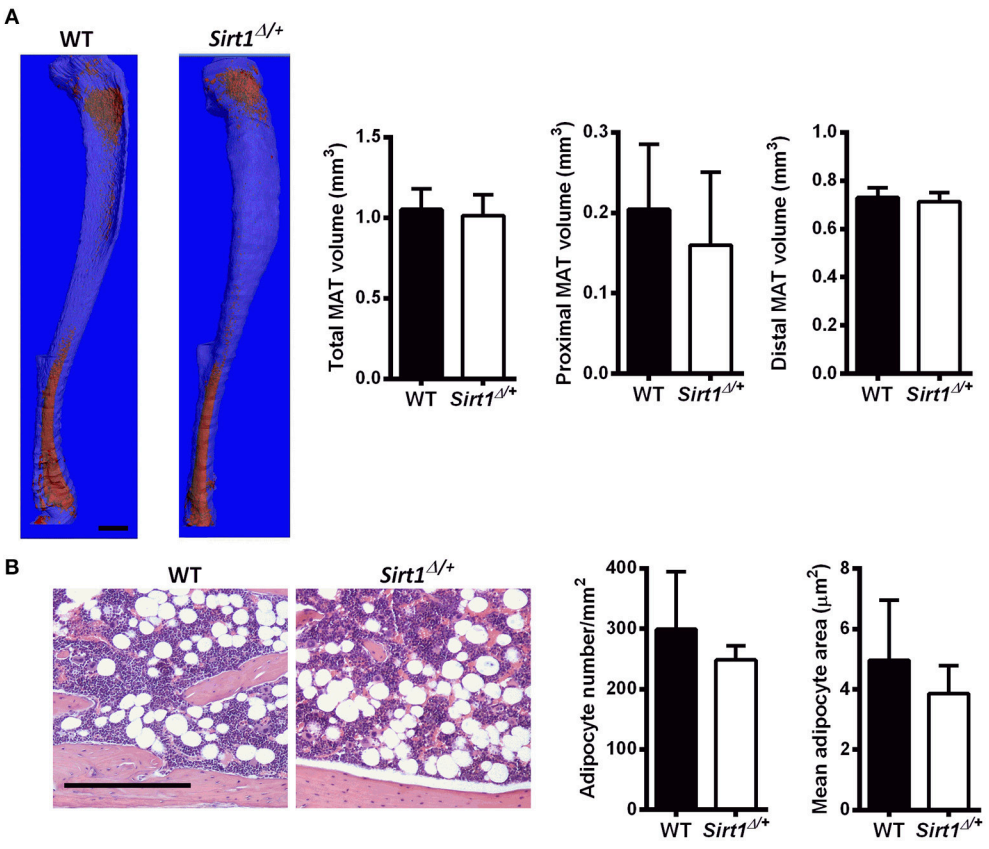

FIGURE 1 | Marrow adipose tissue (MAT) in *Sirt1*$^{\Delta/+}$ and WT female mice **(A)**. Osmium tetroxide staining of tibiae followed by μCT analysis; A representative image (left) and quantification (right). Data is presented as fat volume. Scale bar 1 mm ($n = 8$ mice/group). **(B)** Hematoxylin-stained histological sections of proximal tibiae. Scale bar 200 μm; ($n = 3$ mice/group). Results are Mean ± SEM.

similar in WT and *Sirt1*$^{\Delta/+}$ female mice (**Figure 1B**). Strikingly, gene expression analysis in tibial MAT revealed a dramatic decrease of ∼50% in the thermogenic genes: *β3AR, FoxC2, Prdm16, Pgc1α,* and *Dio2* in *Sirt1*$^{\Delta/+}$ compared to WT female mice (**Figure 2A**). Importantly, *Prdm16* and *Pgc1α* mRNA expression was significantly decreased by over 2 fold in *Sirt1*$^{\Delta/+}$ compared to WT-derived primary BMSC cultures induced to adipogenesis (**Figure 2B**), indicating a cell autonomous effect of Sirt1 on the bone marrow adipocyte. No differences in tibial MAT gene expression were observed in *Sirt1*$^{\Delta/+}$ compared to WT male mice (**Figure 2C**). Thermogenic genes expression in vertebral MAT did not differ between genotypes in both genders (**Figures 3A,B**). Taken together, these results indicate that Sirt1 haplo-insufficiency leads to reduced thermogenic genes expression in tibial MAT in female mice.

We next asked if Sirt1 stimulation reciprocally increases the expression of a thermogenic genes program in BMSCs. To address this question three *in vitro* models were employed: (1) Sirt1 over-expressing C3H10T1/2 cells induced to adipogenesis. (2) Pharmacologic activation of Sirt1 by SRT3025 in C3H10T1/2 cells induced to adipogenesis. (3) Pharmacologic activation of Sirt1 in primary human femoral BM-MSCs.

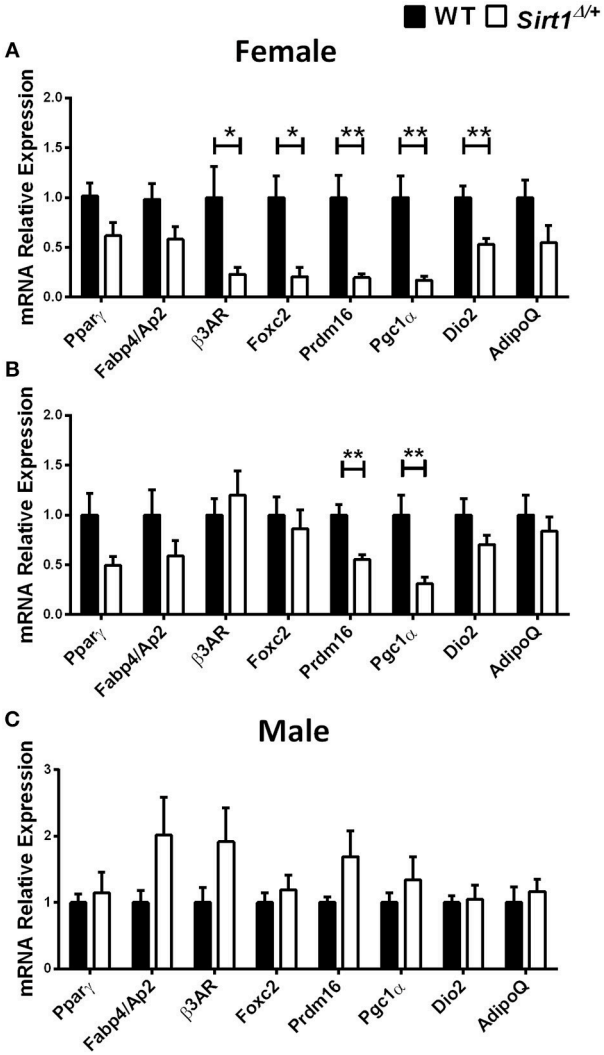

FIGURE 2 | Gene expression analysis of adipocyte markers in tibial MAT and primary bone marrow stromal cell cultures obtained from *Sirt1*$^{\Delta/+}$ and WT mice **(A)**. Gene expression analysis of adipocyte markers in tibial MAT obtained from 5-month old *Sirt1*$^{\Delta/+}$ and WT female mice ($n = 6$–9 mice/group). **(B)** Gene expression analysis of adipocyte markers in primary bone marrow stromal cell cultures induced to adipogenesis, derived from *Sirt1*$^{\Delta/+}$ and WT female mice ($n = 6$ mice/group). **(C)** Gene expression analysis of adipocyte markers in tibial MAT obtained from 5-month old *Sirt1*$^{\Delta/+}$ and WT male mice ($n = 6$–9 mice/group). Results are Mean ± SEM. $^{*}P < 0.05$; $^{**}P < 0.01$ vs. wild type mice (WT).

Increased Thermogenic Markers in Sirt1 Over-Expressing C3H10T1/2 cells

Decreased lipid accumulation was observed in *Sirt1 OE* compared to control C3H10T1/2 cells induced to adipogenesis (**Figure 4A**). Elevated Prdm16 and Pgc1α protein level was observed in *Sirt1 OE* compared to control cells (**Figures 4B,C**). Consistent with these results, pharmacologic activation of Sirt1 by SRT3025 reduced the generation of white adipocytes and stimulated the expression of *Pgc1α, Dio2, FoxC2, Tfam,* and *Cytochrome C* (**Figures 5A–C**). In contrast, Sirt1 inhibition by Ex527 significantly reduced the expression of *Ucp1* (**Figure 5D**).

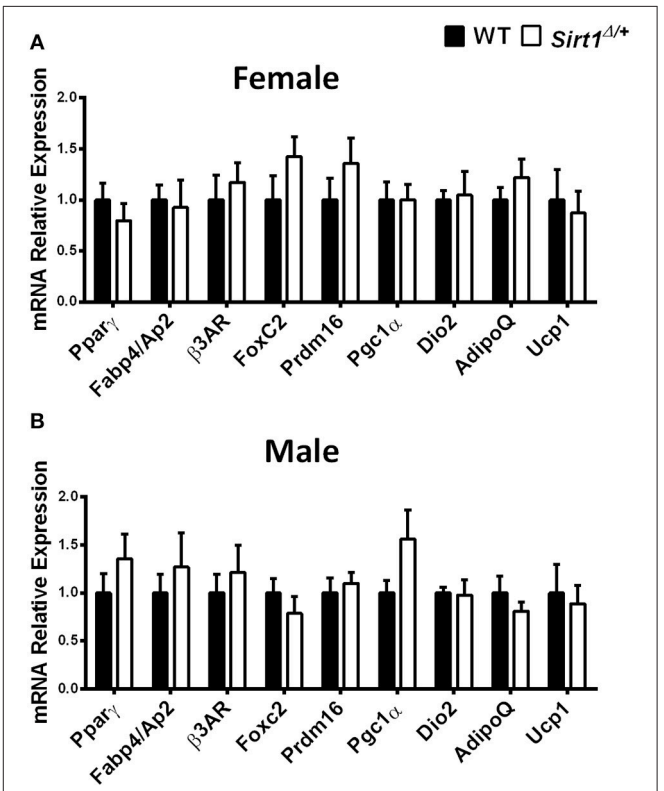

FIGURE 3 | Gene expression analysis of adipocyte markers in vertebral MAT in *Sirt1*$^{\Delta/+}$ and WT female and male mice, Gene expression analysis of adipocyte markers in vertebral MAT obtained from 5-month old *Sirt1*$^{\Delta/+}$ and WT female **(A)** and male **(B)** mice ($n = 10$ mice/group). Results are Mean ± SEM.

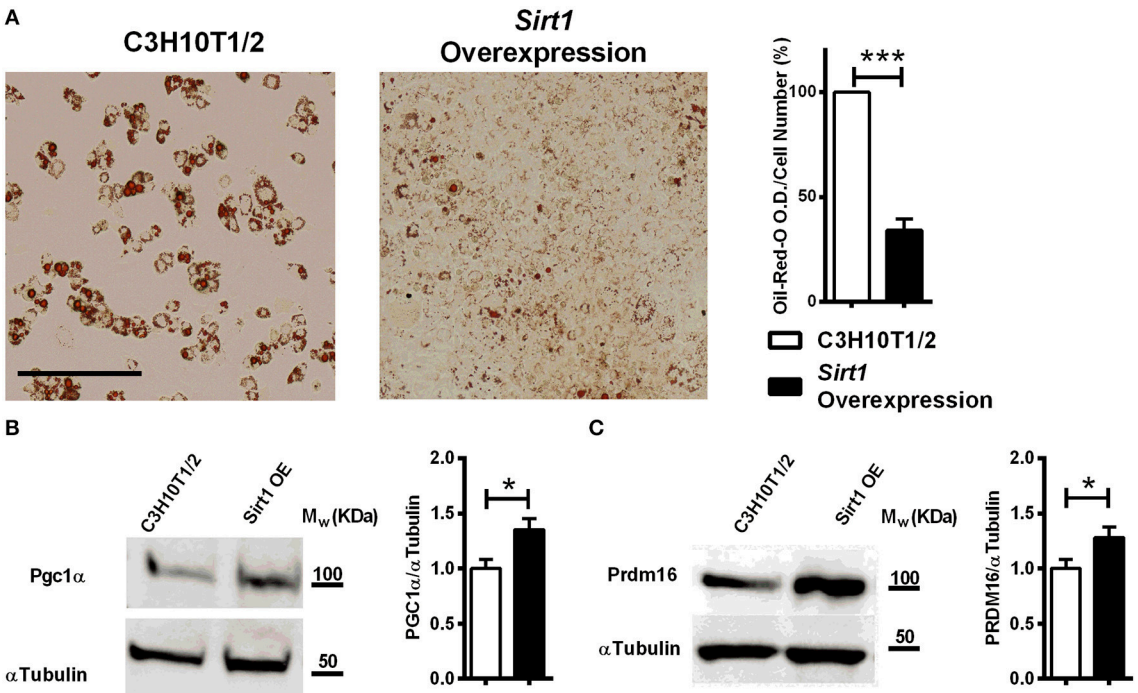

FIGURE 4 | The effect of *Sirt1* over-expression on adipogenesis in C3HT101/2 cells **(A)**. Oil-red-o staining in *Sirt1* over-expressing (*OE*) and C3H10T1/2 cells induced to adipogenesis. Data is presented as optical density (OD) corrected for cell number (crystal violet staining). Scale bar 500μm. **(B,C)** Immunoblot of Pgc1α and Prdm16 in *Sirt1 OE* and C3H10T1/2 cells 7 days post induction to adipogenesis. Results are Mean ± SEM. *$P < 0.05$; ***$P < 0.001$ vs. C3HT101/2 cells.

Sirt1 Activation by SRT3025 Promotes Thermogenic Genes Expression in Human BM-MSCs

To investigate if the effects of Sirt1 on the marrow adipocyte phenotype can be extended to human BM-MSCs, SRT3025 was administered to primary human femoral BM-MSCs. Strikingly, SRT3025-treated human BM-MSCs had increased mRNA expression of *PGC1α* (**Figure 6A**) accompanied by elevated PGC1α protein level (**Figure 6B**), suggesting that Sirt1 activates PGC1α in human femoral BM-MSCs.

Blocking Sclerostin Induces a Thermogenic Gene Program in Human BM-MSCs

We have previously shown that Sirt1 down-regulates sclersotin in mice and human BM-MSCs (28, 29, 41). As sclersotin stimulates marrow adipogenesis (54), we hypothesized that blocking sclerostin will affect human bone marrow adipocyte gene expression profile.

Indeed, blocking sclerostin with the monoclonal antibody Scl-AbII (55, 56) induced a marked increase in gene expression of: *PRDM16*, *β3AR*, *FOXC2*, and importantly an over 2-fold increase in *UCP1* expression, the hallmark of brown and beige adipocytes (**Figure 6C**). These effects were accompanied by a 2-fold increase in PGC1α protein level (**Figure 6D**). Taken together, this data indicate that blocking sclerostin stimulates a thermogenic genes signature in human femoral BM-MSCs.

DISCUSSION

This study reports for the first time a role for Sirt1 in MAT phenotype, demonstrating its stimulatory effect on a thermogenic gene program in marrow adipocytes. *Sirt1* haplo-insufficiency resulted in decreased expression of thermogenic gene markers in MAT in a gender- and site-specific manner. Reduced expression of *β3AR, FoxC2, Prdm16, Pgc1α,* and *Dio2* was found in tibial but not in vertebral MAT derived from adult female but not male $Sirt1^{\Delta/+}$ mice. Similar effects were noted in primary BM-MSC cultures derived from $Sirt1^{\Delta/+}$ mice, indicating a cell autonomous effect of Sirt1 on the BM-MSC. *In vitro*, Sirt1 over-expression in CH310T1/2 cells increased the expression of thermogenic markers characteristic of brown adipocytes Prdm16 and Pgc1α, a mitochondrial biogenesis inducer, and a known Sirt1 target (57). Importantly, in human femoral BM-MSCs pharmacologic Sirt1 activation and blocking its target, sclerostin, had a stimulatory effect on Pgc1α protein level and thermogenic genes expression.

Sirt1 was previously shown to directly deacetylase Pgc1α in liver and muscle, thereby promoting its phosphorylation by 5' adenosine monophosphate-activated protein kinase (AMPK), resulting in its activation (58, 59). The current study demonstrates for the first time that Sirt1 upregulates Pgc1α in murine and human BM-MSCs. Pgc1α was reported to increase thermogenic genes expression in white subcutaneous adipocytes (60) and brown adipocytes (61). Furthermore, Pgc1α is a master

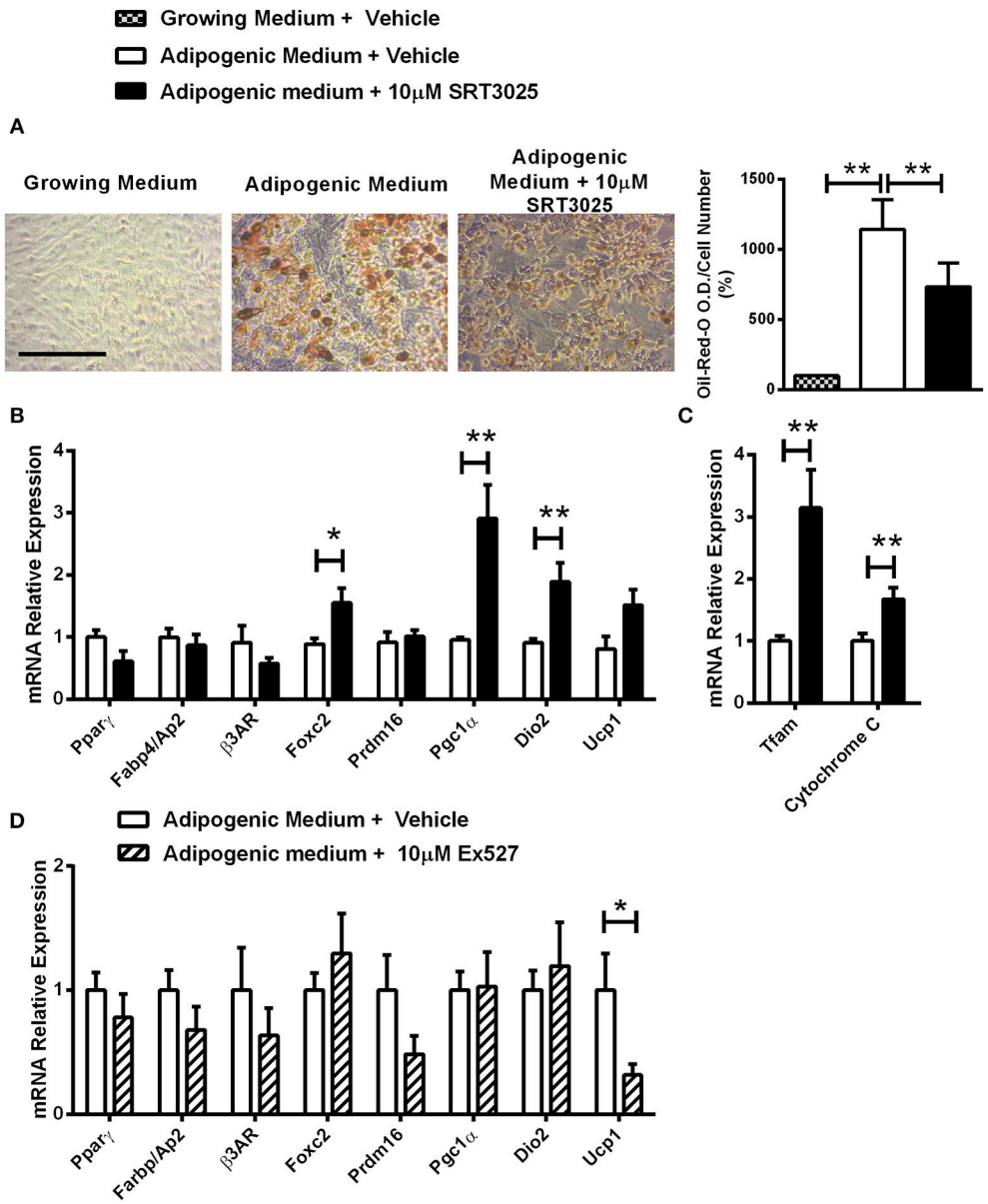

FIGURE 5 | The effects of Sirt1 pharmacologic activation and inhibition on adipogenic markers in C3HT101/2 cells **(A)**. Oil-red-o staining in C3H10T1/2 cells induced to adipogenesis and supplemented with SRT3025 or vehicle (DMSO). **(B,C)** Gene expression analysis of adipocyte **(B)** and mitochondrial markers **(C)** induced to adipogenesis and supplemented with SRT3025 or vehicle (DMSO). **(D)** Gene expression analysis of adipocyte markers induced to adipogenesis and supplemented with Ex527 or vehicle (DMSO). Results are Mean ± SEM. $^{*}P < 0.05$; $^{**}P < 0.01$ vs. vehicle-treated C3HT101/2 cells.

mitochondrial regulator, stimulating mitochondrial biogenesis and inducing the expression of components of the mitochondrial respiratory chain. Indeed, in our study gene expression of *Tfam*, a marker of mitochondrial biogenesis, and *Cytochrome C* was significantly increased with Sirt1 pharmacologic activation *in vitro*. The role of Pgc1α in bone and BM-MSCs was only recently revealed. *In vivo* and *in vitro* gain and loss-of-function studies demonstrated that Pgc1α regulates the skeletal stem cell fate, restraining marrow adipocyte differentiation and promoting osteogenesis (62). Our data indicate that Sirt1 stimulates Pgc1α expression in BM-MSCs thereby leading to induction of a thermogenic gene program.

Interestingly, Prdm16 was also consistently influenced by Sirt1 status in the various *in vivo* and *in vitro* models employed in this study. Prdm16 was significantly decreased in tibial MAT and in primary BM-MSC cultures derived from *Sirt1*$^{\Delta/+}$ compared to WT female mice. Along these lines, it was markedly increased with Sirt1 over-expression or activation *in vitro*. Prdm16 is an activator of Pgc1α expression and transcriptional function through direct protein interaction. It also induces *Pgc1α*, *Ucp1*,

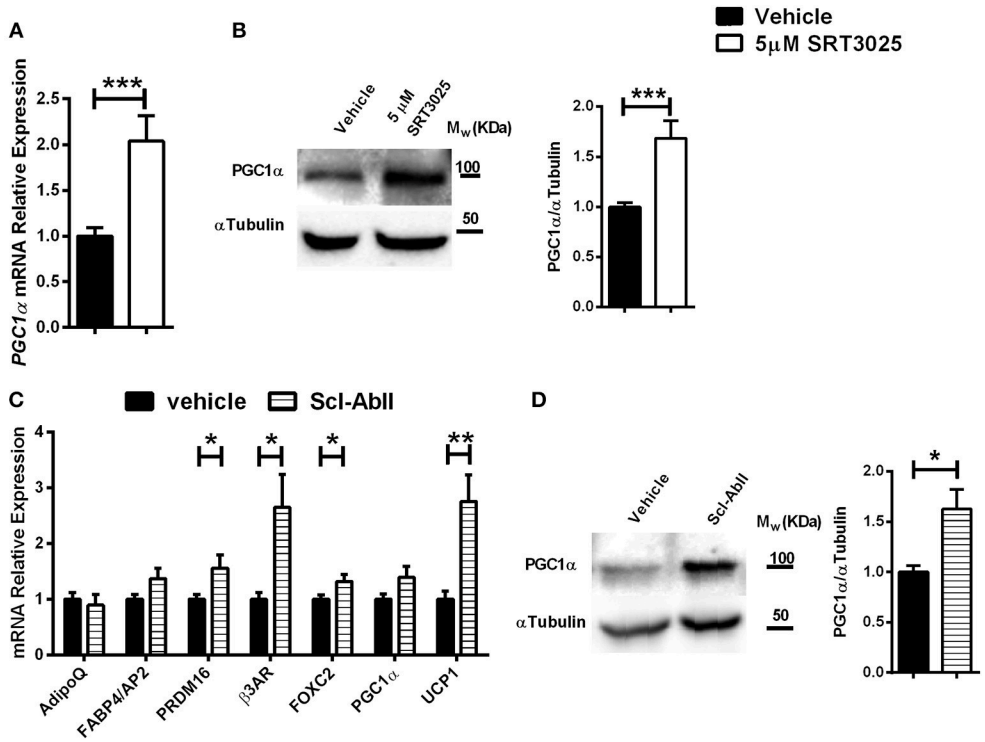

FIGURE 6 | The effects of SRT3025 and anti-Sclerostin antibody on adipogenic markers in human femoral bone marrow mesynchemal stem cells (hBM-MSCs) **(A)**. Gene expression analysis of PGC1α in SRT3025-treated hBM-MSCs. **(B)** Immunoblot of PGC1α. A representative image (left) and densitometry (right) with αTubulin as control. **(C)** Gene expression analysis of thermogenic genes in anti-Sclerostin AbII treated hBM-MSCs **(D)**. Immunoblot of PGC1α in hBM-MSCs treated with anti-Sclerostin AbII. A representative image (left) and densitometry (right) with αTubulin as control. Results are mean ± SEM. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ vs. vehicle-treated hBM-MSCs.

and *Dio2* expression in adipocytes (63). However, regulation of Prdm16 by Sirt1 has not been described before. Only one study demonstrated that Sirt1-dependent Pparγ deacetylation allows Prdm16 recruitment to Pparγ thereby modulating its transcriptional activity, favoring BAT genes expression while repressing WAT genes (27). Thus, the underlying mechanisms governing the observed changes in Prdm16 in this study remain to be elucidated.

We have previously reported that Sirt1 down-regulates sclerostin by deacetylating histones 3 and 4 at its promoter, leading to inhibition of *Sost* gene expression (28). These results were recently confirmed by Stegen et al who demonstrated that conditional deletion of the oxygen sensor prolyl hydroxylase (PHD) 2 in osteocytes resulted in enhanced HIF-1α signaling that stimulated Sirt1-dependent deacetylation of the *Sost* promoter and reduced sclerostin expression (40). Sclerostin, an inhibitor of the WNT/β-catenin pathway, was reported to induce adipocyte differentiation in 3T3-L1 cells (64), primary murine BM-MSCs (54), and human BM-MSCs (54). Along these lines, lower levels of MAT were found in tibiae of sclerostin knock-out (SOST-KO) mice, while sclerostin neutralization with a neutralizing antibody significantly decreased MAT (54). Our results demonstrate for the first time that blocking sclerostin with a neutralizing antibody, currently under advanced investigation for the treatment of osteoporosis, induces a thermogenic gene program in human BM-MSCs, and increases the expression of *UCP1*, a driver of mitochondrial heat generation and energy expenditure. UCP1, a protein located on the inner mitochondrial membrane, uncouples electron transport from adenosine triphosphate (ATP) generation. The resulting energy derived from substrate oxidation is dissipated as heat. UCP1 is expressed in brown and beige adipocytes. Some previous studies failed to detect it in MAT, while others reported low expression levels (11, 44). The metabolic significance of inducing UCP1 in MAT by blocking sclerostin remains to be investigated.

Differences in MAT phenotype between $Sirt1^{\Delta/+}$ and WT female mice were detected in tibiae but not in lumbar MAT. Lumbar vertebrae is a skeletal site in the mouse that has little MAT, whereas proximal tibial MAT was shown to be metabolically responsive to cold exposure (20). Caudal vertebrae was previously shown to have characteristics of constitutive MAT (cMAT) that contains large adipocytes and does not respond to systemic challenges. Thus, lack of difference in vertebral MAT phenotype is not surprising. Of note, most of the studies investigating murine MAT have used the C57BL/6J and C3H/HeJ mouse strains, whereas data in 129/Sv used in this study, is lacking.

This study is not without its limitations. The physiologic significance of our findings could not be evaluated in the mouse model used in this study. Exposure of MSC-specific

Sirt1 knock-out mice to cold temperature or a high fat diet could have provided insight into the contribution of MAT Sirt1 to local and whole body energy metabolism and needs to be performed in future studies. Secondly, gene expression analyses were performed in whole tibiae and vertebrae extracts similar to previously published studies (4, 65), and introduce the bias of contamination by other cell types. However, consistent results were obtained in primary BM-MSCs cultures derived from *Sirt1*$^{\Delta/+}$ and WT mice, supporting the notion of a direct cell autonomous effect of Sirt1 on the marrow adipocyte phenotype. Thirdly, we did not account for regional differences in tibial MAT composition, as was previously suggested (66). Finally, additional Sirt1 targets beyond Pgc1α and sclerostin may have played a role in driving a brown-like adipocyte gene expression program in BM-MSCs.

Whether inducing a thermogenic gene program in marrow adipocytes is beneficial to bone health, hematopoiesis local and whole body energy metabolism begs further investigation. Reduced MAT expression of brown adipocyte markers was previously reported in diabetic and aged mice, conditions associated with both increased skeletal fragility and impaired energy metabolism (44). Due to its wide favorable physiologic effects, Sirt1 has been considered an attractive therapeutic target for drug discovery. Sirtuin1 activating compounds (STACs) were generated, amongst them SRT3025, used in this study. SRT3025 was previously shown by us to restore bone mass and strength in OVX mice (29), but also to have off-target effects (67). In humans SRT3025 was shown to prolong QTc and its development was discontinued (68). As NAD^+ is an indispensable co-substrate required for Sirt1 and other sirtuins activity, there has been an increasing interest in small molecules that raise NAD+ levels as a mechanism to stimulate sirtuins activity (69).

In conclusion, this study shows that Sirt1 regulates the bone marrow adipocyte phenotype inducing a thermogenic gene program in mouse and human BM-MSCs. Inducing BAT-like features in subcutaneous and visceral fat depots is a much desired goal in combating obesity. Whether browning of MAT by Sirt1 activation, sclerostin inhibition or other mechanisms is a plausible novel approach to serve this goal while improving skeletal health remains to be elucidated.

ETHICS STATEMENT

This study was carried out in accordance with the recommendations of IHC-GCP, Public Heath Regulation, the Governing regulations of Ministry of Health. The protocol was approved by The Ethics (Helsinki) Committee at Hadassah University Hospital. All subjects gave written informed consent in accordance with the Declaration of Helsinki.

AUTHOR CONTRIBUTIONS

HA designed and performed most of the experiments and analyzed the data. IG designed and conducted some of the *in vitro* experiments. ME-H, LK, and OS were responsible for studies in humans. ME-H obtained the human samples and performed the experiments. RM and GK designed and conducted the marrow fat μCT quantification studies. GB performed experiments in murine BM-MSCs. EC-K designed and performed the experiments in human BM-MSCs. EA performed some *in vitro* experiments with SRT3025. RD-P conceived and designed the study, prepared the manuscript, and takes full responsibility for the work as a whole.

FUNDING

This study was funded by the Israel Science Foundation Grant 621/16 (to RD-P) and the Joint Research Fund of the Hebrew University (to ME-H).

ACKNOWLEDGMENTS

We thank Frederick W. Alt of Harvard University for providing the *Sirt1*$^{\Delta/+}$ mice, SIRTRIS/GSK and Amgen for providing SRT3025 and Scl-AbII, respectively. We thank the late Raymond Kaplan and the Bnai Brith Leo Baeck London Lodge for their support of osteoporosis research. The osmium tetroxide staining was performed at the SCOPEM (Scientific Center for Optical and Electron Microscopy), ETH, Zurich. We thank Dr. Stephan Preibisch SCOPEM for his assistance.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2019.00126/full#supplementary-material

Supplementary Figure 1 | Metabolic parameters in female and male *Sirt1*$^{\Delta/+}$ and WT mice **(A–D)**: females; **(E–H)**: males. **(A,E)**: Body weight. **(B,F)**: Daily food intake. **(C,G)**. Fasting blood glucose. **(D,H)**. Glucose challenge test (GCT) Results are Mean ± SEM (n = 5–8 mice/group).

Supplementary Table 1 | Mouse and human primer sequences.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Endocrinology

REVIEW
published: 07 January 2019
doi: 10.3389/fendo.2018.00783

The Roles of Mitochondrial SIRT4 in Cellular Metabolism

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Sirtuins comprise a family of nicotinamide adenine dinucleotide (NAD^+)-dependent lysine deacylases that regulate the life span, aging, and metabolism. Seven sirtuin family members (SIRT1-7) have been identified in mammals, including humans. Despite the indispensable role of mitochondrial sirtuin 4 (SIRT4) in metabolic regulation, the primary enzymatic activity of SIRT4 remains enigmatic. SIRT4 possesses ADP-ribosyltransferase, lipoamidase and deacylase activities. Interestingly, the enzymatic activities and substrates of SIRT4 vary in different tissues and cells. SIRT4 inhibits insulin secretion in pancreatic β cells and regulates insulin sensitivity as a deacylase in the pancreas. SIRT4 represses fatty acid oxidation (FAO) in muscle and liver cells differently. SIRT4 has also been identified as a mitochondrial-localized tumor suppressor. A comprehensive understanding of the enzymology of SIRT4 in metabolism is essential for developing novel therapeutic agents for human metabolic diseases. This review will update the roles of SIRT4 in cellular and organismal metabolic homeostasis.

Keywords: SIRT4, mitochondrial, enzymatic activities, insulin secretion, fatty acid metabolism, tumor suppressor

OPEN ACCESS

Edited by:
Yang Yang,
Northwest University, China

Reviewed by:
Brenna Osborne,
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Shingo Kajimura,
University of California, San Francisco, United States

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Specialty section:
This article was submitted to Cellular Endocrinology, a section of the journal Frontiers in Endocrinology

Received: *26 August 2018*
Accepted: *12 December 2018*
Published: *07 January 2019*

Citation:
Min Z, Gao J and Yu Y (2019) The Roles of Mitochondrial SIRT4 in Cellular Metabolism. Front. Endocrinol. 9:783. doi: 10.3389/fendo.2018.00783

INTRODUCTION

Sirtuins include β-NAD^+-dependent deacylases and ADP-ribosyltransferases involved with metabolism and aging (1, 2). Sirtuins (SIRT1-7) can be divided into nuclear (SIRT1, SIRT6, and SIRT7), mitochondrial (SIRT3, SIRT4, and SIRT5) and cytosolic (SIRT2) forms, and some sirtuins are found in more than one compartment (**Figure 1B**). The mitochondrial sirtuins are involved in metabolic regulation and antioxidative defense. In contrast to other members of SIRT, the enzymatic activities of SIRT4 have remained unclear (3, 4). SIRT4 is critical for cellular metabolism and DNA damage responses in mitochondria. SIRT4 contains an N-terminal mitochondrial signal sequence that directs of its localization to mitochondria (4). SIRT4 has the requisite amino acids to participate in deacylase reactions (5), namely, a homologous sirtuin deacylase domain, a conserved catalytic histidine, and a Rossmann fold/NAD^+-binding motif (aa 62–82, 143–146, 260–262, and 286–288) (**Figure 1A**). Previous genetic studies divide human sirtuins into four classes. SIRT4 belongs to the class II human sirtuins (**Figure 1C**). SIRT4 mRNA and protein are detected in human muscle, kidney, testis, and liver cells (6). This expression pattern is consistent with the functions of SIRT4. In this review, we summarize the roles of SIRT4 in cellular metabolic homeostasis, including the regulation of insulin secretion and fatty acid oxidation and the effect on tumor cells of different tissues (**Table 1**).

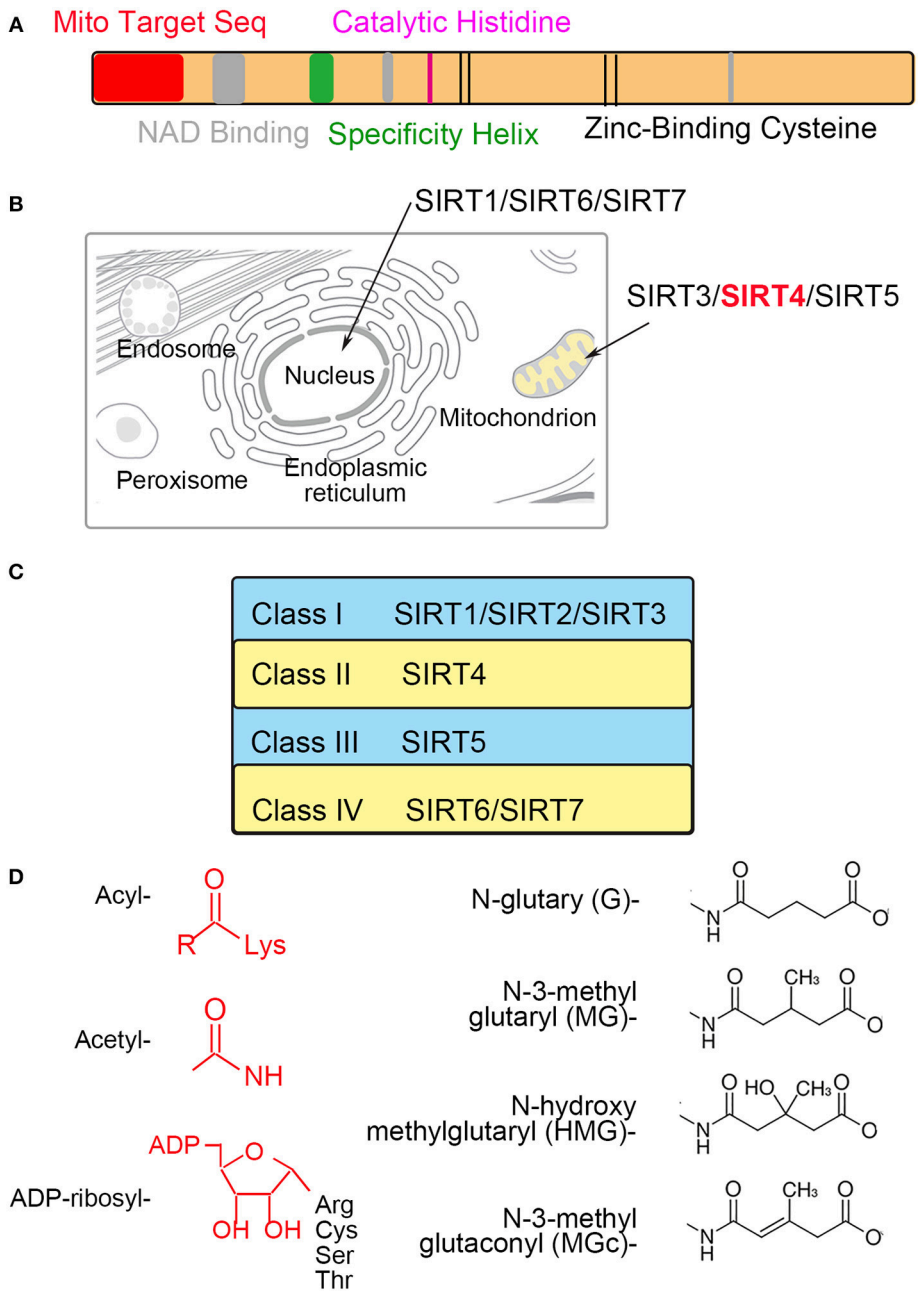

FIGURE 1 | The characteristics of mitochondrial SIRT4 **(A).** Sequence analyses show the amino acid sequence of the mitochondrial localization, NAD^+-binding, substrate specificity, catalytic and Zn^{2+}-binding domains **(B)**. SIRT4 is localized in the mitochondrion **(C)**. SIRT4 is belong to a class II human sirtuins **(D)**. Structure of groups catalyzed by SIRT4.

SIRT4 INHIBITS INSULIN SECRETION IN PANCREATIC β CELLS

SIRT4 was initially found to reduce glutamate dehydrogenase (GDH) activity, thereby inhibiting insulin secretion in pancreatic β cells (7, 9). GDH is known to facilitate glutamine metabolism and ATP production, thus inducing insulin secretion (19, 20). GDH is ADP-ribosylated and inhibited by SIRT4, subsequently repressing leucine-mediated insulin secretion (7, 21). The depletion of SIRT4 in insulinoma cells could activate GDH, thus increasing amino acid-stimulated insulin secretion. Pancreatic β cells derived from SIRT4 knockout mice and those from mice on calorie restriction (CR) as a dietary regimen show a similar effect of insulin secretion (8, 22). Phosphodiesterase (PDE) can be used as a probe for ADP-ribosylation because it cleaves the ADP-ribose and can relieve inhibition. GDH from

TABLE 1 | Summary of roles of SIRT4 in celluar metabolism.

Genotype	Phenotype	Process	Activities	References
SIRT4 OE cells	Inhibition of insulin secretion in pancreas β cells	ADP-ribosylation and inhibition of GDH	ADP-ribosylation	(6–8)
SIRT4 KO cells	Activation of GDH and stimulation of insulin secretion in insulinoma cells	Activation of GDH	ADP-ribosylation	
SIRT4 KO mice	Increased insulin secretion,	Activation of GDH	ADP-ribosylation	
SIRT4 KO mice	Deregulated lecuine metabolism and aging induced IR	Dysregulated leucine oxidation	deacylation	(9–11)
SIRT4 OE cells	Increased lipogenesis and decreased FAO in adipocyte and myocyte cell	Deacetylation of MCD	deacetylation	(12–15)
SIRT4 KO cells	Increased FAO in muscle and WAT cells	Activation of MCD and decreased malony-CoA	Deacetylation	
SIRT4 KO mice	Elevated FAO and resistance to obesity and exercise	Activation of GDH	Deacetylation	
SIRT4 OE cells	Depressed FAO in liver cells	Repression of PPARα transcriptional activation by SIRT1	–	(12, 16–18)
SIRT4 KO cells	Increased FAO rate	Activation of PPARα	–	
SIRT4 KO mice	Increased FAO rate and PPARα pathway	Activation of FAO by PPARα and SIRT1	–	

OE, over expression; KO, knock out.

SIRT4-deficient mice is insensitive to (PDE), and incubation with PDE increases the GDH activity of wild-type lysates to the KO samples. It indicates the absence of the enzymatic cleavage of ADP-ribose would decrease ADP-ribosylation of GDH (6, 8).

A recent study demonstrated that SIRT4 catalyzed the removal of novel lysine modifications: methylglutaryl (MG)-lysine, hydroxymethylglutaryl (HMG)-lysine, and 3-methylglutaconyl (MGc)-lysine (10, 11). SIRT4 participates in leucine oxidation by removing these modifications sequentially. These modifications were discovered and characterized through phylogenetic and structural analysis (**Figure 1D**). The α-helical region containing the catalytic pocket of SIRT4 was associated with an interaction with negatively charged acyl- modifications (23, 24). In a complementary study, a recombinant SIRT4 protein could remove glutaryl-, MG-, HMG-, and MGc-lysine modifications. Further, SIRT4 overexpression in cells resulted in decreased glutaryl-, MG-, and HMG-lysine expression levels. The methylcrotonyl-CoA carboxylase complex (MCCC) associated with leucine catabolism and interacting with SIRT4 was marked with these new modifications *in vivo* (10). The absence of SIRT4 increased and destabilized MCCC acylation, leading to decreased leucine flux. The level of a key allosteric GDH activator, leucine, was changed due to the repression of GDH activity and glutamine metabolism by SIRT4 (25, 26). Knowledge about these new modifications provided new insights into SIRT4 function in further metabolism studies (**Figure 2**).

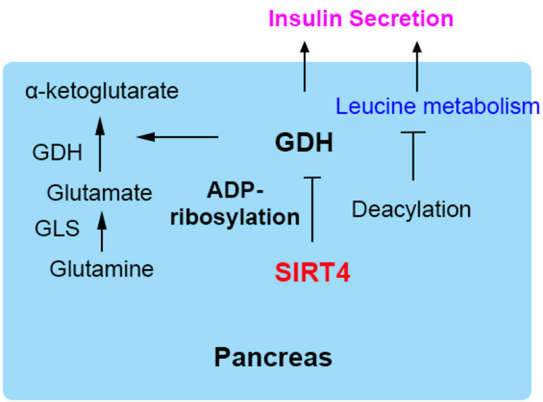

FIGURE 2 | SIRT4 regulates insulin secretion in pancreatic β cells. GDH is ADP-ribosylated and inhibited by SIRT4, repressing insulin secretion.

SIRT4 INHIBITS FATTY ACID OXIDATION IN MUSCLE AND FAT CELLS

The repressive effect of SIRT4 against FAO in muscle cells is regulated by the deacetylation and inhibition of the activity of mitochondrial malonyl-CoA decarboxylase (MCD), leading to an increase in malonyl-CoA (12, 13, 27). Malonyl CoA is a key metabolite that inhibits fat catabolism and promotes fat synthesis (28). There are two enzymes that regulate cellular malonyl-CoA levels. MCD catalyzes the conversion of malonyl-CoA to acetyl-CoA, while acetyl-CoA carboxylase (ACC) converts acetyl-CoA back to malonyl-CoA via a reaction regulated by the phosphorylation of AMPK (14, 29). Cytosolic fatty acids transported into the mitochondrial matrix cross the outer and inner mitochondrial membranes for β-oxidation, and this key step is catalyzed by carnitine palmitoyltransferase (CPT1), which is inhibited by accumulated malonyl-CoA (30, 31). During the nutritional rich state (Fed), when the metabolic intermediates funnel into fat synthesis and energy storage, the steady state is increased malonyl-CoA. This suppresses entry of fatty acids into mitochondria and weakens FAO sequentially (15). Conversely, in the fasted state, the malonyl-CoA is decreased and the FAO is evaluated for energy production (**Figure 3**). Therefore, SIRT4 regulates malonyl-CoA levels of muscle and WAT of mice in

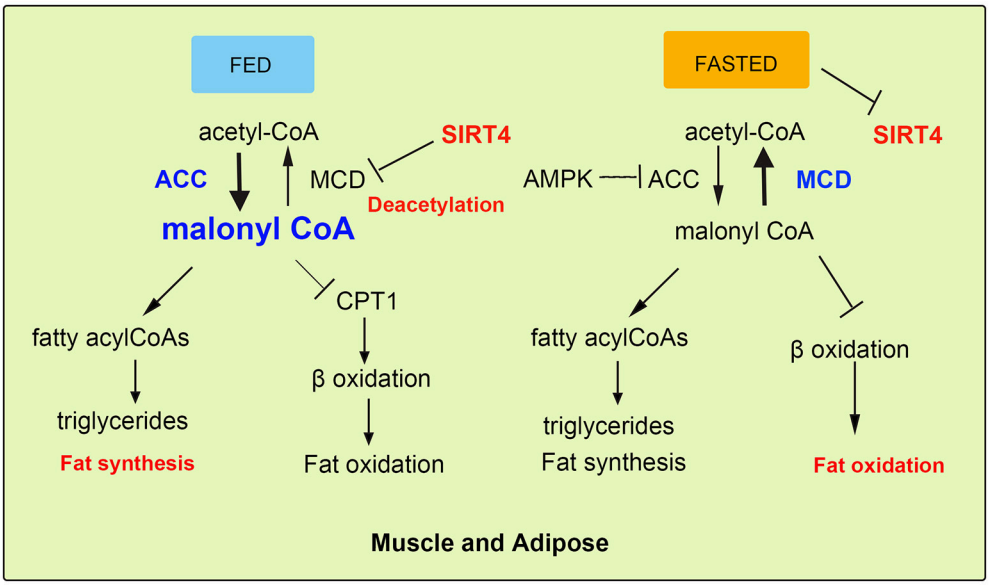

FIGURE 3 | SIRT4 mediates fatty acid oxidation in muscle and adipose cells. In fed state, SIRT4 decreases the activity of the mitochondrial malonyl-CoA decarboxylase (MCD), which can increase malonyl-CoA levels, thus inhibiting FAO. In fasted state, the level malonyl-CoA of is decreased conversely.

the fed and fasted state. In addition, as a unit of fatty acid synthesis, the accumulated malonyl-CoA promotes and provides a carbon skeleton for fatty acid biosynthesis in white adipose tissue (WAT) (32, 33). A previous study reported that SIRT4 overexpression increased lipogenesis and decreased fatty acid oxidation, while SIRT4 knockdown showed the opposite effect on lipid synthesis and catabolism in mouse adipocyte and myocyte cell lines (3, 34). Furthermore, the regulation pattern was confirmed in SIRT4 knockout mice, which showed elevated FAO associated with a resistance to diet-induced obesity and an increased exercise tolerance (24, 35, 36). Additionally, the interaction of MCD and SIRT4 in the mitochondrial matrix was demonstrated by coimmunoprecipitation. SIRT4 lacked detectable histone deacetylase activity, but it deacetylated MCD in a substrate-specific manner (37, 38). Further, mitochondrial located SIRT3 was confirmed and excluded the possibility of deacetylation activity, because interaction between SIRT3 and SIRT4. SIRT3 showed no detectable interaction with MCD indicating the specific function of SIRT4. SIRT4 coordinated lipid homeostasis by promoting lipid anabolism and repressing lipid catabolism (39, 40).

SIRT4 REPRESSES HEPATIC FAO IN LIVER CELLS

Liver plays a role in energy homeostasis by balancing lipid metabolism and energetic demands in organisms (12, 16). Under nutrient-rich conditions, hepatic lipogenesis, and lipoprotein secretion are activated. In contrast, during fasting, hepatic FAO is stimulated to provide the organism with ketone bodies as cellular energy fuel for the brain. One of the key mediators of the hepatic response to fasting is nuclear receptor peroxisome proliferation-activated receptor α (PPARα). SIRT4 negatively mediated fatty acid oxidation in liver cells by suppressing the transcriptional activity of PPARα (17, 41). More specifically, the interaction of SIRT1 and PPARα was disrupted by SIRT4, thus attenuating the activation of PPARα transcriptional activity via SIRT1 to inhibit FAO (18). In normal liver cells, by interacting with PPARα, SIRT1 is recruited to the PPARα response element (PPRE), which catalyzes the N^{ε}-acetyl-lysine deacetylation of the transcriptional coactivator peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) to promote FAO (42). Additionally, SIRT4 competes with other sirtuins including SIRT1 for β-NAD^{+}, leading to decreased SIRT1 activity and a reduced effect of SIRT1 on both the transcriptional activity of PPARα and FAO (43, 44). During fasting, FAO is promoted as a source of cellular energy. The decreased SIRT4 levels in liver cells demonstrate the inhibitory effect of SIRT4 on liver FAO (45).

The roles of SIRT4 in liver cells were supported by knockout and overexpression experiments in cellular and animal models. In SIRT4 knockdown and knockout primary hepatocytes, the expression of mitochondrial and fatty acid metabolism enzyme genes was increased significantly (17). Consistent with increased FAO gene expression, SIRT4 knockdown hepatocytes exhibited higher rates of FAO than wild-type cells. The increase in FAO gene expression in SIRT4 knockout mice was consistent with the results in primary hepatocytes (12). Additionally, SIRT1 mRNA and protein levels were also evaluated both *in vitro* and *in vivo* by the knockdown of SIRT4. In SIRT4/SIRT1 double-knockdown hepatocytes, there was not an increase in FAO compared with that induced by SIRT4 knockdown only (17). These results suggested that SIRT4-modulated FAO was dependent on SIRT1 in primary hepatocytes (22, 46). Moreover, SIRT4 overexpression

repressed SIRT1 activation of PPARα and consequently inhibited hepatic FAO (**Figure 4**).

SIRT4 PARTICIPATES IN CELLULAR ATP HOMEOSTASIS

In addition to regulating FAO, SIRT4 was shown to contribute to cellular ATP homeostasis and mitochondrial biogenesis. Deletion of SIRT4 decreased ATP levels, and overexpression of SIRT4 increased ATP levels (3, 47). ATP/ADP translocase 2 (ANT2a, transmembrane protein spanning the inner mitochondrial membrane) helped SIRT4 mediate cellular ATP homeostasis. Mechanistically, ANT N^{ε}-acylation facilitated mitochondrial respiration by enhancing mitochondrial uncoupling and decreasing ATP production (48). Therefore, SIRT4 catalyzed the N^{ε}-acyl-lysine deacylation of ANT2 to reduce mitochondrial uncoupling, leading to enhanced ATP production. In the mitochondrial signaling pathway, the depletion of SIRT4 activated AMPK to decrease ATP levels. Activated AMPK phosphorylates and inhibits cytosolic ACC, leading to a reduction in MCA and the promotion of FAO as mentioned above.

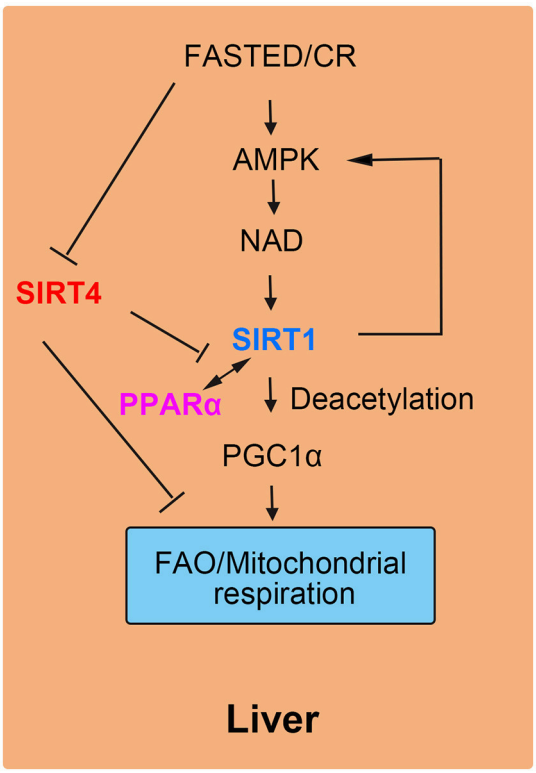

FIGURE 4 | SIRT4 mediates fatty acid oxidation in liver cells. The interaction of SIRT1 and PPARα is blocked by SIRT4, leading to a decrease in FAO.

SIRT4 ACTS AS A TUMOR SUPPRESSOR

SIRT4 is necessary for the regulation of FAO in normal cells but is also identified as a mitochondrial-localized tumor suppressor (49) (**Table 2**). SIRT4 has been recognized to possess tumor-suppressive effects due to the crucial regulatory role of mitochondrial metabolism in tumourigenesis (34, 50).

SIRT4 plays an important role in the response to DNA damage by regulating the DNA damage-induced inhibition of glutamine catabolism (4, 57, 59). DNA damage increases the flux through the pentose phosphate pathway and decreases glutamine uptake and the levels of TCA cycle intermediates. Mitochondrial glutaminase can catabolize glutamine to form glutamate via mitochondrial GDH and aspartate aminotransferase activity (60). Previous research showed that SIRT4 ADP-ribosylated and inhibited GDH and was thus involved in DNA damage-induced inhibition of glutamine metabolism and anaplerosis (33, 39).

SIRT4 possesses a tumor suppressive effect because of its inhibitory effect on glutamine catabolism and its antiproliferative

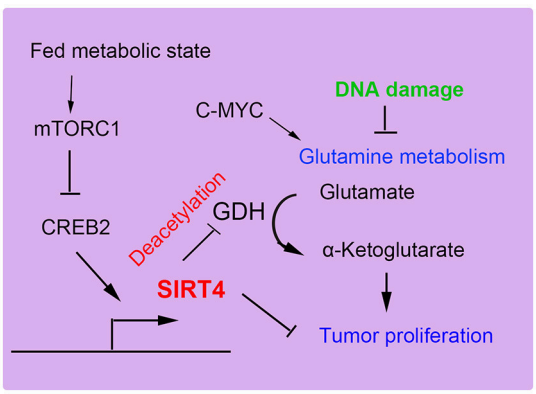

FIGURE 5 | SIRT4 plays a role in tumor suppression. SIRT4 suppresses tumor proliferation by inhibiting glutamine metabolism and DNA damage repair.

TABLE 2 | Summary tumor repressor roles of SIRT4.

Phenotype	Type of tumor	Roles	References
SIRT4 is decreased	Acute myeloid leukemia	Inhibiting proliferation of tumor	(50, 51)
SIRT4 is decreased	Burkitt lymphoma	Inhibiting proliferation of tumor	(52, 53)
SIRT4 is decreased	Colorectal cancer	Inhibiting proliferation and migration of tumor	(54, 55)
SIRT4 is decreased	Gastric cancer	Inhibiting proliferation and migration of tumor	(56)
SIRT4 is decreased	Lung tumor	Inhibiting proliferation and migration of tumor	(57, 58)

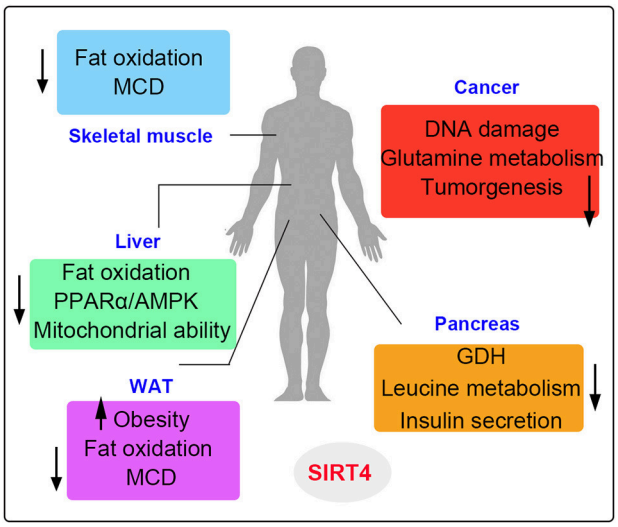

FIGURE 6 | The metabolic roles of SIRT4 in various organs. SIRT4 is mainly involved in the metabolism of muscle, liver, fat, pancreatic, and cancer cells. The up arrows indicate the increased regulation pathways and the down arrows indicate the decreased regulation pathways induced by SIRT4.

effect on cells with DNA damage (61, 62). Indeed, SIRT4 expression is upregulated by DNA damage and is downregulated in many types of human tumor tissues and cells (44). SIRT4 depletion leads to both elevated glutamine-dependent proliferation and stress-induced genomic instability, resulting in tumourigenic phenotypes. SIRT4 inhibits glutamine catabolism, especially replenishment into the citric acid cycle, creating a cellular state promoting DNA damage repair (63). In the absence of SIRT4, DNA damage results in delayed DNA repair and increased chromosomal aneuploidies, suggesting that SIRT4 could protect cells from spontaneous damage. Furthermore, SIRT4 null mice spontaneously develop lung tumors (19, 64).

SIRT4 can specifically repress the growth of B cells induced by the transcriptional factor c-Myc through inhibiting glutamine metabolism induced by the abnormal activation of c-Myc in c-Myc-dependent cancers (52, 65). In human Burkitt lymphoma cells, the overexpression of SIRT4 repressed glutamine metabolism and glutamine-dependent cell proliferation, as observed in cells treated with glycolysis inhibitors, promoting cell death (66). Consistent with these findings, the depletion of SIRT4 resulted in increased glutamine-absorbing and GDH enzymic activity in a mouse model of Burkitt lymphoma induced by c-Myc dysregulation. Additionally, SIRT4 suppressed Burkitt lymphoma driven by c-Myc, independent of c-Myc activity. Furthermore, SIRT4 was found to be overexpressed in colorectal cancer cell lines and to increase E-cadherin expression, which resulted in the suppression of cell proliferation and invasion (59). During the development of colorectal cancer invasion, SIRT4 expression was decreased. The suppressive role of SIRT4 is also mediated by inhibiting glutamine metabolism in colorectal cancer (54). Moreover, SIRT4 could enhance the sensitivity of colorectal cancer cells to the chemical drug 5-fluorouracil by inhibiting the cell cycle, thus showing the antiproliferative effect of SIRT4 overexpression (55). Recent reports have demonstrated that mammalian target of rapamycin complex1 (mTORC1) is correlated with increased nutrient uptake and metabolism. mTORC1 promotes glutamine supplement by activating GDH required transcriptional repression of SIRT4. Specifically mTORC1 suppresses SIRT4 by destabilizing of cAMP-responsive element binding 2 (CREB2) (67). Overexpression of SIRT4 decreases cell growth, transformation and development of tumor. Furthermore, leucine is an important positive regulator of mTORC1, which activity may be regulated by SIRT4 by reducing intracellular leucine levels (**Figure 5**). Therefore, as a glutamine steward, SIRT4 acts as a necessary component of the DNA damage response pathway that manages the metabolic blockade of glutamine metabolism, the cell cycle, and tumor suppression.

CONCLUSION

In this review, we summarize different roles of SIRT4 in terms of enzymatic activities and functions in specific tissues and cells. SIRT4 possesses ADP-ribosyltransferase, lipoamidase and deacylase activities (46, 68, 69). Importantly, the enzymatic activities, and substrates of SIRT4 vary in different tissues and cells (**Figure 6**). SIRT4 inhibits insulin secretion as an ADP-ribosyltransferase and regulates insulin sensitivity as a deacylase in the pancreas. SIRT4 represses fatty acid oxidation (FAO) in the muscles and livers differently. SIRT4 has also been identified as a mitochondrial-localized tumor suppressor. SIRT4 is a less well-understood mammalian sirtuin, its cellular functions require further discovery. Knowledge of roles SIRT4 in cellular metabolism is helpful to provide new insights in the potential development of therapeutic agents for human diseases by targeting enzymatic activities (36).

AUTHOR CONTRIBUTIONS

ZM and JG draft the original manuscript. YY proofed and edited the finalized manuscript.

FUNDING

This work was supported in part by the National Key R&D Program of China (2016YFC1000601) and the National Natural Science Funds for the general program (81571400, 81771580).

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Endocrinology

ORIGINAL RESEARCH
published: 19 December 2018
doi: 10.3389/fendo.2018.00770

17β-Estradiol Promotes Apoptosis in Airway Smooth Muscle Cells Through CD38/SIRT1/p53 Pathway

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17β-Estradiol (E2) is the major estrogen secreted by the premenopausal ovary and shows dual effects on cell apoptosis under pathological conditions. E2 was previously shown to increase CD38 mRNA and protein expression in myometrial smooth muscle, but its function and mechanism remain largely unknown. Here we investigated the role of E2 in hypoxia-induced apoptosis in mouse airway smooth muscle cells (ASMCs) and explored the underlying mechanisms. Results showed that E2 significantly increased CD38 expression at both mRNA and protein levels, accompanied with decreased SIRT1 levels in ASMCs. By using primary ASMCs from the wild type (WT) and the smooth muscle-specific CD38 knockout (CD38 KO) mice, we found that the down-regulation of SIRT1 induced by E2 was abolished in CD38 KO AMSCs. E2 promoted the acetylation of p53 in WT cells, and this effect was also diminished in the absence of CD38. In addition, E2 further activated CD38/SIRT1/p53 signal pathway and promoted cell apoptosis during hypoxia. However, these effects were reversed in CD38 KO ASMCs and by the specific SIRT1 activator Resveratrol. We also found that E2 enhanced CD38 expression through estrogen receptor. The data suggested that CD38 is a direct target for E2 which promotes hypoxia-induced AMSC apoptosis through SIRT1/p53 signal pathway.

Keywords: 17β-estradiol, CD38, SIRT1, hypoxia, apoptosis

OPEN ACCESS

Edited by:
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Specialty section:
This article was submitted to Cellular Endocrinology, a section of the journal Frontiers in Endocrinology

Received: *30 July 2018*
Accepted: *06 December 2018*
Published: *19 December 2018*

Citation:
Liu Y, Guo Y, Huang W, Deng K-Y, Qian Y and Xin H-B (2018) 17β-Estradiol Promotes Apoptosis in Airway Smooth Muscle Cells Through CD38/SIRT1/p53 Pathway. Front. Endocrinol. 9:770. doi: 10.3389/fendo.2018.00770

INTRODUCTION

Hypoxia is recognized as a critical contributor to pulmonary diseases including asthma, airway obstruction and pulmonary hypertension (1–3). Hypoxia stimulates airway inflammation and remodeling, and subsequently induces apoptosis in airway smooth muscle cells (ASMCs) during airway remodeling (4). There is emerging evidence for sex differences in the incidence and progression of lung diseases, and sex hormones play crucial roles in these pathological processes (5). Especially, estrogen regulates ASMCs in various manners. Estrogens reduce $[Ca^{2+}]i$ and promote human ASM relaxation via activation of cAMP and PKA, thereby facilitating bronchodilation (6). In addition, testosterone and E2 exhibit mitogenic effects in ASMCs, probably through estrogen receptors and the MAPK and PI3K signaling pathways, thus promote ASMC proliferation and airway remodeling (7). Estrogen signaling is also involved in allergic inflammation and contributes to sex differences in asthma and allergy (8). However, the effect of estrogen on ASMCs apoptosis during hypoxia remains largely unknown.

CD38 is a type II membrane-bound glycoprotein and functions as the major NADase responsible for the regulation of NAD-dependent deacetylase such as SIRT1 (9). In addition, CD38

is an NAADP synthase required for NAADP-mediated Ca^{2+} release from lysosomal stores (10). CD38/cyclic ADP-ribose (cADPR)-mediated calcium signaling plays critical roles in the regulation of intracellular calcium in a variety of smooth muscle cells, including that of the airway smooth muscle (11–13). Estrogens were shown to increase CD38 gene expression and leads to increased calcium mobilization and contractility of the myometrium (14, 15). It has also been recently reported that E2 downregulated SIRT1 expression in vascular smooth muscle cells, with increased apoptosis, reduced proliferation and migration, which were reversed by the SIRT1 activator Resveratrol (16). SIRT1 regulates p53-dependent apoptosis by deacetylating the Lys382 residue of p53, thus enhancing the transcriptional activity of p53 and inhibiting p53-induced apoptosis (17). However, whether E2 modulates the expression of CD38 and SIRT1 in ASMCs and the detailed mechanisms of E2 in the regulation of hypoxia-induced apoptosis have not been addressed.

In this study, we investigated the role of E2 in apoptosis during hypoxia by using primary ASMCs from the wild type (WT) and the smooth muscle-specific knockout of CD38 (CD38 KO) mice. CD38-mediated SIRT1/p53 signal pathway was also detected, with the purpose to elucidate the mechanism by which E2 promotes apoptosis in ASMCs.

MATERIALS AND METHODS

Materials

E2 and Resveratrol were purchased from Sigma-Aldrich (St. Louis, MO). ICI182,780 was from Abcam (Cambridge, MA). The anti-CD38 antibody was obtained from R&D Systems, Inc. (Minneapolis, MN); the anti-SIRT1 antibody was from EMD Millipore Corp. (Temecula, CA); the anti-p53, anti-Acetyl-p53 (K379), anti-Bax and anti-Bcl-2 antibodies were from Cell Signaling Technology, Inc., (Danvers, MA), and the anti-glyceraldehyde phosphate dehydrogenase (GAPDH) antibody was obtained from KangChen Bio-tech Inc., (Shanghai, China).

Preparation of Smooth Muscle-Specific CD38 Knockout Mice

Mice with LoxP flanking of exon 2 and exon 3 of the CD38 gene (CD38-fl/fl, produced by Cyagen Inc., Suzhou, China) were bred with mice expressing Cre recombinase under the control of a smooth muscle-specific promoter (SMA-Cre, from Collaborative Innovation Center of Model Animal, Wuhan University). The progeny with the genotype SMA-Cre-CD38-fl/fl is the homozygote used in the experiment.

Isolation, Culture, and Characterization of ASMCs

Primary mouse ASMCs were prepared as previously described (18), with some modifications. Male, 8–10 weeks old WT or CD38 KO mice were anesthetized and the tracheas were aseptically excised and placed in Ca^{2+}, Mg^{2+}-free Hanks' balanced salt solution (HBSS). The isolated tracheas were cleaned of connective tissues, cut longitudinally through the cartilage, and enzymatically dissociated with HBSS containing elastase type I (2 mg/ml) and BSA (2.5 mg/ml) for 1 h in a water bath at 37°C. Dissociated cells in suspension were centrifuged and resuspended in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin, 100 μg/ml streptomycin, and 2.5 μg/ml amphotericin β. Cells were plated on culture flasks and grew until confluence at 37°C in humidified air containing 5% CO_2. The confluent cells were passaged with 0.25% trypsin-0.02% EDTA solution. The cultures typically contained more than 98% ASMCs as assessed by immunocytochemical staining for the smooth muscle-specific marker α-actin. Cells at passages 3–5 were used for the experiments.

Cell Culture Treatment and Hypoxia Exposure

For the concentration response assay, ASMCs were pre-treated with various concentrations of E2 (0.1, 1, 10, and 100 nM) for 24 h. For the time course assay, 10 or 100 nM of E2 were added to the cultures for 24 or 48 h incubation. In the subsequent experiments, WT and CD38 KO ASMCs were pre-treated with 10 nM of E2 for 48 h respectively, followed by the exposure of sustained hypoxia. Cells were maintained in a hypoxia chamber (1% O_2, 5% CO_2; balance N_2 and water vapor) for 6 h to induce sustained hypoxia as described previously (19). A normoxic control experiment was performed in parallel by maintaining the cells under normoxia (21% O_2, 5% CO_2; 37°C). The specific SIRT1 activator Resveratrol (RSV, 10 μM) or the estrogen receptor antagonist ICI182,780 (ICI, 10 nM) was added to the cultures 2 h before E2 incubation.

Real-Time PCR

Total RNA was isolated from ASMCs using the TRIzol™ reagent (Life Technologies, CA, USA) according to the manufacturer's instructions. One microgram of total RNA was reverse-transcribed using a One Step PrimeScript™ RT-PCR Kit (Takara, Dalian, China) with a thermocycler. Real-time PCR was performed using the ABI ViiA™ 7 system with a reaction mixture that consisted of SYBR Green 2×PCR Master Mix (Applied Biosystems, CA, USA), cDNA template (0.5 μg), forward primer and reverse primer. Primer sequences were as follows: 5′-GAGCCTACCACGAAGCACTTTT-3′ and 5′-GGC CGGAGGATCTGAGTGTA-3′ (CD38), 5′-GCCAAACTTTGT TGTAACCCTGTA-3′ and 5′-TGGTGGCAACTCTGATAAATG AA-3′ (SIRT1), and 5′-ACATGGCCTCCAAGGAGTAAGAA-3′ and 5′-GGGATAGGGCCTCTCTTGCT-3′ (GAPDH). The PCR protocol consisted of 40 cycles of denaturation at 95°C for 15 s followed by 60°C for 1 min to allow extension and amplification of the target sequence. Data were analyzed using ABI 7500 sequence detection system software. The amount of mRNA was normalized to GAPDH using the $2^{-\Delta\Delta CT}$ method. The results were from three independent experiments performed in triplicate.

Western Blot

The cells were collected and lysed in RIPA lysis buffer. Equal amounts of protein per sample were loaded in each lane, separated by SDS-PAGE, and transferred to PVDF membranes.

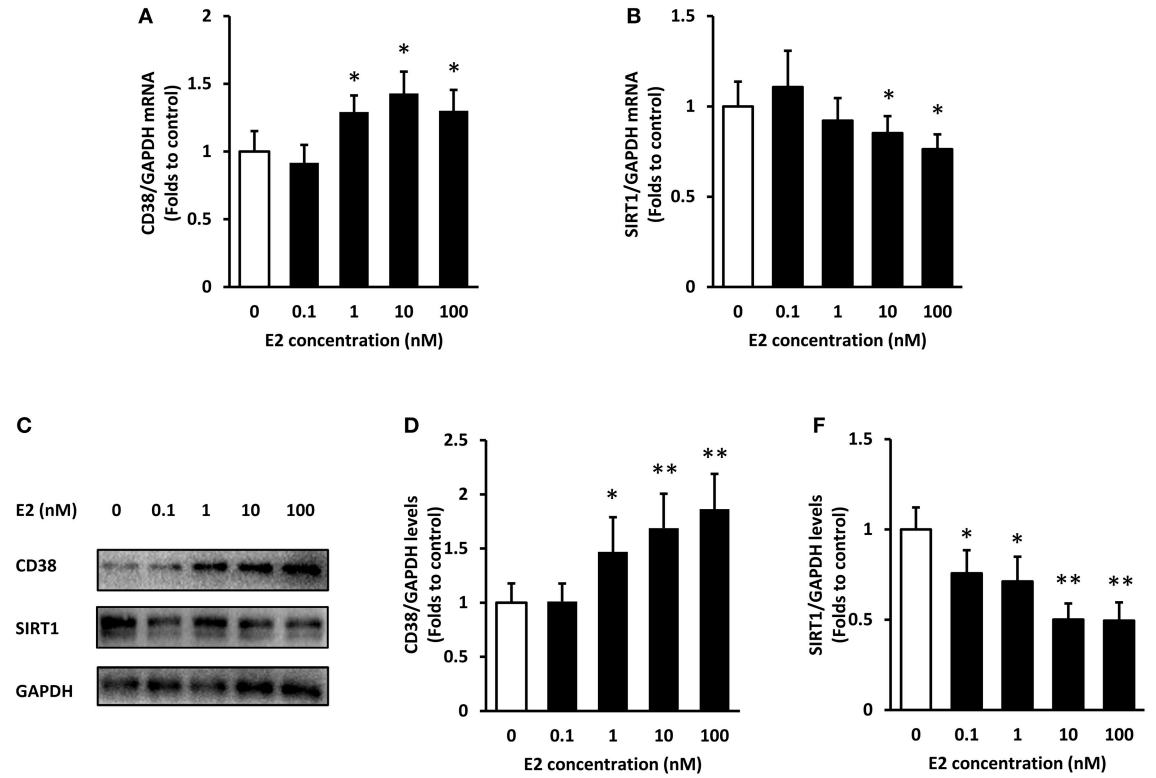

FIGURE 1 | Expression of CD38 and SIRT1 in ASMCs after E2 treatment. ASMCs were pre-treated with the indicated concentrations of E2 for 24 h. **(A)** CD38 and **(B)** SIRT1 mRNA levels were detected by real-time PCR. **(C)** CD38 and SIRT1 protein levels were determined by western blot and quantitative analysis of **(D)** CD38 and **(E)** SIRT1 levels was normalized to GAPDH levels. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. the control group. $N = 3$.

The membranes were blocked with skimmed milk for 1 h, washed in Tris buffered saline containing 0.1% Tween-20 (TBST) and incubated overnight with the primary antibodies. After washing three times with TBST, the membranes were incubated for 1 h at room temperature with horseradish peroxidase-conjugated goat anti-rabbit or anti-mouse IgG and donkey anti-sheep IgG. Bands were visualized using the SuperSignalWest Pico Chemiluminescent Substrate Trial Kit (Pierce, Rockford, IL, USA). Images were taken using the ChemiDoc XRS system with Quantity One software (Bio-Rad, Richmond, CA, USA).

Hoechst 33258 Staining

Cell apoptosis was detected with DNA staining by Hoechst 33258. At the end of the treatment, cells were rinsed with phosphate-buffered saline (PBS, pH 7.4) and fixed with 4% paraformaldehyde for 30 min at room temperature, followed by incubation with Hoechst 33258 (5 μM, final concentration) at room temperature for 20 min. Fluorescence images were examined under the fluorescence microscope (Olympus IX71, Tokyo, Japan).

Caspase-3 Activity Assay

Caspase-3 activity was measured in lysates of AMSCs using the CaspACE^{TM} Assay System, Colorimetric (Promega, Madison, WI) following the instructions of the manufacturer. Briefly, cells were lysed by freeze-thaw, and then incubated on ice for 20 min to ensure complete cell lysis. Cell lysates were centrifuged at 12 000 rpm for 10 min at 4°C, and the supernatant fraction was collected for the determination. An aliquot of culture supernatant was incubated with 200 mM of DEVDpNA substrate at 37°C for 4 h. The absorbance was measured at 405 nm. The luminescence was measured in a microplate reader and the protein levels in the lysates were determined by the method of Bradford. Results were expressed as a percentage of the control cells.

Statistical Analysis

All values are expressed as the mean ± SD of at least three independent preparations. Differences among the groups were compared using one-way ANOVA analysis followed by a Tukey *post-hoc* test. A difference with $P < 0.05$ was considered statistically significant.

RESULTS

E2 Increases CD38 Expression and Decreases SIRT1 Levels in ASMCs

The expression of CD38 and SIRT1 at mRNA and protein levels were detected by real-time PCR and western blot, respectively. Non-normalized Ct values and non-cropped non contrasted western-blot images were provided in **Supplementary Material**.

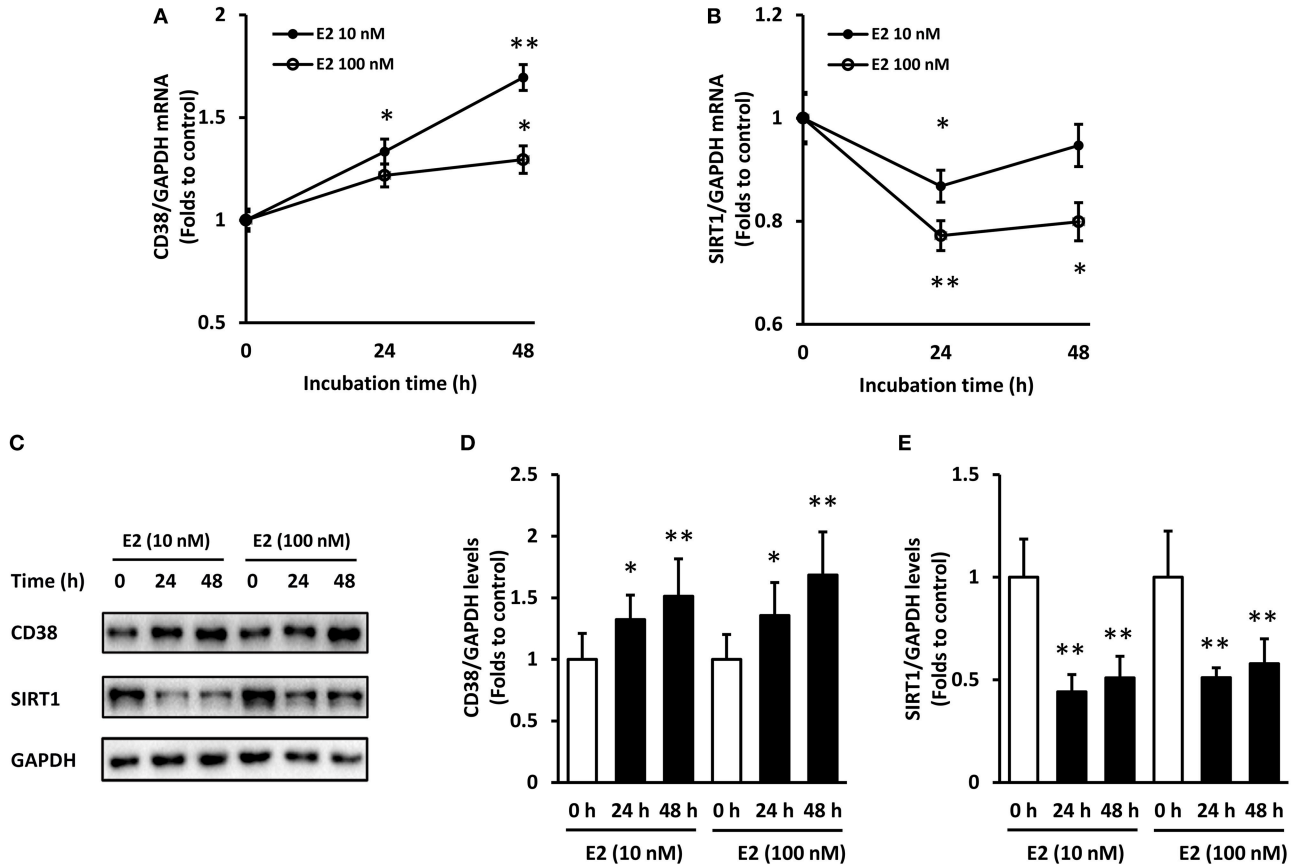

FIGURE 2 | Time course of CD38 and SIRT1 expression in ASMCs with E2 treatment. ASMCs were pre-treated with 10 or 100 nM of E2 for 24 h and 48 h respectively. **(A)** CD38 and **(B)** SIRT1 mRNA levels were detected by real-time PCR. **(C)** CD38 and SIRT1 protein levels were determined by western blot and quantitative analysis of **(D)** CD38 and **(E)** SIRT1 levels was normalized to GAPDH levels. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. the control group. $N = 3$.

Firstly, ASMCs were pre-treated with various concentrations (0.1, 1, 10, and 100 nM) of E2 for 24 h. The mRNA levels of CD38 raised with the increase of E2 concentration, and the expression achieved maximum at 10 nM. There were no significant differences in CD38 expression between the 10 and 100 nM group (**Figure 1A**). By contrast, SIRT1 mRNA levels significantly decreased by the treatment of E2 at 10 and 100 nM (**Figure 1B**). In accordance with the PCR results, CD38 protein levels elevated whereas SIRT1 levels dropped in the presence of E2 in a concentration-dependent manner (**Figures 1C–E**).

In time course experiments, E2 at 10 and 100 nM were added to ASMCs for 24 and 48 h incubation, respectively. Results showed that CD38 mRNA continued to increase within 48 h and the effect was stronger in the 10 nM E2-treated group (**Figure 2A**). SIRT1 mRNA levels decreased at 24 h but partly restored at 48 h time point (**Figure 2B**). The protein levels of CD38 and SIRT1 showed a significant negative correction at 24 and 48 h, and there were no statistical differences between the 10 and 100 nM groups (**Figures 2C–E**). Therefore, pre-treatment with 10 nM of E2 for 48 h were selected for the subsequent experiments.

E2 Acts on SIRT1/p53 Signaling Through CD38 in ASMCs

We used the WT and CD38 KO ASMCs to confirm whether E2 affects SIRT1 expression through CD38. E2 promoted CD38 expression in WT ASMCs as expected (**Figures 3A,B**). The levels of SIRT1 were down-regulated by E2 in WT group compared with the vehicle treated cells. CD38 deficiency induced a marked increase in SIRT1 protein levels compared with the WT group, but this increase was not reversed by E2 treatment (**Figures 3A,C**).The acetylation of p53, one of the downstream targets of SIRT1, was assayed. In WT ASMCs, E2 increased the Ac-p53 levels, which were not changed in CD38 KO cells. The expression of p53 was not significantly altered (**Figures 3A,D**). These results indicated that E2 suppressed SIRT1/p53 signaling directly through CD38.

CD38 Deficiency Reverses the Effect of E2 on SIRT1/p53 Pathway During Hypoxia

We further investigated the role of E2 during hypoxia in WT and CD38 KO ASMCs. Hypoxia exposure induced an obvious down-regulation of CD38 mRNA, which was inhibited by E2

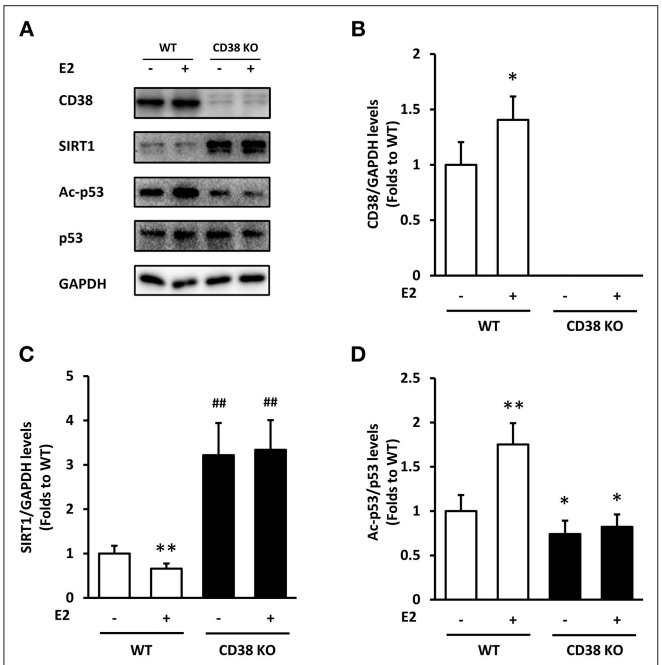

FIGURE 3 | The effects of E2 on SIRT1/p53 signal pathway in WT and CD38 KO ASMCs. **(A)** CD38, SIRT1, p53, and Ac-p53 levels were determined by western blot. Quantitative analysis of **(B)** CD38 and **(C)** SIRT1 levels was normalized to GAPDH levels, and **(D)** Ac-p53 levels were normalized to total p53 levels. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. the WT control group. $^{\#\#}P < 0.01$ vs. the E2-treated WT group. $N = 3$.

treatment in WT cells (**Figure 4A**). SIRT1 mRNA reduced after hypoxia, and E2 further suppressed its expression in WT ASMCs. However, the effect of E2 on SIRT1 expression was abolished in the absence of CD38 (**Figure 4B**). At protein levels, hypoxia resulted in an increase in CD38 and a decrease in SIRT1. E2 further promoted CD38 expression and suppressed SIRT1 levels. By contrast, the effect of E2 on SIRT1 disappeared in CD38 KO cells (**Figures 4C–E**). Hypoxia induced the acetylation of p53, which was also aggravated by E2 treatment. Accordingly, this effect was diminished in CD38 KO ASMCs (**Figures 4C,F**).

CD38/SIRT1 Signaling Attenuates E2-Mediated ASMC Apoptosis After Hypoxia

We examined the apoptosis in ASMCs following hypoxia exposure. Hoechst 33258 staining was employed to evaluate the nuclear condensation and characteristic features of apoptotic cells. In WT ASMCs, control cells showed intact, light blue nuclei whereas cells exposed to hypoxia displayed typical nuclear apoptotic morphology, as indicated by bright, condensed and rounded nuclei. The apoptotic cells significantly increased after E2 treatment. In addition, E2 did not induce ASMC apoptosis under normoxia. However, CD38 deficiency showed an obvious protection against hypoxia exposure, with a marked reduction in apoptosis, and E2 did not further promote apoptosis in CD38 KO AMSCs (**Figure 5A**). The percentage of apoptotic cells was quantified in **Figure 5B**. Bax and Bcl-2 are the major members of Bcl-2 family which play a key role in promoting and inhibiting intrinsic apoptotic pathway. Bax promotes cell death while Bcl-2 prevents apoptosis by inhibiting the activity of Bax (20). The Bax/Bcl-2 ratio was significantly increased after hypoxia and E2 further aggravated the ratio. However, CD38 deficiency showed a lower Bax/Bcl-2 ratio compared with WT and the pro-apotic effect of E2 was antagonized in CD38 KO cells (**Figures 5C,D**). The effects of E2 on caspase-3 activation was further measured following hypoxia exposure. The activity of caspase-3 was comparable between the control and E2-treated cells under normoxia. Hypoxia induced a 2.05-fold increase in caspase-3 activity, and E2 further promoted caspase-3 activation in hypoxic WT AMSCs. However, in CD38 KO ASMCs, a lowed caspase-3 activity was observed after hypoxia exposure both in the absence and presence of E2 (**Figure 5E**).

The specific SIRT1 activator Resveratrol (RSV) was employed to verify whether SIRT1 is essential in E2-induced ASMC apoptosis. Single treatment with E2 or E2 combined with RSV did not affect apoptosis under normal conditions. However, E2 induced-Bax/Bcl-2 ratio change and caspase-3 activation were significantly reversed by RSV following hypoxia (**Figure 6**). The above data confirmed that E2 promoted ASMC apoptosis via CD38/SIRT1 signaling.

E2 Enhanced CD38 Expression Through Estrogen Receptor

To verify whether estrogen receptors (ER) mediate the action of E2 on CD38 expression, we used compound ICI 182,780, an estrogen receptor antagonist with no partial agonist activity (21).Treatment with ICI182,780 significantly decreased CD38 mRNA and protein levels, and E2-indcued CD38 expression was completely abolished in the presence of ICI182,780 (**Figure 7**). This result suggested that E2 promotes CD38 expression through ER.

DISCUSSION

Here, we demonstrated that pretreatment with E2 significantly up-regulated CD38 expression and suppressed SIRT1 activation, thus increasing the acetylation of p53 in mouse ASMCs. E2 further exaggerated hypoxia-induced AMSC apoptosis while this effect disappeared in CD38 KO cells and in the presence of SIRT1 activator. By using the ER antagonist we also found that E2 enhanced CD38 expression through ER. These results suggested that E2 promotes apoptosis through CD38/SIRT1/p53 signaling pathway.

There is increasing evidence that sex differences exist in a variety of lung diseases including asthma and COPD, and sex steroids have complex effects in modulating the processes. For example, in adult women, the cyclical variations in sex steroid levels with the menstrual cycle may influence asthma symptoms. Worsening of symptoms usually occurs when estrogen levels reduce, suggesting that estrogens may be protective for asthma (22). However, Use of estrogen by hormone replacement therapy increases asthma symptoms and the risk of asthma onset (23). The confounding effects request much more research in

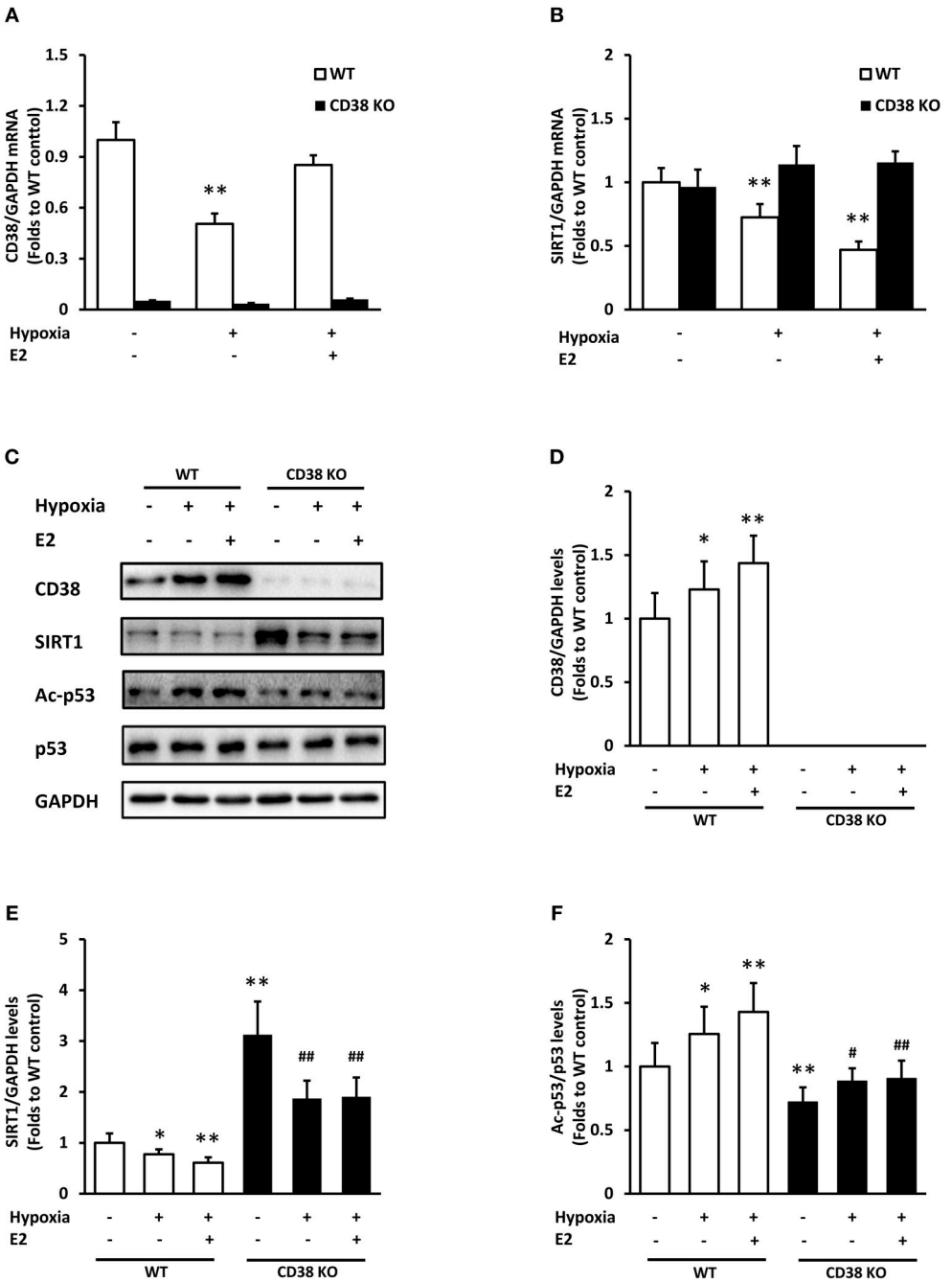

FIGURE 4 | The effects of E2 on SIRT1/p53 signal pathway in WT and CD38 KO ASMCs after hypoxia exposure. **(A)** CD38 and **(B)** SIRT1 mRNA levels were detected by real-time PCR. **(C)** CD38, SIRT1, p53, and Ac-p53 levels were determined by western blot. Quantitative analysis of **(D)** CD38 and **(E)** SIRT1 levels was normalized to GAPDH levels and **(F)** Ac-p53 levels were normalized to total p53 levels. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. the WT control group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ vs. the corresponding WT group. $N = 3$.

modulation of asthma and other lung diseases to elucidate the mechanisms underlying sex differences.

Sex steroids modulate airway smooth muscle contractility in a variety of manners. Estrogens potentiate bronchodilation through prostaglandin synthesis and cGMP modulation, and further influence Ca^{2+} influx channels (24). The mechanism by which estrogens decrease Ca^{2+} responses probably involve ERα (25), inhibition of L-type channels and store-operated calcium channels (26). CD38 is a critical regulator for intracellular Ca^{2+} homeostasis. CD38 is capable of cleaving nicotinamide adenine dinucleotide (NAD) to cyclic ADP ribose (cADPR) which is a trigger for intracellular Ca^{2+} release and hydrolyzing cADPR

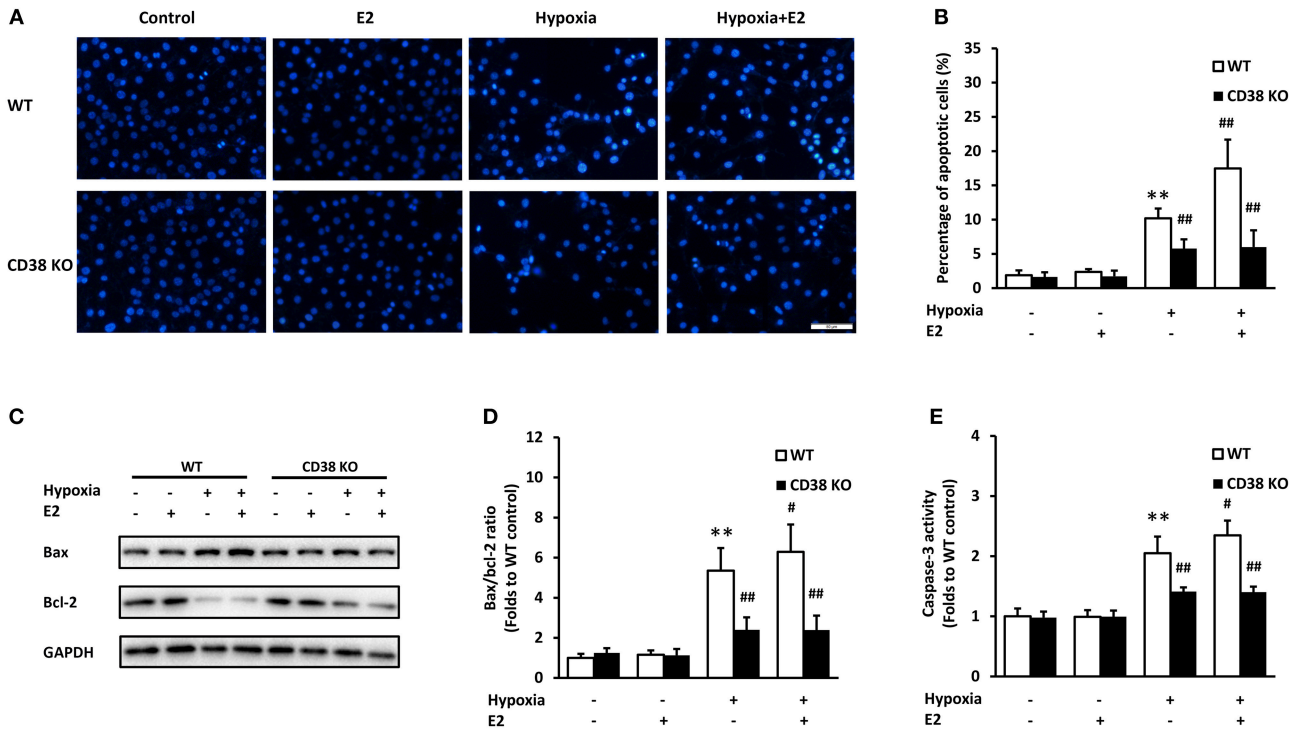

FIGURE 5 | The effects of E2 on apoptosis in WT and CD38 KO ASMCs after hypoxia exposure. **(A)** Representative images of Hoechst 33258 staining in ASMCs. Scale bar, 50 μm. **(B)** Quantitative analysis of apoptosis expressed as the percentage of total cell count. **(C)** Bax and Bcl-2 levels were determined by western blot and **(D)** the Bax/Bcl-2 ratio were quantitatively analyzed. **(E)** The activity of caspase-3 was measured by colorimetry. $^{**}P < 0.01$ vs. the WT control group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ vs. the WT hypoxia group. $N = 3$.

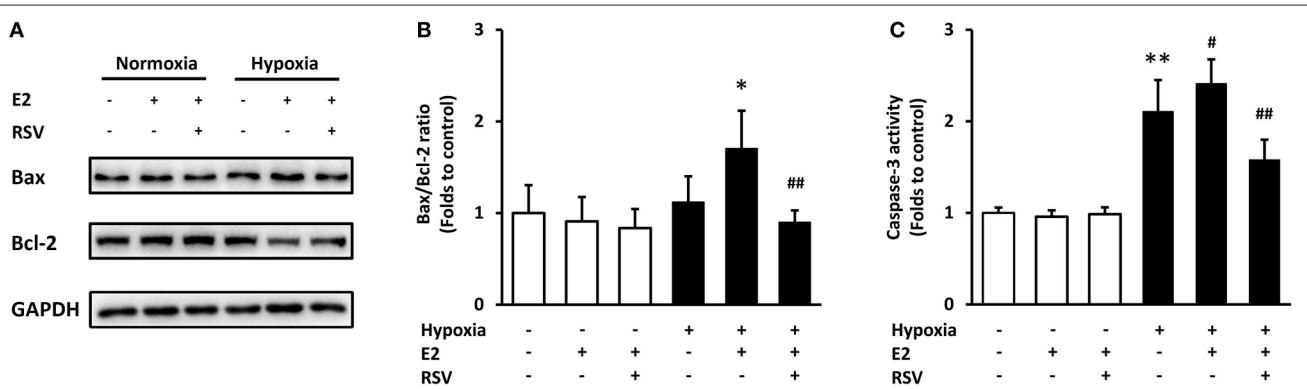

FIGURE 6 | The effects of combined treatment with E2 and SIRT1 activator Resveratrol (RSV) on apoptosis after hypoxia exposure. RSV (10 μM) was added to ASMCs for 2 h incubation followed by 24 h of E2 treatment. **(A)** Bax and Bcl-2 levels were determined by western blot and **(B)** the Bax/Bcl-2 ratio were quantitatively analyzed. **(C)** The activity of caspase-3 was measured by colorimetry. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. the control group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ vs. the E2-treated group under hypoxia. $N = 3$.

to ADPR (27). In addition, CD38 is an NAADP synthase required for NAADP-mediated Ca^{2+} release from lysosomal stores (10). CD38 KO mice exhibit very low cADPR levels in the lungs, attenuated $[Ca^{2+}]i$ responses to spasmogens, and decreased airway responsiveness (28). Cytokines such as IL-13 or TNF-α caused significantly lower inflammation and hyperresponsiveness in the CD38 KO mice compared to WT controls (29, 30), suggesting the crucial roles of CD38 in the contractility of airway smooth muscle and airway hyperresponsiveness. Studies showed that E2 increased CD38 mRNA and protein expression, resulting in increased cADPR synthesis, which may contribute to calcium regulation and myometrial contractility in rat myometrium (31). However, there has no data for CD38 expression in airway smooth muscle.

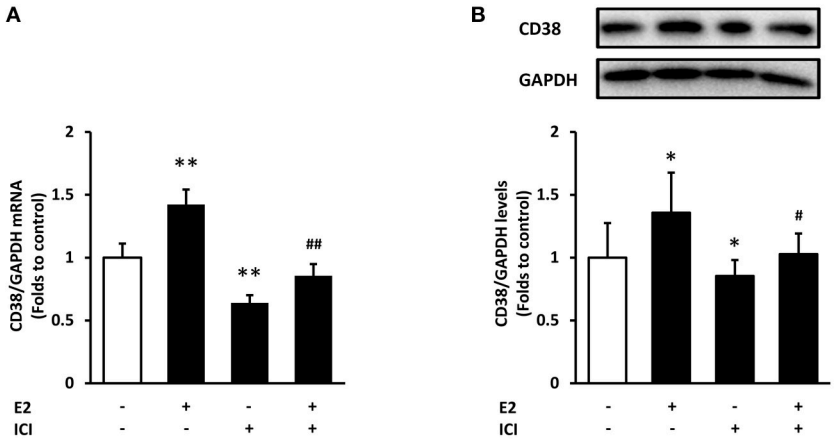

FIGURE 7 | The effects of estrogen receptor antagonist on CD38 expression. The estrogen receptor antagonist ICI182,780 (ICI, 10 nM) was added to ASMCs for 2 h incubation followed by 24 h of E2 treatment. **(A)** CD38 mRNA levels were detected by real-time PCR. **(B)** CD38 protein levels were determined by western blot and quantitative analysis of CD38 levels was normalized to GAPDH levels. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. the control group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ vs. the E2-treated group. $N = 3$.

Therefore, we investigated the effect of E2 varying from 0.1 to 100 nM on CD38 mRNA and protein expression in ASMCs. In accordance with the result from myometrium, E2 showed a concentration-dependent increase in CD38 mRNA and protein levels. The time course assay revealed that CD38 mRNA and protein maintained a high level till 48 h with the treatment of physiological concentration of E2 (10 and 100 nM).

CD38 functions as the primary NAD^+ hydrolase that maintains low intracellular NAD^+ levels with a consequent low sirtuin activity (32) There is an increased NAD^+ levels as well as SIRT1 enzymatic activity in CD38 knockout mice, which is responsible for the deacetylation of the SIRT1 substrate p53 (9). This non-genomic regulation may explain our current results that CD38 gene deletion markedly increases SIRT1 protein levels without significant effect on its mRNA expression. Several studies demonstrated the down-regulation of SIRT1 protein levels by E2 treatment in vascular smooth muscle cells (16, 33), but there was no data showing that E2 had effects on SIRT1 gene expression. In the present study, E2 induced obvious decrease in SIRT1 mRNA and protein levels in WT ASMCs but not in CD38 KO cells, suggesting that other unknown mechanisms may exist in E2's actions associated with CD38 and warrants further investigation in our feature work. Taken together, these results suggested that CD38 is necessary for the modulation of SIRT1/p53 signaling pathway by E2. We for the first time demonstrated that E2 modulates the CD38/SIRT1/p53 signal pathway in mouse AMSCs.

The SIRT1/p53 pathway mediated cell apoptosis in many pathological processes. It has been reported that high concentration of glucose results in neuronal apoptosis through downregulation of SIRT1 and increased acetylation of p53, which likely contribute to the development of cognitive impairment in diabetes (34). In another study, rotenone treatment promotes p53 transcription and apoptosis through targeting SIRT1 and H3K9 SH-SY5Y cells, leading to nigrostriatal degeneration in Parkinson's disease (35). Here we investigated whether E2 acts on CD38/SIRT1/p53 signal pathway during hypoxia in ASMCs. Results showed that hypoxia caused a marked decrease in CD38 mRNA levels, which is consisted with the previous study (36). However, CD38 protein levels was up-regulated after hypoxia exposure. Although both *in vivo* and *in vitro* studies have confirmed that CD38 is activated during the process of hypoxia or ischemia, triggering CD38-mediated NADP(H) depletion with loss of eNOS-mediated NO generation and increased eNOS uncoupling (37, 38), no studies showed the changes of CD38 protein levels under hypoxia. We speculated that different time course changes may exist in mRNA and protein expression following hypoxia, or there might be a negative feedback regulation that inhibits CD38 mRNA expression. E2 exacerbated hypoxia-induced SIRT1 suppression and p53 acetylation, and these effects were abolished in CD38 KO cells, suggesting that CD38 is an upstream signaling molecule that regulates hypoxia-induced SIRT1/p53 activation.

Bcl-2 and Bax are two main proteins of Bcl-2 family, which is notable for the regulation of cell apoptosis. Bcl-2, an anti-apoptotic protein, inhibits the accumulation of cytochrome c in the cytosol, thereby preventing caspase-3 activation and blocking the apoptotic cascade, whereas Bax was identified as the proapoptotic member that triggers the release of caspases. Therefore, the Bax/Bcl-2 ratio is a determining factor in the regulation of apoptotic cell death (39). The ratio of Bax to Bcl-2 increased following hypoxia exposure in different models (40, 41). The expression of Bcl-2 and Bax is regulated by p53. The activation of p53 induces the expression of bcl-2 while simultaneously stimulates the expression of bax (42). By measuring the fluorescence of Hoechst 33258, the Bax/Bcl-2 ratio and the activity of caspase-3, we demonstrated that E2 aggravated cell apoptosis following hypoxia stimulation. The application of CD38 KO cells and the SIRT1 activator further confirmed the direct role of CD38/SIRT1 in E2-mediated AMSC apoptosis.

Finally, we explored the underlying mechanism by which E2 modulates CD38 expression. It has been widely considered

that estrogens perform physiological function through receptor-mediated signaling pathways. The nuclear ERs exist in two main isoforms termed ERα and ERβ, and the classical mechanism of estrogen action involves ligand-induced dimerization of ER which interacts with estrogen responsive elements (EREs) in target gene promoters and results in transcriptional activation (43). Here we determined whether E2 mediated CD38 transcription through ER, by using ICI 182,780. ICI 182,780 is a selective estrogen antagonist that has been used for assessing ER-mediated actions of estrogens (21). Results showed that ICI 182,780 suppressed CD38 mRNA levels and counteracted the effect of E2, indicating the involvement of ER in the regulation of CD38. However, much more work is needed to elucidate the molecular mechanism, including the identification of the isoform (ERα or ERβ) which mediates the effect, and exploring possible ERE on CD38 promoters.

According to our results, the physiological concentration of E2 affects CD38 expression and promotes apoptosis, indicating that E2 have adverse effects on ASMCs. This may probably explain why women are more susceptible to respiratory diseases and the clinical application of estrogens should be more cautious. Further research into the effects of estrogen on the proliferation and inflammatory response in ASMCs are necessary, and the animal models of specific pulmonary diseases such as asthma and pulmonary hypertension in the smooth muscle-specific CD38 KO mice will also provide essential tools for elucidating the function of E2 on ASMCs. These studies are now ongoing in our laboratory.

In summary, the estrogen E2 acts on CD38/SIRT1/p53 signal pathway, resulting the acetylation of p53 and pro-apoptotic effects in mouse ASMCS following hypoxia. The findings may provide novel evidence for the prevention and treatment of respiratory disease through CD38 inhibition.

ETHICS STATEMENT

All experimental procedures involving animal and their care were carried out in accordance with the National Institutes of Health Guidelines. All experimental protocols were approved by Institutional Animal Care and use Committee of Nanchang University. All efforts were made to minimize animal suffering and reduce the number of animals used.

AUTHOR CONTRIBUTIONS

H-BX, K-YD, and YQ designed the experiments. The experimental procedures were performed by YL, YG, and WH. YQ and YL prepared the manuscript. H-BX revised the manuscript.

FUNDING

This study was supported by the National Natural Science Foundation of China (91639106 and 81860020).

ACKNOWLEDGMENTS

We are grateful to Dr. Ling-Fang Wang, Dr. Xiao-Hui Guan, and Ms. Shui-Zhen Shi for their skilled technical assistance and good proposals.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2018.00770/full#supplementary-material

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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REVIEW
published: 06 December 2018
doi: 10.3389/fendo.2018.00748

Sirtuins and Insulin Resistance

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The mammalian Sirtuins (SIRT1-7) are an evolutionarily conserved family of NAD^+-dependent deacylase and mono-ADP-ribosyltransferase. Sirtuins display distinct subcellular localizations and functions and are involved in cell survival, senescence, metabolism and genome stability. Among the mammalian Sirtuins, SIRT1 and SIRT6 have been thoroughly investigated and have prominent metabolic regulatory roles. Moreover, SIRT1 and SIRT6 have been implicated in obesity, insulin resistance, type 2 diabetes mellitus (T2DM), fatty liver disease and cardiovascular diseases. However, the roles of other Sirtuins are not fully understood. Recent studies have shown that these Sirtuins also play important roles in inflammation, mitochondrial dysfunction, and energy metabolism. Insulin resistance is the critical pathological trait of obesity and metabolic syndrome as well as the core defect in T2DM. Accumulating clinical and experimental animal evidence suggests the potential roles of the remaining Sirtuins in the regulation of insulin resistance through diverse biological mechanisms. In this review, we summarize recent advances in the understanding of the functions of Sirtuins in various insulin resistance-associated physiological processes, including inflammation, mitochondrial dysfunction, the insulin signaling pathway, glucose, and lipid metabolism. In addition, we highlight the important gaps that must be addressed in this field.

Keywords: sirtuins, insulin resistance, senescaging, inflammation, mitochondrial dysfunction

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Specialty section:
This article was submitted to Cellular Endocrinology, a section of the journal Frontiers in Endocrinology

Received: *30 August 2018*
Accepted: *26 November 2018*
Published: *06 December 2018*

Citation:
Zhou S, Tang X and Chen H-Z (2018) Sirtuins and Insulin Resistance. Front. Endocrinol. 9:748. doi: 10.3389/fendo.2018.00748

INTRODUCTION

The increasing prevalence of obesity and associated metabolic syndrome (including type 2 diabetes mellitus [T2DM], nonalcoholic fatty liver disease [NAFLD], atherosclerosis and atherosclerotic heart disease) is an increasingly severe challenge in public health (1). Insulin resistance is the critical, universal pathological feature of these diseases, especially T2DM. Insulin is a major hormone secreted by pancreatic β cells after nutrient stimulation and plays a critical role in reducing blood glucose concentration by facilitating glucose uptake by skeletal muscle and adipose tissue and inhibiting endogenous glucose production in the liver (2–4). Insulin resistance occurs when cells are incapable of efficiently responding to a normal dose of insulin (2–4). The development of insulin resistance is a multistep, complex process influenced by genetics and environments (3). Although the precise pathogenesis of insulin resistance remains incompletely understood, several mechanisms are proposed to be involved, including defects in the insulin signaling pathway, ectopic lipid accumulation, systemic inflammation, mitochondrial dysfunction, oxidative stress, and endoplasmic reticulum (ER) stress, as reviewed elsewhere (1, 5, 6).

The mammalian Sirtuins are a family of NAD^+-dependent deacetylases (7). This family consists of seven members (SIRT1-SIRT7), which share the conserved Sirtuin domain conferring NAD^+-dependent deacetylase activity but have variable amino- and carboxy-terminal extensions and display distinct subcellular localization and biological functions (8, 9). SIRT1 is mainly localized to the nucleus (10), but it shuttles between the nucleus and cytoplasm during development and in response to physiological and pathological stress (11). In contrast to SIRT1, mammalian SIRT2 is mainly localized to the cytoplasm (10, 12) but is also found in the nucleus (13). SIRT3, SIRT4, and SIRT5 are localized to mitochondria (10, 14), whereas SIRT6 and SIRT7 are found in the nucleus (10). SIRT6 is a chromatin-associated protein, and SIRT7 resides in the nucleolus (15, 16). Deacetylase activity was initially reported as conserved in mammalian Sirtuins, but different Sirtuins exhibit different acyl group preferences (17). Among the seven Sirtuins, SIRT4-7 exhibit weak or undetectable deacetylation activity *in vitro* (10, 18). SIRT2 reportedly possesses efficient demyristoylase activity (18). SIRT5 is reportedly an efficient NAD^+-dependent protein, lysine desuccinylase and demalonylase (19), while SIRT4 and SIRT6 reportedly possess ADP-ribosyltransferase activity (20, 21).

Mammalian Sirtuins regulate a wide variety of cellular processes, including metabolism, mitochondrial homeostasis, oxidative stress, inflammation, autophagy and apoptosis (22). Sirtuins also play important roles in aging and aging-related diseases, such as obesity, T2DM, cardiovascular disease, cancer, neurodegenerative diseases (23). SIRT1 and SIRT6 are the most extensively characterized Sirtuins. A large body of literature indicates that these two Sirtuins play an important role in metabolism, and they have also recently attracted increased attention with regard to their protective roles in maintaining insulin sensitivity, as reviewed elsewhere (24–26). Compared to the metabolic roles of SIRT1 and SIRT6, the metabolic roles of other Sirtuins remain poorly understood. Here, we review the recent advances in the understanding of the roles of Sirtuins in inflammation, mitochondrial dysfunction, and oxidative stress and discuss their possible roles in insulin resistance.

SIRTUINS IN INFLAMMATION

Obesity-induced chronic, low-grade inflammation is one of the most important contributors to the pathogenesis of insulin resistance (27–29). Adipose tissue is not only an insulin-targeting organ for lipid metabolism but also an endocrine organ that secretes hormones, cytokines, and chemokines to influence insulin sensitivity. For instance, adipocytes secrete adipokines, such as leptin and adiponectin, to promote insulin sensitivity (30, 31), and resistin and retinol-binding protein 4 (RBP4) to impair insulin sensitivity (32, 33). Importantly, adipose tissue is a critical initiator of the inflammatory response to obesity (28). In obesity, metabolism and gene expression of adipocytes change, resulting in increased lipolysis of adipocytes, the release of free fatty acids and proinflammatory cytokines and activation of M1 macrophages (27–29). M1 macrophages produce a large number of proinflammatory mediators, such as TNF-α, IL-1β, and resistin, that act on adipocytes to induce an insulin-resistant state and activate inflammatory pathways in insulin-targeting cells. Ultimately, ectopic lipid deposition and increased expression of inflammatory mediators in the liver and skeletal muscle lead to impaired insulin signaling and exacerbate systemic insulin resistance (29). Signals from all the proinflammatory mediators converge on inflammatory signaling pathways, including jun-n-terminal kinase (JNK) and inhibitor of nuclear factor κB (NF-κB) kinase (IKK) (27, 34, 35). Inhibition of insulin receptor downstream signaling is the primary mechanism for inflammation-induced insulin resistance (27). Activated JNK or IKK can phosphorylate the insulin receptor (IR) and insulin receptor substrate (IRS) proteins and decrease their tyrosine phosphorylation, thus leading to decreased activation of PI3-kinase and Akt and resistance to the metabolic actions of insulin (34, 36). In addition, activation of the JNK and IKK pathways can induce the production of inflammatory mediators, while the Sirtuin family plays essential roles in inflammation, which comprehensively contributes to insulin resistance.

Inflammatory Transcriptional Factor

NF-κB is a key transcriptional factor that mediates the expression of multiple inflammatory factors, including TNF-α, IL-1β, and IL-6. The acetylation of NF-κB promotes its nuclear translocation and activation. SIRT1 has been demonstrated to repress inflammation in multiple tissues and cells (37–40). In particular, SIRT1 suppresses inflammation in both adipocytes (39, 41) and macrophages (42), which leads to a reduction of adipose tissue inflammation. SIRT1 deacetylates p65 subunit of NF-κB at lysine 310 (K310) and inhibits the transcriptional activity of NF-κB (43). Moreover, SIRT1 interacts with transducing-like enhancer of split 1 (TLE1), a co-repressor of NF-κB, to inhibit NF-κB-mediated transcription (44). In addition, SIRT1 deacetylates activator protein-1 (AP-1) to reduce the expression of COX-2 in macrophages and deacetylates p53 to repress macrophage activation (45, 46). Similar to SIRT1, SIRT2 also binds to NF-κB and mediates the deacetylation of NF-κB subunit p65 at K310, which leads to the inhibition of the expression of NF-κB target inflammatory genes in fibroblasts, macrophages and microglial cells (47–49). SIRT2-mediated inhibition of NF-κB and inflammation contributes to its anti-inflammatory function in an experimental colitis mouse model (49), neuroinflammation (48, 50, 51), collagen-induced arthritis (52), and microvascular inflammation in *ob/ob* septic mice (53). In addition to SIRT2, SIRT4 can regulate the activation of NF-κB. SIRT4 has been shown to negatively regulate cigarette smoke extract (CSE)-induced NF-κB activation by inhibiting the degradation of IκBα and inhibiting NF-κB target gene expression, including the proinflammatory cytokines IL-1β, TNF-α, and IL-6, resulting in inhibition of CSE-induced mononuclear cell adhesion to human pulmonary microvascular endothelial cells (54). SIRT4 can prevent NF-κB nuclear translocation as well as the transcriptional activity of NF-κB, thereby suppressing inflammation in human umbilical vein endothelial cells (55). Interestingly, the role of SIRT5 in inflammation is controversial.

Recently, Qin and colleagues showed that SIRT5 deficiency decreased toll-like receptor (TLR)-triggered inflammation in both acute and immunosuppressive phases of sepsis (56). Mechanistically, SIRT5 competes with SIRT2 to interact with NF-κB p65 in a deacetylase activity-independent manner and thus blocks the deacetylation of p65 by SIRT2, which consequently leads to the activation of the NF-κB pathway and induction of its downstream cytokines in macrophages (56). However, Wang and colleagues found that SIRT5 desuccinylates and actives pyruvate kinase isoform M2 (PKM2) by promoting its dimerization and nuclear accumulation, thereby decreasing proinflammatory cytokine IL-1β production in LPS-activated macrophages (57). As hyperproduction of IL-1β contributes to increased susceptibility to inflammatory bowel disease, *Sirt5*-deficient mice are more susceptible to dextran sulfate sodium (DSS)-induced colitis (57). Interestingly, *Sirt6* deficient mice display increased expression of NF-κB-dependent genes in multiple tissues (58). *Sirt6* deletion increases inflammation in the mice adipose tissue and promotes HFD-induced insulin resistance (59, 60). Mechanistically, SIRT6 binds to the NF-κB subunit RelA and deacetylates histone H3 lysine 9 (H3K9) at NF-κB target gene promoters, which leads to a reduction of NF-κB target gene expression (58). These findings suggest that the Sirtuins target inflammatory transcriptional factors (e.g., NF-κB and AP1) directly or indirectly to contribute to insulin resistance comprehensively.

Inflammasome

The anti-inflammatory role of Sirtuins involves other mechanisms. The Nod-like receptor family, pyrin domain-containing 3 (NLRP3) inflammasome is a multiprotein complex that orchestrates the innate immune responses of macrophages by controlling the activation of caspase-1 and the release of the proinflammatory cytokines IL-1β and IL-18 (61–63). Obesity-related inflammation is partly mediated by the NLRP3 inflammasome, and NLRP3 activation exacerbates obesity-linked diseases (64, 65). Resveratrol, a SIRT1 activator, inhibits ionizing irradiation-induced inflammation in mesenchymal stem cells *via* suppressing NLRP3 inflammasome activation (66). In a murine model of sepsis, *Sirt1* deletion results in increasing lung inflammasome activation and inflammatory lung injury (67). A recent study demonstrated that silybin prevents NLRP3 inflammasome activation during NAFLD through SIRT2 (68). However, further studies are needed to clarify the mechanism underlying SIRT2-mediated regulation of NLRP3 inflammasome activity. In a human fasting/refeeding study, Traba and colleagues observed that fasting leads to a reduction in NLRP3 inflammasome activation (69). SIRT3 deletion in a human macrophage line increases NLRP3 inflammasome activation, accompanied by excessive mitochondrial ROS production (69). Pharmacologic and genetic SIRT3 activation enhances mitochondrial function and suppresses NLRP3 activity in THP-1 monocyte cells and in leukocytes extracted from healthy volunteers and from refeeding individuals (69). The authors concluded that nutrient levels regulate the NLRP3 inflammasome partly through SIRT3-mediated mitochondrial homeostatic control. Similarly, Chen *et al.* reported that trimethylamine-N-oxide (TMAO) increases ROS production by inhibiting the SIRT3-SOD2-mitochondrial signaling pathway, which leads to NLRP3 inflammasome activation and consequently promotes vascular inflammation (70). Defective autophagy in monocytes or macrophages might result in NLRP3 inflammasome activation and cause vascular metabolic inflammation (71–73). Acetylation of ATG5, an autophagy-related protein, inhibits autophagosome maturation and induces NLRP3 inflammasome activation (74). Recently, Liu and colleagues demonstrated that SIRT3 binds with ATG5 and deacetylates it, while SIRT3-deficient macrophages display impaired autophagy, leading to accelerated NLRP3 inflammasome activation and endothelial dysfunction (73). These studies suggest that SIRT3 may inhibit NLRP3 inflammasome activation by regulating mitochondrial function, ROS production, and autophagy. As SIRT2 has also been shown to regulate NLRP3 inflammasome activation (68), the potential synergistic effect on regulation of NLRP3 inflammasome activation between SIRT3 and SIRT2 needs further study. Previous studies have highlighted the anti-inflammatory role of SIRT3 in obesity-related diseases, including insulin resistance. The function of SIRT3 in inflammasome regulation largely depends on SIRT3-mediated activation of MnSOD and suppression of oxidative stress. These findings implicate that the SIRT1-SIRT3 indirectly regulate the activation of the NLRP3 inflammasome, which may be involved in the modulation of insulin resistance. However, whether Sirtuins directly regulate inflammasome remains unknown.

Sirtuins, Inflammation and Insulin Resistance

The roles of Sirtuins in inflammation significantly contribute to their functions during insulin resistance. For instance, activation of SIRT1 leads to the repression of JNK and IKK inflammatory pathways greatly and subsequently improves glucose tolerance, reduced hyperinsulinemia, and enhanced systemic insulin sensitivity (40). SIRT1 also controls the inflammatory status of macrophages and T lymphocytes to regulate the metabolism (insulin sensitivity) and inflammation of adipose tissues in obese mice (41, 75–77). In addition, SIRT6 is important for macrophage activation and TNFα production (78). Myeloid *Sirt6* deficiency causes insulin resistance in HFD–fed mice by eliciting macrophage polarization toward an M1 phenotype (79), and facilitates the development of HFD-induced atherosclerosis (80). Deletion of *Sirt6* in T cells or myeloid-derived cells is sufficient to induce liver inflammation and fibrosis (81). Interestingly, SIRT1 and SIRT6 can coordinate a switch from glucose to fatty acid oxidation during the acute inflammatory response (82). Therefore, Sirtuins not only regulate the inflammatory pathways within the target cells (e.g., hepatocytes, skeletal muscle cells, adipocytes) to affect their insulin sensitivity but also regulate inflammatory cells infiltrated in the organs, where the Sirtuins respond to inflammatory and metabolic insults and subsequently regulate insulin sensitivity and disease by targeting inflammatory cell activation and differentiation. Notably, the Sirtuins may also

cooperate in diverse types of cells or within the same type of cell during inflammation-associated insulin resistance.

SIRTUINS IN MITOCHONDRIAL DYSFUNCTION

Mitochondria are the primary site for ATP generation and ROS production. Mitochondrial dysfunction results in decreased ATP production, increased ROS production and accumulated mitochondrial DNA damage, which contribute to insulin resistance (5). Cells eliminate ROS by expressing endogenous antioxidant enzymes, including manganese superoxide dismutase (MnSOD), catalase, glutathione peroxidase (GPX) and glutathione reductase (GRx) (83, 84). An imbalance between the production of ROS and antioxidant enzymes leads to oxidative stress, which has been implicated in the pathogenesis of insulin resistance, obesity, and diabetes (1, 85).

SIRT1

There are fewer mitochondria in muscles of T2DM patients than those of insulin-sensitive individuals (86). Marked reduction of oxidative phosphorylation in the mitochondria can be detected in the liver and skeletal muscle of T2DM patients and insulin-resistant individuals (87, 88). Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) is a transcriptional coactivator that regulates mitochondrial biogenesis and respiration (89, 90). SIRT1 has been shown to deacetylate PGC-1α at several lysine residues and regulates PGC-1α activity, which activates the transcription of genes involved in mitochondrial biogenesis (91). SIRT1 activator resveratrol promotes PGC-1α activity and increases the number of mitochondria in muscle cells, which improves mitochondrial function and protects mice against diet-induced obesity and insulin resistance (92). In addition to mitochondria biogenesis, SIRT1 regulates mitochondrial function through clearance of damaged mitochondria (93). Mechanistically, SIRT1 binds to and deacetylates autophagy regulators (including ATG5, ATG7, and ATG8) to induce mitochondria autophagy or mitophagy (93). SIRT1-mediated deacetylation of FoxO1 and FoxO3a is also known to induce the expression of autophagy pathway components (94, 95). Accumulating evidence has shown that SIRT1 safeguards cells from oxidative stress. SIRT1 reduces ros production by deacetylating and activating FoxO3a to upregulate expression of MnSOD and catalase (95, 96). SIRT1 promotes the transcriptional activity of Nuclear factor (erythroid-derived 2)-like 2 (NRF2) by deacetylating it and upregulates the expression of NRF2 target antioxidant genes, including *MnSOD*, *catalase*, *glutathione,* and *heme oxygenase-1* (*HO-1*) (97).

SIRT2

Oxidative stress increases SIRT2 expression *in vivo* and *in vitro* (98, 99). SIRT2 can bind to FoxO3a and deacetylate it, leading to an increase in FoxO3a transcriptional activity, upregulation of the expression of FoxO target genes such as *MnSOD, Bim,* and $p27^{Kip1}$, and a consequent decrease in ROS generation (98). Nicotinamide adenine dinucleotide phosphate (NADPH) is a functionally important metabolite that is required to generate the reduced form of glutathione (GSH) to maintain cellular redox potential. In response to oxidative stimuli, SIRT2 deacetylates and activates glucose 6-phosphate dehydrogenase (G6PD), a key enzyme in the pentose phosphate pathway (PPP), resulting in increased cytosolic NADPH to attenuate oxidative damage (100). Similarly, oxidative stress increases the glycolytic enzyme phosphoglycerate mutase (PGAM)-SIRT2 interaction, leading to deacetylation and activation of PGAM, which increases the cellular NADPH level to counteract oxidative damage (101). Lysine 4-oxononanoylation (4-ONylation) is a newly discovered histone posttranslational modification that disrupts the interaction between histone H3 and DNA, thereby preventing nucleosome assembly under oxidative stress (102). SIRT2 was reported to remove the 4-oxononanoyl (4-ONyl) lysine groups on histones and attenuate the negative impact of protein 4-ONylation caused by oxidative stress (103). This study provides novel evidence that SIRT2 may exert antioxidation effects through epigenetic modification. Oxidative stress is not completely caused by mitochondria, and whether SIRT2 can influence oxidative stress by regulating mitochondrial function is unclear.

Interestingly, a recent work by Liu and colleagues observed that SIRT2 can localize to the inner mitochondrial membrane of mouse central nervous system cells and that the acetylation of several metabolic mitochondrial proteins is altered in *Sirt2*-deficient mice. In mice, deletion of *Sirt2* causes mitochondrial morphological changes, increases oxidative stress and decreases ATP production in MEFs and brain tissues (104), indicating that SIRT2 may deacetylate antioxidant enzymes in the mitochondria directly.

SIRT3

SIRT3, a central mitochondrial deacetylase, deacetylates, and activates mitochondrial enzymes to regulate mitochondrial metabolism, oxidative stress, cell survival, and longevity (105). SIRT3 has been shown to play a pivotal role in maintaining mitochondrial function and ROS homeostasis. For example, SIRT3 deacetylates complex I and complex II of the electron transport chain to promote electron transport and regulate energy homeostasis (106, 107). SIRT3 deacetylates cyclophilin D (CypD), the regulatory component of the mitochondrial permeability transition pore (mPTP), preventing opening of the mPTP and mitochondrial dysfunction (108). SIRT3 binds to and deacetylates 8-oxoguanine-DNA glycosylase 1 (OGG1), a DNA repair enzyme that excises 7,8-dihydro-8-oxoguanine from the damaged genome, resulting in the repair of mitochondrial DNA (mtDNA) damage, protection of mitochondrial integrity, defense against mitochondrial dysfunction and prevention of stress-induced cellular apoptosis (109). In contrast, *Sirt3* deficiency has been linked to increased ROS production (110, 111). The antioxidant action of SIRT3 involves MnSOD and mitochondrial isocitrate dehydrogenase 2 (IDH2). SIRT3 has been shown to block cardiac hypertrophy by deacetylating FOXO3a and upregulating the expression of FOXO3a target genes such as *MnSOD* and *catalase*, decreasing ROS generation (112). Notably, the antioxidative action of SIRT3 may also be attributed to its direct deacetylation and promotion of the enzyme activity of

MnSOD. SIRT3 deacetylates two critical lysine residues (K53 and K89) of MnSOD that promote MnSOD antioxidation activity, thereby reducing cellular ROS (113). Direct deacetylation of lysine residues, including K68 and K122 by SIRT3, also increases the enzyme activity of MnSOD and decreases ROS production (114, 115). In addition to MnSOD, SIRT3 directly deacetylates and activates mitochondrial IDH2, which results in increased NADPH levels and an increased ratio of reduced to oxidized GSH in mitochondria, thus protecting cells from oxidative stress-induced damage (110, 116). These studies suggest that SIRT3 activity is necessary to prevent mitochondrial dysfunction and reduce oxidative stress.

SIRT4

SIRT4 functions as an efficient mitochondrial ADP-ribosyl transferase that negatively impacts gene expression and various metabolic processes in mitochondria. Our previous work demonstrated that SIRT4 promotes angiotensin II-induced development of cardiac hypertrophy by inhibiting the interaction of SIRT3 and MnSOD, which increases MnSOD acetylation levels, decreases its activity and leads to elevated ROS accumulation (117). SIRT4 may play a role different from that of SIRT3 in regulating mitochondrial function and oxidative stress. *Sirt4* deficiency *in vivo* and *in vitro* increases the expression of genes involved in fatty acid β-oxidation and oxidative phosphorylation, thus enhancing fatty acid oxidation and mitochondrial respiration in liver and muscle (118, 119). SIRT4 increases stress-induced mitochondrial ROS production and interacts with the long form of GTPase optic atrophy 1 (L-OPA1) to promote mitochondrial fusion, thereby inhibiting mitophagy and decreasing the removal of dysfunctional mitochondria (120).

SIRT5

SIRT5 functions to deacetylate, demalonylate, and desuccinylate multiple proteins in mitochondria (19). SIRT5 is involved in the regulation of mitochondrial fatty acid β-oxidation, the urea cycle, and cellular respiration (84, 121). SIRT5 binds to, desuccinylates and activates copper-zinc superoxide dismutase 1 (SOD1) to eliminate ROS (122). Moreover, SIRT5 desuccinylates IDH2 and deglutarylates G6PD, thus activating both NADPH-producing enzymes to scavenge ROS (123). SIRT5 also binds, desuccinylates and inhibits the activity of the glycolysis enzyme PKM2, which facilitates the diversion of glucose metabolites into the pentose phosphate shunt and then produces sufficient NADPH to eliminate ROS (123). Interestingly, a very recent study showed that SIRT5 is present in peroxisomes and can bind, desuccinylate and inhibit ACOX1, the first and rate-limiting enzyme in fatty acid β-oxidation and a major producer of H_2O_2, attenuating peroxisome-induced oxidative stress (124).

SIRT6

SIRT6 plays an important role in DNA repair, genomic stability, and cellular senescence, however, the role of SIRT6 in oxidative stress has not been well clarified. According to recent studies, SIRT6 is believed to protect cells against oxidative stress. Pan et al. (125) found that SIRT6 serves as an NRF2 coactivator by interacting with NRF2 and RNA polymerase II to transactivate NRF2-regulated antioxidant genes, including HO-1. SIRT6 activates AMPK-FoxO3a axis to initiate expression of *MnSOD* and *catalase* and protects cardiomyocytes against ischemia/reperfusion-induced injury (126).

SIRT7

Sirt7 deficiency in mice induces multisystemic mitochondrial dysfunction (127). SIRT7 deacetylates GABPβ1, a master regulator of nuclear-encoded mitochondrial genes, enables it to form the transcriptionally active GABPa/GABPβ heterotetramer, and promotes mitochondria function (127). Additionally, the mitochondrial unfolded protein response [UPR(mt)] is mediated by the interplay of SIRT7 and NRF1 and is coupled to cellular energy metabolism and proliferation (128).

SIRTUINS IN INSULIN SIGNALING PATHWAYS

Insulin Secretion

Under the condition of insulin resistance, normal pancreatic β cells increase the production of insulin to maintain blood glucose levels. However, this compensatory response fails, and relative insulin insufficiency develops. Then, glucose tolerance is impaired, and T2DM eventually occurs. SIRT1, SIRT4, and SIRT6 reportedly regulate pancreatic β cell function (**Figure 1**) (24, 129, 130). According to accumulating evidence, SIRT1 and SIRT6 repress pancreatic β cell dysfunction, attenuating the development of T2DM. β cell-specific SIRT1 transgenic mice exhibit enhanced insulin secretion and improved glucose tolerance to high glucose stimulation (131). Mechanistically, through repressing UCP2 expression, SIRT1 enhances ATP production in pancreatic β cells, to shut down the potassium channel, resulting in the influx of calcium and finally the secretion of insulin (131, 132). SIRT1 can induce *NeuroD* and *MafA* expression *via* deacetylating and activating FoxO1, which protects pancreatic β cells against oxidative damage and preserves pancreatic β cells function (133). In contrast to SIRT1 and SIRT6, SIRT4 functions as a negative regulator of insulin secretion in β cells. Glutamate dehydrogenase (GDH) promotes the metabolism of glutamate and glutamine, generating ATP to further promote insulin secretion. In pancreatic β cells, SIRT4 represses the activity of GDH by ADP-ribosylation, thereby downregulating insulin secretion in response to amino acids under calorie-sufficient conditions (20). SIRT4 also controls leucine oxidation to regulate insulin secretion (134). Given that SIRT3 deacetylases GDH and increases its activity in hepatocytes (135), SIRT3 may function in β cell mitochondria to promote insulin secretion. Recent studies have provided evidence to support this notion. Caton *et al.* observed that SIRT3 expression markedly decreases in islets isolated from T2DM patients, as well as in mouse islets or INS1 cells (136). *Sirt3* knockdown in INS1 cells results in increased production of cellular ROS and IL-1β, increased β cell apoptosis and reduced insulin secretion (136). SIRT3 deficiency predisposes pancreatic β cells to oxidative stress-induced dysfunction and reduces glucose-induced insulin secretion (137). By contrast, SIRT3 overexpression inhibits ER stress and attenuates palmitate-induced pancreatic β cell

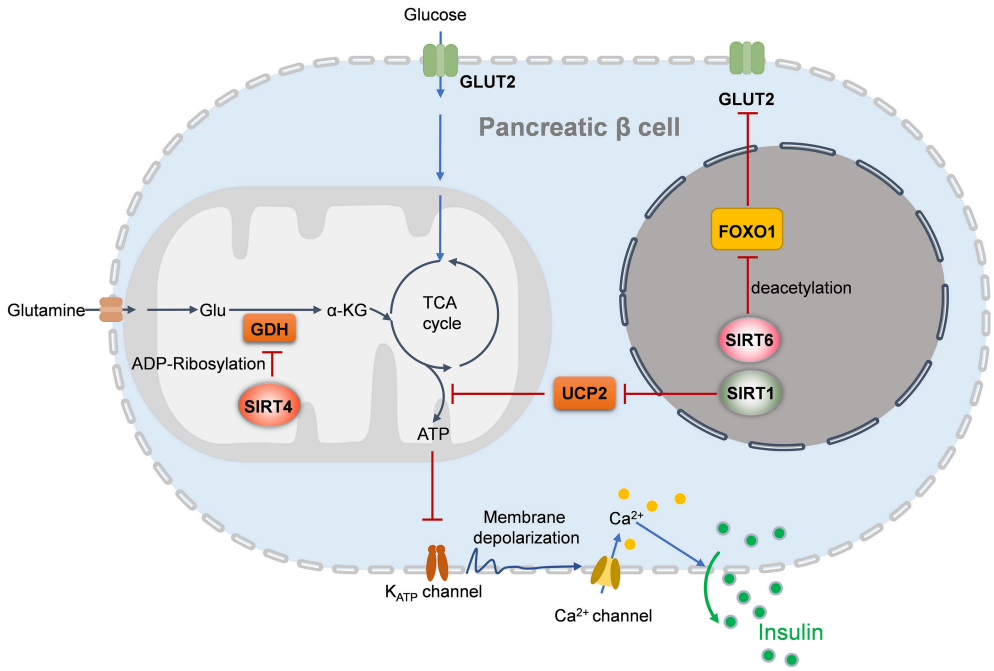

FIGURE 1 | Sirtuins regulates insulin secretion of pancreatic beta cells. In the nucleus, SIRT1 induces insulin secretion through the reduction of UCP2 expression and the enhancement of depolarization in pancreatic β cells, while SIRT6 deacetylates FOXO1 and promotes the expression of GLUT2, which facilitates glucose uptake and insulin secretion. In the mitochondria, SIRT4 promotes the ADP-ribosylation and inactivation of GDH, leading the repression of ATP generation and inhibition of insulin secretion. UCP2, uncoupling protein 2; Glu, glutamate; GDH, glutamate dehydrogenase; GLUT2, glucose transporter 2; α-KG, alpha-ketoglutarate.

dysfunction (138, 139). Therefore, SIRT3 and SIRT4 play opposing roles in regulating insulin secretion in pancreatic β cells. In addition, insulin secretion impairment is observed in *Sirt6* knockout pancreatic β cells, which is mediated by suppression of the FoxO1-Pdx1-Glut2 pathway (140). *Sirt6* deletion in pancreatic β cells also reduces ATP production and increases mitochondrial damage which induces cell apoptosis and impairs glucose-stimulated insulin secretion (129, 141). β cell-specific *Sirt6*-ko mice are glucose intolerance and are defective in glucose-stimulated insulin secretion, in spite do not show abnormality in endocrine morphology, pancreatic β cell mass or insulin production (130). *Sirt6* deficiency also results in aberrant upregulation of thioredoxin-interacting protein (TXNIP) in pancreatic β cells, which inhibits insulin secretion (130).

Insulin Signaling Pathway

Insulin resistance, the inability of cells to efficiently respond to a normal dose of insulin, is caused by impaired insulin signaling and postreceptor intracellular defects (4). Insulin binding to its receptor results in IR phosphorylating itself and several intracellular substrates. The phosphorylated substrates interact with intracellular effectors, leading to the activation of the PI3K-Akt pathway, which is responsible for most of the metabolic actions of insulin, and the Ras-MAPK pathway, which controls cell growth and differentiation (4, 142). Impaired Akt activation is a key factor in metabolic disorders involving insulin resistance. Accumulating evidence suggests that Sirtuins participate in insulin signaling in target cells (**Figure 2**).

SIRT1 positively regulates insulin signaling and Akt activation at multiple levels. SIRT1 represses transcription of *PTPN1,* a negative regulator of the insulin signal transduction cascade, at the chromatin level and improves insulin sensitivity (3). Knockdown of *Sirt1* in 3T3-L1 adipocytes increases phosphorylation of JNK, as well as serine phosphorylation of insulin receptor substrate 1 (IRS-1), which leads to decrease tyrosine phosphorylation of IRS-1, and then inhibit phosphorylation of Akt (39). Inhibition of SIRT1 activity reduces insulin-induced IRS-2 deacetylation, which prevents insulin-induced IRS-2 tyrosine phosphorylation (143). SIRT1 mediates deacetylation of Akt regulates binding of Akt to phosphatidylinositol 3,4,5-trisphosphate (PIP3) which is necessary for Akt membrane localization and activation (144).

SIRT2 can directly regulate the insulin signaling pathway, but its role is controversial. SIRT2 can deacetylate and activate Akt through the Akt/glycogen synthase kinase-3β (GSK3β)/β-catenin signaling pathway, finally resulting in aberrant proliferation and survival of myeloid leukemia cells and epithelial-mesenchymal transition of HCC (145, 146). Interestingly, Ramakrishnan and colleagues showed that SIRT2 is a novel Akt interactor and is required for optimal Akt activation under normal conditions (147). Pharmacological or genetic inhibition of SIRT2 decreases Akt activation in 3T3-L1 preadipocytes and HeLa cells, whereas SIRT2 overexpression enhances the activation of Akt and its

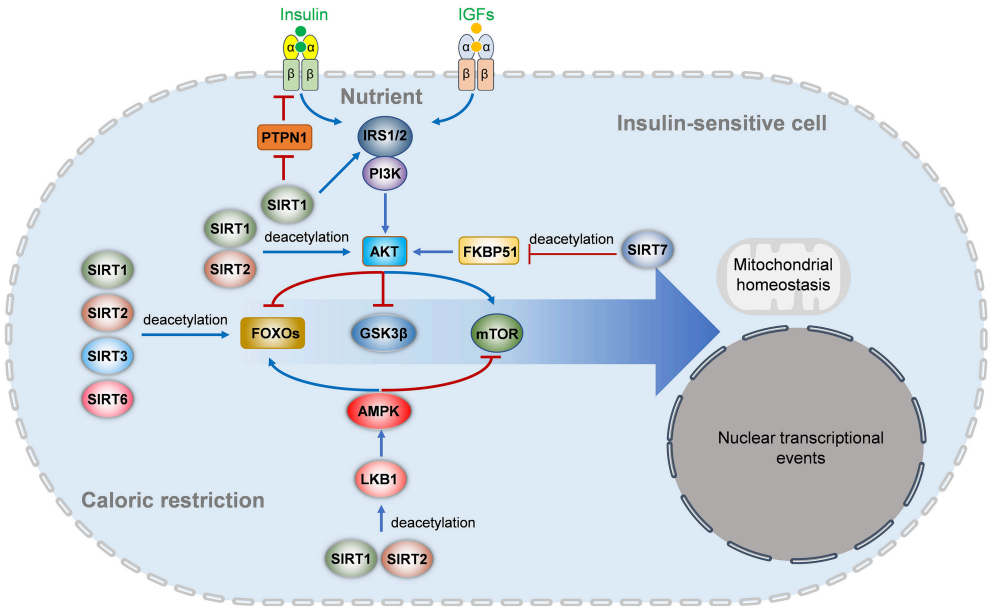

FIGURE 2 | Sirtuins regulates insulin signaling pathways. In nutrient enough conditions, insulin and insulin-like growth factors activate the IRS-PI3K-AKT signaling and downstream FOXO and mTOR signaling to regulate multiple aspects of metabolism, survival, mitochondrial homeostasis, nuclear transcriptional events, and other cellular behaviors. The insulin receptor activation is inhibited by PTPN1, which is repressed by SIRT1. SIRT1 also deacetylates IRS2 and represses IRS1 phosphorylation and PI3K-AKT activation. SIRT1 and SIRT2 also deacetylate AKT to activate its activation directly, while SIRT7 indirectly inhibits AKT activation by deacetylating FKBP51. Under energetic stress or caloric restriction, multiple members of the Sirtuins family are activated. SIRT1, SIRT2, SIRT3, and SIRT6 can directly deacetylate FOXOs (FOXO1 and FOXO3a), while SIRT1 and SIRT2 activate the LKB1-AMPK signaling to activate FOXO and inhibit mTOR signaling. IGF, insulin-like growth factor; IRS, insulin receptor substrate; PTPN1, tyrosine-protein phosphatase non-receptor type 1; PI3K, phosphatidylinositol-4,5-bisphosphate 3-kinase; mTOR, mammalian target of rapamycin; AMPK, AMP-activated protein kinase; LKB1, liver kinase B1; FKBP51, FK506-binding protein 51; GSK3β, glycogen synthase kinase-3 beta.

downstream targets, such as GSK3 and p70-S6-kinase (147). Insulin-induced Akt activation requires Akt binding to inositol 1,4,5-trisphosphate, which leads to Akt conformational changes and facilitates its phosphorylation by PDK1 and mTORC1 (147). Acetylation at Lys20 blocks Akt activation by restricting the binding of Akt to inositol 1,4,5-trisphosphate (144). However, the authors were unable to detect Akt acetylation in the experiment, and they could not determine whether the effects of SIRT2 on Akt are dependent on changing the acetylation level of Akt (147). However, the opposite results have also been reported. Arora et al. reported that SIRT2 is upregulated in insulin-resistant skeletal muscle cells, and inhibition of SIRT2 by pharmacological or genetic means improves phosphorylation of Akt and GSK3β and increases insulin-stimulated glucose uptake (148). Similarly, in insulin-resistant neuro-2a cells, inhibition of SIRT2 by pharmacological or genetic means also enhances the activity of Akt and increases insulin-stimulated glucose uptake (149). Therefore, SIRT2 may regulate Akt in both direct (activity-dependent) and indirect (activity-independent) manners, which may largely rely on the metabolic status of the cells. SIRT2 can deacetylate and regulate the function of FOxO transcription factors, which are direct Akt targets (98, 150). Interestingly, the Akt-independent pathway also contributes to the function of SIRT2 in the regulation of insulin sensitivity. TUG acetylation modulates its interaction with Golgi matrix proteins and enhances its function to trap GLUT4 storage vesicles in intracellular (151). Insulin mobilizes the exocytic translocation of GLUT4 glucose transporters by triggering TUG proteolysis to accelerate glucose uptake in fat and muscle. SIRT2-mediated TUG deacetylation controls insulin sensitivity *in vivo* and *in vitro* (151).

The role of SIRT6 in the insulin signaling pathway is controversial. *Sirt6*-deficient mice die about 4 weeks of age, exhibiting severe metabolic defects, including low insulin and hypoglycemia (15). Xiao et al. found that *Sirt6* deficiency increases Akt phosphorylation through modulating insulin signaling upstream of Akt, including insulin receptor, IRS1, IRS2, and enhances insulin signaling, leading to hypoglycemia (152). On the contrary, *Sirt6* transgenic mice show increased insulin sensitivity in skeletal muscle and liver and exhibit enhanced insulin-induced Akt activation in gastrocnemius (153).

In addition, SIRT7 can regulate Akt signaling. SIRT7 regulates the acetylation of FKBP51, which then regulates Akt activation. Acetylated FKBP51 enhances Akt activity by blocking its interaction with PHLPP-Akt. SIRT7 deacetylates FKBP51 at two major lysine residues. SIRT7 suppresses Akt activation and modulates cell sensitivity to genotoxic agents (154). The inhibitory effects of SIRT7 on Akt activation were also observed in murine hearts.

SIRTUINS IN INSULIN-SENSITIVE ORGANS

Adipose tissue, liver, and muscle are the primary insulin-responsive organs. Insulin regulates blood glucose concentrations by suppressing hepatic glucose output and stimulating glucose uptake by muscle and adipose tissue. In addition, insulin promotes energy storage in adipose tissue, liver, and muscle by stimulating lipogenesis, glycogen, and protein synthesis but inhibiting lipolysis, glycogenolysis and protein catabolism (155).

The impaired lipogenic/adipogenic capacity of adipose tissue leads to increased body fat mass and adverse metabolic consequences (4, 156). Adipose tissue is not only an excessive energy storage pot but also a highly active endocrine organ that secretes proteins, adipokines, cytokines and chemokines to influence insulin sensitivity. Circulating FFAs derived from adipocytes are involved in the accumulation of triglycerides and fatty acid-derived metabolites in muscle and liver, which is a contributing factor to insulin resistance (155). In addition, adipose tissue is an important initiator of the inflammatory response to obesity (28), and FFAs from adipose tissue are important ER stress-triggering factors (28).

The liver, as the central organ responsible for maintaining lipid and glucose hemostasis in the body, plays a crucial role in insulin sensitivity and metabolic diseases. During prolonged fasting or starvation, the liver converts lipids to available energy through fatty acid oxidation and provides glucose to maintain normal blood glucose, initially by glycogenolysis and then by switching to gluconeogenesis (157, 158). Under energy abundance conditions, the liver promotes glycogenesis and lipogenesis to store energy. Insulin stimulates glycogen accumulation and blocks gluconeogenesis and glycogenolysis in the liver to suppress hepatic glucose output (155, 158, 159). In the condition of insulin resistance, suppression of hepatic glucose output is impaired, while increased FFA from adipocytes leads to ectopic lipid accumulation in the liver, which exacerbates insulin resistance (29).

Skeletal muscle is the major site for insulin-stimulated glucose disposal *in vivo* as well as the main energy consumer of lipid catabolism that strongly influences whole-body lipid metabolism (155, 160). The ability to switch between glucose and lipid oxidation is crucial for skeletal muscle to maintain physiological function and metabolic hemostasis (161). Intramuscular fatty acid metabolite accumulation may cause insulin resistance (162).

The functions of Sirtuins in regulating glucose and lipid metabolism as well as insulin sensitivity have been widely investigated in adipose tissue, liver and skeletal muscles (**Figure 3**).

SIRT1 and Insulin-Sensitive Organs

SIRT1 Regulates Fatty Acid and Glucose Metabolism in the Liver

The role of SIRT1 in regulating hepatic gluconeogenesis is controversial under the condition of calorie restriction. Resveratrol has been shown to improve glucose homeostasis in insulin-resistant mice by reducing hepatic gluconeogenesis and increase insulin sensitivity in adipose tissue, skeletal muscle and liver (163). On the contrary, resveratrol causes nuclear translocation of FoxO1 in hepatocytes *via* SIRT1-dependent deacetylation, which leads to activation of gluconeogenesis and increased hepatic glucose output (164). Pyruvate induces SIRT1 protein in the liver during fasting, and once SIRT1 is induced, which can increase gluconeogenic genes expression and promote hepatic glucose output through interacting and deacetylating PGC-1α (165). Conversely, during prolonged fasting, SIRT1 deacetylates CREB regulated transcription coactivator 2 (CRTC2) and promotes its ubiquitin-dependent degradation to inhibit gluconeogenic gene expression, leading to decreased hepatic glucose output (166). On the other hand, upregulation of FOXO1 and PGC-1α activity by SIRT1 leads to activation of gluconeogenic gene expression in hepatic cells and increase hepatic glucose production (157, 166). In addition, SIRT1 regulates the activity of PGC-1α, and glycolytic enzyme phosphoglycerate mutase-1 (PGAM1), inducing repression of glycolytic genes in response to fasting (165, 167). These studies suggest an important role of SIRT1 in maintaining energy balance under fasting. Liver-specific *Sirt1* ko mice develop hepatic steatosis when underwent fasting and obesity when fed with HFD in insulin-dependent and independent manners (38, 168). *Sirt1* transgenic mice show better glucose tolerance and insulin sensitivity and are almost entirely protected from hepatic steatosis with HFD treatment (37, 169). On one hand, SIRT1 deacetylates and activates PGC-1α, increasing fatty acid β-oxidation in the liver (38). On the other hand, SIRT1 plays an important role in inhibiting lipogenesis in the liver. For example, resveratrol can increase the levels of sterol regulatory element-binding protein (SREBP), a critical regulator of lipid and sterol homeostasis in eukaryotes, in livers of alcohol-treated mice and alleviate alcoholic fatty liver (170). SRT1720, a SIRT1 activator, ameliorates fatty liver through suppressing the expression of lipogenic enzymes, including SREBP-1c, acetyl-CoA carboxylase, and fatty acid synthase, in obesity and insulin resistant mice (171). SIRT1 can directly deacetylate SREBP, and control SREBP protein stability *via* SREBP ubiquitination, leading to attenuating SREBP target lipogenic gene expression and inhibiting lipid synthesis and fat storage (172). Otherwise, SIRT1 activation by polyphenols acts as an upstream regulator in the LKB1/AMPK signaling axis, resulting in repression expression of acetyl-CoA carboxylase and fatty acid synthase and reduction of lipid accumulation in hepatocytes (173).

SIRT1 Regulates Adipocyte Differentiation and Adipogenesis

PPARγ and CCAAT/enhancer-binding protein α (C/EBPα) are master regulators of adipogenesis (156, 174). SIRT1 overexpression inhibits the expression of PPARγ and C/EBPα in 3T3-L1 adipocytes (175). SIRT1 represses PPARγ by docking with its cofactors nuclear receptor co-repressor (NCoR) and silencing mediator of retinoid and thyroid hormone receptors (SMRT) and suppresses adipogenesis (175). In differentiated fat cells, upregulation of SIRT1 by resveratrol triggers lipolysis and loss of fat, but SIRT1 inhibitor nicotinamide reduces the release of free fatty acid (175).

	Liver				Adipose tissue				Skeletal Muscle	
	Fatty acid oxidation	Lipogenesis	Glycolysis	Gluconeogenesis	Thermogenesis	Browning	Adipogenesis	Fatty acid oxidation	Glycolysis	Fatty acid oxidation
SIRT1	FOXO1(+), PGC1α(+), CRTC2(-), PPARα(+)	SREBP1c(-), AMPK(+)	HIF1α(-), FOXO1(+), PGC1α(+), PGAM-1(-)	FOXO1(+), PGC1α(+), PPARα(+), CRTC2(-)		PPARγ(-), Prdm16(+)	FOXO1(+), PPARγ(-)		PGC1α(+)	PGC1α(+), AMPK(+), PPARα(+)
SIRT2	PGC1α(+)	ACLY(-)	GKRP(+)	PEPCK1(+)			FOXO1(+)	PGC1α(+)		
SIRT3	LCAD(+)		PDH(+), IDH2(+), NDUFA9(+)		PGC1α(+)	PGC1α(+), SDH(+)			PDH(+), HK2(+)	
SIRT4	PPARα(+), AMPK(+)		PDH(-)				MCD(-)			MCD(-)
SIRT5	ECHA(+), HCDH(+)		GAPDH(+)	GAPDH(+)						
SIRT6	CPT1(+), AOX(+)	SREBP-2(-), ACC(-), SCD(-), FAS(-)	GK(-)	GCN5(+)	PGC1α(+)	PGC1α(+)	KIF5C(-)		AMPK(+)	AMPK(+)
SIRT7	GABPβ1(+)	DCAF1/DDB1/CUL4B (-)	PGK1(-)	G6PC(+)			SIRT1(-), PPARγ(+)			

FIGURE 3 | Sirtuins regulates metabolism in insulin-target organs. The functions of Sirtuins in the regulation of glucose and fatty acid metabolism in the liver, adipose tissue, and skeletal muscle. The (–) indicates Sirtuin represses the activation/expression of this target, whereas (+) indicates Sirtuin promotes the activation/expression of the target. The green background indicates Sirtuin promotes the biological process whereas the pink background indicates the Sirtuin represses the biological process. PGC-1α, peroxisome proliferator-activated receptor gamma coactivator 1-alpha; TORC2, CREB regulated transcription coactivator 2; PPARα, peroxisome proliferator-activated receptor alpha; SREBP1c, Sterol response element-binding protein 1c; AMPK, AMP-activated protein kinase; HIF1α, hypoxia-inducible factor 1 alpha; PGAM-1, phosphoglycerate mutase 1; CRTC2, CREB regulated transcription coactivator 2; PPARγ, peroxisome proliferator-activated receptor gamma; Prdm16, PR domain containing 16; PEPCK1, phosphoenolpyruvate carboxykinase 1; LCAD, long-chain acyl-CoA dehydrogenase; PDH, pyruvate dehydrogenase; IDH2, isocitrate dehydrogenase; NDUFA9, NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 9; SDH, succinate dehydrogenase; HK2, hexokinase 2; MCD, malonyl-CoA decarboxylase; ECHA, trifunctional enzyme subunit alpha; HCDH, hydroxyacyl-Coenzyme A dehydrogenase; GAPDH, Glyceraldehyde 3-phosphate dehydrogenase; SREBP-2, sterol regulatory element-binding protein 2; ACC, acetyl-CoA carboxylase; SCD, stearoyl-CoA desaturase; FAS, fatty acid synthase; GK, glucokinase; GCN5, general control non-repressed protein 5; KIF5C, kinesin heavy chain isoform 5C; GABPβ1, GA binding protein β1; DCAF1/DDB1/GUL4B, DDB1-CUL4-associated factor 1 (DCAF1)/damage-specific DNA binding protein 1 (DDB1)/cullin 4B (CUL4B) complex; PGK1, phosphoglycerate kinase 1; G6PC, glucose-6-phosphatase, catalytic subunit; GKRP, glucokinase regulatory protein.

SIRT1 Regulates Lipid Metabolism in Skeletal Muscle

There is a strong correlation between the presence of intramyocellular lipid in skeletal muscle and liver and progression of T2DM (176, 177). Fasting induces PGC-1α deacetylation by SIRT1 in skeletal muscle, and that is required for activation of mitochondrial fatty acid oxidation genes (178). SIRT1 overexpression protects C2C12 myotubes against fatty acid-induced insulin resistance through transcriptional repression of PTP1B (3). In skeletal muscle, SIRT1 acts downstream of AMPK signaling, deacetylates and modulates the activity of PGC-1α, FOXO1, and FOXO3a to inhibit lipogenesis and promote energy consumption (179). SIRT1 overexpression or resveratrol treatment increases insulin-induced Akt phosphorylation and activation *via* interacting with the PI3K adapter subunit p85 (180). These studies suggest SIRT1 plays a positive role in ameliorating insulin sensitivity in skeletal muscle. However, several studies have demonstrated that skeletal muscle-specific overexpression of SIRT1 does not enhance whole-body energy expenditure or skeletal muscle insulin sensitivity under normal or overfeeding conditions (181, 182). These controversial results suggest SIRT1 in other metabolic tissues, such as adipose tissue, liver or intestinal tissue, may play a role in the metabolic benefits of SIRT1 activation (183). Notably, high-intensity interval training increases SIRT1 activity in human skeletal muscle and mice with muscle-specific inactivation of the SIRT1 deacetylase domain displayed reduced myofiber size, impaired muscle regeneration, and derepression of muscle developmental genes (184, 185). Therefore, SIRT1-mediated metabolic balance is important for skeletal muscle homeostasis and regeneration.

SIRT2 and Insulin-Sensitive Organs

SIRT2 Regulates Adipocyte Differentiation and Adipogenesis

SIRT2 is widely distributed and has been detected in a wide range of metabolic tissues, including the brain, muscle, liver, pancreas and adipose tissue. SIRT2 expression is regulated by metabolic status. For instance, the expression of SIRT2 is elevated in the white adipose tissue (WAT) of cr mice (98). SIRT2 gene expression increased in the peripheral blood mononuclear cells of obese subjects following an 8-week hypocaloric diet (186). By contrast, SIRT2 protein expression in visceral WAT from human obese subjects and a mouse model of diet-induced obesity is downregulated compared with that in WAT from lean controls

(187). SIRT2 gene expression is significantly lower in peripheral blood mononuclear cells of obese children with insulin resistance than in those without insulin resistance (188). A growing body of literature has indicated that SIRT2 is involved in regulating various metabolic processes, including adipocyte differentiation, hepatic gluconeogenesis, and insulin action. SIRT2 mRNA is more abundant than other Sirtuins in adipose tissue *in vivo* and preadipocytes in culture (150), implicating the possible important role of SIRT2 in adipose tissue. FOXO1 acts as an adipogenesis inhibitor (189). In adipose tissue, FOXO1 can interact with PPARγ and negatively regulate its transcriptional activity (190) or bind to the PPARγ promoter region and suppress its expression (191). In mouse 3T3-L1 preadipocytes, SIRT2 interacts with and deacetylates FOXO1, which antagonizes FOXO1 phosphorylation and promotes nuclear retention of FOXO1, leading to repression of the expression of *PPARγ* and *C/EBPα* as well as genes marking terminal adipocyte differentiation, such as *Glut4*, *aP2*, and *fatty acid synthase* (150). SIRT2 also suppresses adipogenesis by deacetylating FOXO1 to promote the binding of FOXO1 to PPARγ and subsequent repression of PPARγ transcriptional activity (192). Increased *de novo* lipogenesis is an important contributor to increased adipose mass (193). ATP-citrate lyase (ACLY) is the building block for *de novo* lipid synthesis, which converts glucose-derived citrate into acetyl-CoA (194). ACLY is acetylated on multiple lysine residues in response to high glucose and promotes lipogenesis, while SIRT2 deacetylates and destabilizes ACLY, leading to reduced lipogenesis (195). Transcriptional regulators such as PPARs and the coactivator PGC-1α play key roles in the process of fatty acid β-oxidation, which determines whole-body energy expenditure (196). Through transcriptional repression of SIRT2, hypoxia-inducible factor 1α (HIF1α) decreases deacetylation of PGC-1α and further diminishes fatty acid β-oxidation in WAT. Adipocyte-specific HIF1α inactivation leads to increased expression of SIRT2 and attenuates dietary-driven obesity in mice (187). These studies suggest that SIRT2 contributes to the control of adipose tissue mass by inhibiting adipogenesis and lipogenesis but promoting fatty acid β-oxidation.

SIRT2 Participates in Gluconeogenesis in the Liver

SIRT2 deacetylates and subsequently increases the stability of PEPCK1, the gluconeogenic rate-limiting enzyme under conditions of glucose deprivation, leading to increased gluconeogenesis (197, 198). FOXO1 and PGC-1α reportedly activate the process of gluconeogenesis in the liver by increasing the transcription of gluconeogenic enzyme genes and are considered negative regulators of insulin sensitivity in the liver (199–201). Insulin suppresses gluconeogenesis by regulating the FOXO1-PGC-1α interaction (199). SIRT2 deacetylates and activates FOXO1/PGC-1α in adipocytes (150, 187, 192), which implies that SIRT2 may enhance gluconeogenesis through the FOXO1-PGC-1α pathway. However, whether the role of SIRT2 in gluconeogenesis depends on different nutrient conditions must be elucidated. In addition, the roles of SIRT2 in metabolic diseases are largely unknown.

SIRT3

Human SIRT3 is expressed in a variety of metabolically active tissues, including muscle, liver, kidney, heart, brain, and BAT (202–204). The expression of SIRT3 in the liver and adipose tissue of mice increases during cr (205–208). A single nucleotide polymorphism in the human *SIRT3* gene has been correlated with the reduced enzymatic efficiency of SIRT3 and the development of metabolic syndrome (209). Compared with WT mice, *Sirt3*-KO mice fed an HFD show accelerated obesity, insulin resistance, hyperlipidemia, and hepatic steatosis (209).

SIRT3 Participates in WAT/BAT Metabolism and Thermogenesis

High levels of SIRT3 occur in the BAT. Cold exposure upregulates SIRT3 expression in the BAT. Increasing the expression of PGC-1α and uncoupling protein 1 (UCP1) by sustained expression of SIRT3 in brown adipocytes leads to increased thermogenesis (7, 206). Fatty acid β-oxidation in the BAT of *Sirt3*-KO mice is significantly reduced (210). Although SIRT3 maintains a low level in WAT (206), several studies refer to the role of SIRT3 in regulating WAT lipid metabolism. cr activates SIRT3 expression in both white and brown adipose tissue; SIRT3 expression decreases in the BAT of several lines of genetically obese mice (206). In a human study, SIRT3 gene expression was decreased in VAT from morbid subjects (211) and WAT from children with obesity (212). Although the role of SIRT3 in WAT lipid metabolism is intricate and unclear, these studies suggest that SIRT3 may play a protective role in obesity.

SIRT3 Regulates Fatty Acid Oxidation in the Liver

Sirt3-deficient mice show higher levels of fatty acid β-oxidation intermediate products and triglycerides in the liver during fasting and develop hepatic steatosis (210). Metabolomic analyses of fasted *Sirt3*-deficient mice revealed that SIRT3 is involved in fatty acid β-oxidation and modulates fatty acid β-oxidation at multiple points, such as short-chain L-3-hydroxy acyl-CoA dehydrogenase (SCHAD), very-long-chain acyl-CoA dehydrogenase (VLCAD) and 3-ketoacyl-CoA thiolase, in addition to LCAD (208). LCAD is a key enzyme in mitochondrial fatty acid β-oxidation, and LCAD deficiency causes hepatic steatosis and hepatic insulin resistance (213, 214). SIRT3 promotes hepatic fatty acid β-oxidation through deacetylation and activation of LCAD (210). However, hepatocyte-specific *Sirt3*-KO mice do not show any obvious metabolic phenotype under either chow or HFD conditions, despite a marked global hyperacetylation of mitochondrial proteins (215). These conflicting findings from global *Sirt3*-KO mice and tissue-specific KO mice suggest that the roles of SIRT3 in other cell types may be important for SIRT3-mediated metabolic effects in the liver.

SIRT3 Regulates Glucose Metabolism in Skeletal Muscle

SIRT3 expression decreases in the skeletal muscle of diabetic and HFD-fed mice (36, 216). CR and exercise upregulate SIRT3 expression in mouse skeletal muscle (162). These findings suggest that SIRT3 is involved in skeletal muscle metabolism. Although muscle-specific *Sirt3* KO in mice shows no obvious effects on

global metabolic hemostasis under normal conditions (215), striking results have been shown in global *Sirt3*-KO mice. Global *Sirt3*-KO mice exhibit decreased oxygen consumption and enhanced oxidative stress in skeletal muscle that leads to impaired insulin signaling (36). The deletion of *Sirt3 in vivo* and *in vitro* induces hyperacetylation of the pyruvate dehydrogenase (PDH) E1α subunit and leads to decreased PDH enzymatic activity (161). Inhibition of PDH activity reduces glucose oxidation and results in a switch to fatty acid β-oxidation, thus leading to a loss of skeletal muscle metabolic flexibility (161). In addition, HFD-fed *Sirt3*-KO mice exhibit increased insulin resistance due to defects in skeletal muscle glucose uptake (217). These studies suggest that SIRT3 may protect insulin sensitivity in skeletal muscle.

SIRT4

SIRT4 Regulates Lipogenesis

The expression of SIRT4 is upregulated in the liver and adipose tissues in rodents fed an HFD (218, 219). SIRT4 deacetylates and inhibits malonyl CoA decarboxylase (MCD), an enzyme producing acetyl-CoA from malonyl CoA, consequently repressing fatty acid oxidation but promoting lipogenesis in WAT and skeletal muscle under nutrient abundance conditions. *Sirt4*-KO mice display increased exercise tolerance and protection against diet-induced obesity (220).

SIRT4 Regulates Fatty Acid Oxidation

SIRT4 inhibition in mouse primary hepatocytes increases fatty acid oxidation gene expression, leading to increased fat oxidative capacity in liver (118). The same result is obtained in muscle (118). Similarly, primary hepatocytes from *Sirt4*-KO mice exhibit higher rates of fatty acid oxidation. SIRT4 suppresses PPARα activity and inhibits hepatic fatty acid oxidation by modulating SIRT1 activity (221). Livers from NAFLD patients exhibit increased SIRT4 and lipogenic gene expression (222). These results support the notion that SIRT4 is likely to inhibit fatty acid oxidation and potentiate ectopic lipid storage in liver and skeletal muscle.

SIRT5

SIRT5 in the Regulation of Fatty Acid Metabolism

SIRT5 is highly expressed in metabolic tissues, including the heart, skeletal muscle, brain, liver, and kidney (121). Using a label-free quantitative proteomic approach, Rardin et al. characterized the lysine succinylome in liver mitochondria and revealed a major role for SIRT5 in regulating many metabolic pathways, including β-oxidation and ketogenesis (223). Park et al. revealed that SIRT5 desuccinylates a set of metabolic enzymes in mitochondria that are involved in amino acid degradation, the TCA cycle and fatty acid metabolism (224). In contrast to the other two mitochondrial Sirtuins, SIRT5 protein levels do not change during CR (121, 207). However, similar to *Sirt3*-KO and *Sirt4*-KO mice, *Sirt5*-KO mice do not show any overt metabolic abnormalities under either normal chow or HFD conditions (225). Sirt5 deficiency does not protect or sensitize mice to the development of HFD-induced obesity, hypertension, and insulin resistance (225). The results from *Sirt5*-KO mice suggest that SIRT5 is not dispensable for cellular metabolism, at least under normal conditions. Subsequent studies have shown promising results. In humans, *SIRT5* gene expression decreases in the liver of NAFLD patients (222), and the expression of *SIRT5* in adipose tissue is positively correlated with insulin sensitivity (226). Using affinity enrichment and label-free quantitative proteomics, Nishida *et al.* characterized the SIRT5-regulated lysine malonylome (227). Pathway analysis identified gluconeogenesis and glycolysis as the pathways most enriched in SIRT5-regulated malonylated proteins (227). SIRT5 regulates glyceraldehyde phosphate dehydrogenase (GAPDH), a glycolytic enzyme, through demalonylation of lysine 184 (227). According to these results, SIRT5 may play a critical role in regulating glucose and lipid metabolism and preserving insulin sensitivity. Mitochondria-specific Sirtuin knockout mice show no obvious metabolic abnormalities, indicating that mitochondrial Sirtuins serve as nutrient sensors to maintain energy homeostasis.

SIRT6 and Insulin-Sensitive Organs

SIRT6 Regulates Adipogenesis, Lipid Metabolism and Thermogenesis in Adipose Tissue

SIRT6 expression is decreased in adipose tissue of *db/db* mice but increased in adipose tissue of human individuals with weight loss (228, 229), suggesting that SIRT6 plays a role in adipose tissue. Chen et al. (230) demonstrated that SIRT6 is required for mitotic clonal expansion during adipogenesis by inhibiting expression of kinesin family member 5C (KIF5C) and subsequent increasing CK2 kinase activity. *Sirt6* transgenic mice exhibit resistance to HFD-induced obesity and insulin resistance (231). Conversely, fat-specific *Sirt6* knockout increases blood glucose levels and hepatic steatosis, and sensitizes mice to HFD-induced obesity and insulin resistance (59, 60, 232). SIRT6 overexpression downregulates a set of PPARγ target genes that are involved in lipid metabolism, lipid transport and adipogenesis (231). Especially, SIRT6 decreases expressions of ANGPTL4, a negative regulator of lipoprotein lipase, and diglyceride acyltransferase 1 (DGAT1), a key enzyme in triglycerides synthesis, leading to the increased serum triglyceride clearance and reducing triglyceride synthesis in adipose tissues (231). *Sirt6* deletion decreases FoxO1 transcriptional activity by increasing its acetylation and phosphorylation and reduces expression of adipose triglyceride lipase (ATGL), a key lipolytic enzyme, reducing lipolysis (59). Fat-specific *Sirt6* knockout not only induces obesity and insulin resistance but also impairs the thermogenic function of brown adipocytes (232). Yao et al. (232) found *Sirt6* deletion decreases ATF2 binding to the PGC-1α promoter, leading to reducing the expression of PGC-1α and PGC-1α target thermogenic genes.

SIRT6 Represses Gluconeogenesis and Lipid Accumulation in the Liver

The hepatic SIRT6 level is reduced in obese/diabetic mice and gluconeogenic genes were higher in *Sirt6*-deficient livers whereas ectopic re-expression of SIRT6 suppressed gluconeogenesis and normalizes glycemia (228, 233). Mechanistically, SIRT6 interacts with and increases the activity of general control non-repressed protein 5 (GCN5), an acetyltransferase, which, in turn, catalyzes the acetylation of PGC-1α, suppressing gluconeogenic

gene expression such as *phosphoenolpyruvate carboxykinase C (PEPCK-C)* and *glucose 6-phosphatase*, and resulting in repression of hepatic glucose output (228). p53 directly activates expression of SIRT6, which subsequently interacts with and deacetylates FoxO1, leading to FoxO1 export to the cytoplasm, and finally, reduce the expression of gluconeogenetic genes such as *glucose 6-phosphatase alpha* and *phosphoenolpyruvate carboxykinase 1* (234). Human fatty liver samples exhibited significantly lower levels of SIRT6 than normal controls and liver-specific deletion of *Sirt6* in mice causes increased glycolysis, triglyceride synthesis, reduced β-oxidation, and leads to liver steatosis (235). Rosiglitazone, an agonist of PPARγ, increases the expression of SIRT6, PGC-1α, and FoxO1, and AMPK phosphorylation in rat liver and ameliorates hepatic lipid accumulation (236). *Sirt6* knockdown abolished the effects of rosiglitazone (236), suggesting *Sirt6* at least partly mediates the metabolic effects of rosiglitazone. Altogether, those evidence suggest that SIRT6 significantly participates in glucose and lipid metabolism in the liver.

SIRT6 Increases Insulin Sensitivity in the Skeletal Muscle

SIRT6 also regulates metabolic homeostasis in the skeletal muscle. *Sirt6* transgenic mice show enhanced insulin sensitivity in skeletal muscle and exhibit enhanced insulin-induced activation of Akt in the gastrocnemius (153). By contrast, skeletal muscle-specific *Sirt6* ko mice exhibit impaired glucose homeostasis and insulin sensitivity, attenuating whole-body energy expenditure (237). Mechanistically, *Sirt6* deletion decreases AMPK activity and subsequently decreases the expression of genes involved in glucose and lipid uptake, fatty acid oxidation, and mitochondrial oxidative phosphorylation (237). Further studies are needed to elucidate the direct mechanism underlying SIRT6 function in skeletal muscle.

SIRT7

SIRT7 Regulates Fatty Acid Metabolism in Adipose Tissues

SIRT7 is the least characterized Sirtuin of the seven mammalian Sirtuins. SIRT7 protein levels are high in the liver, spleen, and testis, whereas are low in the muscle, heart, and brain of mice (16). In human, *Sirt7* mRNA is expressed in various tissues (10). Recent reports clarify the important roles of SIRT7 in a variety of biological processes including DNA repair, chromatin assembly, and aging. However, the role of SIRT7 in metabolism remains largely unknown. The expression of *Sirt7* mRNA level is upregulated in adipose tissues of obese patients (238). In HFD-fed mice, *Sirt7* knockout decreased the expression of the fatty acid transporter CD36 in WAT (239). In addition, *Sirt7* knockout led to an increase of thermogenesis along with increased expression of UCP1 and DIO2 in BAT (239). These results suggest SIRT7 regulates lipid metabolism in adipocytes. Recently, Fang *al et.* found that SIRT7 restricts SIRT1 activity by preventing SIRT1 auto-deacetylation, and increasing SIRT1 activity in *Sirt7*-KO mice blocks PPARγ and adipocyte differentiation, thereby decreases the accumulation of white fat (240, 241). Together, these findings implicate the important role of SIRT7 in the regulation of fatty acid metabolism.

SIRT7 Regulates Fatty Acid and Glucose Metabolism in the Liver

Up to now, there are three studies linking SIRT7 to the liver lipid metabolism using independently generated mouse models. Shin et al. reported that *Sirt7*-KO mice developed steatosis resembling human fatty liver disease (242). Selectively overexpression of SIRT7 in the liver of *Sirt7*-KO mice *via* adeno-associated virus 8 (AAV8)-mediated gene transfer prevents the development of fatty liver (242). The authors found expressions of inflammatory markers and lipogenic genes are increased in *Sirt7*-deficient livers, and they clarified the underlying mechanism as SIRT7 repressing the expression of ribosomal proteins through decreasing Myc activity and further suppressing ER stress (242). Ryu et al. generated a different *Sirt7*-KO mouse by deleting exons 6-9, and observed more general metabolic defects including hepatic microvesicular steatosis, increased blood lactate levels, reduced exercise performance, cardiac dysfunction and age-related hearing loss induced by multisystemic mitochondrial dysfunction (127). Mechanistically, SIRT7 deacetylates GABPβ1, thereby enables it to form the transcriptionally active GABPα/GABPβ heterotetramer, and then promotes mitochondria function (127). Another study has the opposite result. Yoshizawa *et al.* reported that *Sirt7*-KO mice, deleting exons 4-9, are resistant to HFD induced fatty liver, obesity, and glucose intolerance (239). TR4 is a nuclear receptor involved in lipid metabolism and its target genes increase fatty acid uptake and triglyceride synthesis and storage (243). Hepatic SIRT7 was reported to increase TR4 expression through binding with DCAF1/DDB1/CUL4B E3 ubiquitin ligase complex and inhibiting TR4 degradation (239). It is difficult to explain the divergence of three *Sirt7*-KO mouse models with different genetic background. Liver-specific knockout or *Sirt7* transgene mouse model may be helpful to clarify the role of SIRT7 in liver lipid metabolism (239, 242). In addition to lipid metabolism, SIRT7 is involved in glucose metabolism. SIRT7 regulates acetylation at the K323 site of phosphoglycerate kinase 1 (PGK1), an important enzyme in glycolysis, decreases PGK1 enzyme activity and inhibits glycolysis in liver cancer cells (244). Yoshizawa *et al.* Found that *Sirt7*-KO mice show decreased expression of the hepatic glucose-6-phosphatase catalytic subunit (G6PC), a key gluconeogenic enzyme, and resistance to glucose intolerance (239). Mechanistically, glucose deprivation stimulates SIRT7 binding to the promoter of G6PC, and deacetylating H3K18 in the G6PC promoter, which results in elevated G6PC expression and promotion of hepatic gluconeogenesis (245).

SIRTUINS IN AGING-RELATED METABOLIC DEFECTS

Aging is a complex process accompanied by the declines in basal metabolic rate and physical activity. Aging is one of the major risk factors contributing to the development of insulin

resistance, obesity, T2DM and metabolic syndrome (246). During the aging process, chronic inflammation and mitochondria dysfunction in pancreatic β cells and insulin-sensitive organs have been demonstrated to be major mechanisms linking aging and insulin resistance (247–250). As mentioned above, Sirtuins play important roles in regulating inflammation and mitochondria function. Sirtuins are critically involved in lifespan and healthspan. Deficiency of Sirtuins (SIRT1, SIRT6, and SIRT7) is associated with shortened lifespan and metabolic diseases (251). Our recent evidence also demonstrated that SIRT2 deficiency also facilitated the aging-related development of cardiac dysfunction, including hypertrophy and fibrosis (252). By contrast, germline or cell-specific overexpression of SIRT1 or SIRT6 were reported to expand lifespan and defense metabolic diseases in insulin-dependent and independent manners (253–255).

Sirtuin-targeted strategies show promising in repressing aging-related insulin resistance and metabolic diseases. For instance, the SIRT1 activator SRT1720 extends lifespan and improves the health of mice fed a standard diet (163, 256). It is well established that caloric restriction (CR), the Sirtuin activator, is an effective and reliable means to defense against aging and extend the lifespan and healthspan of mammals, including monkeys (257). Activation of vascular SIRT1 by CR leads to the repressing of aging-related metabolic vascular diseases, including atherosclerosis and aortic aneurysm (258–260). In human studies, CR also can reduce insulin resistance significantly and delay the onset of metabolic diseases (261, 262). Although the mechanisms by which CR extend lifespan are not fully understood, Sirtuins have been implicated to mediate beneficial effects of CR on aging (263). Notably, the CR mimetics (metformin, resveratrol, rapamycin) could expand lifespan and repress diseases related to insulin resistance in rodents partially through activation of Sirtuins (257, 264). Our data showed that SIRT2 contributes to the effects of metformin on aging-related diseases, including cardiac remodeling (252). Currently, clinical trials investigating the anti-aging effects of metformin is undergoing (ClinicalTrials.gov Identifier: NCT02432287).

Importantly, the Sirtuins do not function in individual metabolic organs or cell types alone during aging. Instead, the Sirtuins orchestrate the crosstalk between different organs or between different cell types within the local microenvironmental niche to maintain metabolic homeostasis and prevent against insulin resistance. The SIRT1 activator SRT3025 provides atheroprotection in *Apoe*$^{-/-}$ mice by reducing hepatic *Pcsk9* secretion and enhancing *Ldlr* expression (265). Resveratrol activates duodenal SIRT1 to initiate a gut-brain-liver neuronal axis that improves hypothalamic insulin sensitivity in rats (183). SIRT1 in intestinal stem cells also contributes to the protection roles of caloric restriction on aging (266). In addition, SIRT3 activation by nitrite and metformin improves insulin sensitivity in skeletal muscle and normalizes pulmonary hypertension associated with heart failure with preserved ejection fraction (267). Sirtuins also regulate inflammatory cells within the local microenvironmental niches to regulate insulin resistance in an autocrine or paracrine manner (41, 79, 80).

Therefore, targeting Sirtuins could be a promising strategy for improvement of insulin sensitivity and metabolic status of the whole body. However, activation of Sirtuins alone may not archive the biggest benefits because of the exhaustion of the endogenous NAD. Sirtuin activator in supplement with NAD precursor may represent a better therapeutic strategy for repressing aging-related insulin resistance and metabolic diseases.

CONCLUDING REMARKS

Insulin resistance is a critical pathological feature of obesity and metabolic syndrome and plays a key role in the pathogenesis of T2DM and attendant cardiovascular complications. Moreover, insulin resistance provides a therapeutic strategy to prevent, delay or treat T2DM, obesity, and metabolic syndrome by improving insulin sensitivity. Although insulin resistance is a complex metabolic disorder that has remained poorly understood, Sirtuin family members are involved in the potential cellular mechanisms of the pathogenesis of insulin resistance. According to accumulating evidence in the past decades, Sirtuin family members have emerged as a nutrient sensor to maintain energy homeostasis. Cellular and animal studies have demonstrated that Sirtuins play an important role in regulating glucose and lipids by modulating crucial enzymes in metabolic pathways and interfering with inflammation, oxidative stress, mitochondrial dysfunction, ER stress, and the insulin signaling pathway.

Sirtuins respond to environmental (diet and lifestyle) or metabolic (obesity, fasting, and diabetes) insults at mRNA and protein levels in insulin-sensing organs (268, 269). The roles of Sirtuins in regulating glucose and lipid metabolism as well as insulin resistance in liver, adipose tissue, and skeletal muscle make their importance in regulating metabolic diseases, including T2DM and diabetic complications (269).

Nevertheless, many additional studies are needed.

1. Different Sirtuins may have the same downstream targets, such as FoxO3a, FoxO1, PGC-1α, and GDH, and there is cross-talk among Sirtuin family members (56, 117, 270). How do different Sirtuin members coordinate to regulate the same downstream targets?
2. The Sirtuin family comprises NAD^+-dependent histone deacetylases; however, recent results have revealed that Sirtuin members can act in a deacetylase-independent manner (117). How can we determine the functions and activities of Sirtuins in addition to their deacetylation function in insulin resistance?
3. In addition to Sirtuins, there are other epigenetic modification enzymes, including SUV39H1 and EZH2 are involved in insulin resistance and T2DM (271–276). There is an interaction between SUV39H1 and Sirtuins, including SIRT1, SIRT3, and SIRT7 (277–281). EZH2 is reportedly the deacetylating substrate of SIRT1 (282, 283). SIRT2 negatively regulates JMJD2A expression in human non-small cell lung cancer tissues (284). Is JMJD2A involved in insulin resistance? In the condition of insulin resistance, how do these epigenetic modification enzymes influence each other and consequently act on insulin sensitivity?
4. Obesity, insulin resistance, and T2DM are aging-related abnormalities. Sirtuins, especially SIRT1, SIRT2, and SIRT6,

are characterized as protectors of aging and aging-related diseases. Whether the promotive effect of aging by the decline of Sirtuin activity is involved in insulin resistance deserves further investigation. In addition, cell senescence-induced organ dysfunction and aging (senescaging) are common during physiological and pathological aging processes (268). Selective elimination of senescent cells, or senolysis, was reported to delay aging and aging-related metabolic diseases including congestive decline, atherosclerosis, cardiac diseases, and osteoarthritis (285–291). However, it remains to elucidate that what are the physiological and pathological functions of cellular senescence in organs during aging and that whether Sirtuins regulate senescaging in insulin resistance and healthy conditions.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work. SZ is responsible for literature collection and article draft. XT designed the Figures and revised the manuscript. H-ZC is the leading principal investigator who directed the study and data analysis, and prepared the manuscript. All authors approved publication of this work.

ACKNOWLEDGMENTS

This work was supported by the National Natural Science Foundation of China (Grant Nos. 81600038, 31571193 and 81800273). H-ZC is also supported by the Youth Top-notch Talent Support Program and the Youth Yangtze River Scholar Program in China. XT is also supported by the Postdoctoral Innovative Talents Support Program (BX20180206), the China Postdoctoral Science Foundation (2018M631084) and Full-time Postdoctoral Research and Development Fund of Sichuan University (2018SCU12010). We apologize to those scientists whose work we could not highlight owing to space limitations. We thank Miss. Xiao-Feng Chen for reading the manuscript and helpful suggestions.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Endocrinology

REVIEW
published: 29 November 2018
doi: 10.3389/fendo.2018.00724

Distinctive Roles of Sirtuins on Diabetes, Protective or Detrimental?

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Dysregulation of metabolic pathways leads to type 2 diabetes, characteristic of high glucose concentration caused by insulin resistance. The histone deacetylases sirtuins exhibit remarkable enzymatic activities. Accumulating evidence indicates that sirtuins can be pharmacologically activated to ameliorate diabetes. Here, we evaluated different roles of sirtuins (SIRT1-SIRT7) in diabetes progression and described their involvement in metabolic pathways of skeletal muscle, adipose tissue and liver. The nuclear sirtuins, SIRT1, SIRT6, and SIRT7, regulate the activity of key transcription factors and cofactors in almost all tissues with the cellular responses to energy demands. The mitochondrial sirtuins, SIRT3, SIRT4, and SIRT5, regulate the activity of mitochondrial enzymes in response to fasting and calorie restriction. Moreover, genetic polymorphisms of SIRT1 and SIRT2 have been reported to associate with diabetes development. It's worth noting that SIRT1, SIRT2, SIRT3, and SIRT6 are positive regulators of insulin resistance in most cases. In the opposite, SIRT4 and SIRT7 inhibit insulin secretion and fatty acid oxidation. Identification of SIRT1 activators for diabetes has gained wide attention, such as metformin, resveratrol, and resveratrol derivatives. Randomized, prospective, and large-scale clinical trials are warrant to uncover the responsibilities of SIRTs modulators on diabetes progress.

Keywords: SIRTs, diabetes, insulin resistance, glucose uptake, fatty acid oxidation

OPEN ACCESS

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Specialty section:
This article was submitted to Cellular Endocrinology, a section of the journal Frontiers in Endocrinology

Received: *23 August 2018*
Accepted: *15 November 2018*
Published: *29 November 2018*

Citation:
Song J, Yang B, Jia X, Li M, Tan W, Ma S, Shi X and Feng L (2018) Distinctive Roles of Sirtuins on Diabetes, Protective or Detrimental? Front. Endocrinol. 9:724. doi: 10.3389/fendo.2018.00724

INTRODUCTION

Sirtuins belong to class III histone deacylases, and in each deacylation cycle one molecule NAD^+ is consumed (1). SIRTs isoforms have been defined in mammals, such as SIRT1–7. Although they are equipped with a highly conserved structure of about 275 amino acids, just like the silent information regulator 2 in yeast (2), the C-and N-terminal extensions are distinctive, which are the predominant factor of sirtuins subcellular localization (3). SIRT1, SIRT6, and SIRT7 are principally found in the nucleus. SIRT2 is mainly located in the cytoplasm, and SIRT3-5 are located in the mitochondria (**Figure 1**). The catalytic core is made up of a small zinc-binding domain, a large Rossmann-fold domain, and a few flexible loops which bind these domains together. The large domain of most sirtuins resembles each other, characteristic of a β-sheet encircled by six α-helices, excluding SIRT2, which has seven α-helices (4). In the small domain, diversities are observed in the principal sequence. Firstly, the helix bundle is only absent in SIRT7 and SIRT6. Next, SIRT5 and SIRT4 have a loop and a short helix, yielding an insertion in the small domain. This feature

might be essential for the mitochondrial localization (5). Lastly, SIRT1 has a 5-residue loop in this domain, neighboring to the zinc-binding cysteine. Those dissimilarities in the catalytic core might closely relate with their key properties.

SIRT1-3 and SIRT6 exhibit remarkable demyristoylase activity (6, 7). Except for functioning as an ADP-ribosyltransferase (8, 9), SIRT4 also possesses a lipoamidase effect (10). By targeting carbamoyl phosphate synthetase (CPS1), SIRT5 can remove malonyl or succinyl groups in a manner very similar to deacetylation (11, 12). SIRT6 deacetylates histone H3K9 & H3K56, and mono-ADP-ribosyltransferate long-chain acyl and acetyl groups (13). SIRT7 is distributed in all organs and tissues (14) and activates RNA polymerase I transcription. Although several of its molecular substrate have been identified, including WSTF–ISWI chromatin remodeling complex (WICH), rDNA transcription factor UBF (the nucleolar upstream-binding factor) and RNA polI (15), SIRT7's catalytic activity remains elusive.

Diabetes is a global epidemic problem growing exponentially, posing a serious threat. Type 2 diabetes mellitus (T2DM) is a multifactorial metabolic and endocrine disorder for human beings, characteristic of abnormal glucose level in blood. T2DM individuals are estimated to be 642 million by 2,040 globally (16). T2DM is predominately attributed to insulin resistance and pancreatic β-cell dysfunction (17, 18). Insulin resistance, primarily in liver, muscle and adipose tissue as well, spoils glucose disposal, leading to β-cell insulin increase and hyperinsulinemia in a compensatory manner.

Blunted sirtuin activity has been reported to induce diabetes and metabolic syndrome, and aggravate high-fat diet (HFD) effects in mice. Exceptionally, SIRT4 prevents insulin secretion and stimulates T2DM. SIRT4 also negatively regulates fatty acid oxidation (FAO) in muscle and liver cells. A mutation in human SIRT1 caused a familial form of autoimmune diabetes (19–21). SIRT1 can interact with transcription factors and co-activators (RelA/p65, FOXO, and p53). T2DM group has lower SIRT1 mRNA levels compared with healthy group. There is a negative connection between fasting plasma glucose and SIRT1, as shown in the correlation analysis. The expression of SIRT1 in monocytes and granulocytes of T2DM might associate with glucose/lipid metabolism status (22). In both of the kidney and liver of diabetic rats, SIRT1 and SIRT2 gene expressions reduced considerably than blank control group (23). HFD triggers SIRT1 decrement in mice probably via proteolysis (19). SIRT1 expression is also reduced in obese humans (24, 25), and meanwhile diabetes is alleviated in SIRT1-overexpressed mice (26–28). SIRT6 is important for sustaining pancreatic β-cell function in mice. SIRT6 knockout leads to severe hypoglycemia in mice. SIRT6 deficiency results in liver steatosis and accelerates insulin resistance and obesity induced by diet. Overnutrition and aging decreased SIRT6 level as well as irregular lipid and glucose metabolism (29). SIRT7 deficiency in mice induces multi-systemic mitochondrial dysfunction. To fully understand the part SIRTs play in diabetes and to reveal regulatory mechanisms regarding SIRTs is the principal purpose in the current review.

SIRTs IN INSULIN RESISTANCE

Pancreatic β cells secret insulin after nutrient stimulation. In the fed state, glycolysis, glucose uptake, and glycogen formation will be promoted by insulin. The glucose homeostasis in adipose tissue and skeletal muscle can also be regulated by insulin (30). Insulin resistance will give rise to hyperuricemia, visceral adiposity, dyslipidemia, hypertension, and hyperglycemia. About 70% of glucose is disposed in muscle. Free fatty acids accumulation and inflammation in muscle triggeres abundant lipid deposition. Imbalanced muscle uptake promotes extra glucose to returns to the liver, which yields intense circulation of free fatty acids, finally leading to fat accumulation and insulin resistance.

As a major inhibitor of the insulin receptor, protein tyrosine phosphatase 1b (PTP1B) can be inhibited by SIRT1, thus increasing insulin sensitivity (31–33). In insulin-resistant obese mice, PTP1B level was raised, associated with decreased SIRT1 expression in skeletal muscle. SIRT1 overexpression brought PTP1B expression to the baseline and abrogated the insulin-stimulated signaling in skeletal muscle (**Figure 2** and **Table 1**). In β-cells, SIRT1 overexpression boosted insulin secretion and improved glucose tolerance, contributing to glucose homeostasis (34). Uncoupling protein 2 (UCP2) disturbs the electrochemical proton gradient, leading to shrink in ATP production and insulin secretion impairment afterwards (35). SIRT1 overexpression suppressed UCP2, finally enlarging ATP levels and insulin secretion (34, 36). UCP2 expression in MIN6 β-cells was reduced by SIRT1, as demonstrated in microarray assays. SIRT1 RNAi decreased the secretion capability of β-cells, which was renovated by UCP2 RNAi (36). On the other side, oxidative stress-induced hyperglycemia and cytokine toxicity was repressed by SIRT1 via deacetylating forkhead box O1 (FOXO1) and the NF-κB subunit p65 on β-cells, respectively (37, 38). Multiple feedback loops are involved in SIRT signaling network. Via binding to SIRT1 promoter, p53 successfully inhibited SIRT1 transcription activity (39). FOXO3a can block the effect of p53, in consequence SIRT1 promoter will be relieved (39). Peroxisome Proliferator Activated Receptor Gamma (PPARγ) is negatively associated with SIRT1 activity (40, 41), whereas PPARα and PPARβ function in the opposite way (42, 43). MiR-199a, MiR-34a, posttranslational modification, such as phosphorylation, also affect SIRT1 activity or transcription (44–47). Taken together, SIRT1 positively regulated insulin sensitivity.

Recently, SIRT2 has been implicated in sustaining insulin sensitivity and glucose homeostasis (48). In insulin-resistant livers and hepatocytes, SIRT2 expression was lowered, accompanied with mitochondrial dysfunction, extracellular signal-regulated kinase (ERK) activation, and amplified production of reactive oxygen species (ROS). On the contrary, insulin sensitivity and mitochondrial dysfunction was improved, and ROS generation was lessened in SIRT2-overexpressed insulin-resistant hepatocytes (49). In human peripheral blood mononuclear cells (PBMCs), insulin resistance and obesity negatively related with SIRT2 (50). As Protein Kinase B (Akt) substrates, FOXO transcription factors are deacetylated by SIRT2 (51–54). However, the function of SIRT2 in insulin

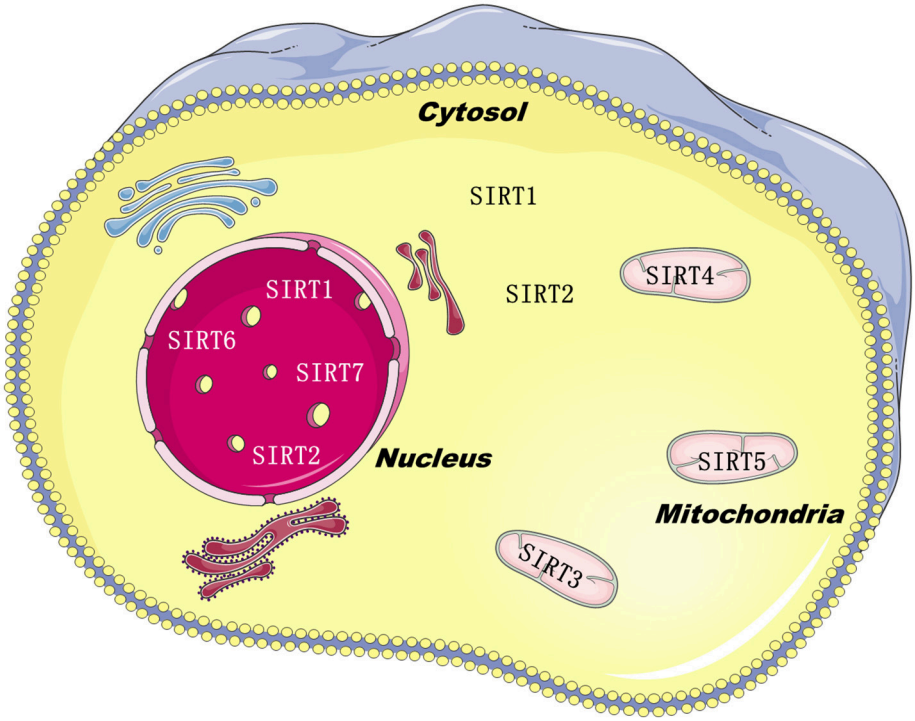

FIGURE 1 | The localization of SIRTs family in the cells.

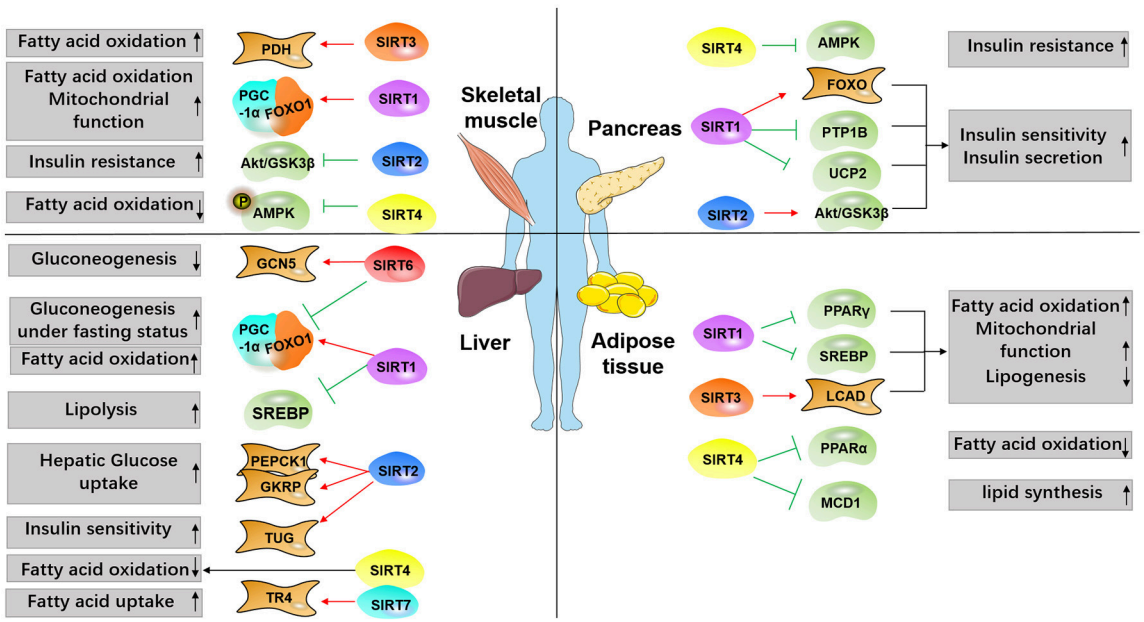

FIGURE 2 | Overview of SIRTs targets involved in diabetes. Major metabolic tissues, including liver, pancreas, adipose, and skeletal muscle are depicted to demonstrate SIRTs functions in the metabolic process modulating insulin resistance, glucose uptake, and lipid synthesis. Red arrows indicate promoting effect, and green arrows indicate inhibiting effect.

TABLE 1 | Summary of sirtuin functions in diabetes development.

Sirtuins	Subcellular localization	Enzyme actions	Substrates	Functions
SIRT1	Nuclear Cytoplasm	Deacetylase	PTP1B, UCP2, PPARα, PPARγ, PPARγ2, p53, FOXO1, PGC-1α, NF-κB, CRTC2, SREBP	Adipogenesis↓ Lipogenesis↓ Lipolysis↑ FAO↑ Glucose uptake↑ Mitochondrial biogenesis↑ Insulin secretion↑ in the fed state Hepatic gluconeogenesis↑ in fasted state Preserve glucose homeostasis under fasted and fed conditions
SIRT2	Cytoplasm (nuclear during interphase and mitosis)	Deacetylase ADP-ribosyltransferase Demyristoylase	ERK1/2, GSK3β, p70S6 Akt, FOXO, TUG, GKRP, PEPCK1, PGC-1α	Adipogenesis↓ Lipolysis↑ FAO↑ HGU↑ Regulation of adipocyte differentiation Insulin sensitivity↑ in insulin-resistant hepatocytes, mitochondrial dysfunction↓ Insulin sensitivity↓ in skeletal muscle cells Skeletal muscle glucose uptake↓
SIRT3	Mitochondrial	Deacetylase Decrotonylase	PGC-1α, AMPK, CREB, PDH, LCAD	FAO↑ Insulin resistance↓ in skeletal muscle Regulate mitochondrial antioxidant defense enzymes, ROS↓ Urea cycle and ketogenesis in liver↑
SIRT4	Mitochondrial	ADP-ribosyltransferase Deacetylase Lipoamidase	PGC-1α, AMPK, Adenine translocator, IDE, Glutamate dehydrogenase, MCD1	Lipogenesis↑ FAO↓ Hepatic lipid accumulation↑ Insulin secretion↓ Mitochondrial biogenesis↓
SIRT5	Mitochondrial	Deacetylase Desuccinylase Deglutarylase Demalonylase	IDH2, G6P, CPS1	Ammonia detoxification↑ Regulates glucose oxidation, FAO, ROS Cellular antioxidant defense↑
SIRT6	Nuclear, associated to chromatin	Deacetylase ADP-ribosyltransferase Demyristoylase	HIF-1α, Akt, FOXO1, GCN5, IGF-1, NF-κB, GLUT1, LDH, PGK1, PFK-1	Insulin resistance↓ Gluconeogenesis↓ Mimics the effect of calorie restriction Maintains glucose homeostasis and repress mitochondrial respiration by acting as an HIF-1α corepressor
SIRT7	Nucleolar	Deacetylase	TR4/TAK1, Cd36, PPARγ,	Adipogenesis↑ Fatty acid uptake↑ Hepatic lipid accumulation↑ Triglyceride synthesis/storage↑ Controversy of fatty liver development in SIRT7 knockout mice

Green shade infers detrimental effect, while red shade infers protective effect. The symbol ↑means increment, and ↓means decrement.

signaling is still controversial. Under standard nutrient conditions, it has been suggested that Akt activation in insulin-responsive cells is mainly attributed to SIRT2, other than SIRT1 (55, 56). However, SIRT2 overexpression strengthened insulin-induced Akt/GSK3β/p70S6 signaling in HeLa cells and 3T3-L1 preadipocytes (56). It's elusive whether the phenomenon was caused by alteration of Akt acetylation status. Further investigations are needed to explore whether Akt deacetylation mediated by SIRT2 disturbs both protein binding and downstream pathway. TUG (tether, containing a UBX domain, for GLUT4) plays a role in the exocytosis of glucose transporter type 4 (GLUT4) (57), and binds with SIRT2. SIRT2 regulated the acetylation of TUG *in vitro* and *in vivo* (58). Enhanced TUG acetylation was observed in the liver of SIRT2 knockout mice, alone with greater glucose uptake and more GLUT4 storage vesicles in response to insulin (58). Altogether, SIRT2 may exert distinctive and even opposing effect in response to insulin in different tissues.

However, SIRT4 has been reported to inhibit insulin secretion (9, 59, 60). SIRT4 overexpression promotes lipogenesis and dyslipidimeia, and meanwhile diminishes FAO. All these will lead to insulin resistance (21). SIRT4 mono-ADPribosylates insulin degrading enzyme and ATP/ADP translocases in β cells, leading to downregulation of insulin secretion induced by glucose (61). SIRT4 deletion declines ATP level and low ATP level will activate 5′adenosine monophosphate-activated protein kinase (AMPK), PGC-1α and its target genes, both of which are involved in mitochondrial biogenesis and FAO. Dysregulation of AMPK signaling leads to autophagy deactivation, oxidative stress, and inflammation which are implicated in pathogenesis of insulin resistance (62).

SIRT6 plays an essential role pancreatic β-cell function and survival in mice (63). SIRT6 protected against insulin resistance and obesity induced by HFD (64). Akt phosphorylation at Ser 473 and Thr 308 were negatively regulate by SIRT6 through interfering with insulin receptors, insulin receptor substrate and

various upstream molecules (65). In SIRT6 deficiency mice, increased Akt phosphorylation and activated insulin signaling is observed, yielding more glucose uptake and even hypoglycemia. Additionally, glucose induced more cell apoptosis and impaired insulin secretion severely in MIN6 β-cells in SIRT6 ablation mice. Contrariwise, SIRT6 overexpression rescued β-cell apoptosis and dysfunction (66, 67).

Hence activating SIRT1, SIRT3, and SIRT6 would be a right option to struggle with T2DM due to the repression on PTP1B and UCP2 and final increment in insulin secretion. But SIRT4 functions in a negative way in diabetes development.

SIRTs IN GLUCOSE METABOLISM AND HOMEOSTASIS

During energy restriction status, glucose will be provided by the liver to sustain normoglycemia, initially in the glycogenolysis manner and then changing to gluconeogenesis (68). In the fed condition, insulin is secreted to suppress gluconeogenic enzymes transcription including phosphoenolpyruvate carboxykinase (PEPCK1), fructose-1,6-bisphosphatase, and glucose-6-phosphatase (G6P). PGC-1α and FOXO1 can upsurge gluconeogenic enzyme genes transcription (69, 70).

SIRT1 motivates hepatic gluconeogenesis in fasting status. In contrast, SIRT1 sensitizes insulin and lowers glucose under insulin-resistant condition (71). SIRT1 also deacetylates PGC-1α, and subsequently improves gluconeogenic genes expression in the liver (71, 72), finally encouraging hepatic glucose output during fasting. SIRT2 deacetylates and stabilizes PEPCK1 under glucose deprivation conditions (73). Compromised hepatic glucose uptake (HGU) is the cause of postprandial hyperglycemia in T2DM patients (74). In diabetic mice fed with HFD, SIRT2 overexpression in liver rises HGU and alleviates glucose tolerance. In liver-specific SIRT2 knockdown mice, HGU was diminished and glucose tolerance was imbalanced. It has been reported that SIRT2 stimulates HGU probably via deacetylating K126 of glucokinase regulatory protein (GKRP) (74).

SIRT5 manipulates protein substrates which are involved in ROS management, FAO, ammonia detoxification, ketone body formation, and glucose oxidation by glutarylation, malonylation, and succinylation (75).

SIRT6 interferes with FOXO1, thus reducing gluconeogenic genes such as G6P and PEPCK (76). Hepatic gluconeogenesis was meaningfully upregulated in SIRT6 knockout mice, suggesting a compensatory reaction to hypoglycemia (77). General control non-repressed protein 5 (GCN5) acetylated PGC-1α and diminished the transcriptional activity of PGC-1α (72). SIRT6 could activate GCN5 (77). A hypoxia-inducible factor 1α (HIF-1α) inhibitor would rescue the hypoglycemia phenotype in SIRT6 deficiency mice. Mice with SIRT6 knockout in brains exhibited lower levels of insulin-like growth factor 1 (IGF-1) and growth hormone than control mice, similar to the effect achieved in whole-body SIRT6 knockout mice (78), suggesting that the central nervous system is critical in glucose metabolism.

SIRTs IN CALORIE RESTRICTION AND EXERCISE

Calorie restriction (CR) has been reported to postpone the onset of diabetes. During the initial phase of CR, liver gluconeogenesis is activated by pancreatic α cells-secreted glucagon, during which the cyclic AMP response-element-binding protein (CREB) and CREB-regulated transcription coactivator 2 (CRTC2) are involved. CR and exercise is beneficial for health and longevity, and genetic ablation of SIRT1, SIRT3, and SIRT6 would block the benefits provided by CR and exercise (79–83).

SIRT1 encourages FOXO1-tirggered gluconeogenesis in fasting (84). SIRT1 activators exert similar effects like CR (85, 86). However, this effect is reversed by CRTC2 deacetylation mediated by SIRT1 (84). SIRT1 then deacetylates and activates PGC-1α to facilitate gluconeogenesis (71). CRTC2 supports gluconeogenesis in the initial stage of fasting. Hepatic SIRT1 reduced CRTC2 level via deacetylation and ubiquitination, at 18 h fasting. FOXO1 accounted for gluconeogenesis after 18 h of fasting (84). Mice exposed to long-term CR (18 months) displayed SIRT2 increment in kidney and white adipose tissue (WAT) but not in brain or liver (51). Likewise, short-term fasting (24 h) also enhances protein and mRNA expression of SIRT2 in WAT (53). SIRT6 also accounted for CR function. SIRT6 knockout eliminated CR-induced life extension. SIRT6 Overexpression mimics the effects of exercise and CR in mice, and extends lifespan and health span, including reduced glucose, insulin, adipokines, cholesterol, and body weight (64, 87, 88). In addition, CR-stimulated SIRT6 repressed NF-κB pathway (89). SIRT1 also transcriptionally activated SIRT6, therefore sirtuins might work in a coordinated way to modulate each phase of calorie restriction (29).

SIRTs IN MITOCHONDRIAL GLYCOLYSIS AND BIOGENESIS

ATP is generated in animal cells by two principal processes, glycolysis and mitochondrial oxidative phosphorylation. T2DM, obesity, and many other aging-related disorders are characteristic of amplified oxidative damage. ROS is generally produced in mitochondria, as superoxide (O_2^-) is a byproduct during electron transport system metabolism. In response to excess glucose, SIRTs will orchestrate the ratio of respiration and glycolysis, consuming energy through proton leak (90).

SIRT1 deacetylates PGC-1α, which is critical for mitochondrial function and gluconeogenesis. SIRT1 directly deacetylates and activates PGC-1α while SIRT3 enhances PGC-1α protein expression indirectly(91), through activating CREB and AMPK which accordingly increases downstream mitochondrial biogenesis targets (92–94). Significant mortality, defective thermogenesis, decreased hypoglycemia, and reduced FAO are obvious in SIRT3 knockout models (95). SIRT3 also deacetylates key genes in oxidative stress and mitochondrial antioxidant defense enzymes. The beneficial effects of SIRT3 on CR can chiefly be ascribed to inhibiting ROS (83). Actually, the

alleviation in cellular oxidative stress that generates during CR is absent in SIRT3 knockout mice (82).

In contrast to SIRT3, SIRT4 inhibits mitochondrial biogenesis by suppressing PGC-1α expression. AMPK and SIRT4 interplay to retrograde PGC-1α signaling, suggesting that SIRT4 negatively manipulates mitochondrial biogenesis (96). SIRT5 has been reported to promote antioxidant defense and sustain NADPH homeostasis in cells by increasing G6P deglutarylation and isocitrate dehydrogenase 2 (IDH2) desuccinylation (97). Interestingly, SIRT6 interacts with HIF-1α to co-repress mitochondrial respiration (98). SIRT6 deficiency promoted HIF-1α activity and glycolysis by enhancing phosphofructokinase 1 (PGK1), glucose-6-phosphate isomerase, phosphoglycerate kinase (PFK-1), lactate dehydrogenase (LDH), and GLUT1 (98, 99).

SIRTs IN THE METABOLIC HOMEOSTASIS OF SKELETAL MUSCLE

Skeletal muscle is a critical tissue to maintain energy homeostasis. Storage of lipid metabolites and fatty acids in muscle prevents glucose uptake, finally leading to T2DM (100, 101). Induction of fatty acid β-oxidation has emerged as a hopeful method to attenuate muscle insulin resistance in muscle.

In skeletal muscle, SIRT1 expression is triggered by fasting. SIRT1 in turn deacetylates both FOXO1 and PGC-1α and facilitates fatty acid β-oxidation (91). Likewise, FAO and mitochondrial biogenesis genes are upregulated owning to SIRT1 activators SRT1720 treatment via deacetylating PGC-1α (27, 102). Moreover, SIRT1 plays a role in mitochondrial biogenesis induced by adiponectin in skeletal muscle (103).

SIRT2 negatively regulates insulin resistance and glucose uptake in C2C12 muscle cells. Akt/GSK3β signaling and glucose uptake which are driven by insulin was enlarged by inhibition of SIRT2 under insulin-resistance conditions (104). SIRT2 knockdown under insulin-resistant status enhanced insulin sensitivity in skeletal muscle cells. Nevertheless, blunt of SIRT3 and SIRT1 in C2C12 cells impairs insulin pathway and stimulates insulin resistance. Despite the fact that SIRTs possess a conserved catalytic domain, they exert a differential regulating effect on insulin resistance. SIRT3 Knockdown in muscle cells impairs insulin action and metabolic flexibility (105, 106), and muscle ability to adjust to fuel oxidation (107). SIRT3 deletion amplified acetylation of pyruvate hydrogenase (PDH), yielding declining PDH activity, and subsequent less glucose oxidation. All these gave rise to a switch to FAO, even with glucose available (106, 108).

SIRT4 negatively regulates mitochondrial biogenesis and FAO in muscles. SIRT4 regulates insulin secretion by modulating glutamate dehydrogenase. As expected, fat acid oxidative capability and mitochondrial metabolism enzymes in muscle and hepatocytes was upregulated in response to SIRT4 knockdown. In primary SIRT4 knockdown myotubes, phosphorylation of AMPK was activated, accompanied with intense cellular respiration and FAO. Moreover, protein and mRNA levels of SIRT1 were enhanced both *in vitro* and *in vivo*, largely attributed to the reduced SIRT4 levels (109).

SIRTs IN FATTY ACID OXIDATION

The development of T2DM and its complications is associated with lipid metabolism disorder. Inadequate FAO gives rise to the initiation of insulin resistance and lipid accumulation (110, 111).

SIRT1 fosters fat metabolism in liver cells, as demonstrated by the formation of fatty livers in mice with SIRT1 deletion in the liver (112, 113). SIRT1 knockout mice hardly suppressed lipogenic genes or increased FAO genes in the background of fasting (112). In normal hepatocytes, SIRT1 interacts with the PPARα response element where it deacetylates PGC-1α and increases PPARα expression, thus stimulating FAO (113, 114). Furthermore, once the fat anabolism-inducing factor PPARγ was deacetylated by SIRT1, the sterol regulatory element binding proteins (SREBPs) will be deactivated and become more susceptible to degradation (115), achieving more lipolysis. Deacetylation of PGC-1α and expression of β-oxidation genes was accordingly diminished due to SIRT2 function impairment (116).

SIRT3 plays an essential role in FAO in the mitochondria. Upon CR or fasting, SIRT3 is activated in mitochondria to stimulate FAO through inducing the deacetylation of long-chain-specific acyl coenzyme A dehydrogenase (LCAD) (95, 117). SIRT3 stimulates ketogenesis and urea cycle as well (118, 119). A chronic HFD reduced SIRT3 levels in mice, associated with LCAD function impairment and mitochondrial hyperacetylation (120).

SIRT4's effect is remarkably different from SIRT3 and SIRT1. Ablation of SIRT4 avoids steatosis during HFD (109). In addition, SIRT4 suppresses PPARα to prevent FAO, in the meantime SIRT4 inhibits malonyl CoA decarboxylase 1 (MCD1) to facilitate the synthesis of lipid (29). SIRT4 interferes with SIRT1-PPARα complex, therefore the activation effect of SIRT1 on PPARα and the inhibiting effect on FAO was abrogated.

Analogous to SIRT4, SIRT7 knockout in liver resulted in blunted triglyceride accumulation. Hepatic SIRT7 facilitated triglyceride storage/synthesis and fatty acid uptake by activating TR4/TAK1, a nuclear receptor participating in lipid metabolism. Moreover, the ubiquitin-proteasome pathway is also involved in the regulating effect of hepatic SIRT7 on lipid metabolism (121). SIRT7 also hinders TR4 degradation. TR4 involves in lipid balance by modulating monoacylglycerol O-acyltransferase 1, *Cidec*, cell death-inducing DFFA-like effector a (*Cidea*), and Cd36. SIRT7 has been reported to upsurge hepatic lipid accumulation owning to increasing Cd36 expression (121, 122). Yoshizawa et al. (121) observed that HFD failed to stimulate glucose intolerance, obesity, or fatty liver in SIRT7 knockout mice. The conclusion is controversial to the result that SIRT7 knockout promoted fatty liver development (123, 124). Compared with SIRT1, SIRT7 displays distinctive effect on maintaining liver lipid homeostasisa. Lipid storage is raised by SIRT7 by suppressing PPARα, like SIRT4 (125).

SIRTs IN THE METABOLIC HOMEOSTASIS OF ADIPOCYTE

Regarded as a storage compartment for fatty lipids, adipose tissue also serves as an important modulator for energy homeostasis, vascular remodeling endocrine functions, insulin sensitivity, and innate immune response (126). Adipokines, such as leptin and adiponectin, are systemically regulated by white adipose tissue (WAT). There is convincing evidence supporting that adiponectin derived from adipocyte plays an essential part in insulin resistance (127, 128). Moreover, adiponectin sustains glucose homeostasis and protects against diabetes and obesity.

Numerous SIRT1 benefits take place in WAT (129). SIRT1 inhibits lipogenesis and stimulates fat mobilization in adipocytes from WAT, via suppressing PPARγ. Eventually, peripheral utilization of the fat storages is raised (113). White fat can be switched to metabolically active brown fat due to SIRT1 deacetylation on PPARγ (130). Conversely, SIRT1 can be cleaved in WAT by caspase I and inflammasome which is activated by HFD (19).

SIRT1 knockdown reduced WAT mass in rats. The mRNA contents of PPARγ2 and PPARγ, both of which were adipogenic genes, are abridged in adipose, driving adipocyte differentiation and adipose loss. Adipocytes-specific SIRT1 knockdown led to lower plasma concentrations of adiponectin and leptin. In adipose of obese individuals, mRNA levels of SIRT1 were lower in than those of control group. In the opposite, obese patients have higher SIRT7 expressions in adipose. SIRT7 and SIRT1 promoters' methylation status are not closely associated with the upregulation or downregulation of their mRNA levels induced by obesity. In visceral adipose tissue (VAT) of obese patients, the content of miR-181a-3p and miR-34a-5p negatively associated with SIRT1 levels. In contrary, the expression of miR-125b-5p and miR-125a-5p negatively correlated with SIRT7 in VAT of slim subjects (131). Furthermore, MiR-93 impedes the metabolic target SIRT7 (132).

SIRT7 has been identified as a chief driver of adipogenesis by inducing differentiation and maturation of early adipocyte precursors. PPARγ is adipogenic and its expression is reduced in the WAT of mice with SIRT7 deletion (132), designating that SIRT7 stimulates adipogenesis. SIRT7 can remove long-chain fatty acyl groups more efficiently than removing acetyl groups (133, 134).

Inhibited SIRT2 expression and amplified HIF-1α expression are observed in VAT from obese individuals. HIF-1α hinders adipocyte-mediated fatty acid catabolism by blocking SIRT2-PGC-1α pathway, thereby favoring the progression of obesity (116). Diet-induced obesity was strengthened in SIRT6 knockout mice, principally attributed to hypertrophy of adipocyte other than differentiation of abnormal adipocyte (135).

SIRTs IN NEURONS UNDER DIABETIC CONDITIONS

In the anorexigenic proopiomelanocortin (POMC) neurons, SIRT1 is critical in preserving normal energy expenditure. POMC neurons-specific SIRT1 knockout mice are vulnerable to diet-induced obesity (136). SIRT1 is also defensive against diabetes and obesity in the steroidogenic factor 1 neurons (137). Additionally, the peptide release of orexigenic agouti is suppressed by SIRT1 via interacting with FOXO1 (138, 139). Nevertheless, SIRT1 ablation in neurons was related with insulin secretion in hypothalamic neurons (140).

SIRT2, PGC-1α, and P-AMPK declined dramatically in diabetic cortex. AMPK/SIRT/PGC-1α pathway, which mediates antioxidant abilities and mitochondrial biogenesis, is involved in cortex neurodegeneration progression under diabetic conditions (141). SIRT6 and SIRT2 expression were meaningfully reduced in the neural stem or embryo cells from pre-gestational maternal diabetes. Superoxide dismutase 1 (SOD1) mimetic and overexpression rescued the decrement of SIRT6 and SIRT2 in the diabetic embryopathy mouse model. Histone acetylation caused by Sirtuin decrement might participates in neural tube defects induced by diabetes.

SIRTs POLYMORPHISMS IN DIABETES

While abundant data point to the essential role of SIRTs activities, there are genetic polymorphisms of SIRT1 and SIRT2 concerning diabetes. rs10509291 and rs7896005 in SIRT1 genes are associated with T2DM development as well as reduced acute insulin response (142). In a report about Japanese patients with T2DM, four single nucleotide polymorphisms (SNPs) in SIRT1 that were positively correlated with diabetic nephropathy, and a haplotype containing the SNPs within SIRT1 locus had a stronger association (143). Moreover, SIRT1 mutation has been reported to link with autoimmune diabetes. Type 1 diabetes mellitus (T1DM) is an autoimmune disease characteristic of autoimmune-mediated β cell destruction. Lately, exome, and direct sequencing recognized a T-to-C exchange in exon 1 of *SIRT1* in a patient diagnosed with T1DM, corresponding to a leucine-to-proline mutation at residue 107. It is speculated that the SIRT1 L107P mutation, located within the N-terminal protein-binding domain, could also disturb the oligomerization and activity (20). Furthermore, the prenatally famine-exposed kids, who have minor alleles of SIRT1 gene (GA and AA of rs1467568 and AG and GG of rs7895833), have a lower risk for T2DM in adult life (144). DNA sequence variants (DSVs), including g.38900237G > A, g.38900359C > T, g.38900561C > T, and g.38900912G > T, might upsurge SIRT2 gene promoter activity and SIRT2 levels, contributing to T2DM as a risk factor (50).

CLINICAL TRIALS OF SIRTs ACTIVATORS IN DIABETES: CURRENT EVIDENCE

As mentioned above, SIRTs activators exert positive effects in neurodegenerative, and metabolism diseases. Identification of SIRT1 activators for T2DM treatment has become immediate areas of research focus, such as metformin, resveratrol, resveratrol derivatives (Resveratrol aliphatic acids, acetylated derivatives, 3,3′,4,4′,5,5′-hexahydroxystilbene) and polyphenols

(quercetin, piceatannol, fisetin, pinosylvin, and butein) (145). Metformin is the recommended first-line oral glucose-lowering drug initiated to control hyperglycemia in T2DM, a synthetic dimethyl biguanid (146). SIRT1 level rises after metformin treatment. SIRT1 is obviously entangled into the mechanism of metformin action (147). In the largest Randomized Controlled Trial inspecting metformin for diabetes prevention (148, 149), 1073 subjects from 27 USA medical centers took metformin (850 mg twice every day) or placebo ($n = 1082$). As compared with placebo, diabetes occurrence was considerably decreased by 31% (95% $CI = 17\%, 43\%$) in the metformin group after almost 3 years follow-up.

When it comes to another potent SIRT activator resveratrol, SIRTris Pharmaceuticals had launched an oral resveratrol formulation (SRT501), which has entered Phase III clinical trials for T2DM therapy (150). Effects of trans-resveratrol extract from Polygonum in patients with type 2 diabetes has completed Phase I clinical trials, with trial number NCT01677611 (151). In a dose-escalation Phase I trial, resveratrol clearance (5 g in a single dose) was rapid, and urine excretion reached 77% within 4 h, signifying that derivatives structure optimization with longer half-life is in great need (152). Moreover, derivatives SRT-2183, SRT-1720, and SRT-1460 are also discovered. But SRT-1720 was terminated owing to limited effect. SRT-2104 was more potent and the Phase II clinical trials has completed successfully (153). The pharmacokinetics and safety study of SRT2379 evaluated in healthy male volunteers has finished the Phase I clinical trials (154).

CONCLUSION

SIRTs play a noticeable role in modulating insulin resistance and glucose uptake in adipose tissue, liver, and muscle. SIRT1, SIRT2, SIRT3, and SIRT6 has been implicated to positively sustain insulin sensitivity and glucose homeostasis, rendering them attractive potential drug targets. While SIRT4 and SIRT7 negatively regulate insulin secretion and FAO.

Specifically, SIRT1 enhances fat catabolism in adipose tissue, skeletal muscle and liver by modifying the activity of PPARα, PPARγ, and PGC-1α. Apart from inducing fat catabolism, SIRT1 also promotes FAO, mitochondrial oxidative capacity and energy expenditure in fat tissue and skeletal muscle, not only through direct activation of PPARα, but also through secondary activation of AMPK and PPARα by SIRT1-mediated adiponectin synthesis. SIRT1 prevents lipogenesis and motivates free fatty acid release by inhibiting SREBP and PPARγ. SIRT1 exhibits conflicting effects on maintaining glucose homeostasis under fed and fasted conditions. In fed condition, SIRT1 reinforces pancreatic insulin secretion. In fasted status, SIRT1 promotes hepatic gluconeogenesis by deacetylating FOXO1 and PGC-1α. SIRT1 exerts insulin-sensitizing effect by inhibiting PTP1B and UCP2 expression and regulating adiponectin synthesis. SIRT2 increases insulin sensitivity in insulin-resistant hepatocytes, while decreases insulin sensitivity in skeletal muscle cells. Nevertheless, there is very limited literature on SIRT5 enzyme activity until the recent finding as it can remove succinyl or malonyl groups, and this action resembles deacetylation. SIRT5 is broadly expressed, but SIRT5-deficient mice are healthy, fertile, and without major clinical phenotype (155), inferring that SIRT5 is not indispensable for metabolic homeostasis at least under basal conditions. SIRT6 supports pancreatic β-cell function and sustains glucose homeostasis by acting as a HIF-1α corepressor. Conversely, SIRT4 and SIRT7 exhibited negative effect on diabetes therapy, such as aggravating lipogenesis, and inhibiting insulin secretion.

The majority of sirtuins isoforms are protective on diabetes and a minority appears to be detrimental, but the antagonism effect on the whole body remains elusive. Although several metabolic pathways and targets have been proposed to mediate SIRTs function on T2DM, some outstanding questions need to be resolved. Moreover, do these SIRTs act independently or synergistically on diabetes? How do they communicate for cooperative actions in cells? SIRTs are regulated by protein-protein interactions and microRNAs at the level of translation and transcription (156), however, little is known about the epigenetic mechanisms modifying sirtuins. In some cases, sirtuins isoforms regulate certain essential enzymes in an opposite direction. For instance, PDH can be activated through deacetylation by SIRT3 (106), while both delipoamidating by SIRT4 or desuccinylating by SIRT5 inhibited PDH activity (157, 158), and the question that which effect will win out is quite a puzzle. Furthermore, when SIRTs display both ADP-ribosyltransferase and deacetylase activity, the circumstances that decide the predominant activity need to be determined.

A plenty of clinical trials has been carried out, including resveratrol, metformin, and other SIRT activators. It is possible that in the foreseeable future one or more SIRT activators will be approved for diabetes therapy. As a well-known pharmaceutical preparation, the widespread usage of metformin facilitates the recruit of a large randomized controlled trial. Metformin has been regarded as the most promising candidate. But it's a little harder to explore the natural compounds in a large scale, such as curcumin, berberine, and genistein. It is attributed to the complexity in reducing batches variability of supplements and the difficulty in evaluating dietary intake in observational studies (159–161). Long-term, outcomes-based placebo-controlled rigorous clinical trials would be crucial to confirm the function of SIRT activators on diabetes.

AUTHOR CONTRIBUTIONS

JS and LF drafted the manuscript. All authors contributed in the discussion section, and approved it for publication.

ACKNOWLEDGMENTS

We acknowledge the financial support from the National Natural Science Foundation Committee of China (No. 81603336, 81703775, 81873055), Jiangsu province high-level health personnel Six-One Project (LGY2017085), Jiangsu Province Youth Medical Key Talent Project (QNRC2016634), Innovative Research Team of Health Development Project with Science and

Education in Jiangsu Province (CXTDB2017003), Program for Innovative Research Team of Six Talent Peaks Project in Jiangsu Province (SWYY-CXTD-004). The Public Welfare Technology Application Research Linkage Project of Anhui Province (grant No. 1704f0704062). TCM for Public Interest Research from Ministry of Finance of China (No. 201507004).

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Endocrinology

REVIEW
published: 23 November 2018
doi: 10.3389/fendo.2018.00702

Brain SIRT1 Mediates Metabolic Homeostasis and Neuroprotection

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Sirtuins are evolutionarily conserved proteins that use nicotinamide adenine dinucleotide (NAD^+) as a co-substrate in their enzymatic reactions. There are seven proteins (SIRT1-7) in the human sirtuin family, among which SIRT1 is the most conserved and characterized. SIRT1 in the brain, in particular, within the hypothalamus, plays crucial roles in regulating systemic energy homeostasis and circadian rhythm. Apart from this, SIRT1 has also been found to mediate beneficial effects in neurological diseases. In this review, we will first summarize how SIRT1 in the brain relates to obesity, type 2 diabetes, and circadian synchronization, and then we discuss the neuroprotective roles of brain SIRT1 in the context of cerebral ischemia and neurodegenerative disorders.

Keywords: Sirt1, obesity, type 2 diabetes mellitus, circadian rhythms, cerebral ischemia, Alzheimer's disease, Parkinson's disease

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Edited by:
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Specialty section:
This article was submitted to Cellular Endocrinology, a section of the journal Frontiers in Endocrinology

Received: *17 August 2018*
Accepted: *06 November 2018*
Published: *23 November 2018*

Citation:
Xu J, Jackson CW, Khoury N, Escobar I and Perez-Pinzon MA (2018) Brain SIRT1 Mediates Metabolic Homeostasis and Neuroprotection. Front. Endocrinol. 9:702. doi: 10.3389/fendo.2018.00702

INTRODUCTION

Sirtuins are homologs of yeast silent information regulator 2 (Sir2). Sir2 has attracted the attention of researchers given its involvement in longevity (1). The mammalian sirtuins (SIRT1-7) have different subcellular localizations. SIRT1, SIRT6, and SIRT7 are mainly localized in the nucleus, whereas SIRT1 is also reported to translocate in the cytoplasm. SIRT2 is predominantly cytoplasmic and shuttles to the nucleus transiently. The mitochondrial sirtuins are SIRT3, SIRT4, and SIRT5 (2). In terms of enzymatic activities, sirtuins share a conserved nicotinamide adenine dinucleotide (NAD^+) binding site and remove acetyl groups from target proteins in an NAD^+-dependent manner. Additionally, some sirtuins have been reported to exhibit demyristoylase (SIRT2), ADP-ribosyltransferase (SIRT4 and SIRT6), and demanlonylase and desuccinylase (SIRT5) activities (2).

The Role of SIRT1 as a Metabolic Sensor

Among all sirtuins, SIRT1 is the most extensively studied and well-characterized. As mentioned above, SIRT1 is an NAD^+ dependent deacetylase that removes the acetyl groups from protein substrates to add to the ADP-ribose, a product from the cleavage of NAD^+. NAD^+ is a dinucleotide with one nucleotide contains an adenine and the other contains nicotinamide (3). In addition to be the rate-limiting co-substrate for NAD^+ dependent enzymes, NAD^+ can be used a coenzyme in the metabolic redox reactions. NAD^+ exists in two forms, the oxidized form as NAD^+, and the reduced form as NADH. NAD^+/NADH plays a critical role in glycolysis and cellular respiration for ATP production. In glycolysis, NAD^+ is reduced to NADH. In oxidative phosphorylation and cellular respiration, NADH is oxidized to NAD^+ by electron transport chain (ETC) (1). As such, NAD^+ concentrations fluctuate with cellular metabolic status and nutrient availability.

NAD^+ levels increased during the energetic crisis, such as calorie restriction and decreased under conditions of high-energy load, such as high-fat diets. The fact that Sirt1 enzymatic activity depends upon NAD^+ levels allows Sirt1 to act as a metabolic sensor that couples cellular metabolic status to regulatory responses (1).

SIRT1 in Metabolism

SIRT1 is widely distributed in the body and plays diverse roles in metabolism in different organs including liver, pancreas, muscle, and adipose tissue (4–6). One of the important aspects associated with increased SIRT1 activity is the caloric restriction (CR) (7, 8). CR has been extensively studied, where it has been demonstrated that SIRT1 plays a central role in CR-induced longevity (8–11). As mentioned, it has been suggested that SIRT1, as a metabolic sensor, coordinates the transcriptional networks with the restricted metabolic status (8, 12, 13). During times of energy reduction, NAD^+ concentrations increase, thereby enhancing the NAD^+ deacetylase activity of SIRT1. The SIRT1 mediates deacetylation of a broad range of protein substrates. Proteins that regulate mitochondrial biogenesis, glucose homeostasis, inflammation, and apoptosis have been identified as SIRT1 substrates (14–16). These biological functions are linked to energy homeostasis and eventually extend lifespan.

SIRT1 in the Brain

SIRT1 is widely expressed in the adult brain. Most of the SIRT1 is localized in the neuronal nuclei. However, SIRT1 is also found in the glial cells of post-mortem human brains, and in neural stem cells, microglia, and astrocytes in culture (17). In the hypothalamus, the control center for homeostasis, SIRT1 mRNA is highly expressed in the arcuate, ventromedial, dorsomedial and paraventricular nuclei of the hypothalamus, which suggests an important role for brain SIRT1 in regulating metabolic status (18). Another function of brain SIRT1 is the regulation of the central circadian clock in the suprachiasmatic nucleus (SCN) of the hypothalamus. In the SCN of the hypothalamus, SIRT1 regulates circadian clock gene expressions by mediating the acetylation status of circadian genes (19). Besides the physiological functions of SIRT1 in the hypothalamus, SIRT1 is reported to exert neuroprotection in neurological dysfunctions (20). In this review, we will focus on the roles of SIRT1 in the brain in metabolism, circadian rhythm, and SIRT1 function in the context of cerebral ischemia and neurodegenerative disorders (**Figure 1**).

SIRT1 AND MITOCHONDRIAL FUNCTIONS

Mitochondrial is one of the most important sources for cellular energy in eukaryotes, producing up to 95% of the ATP through oxidative phosphorylation (21). This provides great significance into the roles of the mitochondrial in the brain, where it is estimated to take up to 20% of the total oxygen consumption of the body energy (22). As such, this dysfunction could affect metabolic efficiency, thus linking to a common pathology ranging from metabolic disorders to neurological diseases (21).

SIRT1 can regulate the transcription of mitochondrial genes encoded in the nucleus that are involved in vital mitochondrial processes related to longevity and aging. While SIRT1 is mainly localized in the nucleus, levels have also been detected in the mitochondrion where it may interact with different substrates (23). In this section, we will discuss SIRT1's involvement in important mitochondrial functions including mitochondrial biogenesis, mitophagy, and energy metabolism.

Regulation of Insulin Secretion by SIRT1 Via UCP2

Uncoupling protein 2 (UCP2) is an inner mitochondrial membrane protein that can uncouple oxidative phosphorylation from respiration/ATP production. This is done via dissipation of the proton gradient, in which protons are returned to the mitochondrial matrix. UCP2 is found in many different tissues, including the brain, and has been shown to be involved in energy balance (24), homeostasis, and longevity (25). SIRT1 has been found to positively regulate insulin production by means of repressing UCP2 (26–28). As a result, cells express higher ATP levels after glucose stimulation, which is essential for inducing insulin secretion (26). In this manner, levels of insulin are regulated commensurate to levels of food intake and if impaired may contribute to obesity-induced diabetes (26). In this respect, SIRT1 can respond to nutrients available in the environment and promote transcriptional changes that may enhance energy metabolism.

PGC-1α and SIRT1 Interact to Induce Mitochondrial Biogenesis and Metabolic Processes

Peroxisome proliferator-activated receptor γ (PPARγ) coactivator-1α (PGC-1α) is a transcriptional coactivator and major regulator of mitochondrial biogenesis and several metabolic processes (29). Studies have shown that SIRT1 interacts with PGC-1α to induce its transcriptional activity via deacetylation (16, 30–32). PGC-1α may activate a wide array of transcription factors (TFs) that include both DNA-binding TFs, such as nuclear respiratory factor 1 (NRF-1), and nuclear hormone receptors, such as PPARγ, thyroid hormone receptors, retinoic acid receptors, glucocorticoid receptors, and estrogen receptors (33, 34).

NRF-1, specifically, can regulate the activation of the nuclear-encoded mitochondrial transcription factor A (TFAM), which can bind to mtDNA and stimulate mitochondrial DNA replication and increase the expression of mitochondrial genes (35). As a result, NRF-1 induces the expression of mitochondrial transporters, components of oxidative phosphorylation, and ribosomal proteins (36). Aquilano et al. found that SIRT1 and PGC-1α also interact with TFAM within the mitochondria (23). In either case, the subsequent increased expression of mitochondrial genes promotes mitochondrial biogenesis—an essential process important for maintaining oxidative capacity and levels of energy production. In some instances, SIRT1 activity is required to stimulate mitochondrial biogenesis, as reported in pulmonary arteriolar smooth muscle cells (37); however, whether

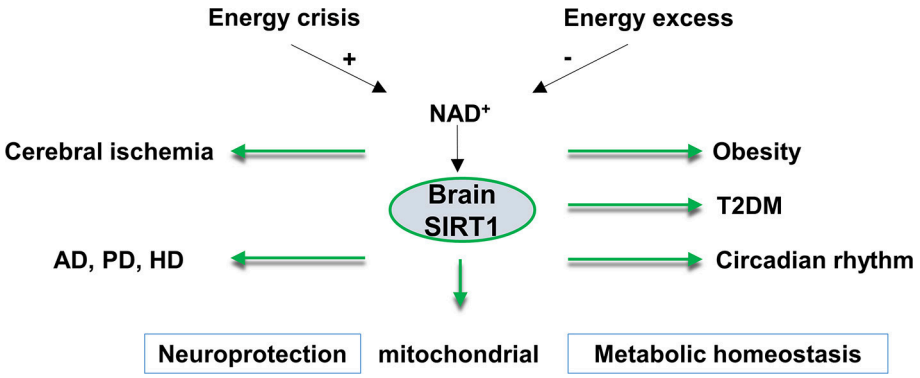

FIGURE 1 | An overview of brain SIRT1 in metabolic and neurological disorders. Brain SIRT1 activity is dependent on NAD^+ levels, which increases under energy crisis, and decline with high energy load. Any dysregulation of brain SIRT1 activity can have devastating consequences in terms of mitochondrial function, metabolic homeostasis, circadian synchronization, and neurological function. A proper function of brain SIRT1 is protective against obesity, diabetes, circadian dysregulation. In addition, brain SIRT1 exerts neuroprotection against ischemic injury and neurodegenerative disorders, such as Alzheimer's disease (AD), Parkinson's disease (PD), and Huntington's disease (HD). T2DM, type 2 diabetes, NAD^+, nicotinamide adenine dinucleotide.

SIRT1 is necessary for mitochondrial biogenesis to occur has recently become controversial (38).

The increase of mitochondrial gene expression also stimulates several metabolic processes depending on the tissue type (33, 36). In the brain and heart, PGC-1α functions as an important regulator of the metabolism of reactive oxygen species (ROS) under normal physiological conditions and certain states of oxidative stress (36). In conditional liver-specific SIRT1 KO mice, PPARα signaling activated by PGC-1α was impaired (39). This lead to a decrease in fatty acid oxidation and ketogenesis, suggesting a vital role for SIRT1 in regulating hepatic lipid homeostasis (39). Dysregulation of any of these processes may contribute to both aging and age-associated metabolic diseases.

SIRT1 and Mitophagy

Maintaining quality mitochondrial pools is essential for cell health and viability. Mitochondrial components are typically damaged by the accumulation of ROS—a byproduct of the mitochondrial electron transport chain—which typically occurs during conditions of stress. Due to its close proximity, ROS overproduction damages mtDNA that may ultimately contribute to neurodegenerative disorders, stroke, cancer, and age-related diseases (40–42). Constant mitochondrial turnover is important to maintain a healthy mitochondrial population. Thus, a quality control mechanism is required to eliminate and replace dysfunctional mitochondria with new and more efficient mitochondria. To achieve this specialized form of homeostasis in response to stress, cells utilize a process known as mitophagy for the selective degradation of mitochondrial components. This elimination process is balanced with mitochondrial biogenesis. While the exact mechanism for mitophagy has yet to be elucidated, some studies indicate PINK1/PARKIN as the key pathway involved (43, 44). As discussed above, SIRT1 is essential for promoting mitochondrial biogenesis; however, it also plays an important role in autophagy.

Several studies have shown that SIRT1 interacts with components of the autophagy machinery including Forkhead box O3 (FOXO3)—a transcription factor heavily associated with autophagy induction (45–47). In the aged kidney, the mitochondrial damage was associated with deficiencies in SIRT1, and under hypoxic conditions, SIRT1 was able to promote cell adaptation by deacetylation of FOXO3 (46). Another study demonstrated that SIRT1 is essential for fully activating autophagy under conditions of starvation (48). Additionally, SIRT1 deficient embryos and neonatal mice displayed an accumulation of abnormal organelles, especially mitochondria, and impaired autophagy (48). PGC-1α is also involved in mitophagy as it regulates the expression of transcription factor EB (TFEB)—a well-known master regulator of autophagy and lysosomal biogenesis (49). It may be possible that SIRT1 may also influence mitophagy given its interaction with PGC-1α. Taken together, SIRT1 is involved in diverse mechanisms for the regulation of mitochondrial functions (**Figure 2**). The regulation of SIRT1 in mitochondrial functions may underlie its importance in regulating energy metabolism and in so doing, may be part of its neuroprotective role.

BRAIN SIRT1 AND OBESITY

The hypothalamus is the control center for homeostasis. In the arcuate nucleus of the hypothalamus, the proopiomelanocortin (POMC) neurons suppress appetite while the activation of agouti-related peptide/neuropeptide Y (AgRP/NPY) neurons stimulate appetite (50). The ventromedial nucleus (VMN) is another nucleus involved in satiety as VMN lesions lead to an increase in food intake and obesity (51). In recent years, a substantial number of studies demonstrated that the hypothalamic SIRT1 is crucially important for the central regulation of food intake and energy expenditure.

SIRT1 in the POMC neurons is required to protect against high calorie-induced obesity. When challenged with a hypercaloric diet, POMC-SIRT1 mutant mice showed reduced energy expenditure and increased body weight (52). Interestingly,

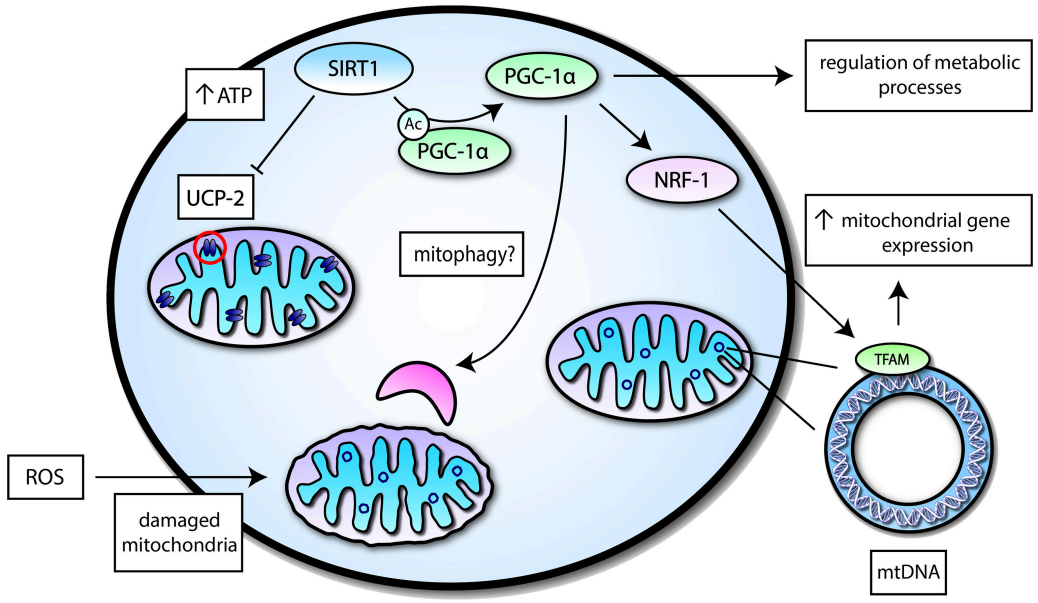

FIGURE 2 | A simplified overview of mitochondrial functions mediated by SIRT1 activity. SIRT1 may interact with transcription factors or mitochondrial proteins to induce different effects related to mitochondrial function—a select few of these proteins are highlighted. SIRT1 can suppress Uncoupling protein 2 (UCP-2) in the inner mitochondrial membrane to increase levels of ATP, which is important for energy metabolism. SIRT1 may also deacetylate Peroxisome proliferator-activated receptor γ (PPARγ) coactivator-1α (PGC-1α) to induce its activation and augment mitochondrial biogenesis by increasing mitochondrial gene expression via Nuclear respiratory factor 1 (NRF-1) and Nuclear-encoded mitochondrial transcription factor A (TFAM). PGC-1α itself can regulate different metabolic processes and may potentially play a role in mitophagy.

these metabolic changes were not due to hypoactivity, as the mutant mice showed unaltered levels of daily activities compared to their control counterparts (52). The reduction in energy expenditure could be explained by a reduction in sympathetic nerve activity in the adipose tissue of the mutant mice (52). Conversely, mice with overexpressed SIRT1 in the POMC neurons exhibited a leaner phenotype compared to their wild-type littermates (53). Age-related weight gain was absent in the POMC-SIRT1 overexpressed mice. The leaner phenotype was attributed to increased sympathetic activity in the adipose tissue with consequently enhanced energy expenditure (53).

Another mechanism in which SIRT1 modulates systemic homeostasis is through deacetylation of Forkhead box protein O1 (FoxO1). FoxO1 is a downstream transcription factor in the insulin signaling pathway. Hypothalamic FoxO1 activation or overexpression inhibits the anorexigenic effects of insulin (54), increases adiposity, and leads to weight gain (55). The overexpression of SIRT1 in POMC neurons was able to rescue FoxO1 activation induced obesity (55). These effects occurred through decreased acetylation and expression of FoxO1 by POMC-SIRT1 overexpression (55). Similarly, FoxO1 mediated hyperphagia was blunted by hypothalamic SIRT1 overexpression (56).

Hypothalamic SIRT1 is also implicated in the leptin-mediated regulation of metabolism. Leptin is a hormone secreted by the adipose tissue that suppresses body weight. In the hypothalamus, leptin binds to its receptor (Ob-Rb) and activates the signal transducer and activator of transcription 3 (STAT3), which further regulates gene expressions to affect energy homeostasis. The leptin-induced protective mechanisms against obesity are dependent on SIRT1 in the POMC neurons. In POMC-SIRT1 deficient mice, leptin-mediated activation of the phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K) signaling pathway and the suppression of food intake were disrupted (52). In addition, when SIRT1 is overexpressed in the hypothalamus, either in POMC or AgRP neurons, non-obese mice exhibited increased sensitivity to leptin, as demonstrated by increased phosphorylation of STAT3 as well as reduced food intake (53). Interestingly, these phenotypes were blunted in mice consuming a high-fat diet, due to decreased expression of SIRT1 and NAD^+ levels in the hypothalamus, suggesting that the metabolic status could influence the function of hypothalamic SIRT1 (53). In support of this, the hypothalamic SIRT1 expression is induced upon feeding in the standard fed mice, whereas diet-induced obesity abrogated this induction (56). In addition to the arcuate nucleus, SIRT1 in steroidogenic factor 1 (SF1) neurons of the VMN also contributes to the physiological function of leptin. The lack of SIRT1 in the SF1 neurons predisposed mice to dietary-induced obesity. SF1-SIRT1 mutant mice exhibited diminished energy expenditure and impaired leptin sensitivity (57). In contrast, SIRT1 overexpression in the SF1 neurons restored oxygen consumption, increased leptin sensitivity and protected mice against high-calorie diet induced weight gain (57).

In the aforementioned studies, the activation of hypothalamic SIRT1 negatively regulated energy balance and protected against obesity. Contrary to these findings, studies also

reported a positive energy regulation by brain SIRT1. Apart from leptin, ghrelin is another peptide released peripherally and acts on the central nervous system (CNS) to regulate metabolism. Ghrelin is produced by the stomach and activates AMPK in the hypothalamus to increase appetite. In rodents, the intracerebroventricular (ICV) infusion of the SIRT1 inhibitor, EX-527, blunted the ghrelin-induced food intake, thus demonstrating that ghrelin is dependent on hypothalamic SIRT1 to stimulate appetite (58, 59). Similar beneficial metabolic effects were seen in two other studies that blocked brain SIRT1 by the ICV infusion of EX-527. One study showed that the inhibition of brain SIRT1 in fasted rats could reduce food intake and decrease weight gain (60). The other study compared the effects of brain SIRT1 inhibition in obese and lean rats. The authors observed a significant decrease in body weight and an increase in energy expenditure in diet-induced obese rats, but not in rats fed normal chow upon brain SIRT1 inhibition (61). The mechanisms were attributed to increased activity of the hypothalamic-pituitary-thyroid axis, resulting in enhanced energy expenditure (61). The discrepancy between the hypophagia and hyperphagia effects by SIRT1 activation may be due to the different animal models used in these studies (i.e., ICV infusion of EX-527 and genetic deletion of SIRT1 in a specific population of neurons). Despite the controversies, these studies demonstrated a crucial role for brain SIRT1 in the systematic regulation of energy homeostasis (**Figure 3**).

BRAIN SIRT1 AND TYPE 2 DIABETES

Obesity is a leading risk factor for type 2 diabetes (T2DM). The accumulation of fat, especially visceral fat, progressively enhances insulin resistance and eventually leads to T2DM (62, 63). Given the crucial role of SIRT1 in obesity, it is no surprise that emerging evidence suggests SIRT1 within the brain controls the systematic regulation of glucose/insulin homeostasis. Mentioned briefly above, SIRT1 in SF1 neurons is required for the defense against dietary-induced obesity. In addition to this, insulin activated PI3K signaling was blunted in the skeletal muscle of SF1-neuron-SIRT1 deleted mice. Conversely, SIRT1 overexpression in SF1 neurons enhanced skeletal muscle insulin sensitivity in these mice (57).

Resveratrol is a potent SIRT1 activator that improves glucose homeostasis. Studies demonstrate that brain SIRT1 at least partially contributes to the resveratrol-mediated glucose balance (64). ICV infusion of resveratrol rescued the hyperglycemia phenotype in diet-induced obese and diabetic mice (64). In support of the former data, hypothalamic or systemic administration of resveratrol increased hepatic insulin sensitivity, which was blunted by the inhibition of SIRT1 in the hypothalamus (65). Collectively, these data suggest SIRT1 activation within the brain is likely to improve insulin resistance and combat against diabetes. In such cases, brain SIRT1 activation leads to suppressed peripheral glucose production.

Two studies that investigated the cell type-specific role of neuronal SIRT1 in glucose metabolism suggested a different regulatory mechanism. Neuronal SIRT1-deficient mice exhibited higher insulin sensitivity in the hypothalamus and peripheral tissue. It was suggested that SIRT1 deacetylates and represses Insulin receptor substrate 1 (IRS-1) and the insulin signaling pathway. In this case, central PI3K signaling was enhanced in neuronal SIRT1 deficient mice (66). Another study demonstrated that neuronal SIRT1 mediates glycolysis in the brain. Pharmacological inhibition or genetic mutation of neuronal SIRT1 caused glycolysis deficits *in vitro* and *in vivo*, whereas resveratrol treatment increased the glycolysis rate in primary neurons (67). It is reported that, in peripheral tissues, SIRT1 inhibits glycolysis to reduce glucose consumption (68). For example, in liver, under metabolic stress, SIRT1 deacetylates and activates PGC-1α to suppresses glycolysis and promote gluconeogenesis. These data demonstrate that SIRT1 regulates glucose metabolism in a tissue-specific and cell type-specific manner (16).

BRAIN SIRT1 AND CIRCADIAN RHYTHM

The circadian rhythm is a 24 h endogenous cycle that allows organisms to synchronize their physiology and behavior to the daily cycle of daylight and darkness (69, 70). The circadian clock is entrainable by internal and external zeitgebers "time givers." In mammals, the circadian clock is found across different tissues, yet the central clock is found in the SCN of the hypothalamus from which it entrains peripheral clocks to regulate oscillatory functions, such as metabolism and the sleep/wakefulness cycle (69, 70).

The molecular mechanism of the circadian rhythm consists of a set of transcriptional activators and repressors involved in positive and negative autoregulatory feedback loops (69, 71). In mammals, the core clock genes are the acetyltransferase CLOCK (Circadian Locomotor Output Cycles Kaput) and its heterodimer BMAL1 (Brain and muscle Arnt-like protein-1). When dimerized, the CLOCK-BMAL1 complex translocates to the nucleus and induces the expression of several downstream genes. Among these genes are their own negative regulators period (PER1, PER2, PER3) and cryptochrome (CRY1 and CRY2) proteins (69, 70). Over the course of the day, PER and CRY start to accumulate and together with the casein kinase 1δ (CK1δ) and CK1ε translocate to the nucleus to repress their own transcription (70). As repression progresses, PER and CRY levels decline and transcription by CLOCK-BMAL1 re-initiates a new cycle (70). PER and CRY are also eliminated by post-translational modifications and degradation (69, 70). The CLOCK-BMAL1 complex also regulates the downstream retinoic acid-related orphan receptors (RORα, RORβ) and the nuclear receptors (Rev-Erbα, Rev-Erbβ), which compete for the regulation of the BMAL1 promoter and reinforce the oscillation (70).

SIRT1 in the Peripheral Clocks

SIRT1 has been shown to be an important regulator of the circadian clock genes in both the central and peripheral clocks (**Figure 4**). In 2008, two independent studies using peripheral tissues were the first to link SIRT1 to the regulation of the clock genes. Using mouse hepatocytes and cultured fibroblasts, it was shown that the protein levels of SIRT1 cycle in a

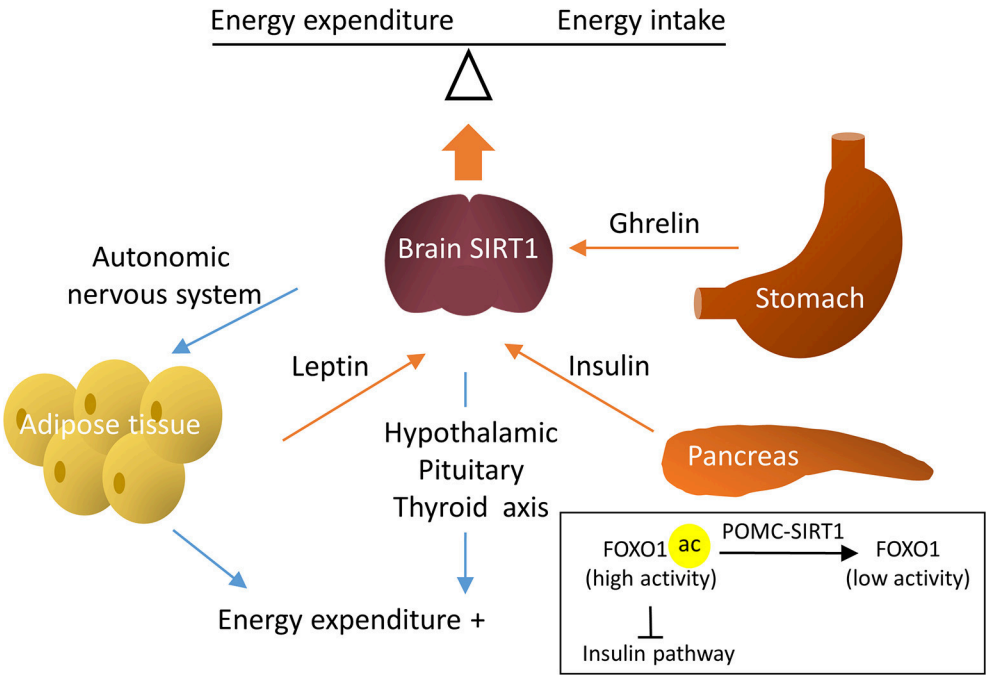

FIGURE 3 | Regulative mechanisms of brain SIRT1 in metabolic homeostasis. Brain SIRT1 increases energy expenditure via the hypothalamic pituitary thyroid axis and increased sympathetic nerve activity in the adipose tissue. In addition, hormones, such as leptin, insulin, and ghrelin through brain SIRT1 to balance energy expenditure and energy intake. For example, SIRT1 in POMC neurons deacetylate Forkhead box protein O1 (FOXO1) to increase the insulin signal pathway.

circadian manner, in turn, is required to promote the circadian transcription of Bmal1, Rorγ, Per2, and Cry1 (19). This study also showed that the binding of SIRT1 to the CLOCK-BMAL1 complex is rhythmic and promotes the deacetylation and degradation of the PER2 protein (19). In the second study using fibroblasts and liver tissues, SIRT1 was reported to be a negative regulator of the CLOCK-BMAL1 complex (72). By antagonizing the acetyltransferase activity of CLOCK, SIRT1 removes acetyl-marks from histone H3 and BMAL1, preventing the CLOCK-BMAL1 heterodimer from activating circadian promoters. This study also revealed that SIRT1's activity rather than levels is regulated in a circadian manner (72). Subsequent studies then revealed that the rhythmic activity of SIRT1 is due to the oscillatory patterns of NAD^+ levels regulated by its rate-limiting enzyme NAMPT, which is positively regulated by the core clock genes CLOCK-BMAL1 (73, 74). The activation of SIRT1 through this NAMPT-mediated NAD^+ biosynthetic pathway, in turn, inhibits the activity of CLOCK-BMAL1, thus forming a negative feedback loop (73, 74). These studies revealed a crucial role for SIRT1 in coupling metabolism to the circadian cycle through its reliance on NAD^+ as a cofactor. Supporting this observation, studies have shown that high-fat diets can disrupt the rhythmicity of circadian clock genes in several tissues and that the administration of SIRT1 activators such resveratrol, can reverse these effects and restore the rhythmicity to the circadian genes (75, 76). Additionally, several other studies have attributed different functions to SIRT1 in the regulation of the circadian clock genes in peripheral tissues thus adding additional levels of complexity to its function (77–79).

SIRT1 in the Central Clock

In the SCN of the hypothalamus, SIRT1 was reported to activate the transcription of the circadian genes BMAL1 and CLOCK through PGC-1α (80) (**Figure 4**). Interestingly, this study also showed that aging reduces the levels of SIRT1 in the SCN, which coincided with reduced BMAL1 and PER2 levels. This, in turn, leads to a longer intrinsic period and disruption in the activity patterns and entrainment of mice to the light schedule. Furthermore, the knockout of *Sirt1* from young mice brains was able to phenocopy these age-dependent disruptions in the circadian cycle, while its overexpression protected old mice from the age-dependent effects (80). Thus, this study revealed a crucial role for SIRT1 in the activation of the central pacemaker and maintenance of robust circadian control in young animals. It also suggested that the age-dependent reduction in SIRT1 led to the observed disruptions in the circadian cycle with aging (80). Another interesting study showed that SIRT1 from the ventromedial hypothalamus (VMH) sends nutrient-time information to the central clock through efferent signals to synchronize the central clock to feeding cues (81). SIRT1 ablation from the SF1 neurons of the VMH disrupted the connection between food intake and circadian rhythm as revealed by deregulated activity behaviors and circadian gene expression in the SCN (81). This study strongly supports the role of SIRT1 as a nutrient sensor that couples metabolism to the circadian rhythm of the central clock.

The regulation of the circadian genes by SIRT1 in the central clock has also been reported to be disrupted in a

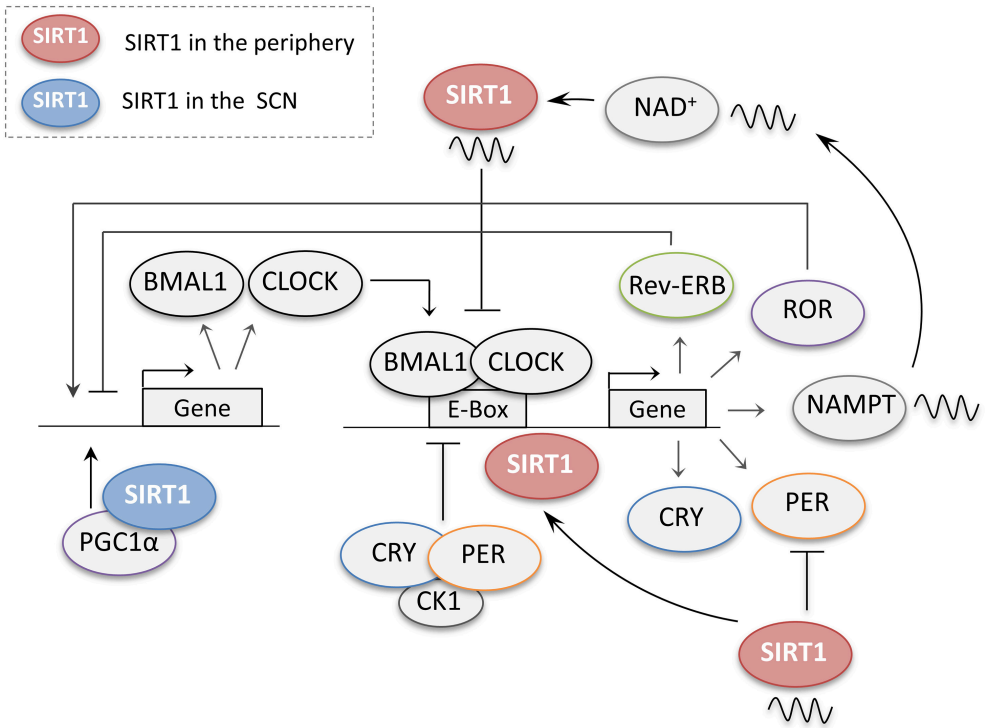

FIGURE 4 | The regulation of central and peripheral clock genes by SIRT1. When dimerized, the core clock genes CLOCK and BMAL1 promote the expression of several downstream genes including their own negative regulators periods (PER) and cryptochromes (CRY). PER and CRY accumulate during the day and together with casein kinase 1 (CK1) then repress their own transcription. The CLOCK-BMAL1 complex also regulates the retinoic acid-related orphan receptors (ROR) and the nuclear receptors (Rev-Erb), which compete for the regulation of the BMAL1 promoter. In the peripheral clocks, SIRT1 regulates the circadian genes at different levels. SIRT1 protein levels cycle in a circadian manner, and through its rhythmic binding to the CLOCK-BMAL1 complex SIRT1 promotes the circadian transcription of Bmal1, Rorγ, Per2, and Cry1. SIRT1 also promotes the deacetylation and degradation of the PER2 protein. SIRT1 activity has also been reported to cycle in a circadian manner owing to the rhythmic expression of NAMPT, a crucial enzyme for NAD^+ biosynthesis, by the CLOCK-BMAL1 complex. In turn, SIRT1 also acts as a negative regulator of the CLOCK-BMAL1 complex thus preventing the activation of circadian promoters. In the suprachiasmatic nucleus (SCN), SIRT1 activates the transcription of the circadian genes BMAL1 and CLOCK through PGC-1α.

number of neurological diseases (**Figure 5**). It was shown that Apolipoprotein E knockout ($ApoE^{-/-}$) mice, a model of Alzheimer's disease, exhibit disruptions in the circadian locomotor activity under dim light and constant darkness along with impairments in re-entrainment to phase change schedules (82). These mice also exhibit an alteration in the expression of SIRT1 and circadian clock genes in the SCN (82). Interestingly, the supplementation with fat or ketone bodies or the intraperitoneal administration of nicotinamide can rescue the circadian clock in these mice by restoring their locomotor rhythmicity and circadian expression of SIRT1 and clock genes (82). Additionally, in triple transgenic Alzheimer's disease (3 × Tg-AD) mice, the patterns of expression of circadian clock genes were also reported to be disrupted in the SCN in response to daylight and darkness. Consistently, these mice also exhibited significantly higher levels of SIRT1 in the SCN compared to non-transgenic after a 12 h exposure to darkness (83). Thus, these studies combined suggest that SIRT1 may be a relevant therapeutic target for the restoration of the circadian rhythm in the SCN, which is disrupted in both aging and neurological disorders.

SIRT1 AND CEREBRAL ISCHEMIA

Researchers have established different roles for brain SIRT1 in different neurological diseases. Evidence from preclinical studies established a neuroprotective role for SIRT1 in ischemic injury. SIRT1 deficient mice, compared to their wild-type littermates, exhibited significantly larger infarct volume and increased impairment of neurological functions after permanent middle cerebral artery occlusion (pMCAO) (84). In a similar line of evidence, pharmacological blockade of SIRT1 activity by SIRT1 inhibitor sirtinol increased the infarct volume following pMCAO (84). In contrast, SIRT1-overexpression protected the brain from cerebral ischemic injury. In a bilateral common carotid artery stenosis (BCAS) model that causes chronic cerebral hypoperfusion, wild-type mice displayed white matter deficits and spatial memory impairments following BCAS (85). Conversely, SIRT1 overexpressed mice showed preserved histological outcome of the corpus callosum and restored spatial working memory (85). Additionally, increased SIRT1 activity by Activator 3, a specific SIRT1 activator, reduced infarct volume in mice (84).

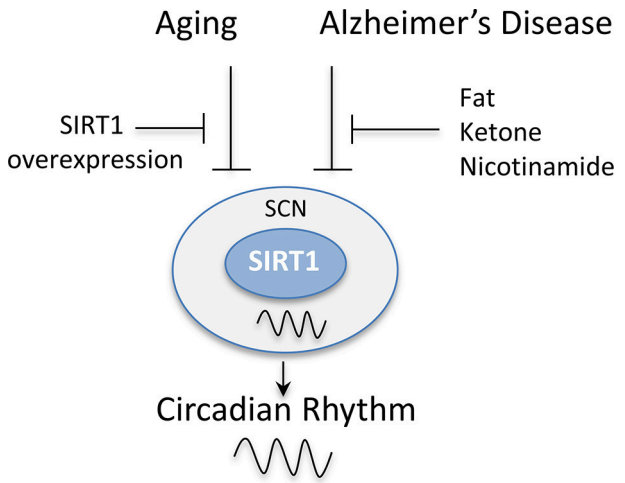

FIGURE 5 | SIRT1 levels in the suprachiasmatic nucleus (SCN) are disrupted by aging and Alzheimer's disease. The rhythmic expression of SIRT1 in the SCN has been reported to be disrupted in animal models of aging and Alzheimer's disease. This, in turn, disrupts the circadian expression of clock genes causing a disruption in the activity patterns of mice and their entrainment to light. The overexpression of SIRT1 protected mice from these age-dependent effects. Similarly, the administration of fat, ketone bodies, or nicotinamide rescued the circadian expression of clock genes in Alzheimer's disease mouse models and restored their locomotor rhythmicity.

The neuroprotection against cerebral ischemia by SIRT1 is achieved through multiple mechanisms. Following ischemia, stressors, such as DNA damage and oxidative stress activate the tumor suppressor gene, p53, which mediates apoptosis (86). Ischemia-induced activation of p53 triggers the mitochondrial apoptotic pathway and facilitates neuronal cell death (86, 87). Inhibition of p53 blocks apoptosis, promotes a survival signaling pathway, and protects neurons against ischemic-induced cell death (88, 89). Genetic deletion or pharmacological inhibition of SIRT1 increased the acetylation of p53 in the peri-infarct area (84). In contrast, SIRT1 activation deacetylated p53 and reduced p53-dependent neuronal apoptosis (90).

SIRT1 dependent endothelial nitric oxide synthase (eNOS) modulation is another beneficial mechanism. Nitric oxide (NO) is a vasodilatory factor that is produced by endothelial nitric oxide synthase (eNOS) in endothelial cells. Acetylated eNOS was significantly increased at 2 h after BCAS in wild-type mice, whereas in SIRT1-Tg mice, the acetylation of eNOS was not observed (85). Increased deacetylation of eNOS is suggested to increase NO production, regulating the vascular tone of blood vessels, and helping to maintain cerebral blood flow during chronic hypoperfusion (85). Consistent with this, in a global cerebral ischemia model of bilateral common carotid artery occlusion (BCAO), SIRT1-Tg mice showed significantly preserved cerebral blood flow during BCAO, which was absent in their wild-type littermates. Similar to the former, pharmacological activation of SIRT1 by resveratrol treatment 1 h after MCAO increased plasma NO and decreased infarction volumes in an eNOS dependent manner (91).

As briefly mentioned above, SIRT1 can also mediate protection by retaining the integrity of white matter (85). White matter lesions are commonly seen in elderly people. One study, which enrolled 1,077 subjects, revealed only 5% were completely free of white matter lesions (92). The prevalence of white matter lesions increased with aging and is associated with cognitive defects. Moreover, the cerebral white matter is highly vulnerable to ischemic injuries (93, 94). Of note, evidence also showed that the degree of white matter lesions relates to infarct volumes and predicts future ischemic incidence after the first stroke attack in patients (95). Supporting the white matter protection by SIRT1 in ischemic stroke, studies in other neurological models demonstrate a similar SIRT1 mediated benefit. In neonatal brain injury, SIRT1 regulates glial progenitor cells to promote white matter regeneration (96). Similarly, SIRT1 mediates neuronal protection in an autoimmune model of white matter injury (97).

Importantly, Sirt1 is required in the neuroprotection elicited by ischemic preconditioning (IPC) or resveratrol preconditioning (RPC) (1, 17, 67). IPC develops when a brief period of sublethal ischemia is followed by a period of recovery. It exerts a neuroprotective state against lethal ischemia in different organs of the body including the brain. Furthermore, IPC has shown promising prophylactic potential in diminishing cerebral ischemic injury as shown in recent translational research studies (98, 99). Similar to IPC, resveratrol treatment is able to protect the brain from a following cerebral ischemic attack (1, 17, 67). Collectively, the evidence gathered here demonstrates the pivotal role SIRT1 plays against cerebral ischemia (**Figure 6**).

SIRT1 AND NEURODEGENERATIVE DISORDERS

In addition to providing neuroprotection against cerebral ischemia, the activation of SIRT1 has been shown to confer protection against neurodegenerative diseases, such as Alzheimer's disease (AD), Parkinson's disease (PD) and Huntington's disease (HD) (20, 100). These diseases are substantial burdens to society and can be debilitating to afflicted individuals, making it imperative to investigate potential therapeutic factors, like SIRT1.

The benefits of SIRT1 in neurodegenerative diseases was first reported by Graff et al. in a CR model (101). Graff et al. studied an inducible neurodegenerative mouse model, called CK-p25. These mice exhibited a substantial neuronal loss, deficits in synaptic density and plasticity, as well as learning and memory impairments under the induction of doxycycline. CK-p25 mice underwent 3 months of CR and after the sixth week of CR the neurodegeneration was induced. The CR group showed preserved synaptic density, synaptic plasticity, and memory capacities. CR neuroprotection was mediated by SIRT1 activation, shown by the deacetylation of p53, in the CR but not control group. Furthermore, the use of a small SIRT1 activator, as well as SIRT1 overexpression, recapitulated the CR neuroprotection (101). This evidence shows SIRT1's neuroprotective capacity against neurodegenerative effects on synaptic function and memory capacities. In this way, SIRT1

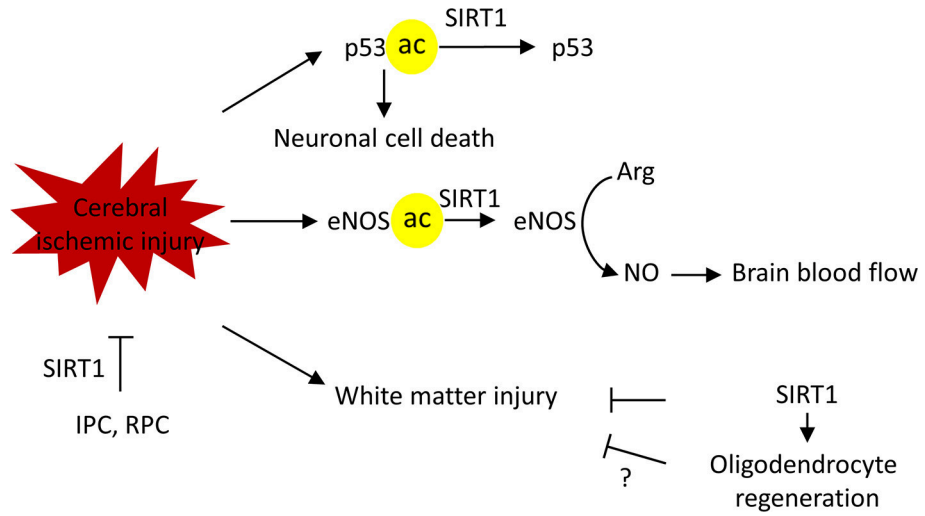

FIGURE 6 | SIRT1 protects against cerebral ischemic injury in multiple mechanisms. SIRT1 deacetylate p53 to block the p53-induced apoptotic pathway, thus, promoting neuronal survival. SIRT1 deacetylates endothelial nitric oxide synthase (eNOS) to regulate vascular tone and maintain brain blood flow. SIRT1protects against white matter injury in ischemic injury, possible via promoting the oligodendrocyte regeneration. Finally, SIRT1 is required for ischemic preconditioning (IPC) and resveratrol preconditioning (RPC) induced ischemic neuroprotection. Arg, L-arginine, NO, nitric oxide.

activation, or perhaps overexpression, may protect against synaptic dysfunction in common forms of neurodegeneration.

Alzheimer's Disease and the Therapeutic Potential of SIRT1

AD is a neurodegenerative disease that can be either early-onset or late-onset. Early-onset is associated with a genetic contribution to the disease's etiology, while the late onset etiology is more complicated and likely multifactorial (102). In the more common, late-onset form of the disease, neuritic senile plaques (NSP) and neurofibrillary tangles (NFT) contribute to neuronal toxicity and death. NSPs originate from the buildup of a protein called β-amyloid (Aβ). β-secretase and γ-secretase are enzymes that cleaves the amyloid precursor protein (APP) to produce Aβ, which is then secreted into the extracellular space, eventually forming toxic aggregates. NFT's are tangles of the cytoskeletal protein tau that receive an aberrant post-translational modification. A common pathological modification of tau is phosphorylation, which forms the toxic p-tau (103). The formation of NSP's and NFT's are pivotal steps in AD pathology.

Recent evidence has linked SIRT1 activity with the interference of the factors and-or processes that produce NSP's and NFT's (**Figure 7**). CR in mice was shown to reduce the expression of β-secretase in part due to the activation of SIRT1 (104). This effect was through the AMPK-SIRT1-PGC-1α pathway in which the transcription factor PGC-1α became upregulated and reciprocally downregulated β-secretase. PGC-1α required SIRT1 deacetylase activity for its transcriptional repression of β-secretase. Thus, CR induced activation of SIRT1 promotes AD neuroprotection through changes in transcription factor activity. As stated before p-tau can contribute to AD. The acetylation of p-tau (acetylated-tau, ac-tau) prevents its degradation and promotes pathological accumulation (105). The overexpression of SIRT1 in HEK293T cells expressing human tau showed a reduction of ac-tau, while SIRT1 deletion results in hyperacetylation. Furthermore, a GST pull-down assay showed a direct interaction of SIRT1 and tau (105). SIRT1 has the capacity to deacetylate ac-tau, which in turn, allows for the degradation of tau and p-tau, potentially reducing the formation of NFT's in AD pathology.

In an oxidative stress model of neuroblastoma SK-N-BE cells, the SIRT1 activator resveratrol was administered to determine its effect on neurodegenerative oxidative stress and protein aggregation (106). Resveratrol treatment prevented toxicity from hydrogen peroxide-induced oxidative stress and prevented Aβ aggregation (106). When applying sirtinol, a non-specific SIRT1 inhibitor, the protection afforded by resveratrol against oxidative stress was lost but not the prevention of Aβ accumulation (106). This evidence indicates that oxidative stress that accompanies AD can be protected against through SIRT1 activation, however, resveratrol protection of Aβ accumulation is SIRT1-independent. SIRT1 overexpression in a transgenic mouse model of AD was investigated by Corpas et al. (107). They studied the CA1 region of the hippocampus to determine if SIRT1 is protective against memory loss and cognitive decline in AD. In the transgenic AD mouse, 6 months of SIRT1 overexpression preserved learning and memory (107). SIRT1 overexpression heavily reduced the presence of Aβ and p-tau in the AD model while increasing the expression of neurotrophic factors, such as brain-derived neurotrophic factor (BDNF) (107). Interestingly, in wild-type mice, SIRT1 overexpression enhanced cognitive function (107). Overexpression of SIRT1 provided protection against pathological protein aggregation and cognitive decline in an AD model while improving cognitive function in the wild-type control (107). SIRT1 is a robust candidate for AD therapies as it has been shown to prevent the accumulation of NSP's and

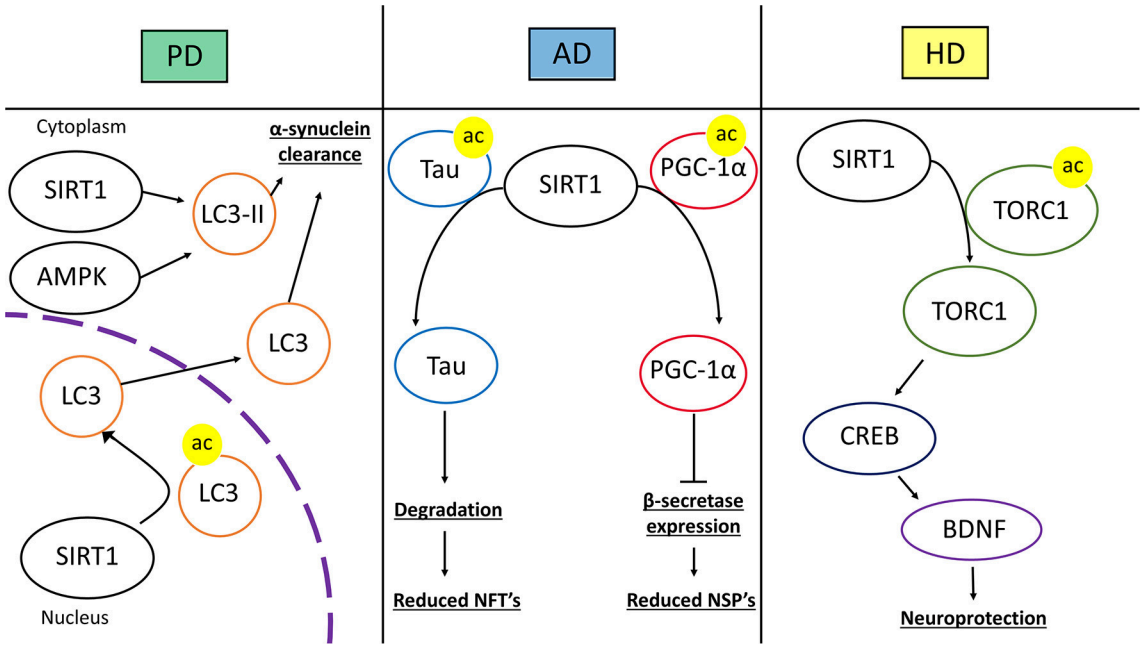

FIGURE 7 | Therapeutic mechanisms of SIRT1 in neurodegenerative disease. The left panel represents SIRT1 in Parkinson's disease (PD). SIRT1 deacetylates microtubule-associated protein 1A/1B-light chain 3 (LC3) in the nucleus which induces the translocation of LC3 into the cytoplasm. In the cytoplasm, SIRT1 and AMP-activated Protein Kinase (AMPK) coordinate to activate LC3-phosphatidylethanolamine (LC3-II). These mechanisms lead to increased autophagic clearance of α-synuclein, reducing α-synuclein deposits. In the middle panel, SIRT1's role in Alzheimer's disease (AD) is represented. SIRT1 can directly deacetylate acetylated-tau protein, increasing its susceptibility to degradation and prevent tau from forming neurofibrillary tangles. SIRT1 can also deacetylate peroxisome proliferator-activated receptor γ (PPARγ) coactivator-1α (PGC-1α), which increases its transcriptional regulation activity. After being deacetylated, PGC-1α can instill transcriptional repression of β-secretase, which in turn can reduce the level of amyloid-β production and neuritic senile plaque accumulation. The right panel represents Huntington's disease (HD). SIRT1 deacetylates CREB-regulated transcription coactivator 1 (TORC1), which allows TORC1 to activate cAMP response element-binding protein (CREB). CREB then transcriptionally upregulates brain-derived neurotrophic factor (BDNF). The increase in BDNF promotes neurotrophic and neuroprotective mechanisms against HD pathology.

NFT's, reduce AD-related oxidative stress, and protect against the cognitive deficits that result from AD pathology. Further investigation into SIRT1's role in AD protection may provide endogenous targets for treating, and potentially preventing, the disease.

Parkinson's Disease and the Therapeutic Potential of SIRT1

PD is a neurodegenerative disease that causes the early and large-scale death of dopaminergic neurons (DA) in the substantia nigra pars compacta (SNpc) (108). The loss of these dopaminergic neurons results in motor deficits and other quality of life diminishing symptoms (109). PD pathology is not exclusive to dopaminergic neurons or the SNpc; thus, making PD therapies difficult to design. DA neuronal death in PD typically results from the aggregation of the protein α-synuclein which forms inclusions called Lewy bodies and Lewy neurites (108). The inclusions of α-synuclein are formed in some familial cases of PD due to mutations in the *SNCA* gene which produces the protein in a misfolded state (110). Another prominent aspect of PD is inflammation and reactive gliosis, both of which may have the capacity to be harmful and protective (111). Overall, the current therapies and understanding of pathology for PD are lacking, making PD a pressing focus of future investigation.

Once again, SIRT1 may play a protective role in neurodegenerative disorders, PD included. There is evidence that suggests there may be genetic correlations between SIRT1 and PD, SIRT1-activated anti-PD signaling, and SIRT1-dependent neuroprotection in various models. Extracellular α-synuclein accumulation leads to mitochondrial dysfunction and a reduction of SIRT1 expression (112). The downregulation of SIRT1 facilitated pathological mechanisms, such as apoptotic cell death. In a genetic study with PD patients and healthy controls, the sequence of the SIRT1 promoter and associated regulatory regions were analyzed to determine if there is a mutational connection between the factor and the disease. Three heterozygous sequence variants within the SIRT1 promoter were identified in PD patients, but not controls (113). These variants may alter the transcription of the SIRT1 gene and could potentially link SIRT1-associated mutations to PD risk. These lines of evidence suggest that the loss or mutation of SIRT1 facilitates PD pathology which highlights SIRT1 as a protective target.

There is evidence that shows that cellular signaling resultant from SIRT1 activation, or pathways that include SIRT1, are

involved in the reduction of α-synuclein and promotion of DA neuron survival (**Figure 7**). The application of an activator for the PPARγ, called GW1929, to an *in vitro* human DA neuronal culture conferred resilience when the cultures were subjected to oxidative stress (114). This resilience was attributed to antioxidant signaling and PGC-1α stimulation. GW1929 treatment increased SIRT1 expression and protein levels. GW1929 also resulted in phosphorylated cyclic-AMP response element binding protein 1 (CREB), a pro-survival transcription factor, which then activated SIRT1 (114). Ultimately, upregulation and activation of SIRT1 activated PGC-1α to confer DA neuron protection against oxidative stress (114). In this way, SIRT1 is indirectly upregulated and activated by PPARγ activation in DA neurons suggesting a key role for SIRT1 in DA neuron vitality. In PD, PGC-1α activity may be altered resulting in downregulation of its target genes (115). A study looked at how resveratrol treatment would affect PGC-1α and metabolic homeostasis in primary fibroblasts from early-onset PD. The treatment of resveratrol helped to regulate metabolic homeostasis through AMPK-SIRT1-PGC-1α signaling. An increase in PGC-1α transcription and improvement in mitochondrial function was observed (115). Again, activated SIRT1, in this study through resveratrol treatment, conferred protection against a PD model by activating PGC-1α. In a mouse model of PD induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), the transgenic overexpression of PGC-1α conferred DA neuronal protection against oxidative stress. Resveratrol treatment recapitulated the protective effects of PGC-1α overexpression in the mouse PD model (116). In a PC12 PD model, the treatment of EGCG, a polyphenol, protected against toxicity through an upregulation of PGC-1α, via SIRT1 activity (117). It is clear that SIRT1 signaling can result in the activation of PGC-1α which is protective against oxidative stress and PD pathology. The evidence supports SIRT1 as a target for future therapeutic approaches in PD treatment due to its induction of neuroprotective cell signaling. Further investigation will provide a greater depth of understanding for PD pathology.

Inflammation within the CNS can exacerbate or potentially initiate PD pathology (118). The cell signaling that occurs from increased inflammation and reactive nitrogen species enhances the dysfunction of neurons and promotes cell death (119). A study looked at how inducible nitric oxide synthase (iNOS), which produces NO that can modify proteins through S-nitrosylation, effects inflammatory signaling in neurodegenerative diseases. S-nitrosylation of SIRT1 inhibits its deacetylase activity. In a rodent model of PD with systemic inflammation, S-nitrosylation of SIRT1 correlated with an increase in p53 and NF-κB acetylation, thereby increasing their activity and promoting further inflammation (119). In SH-SY5Y cells, SIRT1 was shown to directly deacetylate histone residue H3K9 of the p53 promoter, eventually resulting in reduced expression and protecting against apoptosis (120). Additionally, resveratrol-activated SIRT1 regulated p53 and protected against dopaminergic neurodegeneration induced by rotenone, a complex I inhibitor (120). As an inflammatory regulator in PD and potentially other neurodegenerative diseases, SIRT1's deacetylase activity protects against pro-apoptotic inflammatory signaling.

The removal of α-synuclein in a healthy DA neuron entails the use of a few cellular clearance mechanisms, most of which involve some type of autophagy (121). One of these mechanisms utilizes a recruitment protein called LC3, which helps to drive degradation of misfolded α-synuclein that is present in the LC3 bound autophagosome (122). In an MPTP-mouse model, resveratrol or EX-527 were administered to study their effects on motor impairments and autophagic clearance of α-synuclein (122). Resveratrol treatment attenuated MPTP effects on motor deficits and autophagic impairment while EX-527 exacerbated them (122). Furthermore, the beneficial effects of resveratrol treatment were shown to be SIRT1 dependent. SIRT1 was shown to deacetylate nuclear LC3 allowing for its translocation to the cytosol from the nucleus and initiate autophagic clearance (122). Thus, SIRT1 deacetylase activity mediates clearance of α-synuclein through LC3 mediated autophagy to protect against PD pathology. In addition, activation of the AMPK-SIRT1-autophagy pathway was shown to increase LC3-II and enhance α-synuclein clearance after resveratrol treatment (123). Increased clearance of α-synuclein is yet another mechanism by which SIRT1 confers protection against PD pathology.

Considering the multitude of evidence supporting SIRT1's neuroprotective potential against PD and the dynamic range of mechanisms in which that protection is enacted, SIRT1 appears to be an optimal target for the therapeutic treatment of PD. However, recent attempts to enhance SIRT1 expression or activity directly, not through activators, has not shown the same robust results. A study utilized a CNS SIRT1 overexpression mouse model to study the MPTP model of PD. As compared to controls the SIRT1 overexpression mouse did not confer protection against acute toxicity of MPTP in nigrostriatal DA neurons (124). Additionally, in a study that looked at the modulation of SIRT1 expression in multiple human neurodegenerative diseases, there was no significant change found in the SIRT1 expression of patient samples of PD and Lewy bodies dementia (125). This contradictory evidence suggests SIRT1 must operate in a network of cellular signaling and deacetylase activity to confer is neuroprotection against PD pathology.

Huntington's Disease and the Therapeutic Potential of SIRT1

Another neurodegenerative disease in which SIRT1 has been investigated is HD. HD is a genetically autosomal dominant disease in which the HD gene produces a mutant version of the protein. Extended CAG-repeats in the HD gene results in the translated protein acquiring a pathological conformation, affecting its solubility and promoting aggregations (126). Aggregations of the pathological Huntington's protein commonly occur in the axons of neurons, predominantly within the striatum. These axonal aggregations are considered to block anterograde and retrograde axonal transport in affected cells. Post-mortem tissue of HD patients showed cytological features of ballooned cells and shrunken cells within the affected brain

regions (127). The blockage of axonal transport is suggested to result in pathological localization of mitochondria and mitochondrial dysfunction. The combination the cytological aberrations and mitochondrial dysfunction are implicated as leading reasons for neuronal death in HD pathology (128).

Like other neurodegenerative diseases, the role of SIRT1 has been investigated in the context of HD pathology. In the R6/2 mouse model of HD, the levels of metabolic and cell cycle regulators were assessed. SIRT1 mRNA and protein were increased in this model but this increase did not correlate to increased activity as shown by no significant change in p53 acetylation (129). This change in SIRT1 expression suggests that SIRT1 levels are altered as a result of HD pathology. In the same model of HD, treatment with β-Lapachone, a natural compound found in the Lapacho tree's bark, was shown to increase the expression of SIRT1 (130). Increased SIRT1 resulted in PGC-1α deacetylation and CREB phosphorylation, which correlated with reduced reactive oxygen species and improvement of rota-rod performance. β-Lapachone thus showed therapeutic potential for HD, enacted through SIRT1 activation. There may be specific contexts in which increased SIRT1 is therapeutic rather than a feature of HD pathology and this is likely related to increased SIRT1 activity.

Many studies have intentionally augmented SIRT1 in the contexts of HD to elucidate whether it is part of the pathology or potentially therapeutic. In an HD mouse model, SIRT1 was overexpressed, improving motor functions and pathological metabolic functioning (131). SIRT1 overexpression was shown to alleviate the HD associated reduction in BDNF concentrations (**Figure 7**). BDNF signaling via its TrkB receptor was shown to be rescued as well. Interestingly, this study also suggests that mutant HD protein inhibits deacetylase activity of SIRT1, as shown by the hyperacetylation of SIRT1-specific targets in the presence of HD mutant protein (131). Another study looked at the effects of SIRT1 absence in HD pathology by using a brain-specific KO of SIRT1 in a mouse model of HD (132). The loss of SIRT1 exacerbated pathological features of HD. These mice had acceleration of motor deficits and increased mutant HD protein aggregation compared to the HD mice with SIRT1 (132). This study also investigated SIRT1 overexpression, which afforded neuroprotection against HD. SIRT1 neuroprotection was dependent on CREB-regulated transcription coactivator 1 (TORC1), which is deacetylated by SIRT1. This interaction increases BDNF and in the presence of the mutant HD protein, the SIRT1-TORC1 interaction is inhibited, repressing BDNF (132).

The role of SIRT1 in HD certainly warrants further investigation. Though there are pathological increases in SIRT1 in neurons suffering from HD pathology, this may be a compensatory mechanism due to the inhibition of SIRT1 by the mutant HD protein. In studies overexpressing SIRT1, HD pathology has been ameliorated and this protection is dependent on SIRT1's activity. Taken together, these lines of evidence suggest that SIRT1 is inhibited in HD pathology and there may be an increase in its expression for compensatory reasons. Furthermore, the loss of SIRT1 deacetylation activity may contribute to HD pathology and restoration of SIRT1 activity likely possess therapeutic potential against the disease.

CONCLUSIONS

In summary, any dysregulation of brain SIRT1 activity can have devastating consequences in terms of metabolism, circadian synchronization, and neurological function. Given that SIRT1 is highly specialized distributed in the hypothalamic nuclei, it is no surprise that brain SIRT1 is a major contributor to the systemic network of metabolic homeostasis. It should be noted that, nowadays, accumulated evidence supports a reciprocal relationship between brain and peripheral tissues in metabolic benefits, circadian oscillations and neurological functions (133, 134). Although we only discussed SIRT1 in the brain, SIRT1 in various peripheral organs also mediates metabolism and circadian rhythms through sensing environmental cues and feeding back into the homeostatic network (13, 133). Therefore, pharmacological agents that target SIRT1 and its relevant signal pathway in one system could potentially provide pleiotropic benefits.

Although most studies discussed above have used resveratrol as the SIRT1 activator, limitations remain for the resveratrol-induced SIRT1 activation. One study reported the activation of AMPK by high-dose resveratrol, suggesting the indirect effects of SIRT1 through AMPK pathway as well as the off-target effects of resveratrol (135). As a polyphenol activator, resveratrol is poorly water-soluble. Thus, the bioavailability of resveratrol also needs to be taken into consideration, especially when applied in clinical trials. To improve the bioavailability, targeted delivery of resveratrol, such as nanoparticles has been developed (136, 137).

In addition to resveratrol, another promising target to activate SIRT1 is the NAD^+ pathway. As mentioned before, NAD^+ is the rate-limiting co-substrate for SIRT1. Thus, increased NAD^+ levels is presumably to activate SIRT1. So far, to supplement NAD^+ precursor and boost NAD biosynthesis has been the main approaches to alter NAD^+ levels. Experimental studies have reported beneficial effects of NAD^+ precursor supplementation (138–140). Current information from clinical studies is still lacking. One recent study reported promising result that chronic nicotinamide riboside, a NAD^+ precursor, effectively increased NAD^+ levels in elders (141). Again, it is difficult to tell whether the beneficial phenotypes are produced by NAD^+ or NAD^+-induced SIRT1 activation. Therefore, pharmacological agents that are ligand-specific and tissue-specific are warranted to further clarify the functions of SIRT1 in biological and pathological events.

AUTHOR CONTRIBUTIONS

JX, CJ, NK, IE, and MP-P cooperated to write the article and revised the content. MP-P and JX outlined the manuscript. MP-P supervised the work.

FUNDING

This work was financially supported by the NIH/NINDS grants NS45676, NS097658, and NS34773.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Neuroscience

REVIEW
published: 21 November 2018
doi: 10.3389/fnins.2018.00833

The Role of Sirt1 in Ischemic Stroke: Pathogenesis and Therapeutic Strategies

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Silent mating type information regulation 2 homolog 1 (Sirt1), a nicotine adenine dinucleotide (NAD^+)-dependent enzyme, is well-known in playing a part in longevity. Ischemic stroke is a major neurological disorder and is a leading cause of death and adult disability worldwide. Recently, many studies have focused on the role of Sirt1 in ischemic stroke. Numerous studies consider Sirt1 as a protective factor and investigate the signaling pathways involved in the process under ischemic stress. However, the answer to whether upregulation of Sirt1 improves the outcome of stroke is still a controversy. In this review, we discuss the role and mechanisms of Sirt1 in the setting of ischemic stroke.

Keywords: Sirt1, deacetylase, ischemic stroke, neuroprotection, sirtuin

OPEN ACCESS

Edited by:
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Northwest University, China

Reviewed by:
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Specialty section:
This article was submitted to Neuroendocrine Science, a section of the journal Frontiers in Neuroscience

Received: *31 July 2018*
Accepted: *24 October 2018*
Published: *21 November 2018*

Citation:
Zhang J-F, Zhang Y-L and Wu Y-C (2018) The Role of Sirt1 in Ischemic Stroke: Pathogenesis and Therapeutic Strategies. Front. Neurosci. 12:833. doi: 10.3389/fnins.2018.00833

INTRODUCTION

Stroke is the second leading cause of death and a major cause of adult disability worldwide (Cushman et al., 2008; Donnan et al., 2008). Ischemic stroke is the most common type of stroke and accounts for 87% of all stroke cases (Macrez et al., 2011). Vascular recanalization therapies, including tissue plasminogen activator thrombolysis and thromboembolectomy, are currently considered the best therapies but are limited due to a narrow treatment window and several safety concerns (Del Zoppo et al., 2009; Albers et al., 2018; Nogueira et al., 2018; Thomalla et al., 2018). There is no effective treatment for ischemic stroke so far. It is crucial to find new therapies for this major medical problem.

There are two major injuries to the brain. Firstly, a blocked cerebral artery and secondly, the subsequent reperfusion may cause the secondary injury. Penumbra, defined as a zone of tissue surrounding the core of the infarction area, is an important target for researchers and clinicians to find effective therapies (Wang et al., 2012). The occlusion of a cerebral artery can cause the deprivation of oxygen and energy, thereby leading to the dysfunction of cerebral tissue and neuron death. After the early phase of necrosis, the following pathophysiological reactions such as formation of free radicals, changes in gene expression, apoptosis, and inflammation contribute to the delayed phase of tissue damage (Moskowitz et al., 2010; Petegnief and Planas, 2013). However, the ineffectiveness of current therapies indicates that there should be other important mechanisms leading to the pathophysiology of ischemic stroke.

Silent mating type information regulation 2 homolog 1 (Sirt1), also called sirtuin 1, is a nicotine adenine dinucleotide (NAD^+)-dependent enzyme (Kelly, 2010). Among several potential

TABLE 1 | Role and mechanisms of Sirt1 in ischemic stroke.

Compound	SIRT1 role	Mechanism	Reference
IRF9	Anti-apoptosis	IRF9 inhibits Sirt1 deacetylase activity, culminating in the acetylation and activation of p53-mediated cell death signaling in response to acute I/R stress.	Zhang et al., 2017
LKE	Anti-apoptosis, anti-inflammation	LKE mediated, at least in part, through CRMP2 and Sirt1 upregulation and PARP1 inhibition.	Nada et al., 2012
Resveratrol	Anti-oxidation, anti-apoptosis, anti-inflammation	Resveratrol upregulates the Sirt1/PGC-1a, Akt/pCREB, and p38 pathways and downregulates pERK1/2 expression in ischemic injury.	Zhu et al., 2010
	Regulation in glycolytic function	Resveratrol via neuronal Sirt1 promotes glycolytic efficiency to combat energetic stress.	Yue et al., 2008
	Energy regulation	Resveratrol provides neuroprotection by inhibiting PDEs and regulating the cAMP/AMPK/Sirt1 pathway.	Kundu and Thompson, 2008
Resveratrol preconditioning	Anti-oxidation and regulation of neural survival	The mechanism is mediated by Sirt1 through upregulation of BDNF and downregulation of uncoupling protein 2.	Koronowski et al., 2015
HBO-PC	Anti-oxidation	HBO-PC is mediated by the activation of Sirt1 and Nrf2/antioxidant defense pathway.	Xue et al., 2016
Arctigenin	Anti-inflammation	Arctigenin inhibited NLRP3 inflammasome activation through Sirt1 pathway.	Hernandez-Jimenez et al., 2013
Curcumin	Anti-apoptosis	Curcumin activates Sirt1 signaling, resulting in decreased expression of Ac-p53 and Bax and increased Bcl-2 expression.	Miao et al., 2016
Icariin	Anti-oxidation	Icariin protects against brain ischemic injury by increasing the Sirt1 and PGC-1a expression.	Kou et al., 2017
Nampt	Regulation in autophagy	Nampt induces autophagy via TSC2-mTOR-S6K1 signaling pathway in a Sirt1-dependent manner.	Wang et al., 2012
	Energy regulation	Nampt protects against ischemic stroke through rescuing neurons from death via the SIRT1-dependent AMPK pathway.	Dasgupta and Milbrandt, 2007
Leptin	Anti-apoptosis	Leptin increases CB2, Sirt1 and TRPV1 expression as well as expression of the endogenous leptin receptors and reduces the expression of CB1 receptors.	Ashrafi et al., 2017
Magnolol	Anti-apoptosis and anti-inflammation	Magnolol activation of Sirt1 was accompanied by the inhibition of Ac-FOXO1 expression, which decreased the expression of bax and increased Bcl-2 expression.	Chen et al., 2014
Melatonin	Anti-apoptosis	Melatonin increased Sirt1 and reduced Ac-p53 and Ac-NF-κB and was also associated with a rise of Bcl2 and a lowering of Bax.	Wang et al., 2008
Tetrahydroxystilbene glucoside	Anti-oxidation and Anti-apoptosis	The mechanisms are involved with depression of the JNK and Bcl-2 family-related apoptotic signaling pathway, and inhibition of iNOS mRNA expression, which was partly mediated by the activation of Sirt1 and thereby inhibition of NF-κB activation.	Taxin et al., 2014
SalB	Anti-oxidation, anti-apoptosis, anti-inflammation	SalB decreased TNF-α and IL-1 levels in the brain tissue and upregulated the expression of Sirt1 and Bcl-2 and downregulated the expression of Ac-FOXO1 and Bax.	Guo et al., 2017
Estrogen	Energy regulation	Estrogen protects against ischemic stroke via the SIRT1-dependent AMPK pathway.	Wang et al., 2011
HBO-PC	Anti-apoptosis	SirT1 increased Bcl-2 expression and decrease cleaved caspase 3.	Yan et al., 2013
/	Effect on BBB permeability	Sirt1 inhibited Sirt3 expression through the AMPK-PGC1 pathway, causing mitochondrial ROS generation and then increase BBB permeability.	Hurtado et al., 2013
/	Regulation of cerebral blood flow	Sirt1 upregulates the nitric oxide (eNOS–NO) system.	Briski et al., 2014

IRF9, interferon regulatory factor 9; I/R, ischemia/reperfusion; LKE, lanthionine ketimine 5-ethyl ester; CRMP, collapsin response mediator proteins; PARP, poly-ADP-ribose polymerase; PGC-1α, peroxisome proliferator-activated receptor-gamma coactivator; CREB, cyclic AMP response element-binding protein; ERK, extracellular signal-regulated kinase; NLRP, NOD-like receptor family pyrin domains; Bcl-2, B-cell lymphoma 2; TSC2, tuberous sclerosis complex-2; mTOR, mammalian target of rapamycin; CB, cannabinoid receptor type; TRPV, transient receptor potential vanilloid; FOXO, forkhead box protein O; NF-κB, nuclear factor kappa B; AMPK, adenosine monophosphate (AMP)-activated kinase; JNK, c-Jun N-terminal kinase; BDNF, brain-derived neurotrophic factor; eNOS, endothelial nitric oxide synthase; NO, nitric oxide; iNOS, inducible NO synthase; BBB, blood–brain barrier; HBO-PC, hyperbaric oxygen preconditioning.

therapeutic targets, Sirt1 is one of the most valuable candidates because it can modulate gene expression and adapt cell metabolism to ischemic stress. Sirt1 deacetylates numerous transcription factors other than histones and is involved in various biological processes (Chen et al., 2005). A large number of studies consider Sirt1 as a survival factor against aging process, including cardiovascular disease (Li et al., 2011) and neurodegeneration (Donmez, 2012). In recent years, Sirt1 has been found to be neuroprotective against cerebral ischemia/reperfusion (I/R) injury (Herskovits and Guarente, 2014). Activation of Sirt1 alleviates ischemia through several mechanisms (**Table 1**). Although it is still a controversy whether Sirt1 could improve stroke outcome, there have been plenty of studies indicating the potential therapeutic value of Sirt1 for ischemic stroke. In this review, we discuss the role and potential mechanisms by which Sirt1 protects against ischemic stroke (**Figure 1**).

ANTI-INFLAMMATORY AND ANTI-APOPTOTIC EFFECTS

Sirt1 plays an important role in endogenous neuroprotection, which is demonstrated using pharmacological and genetic gain of function and loss of function experiments. Activating or inhibiting enzymatic activity of Sirt1 causes a decrease or an increase in infarct volume. Sirt1 is considered to be related to anti-inflammatory and anti-apoptotic effects in cerebral ischemia because its inhibition exacerbated ischemic injury accompanied by increased acetylation of p53 and NF-κB (nuclear factor-kappa B p65), which are important factors mediating inflammatory and apoptotic pathways causing brain damage (Hernandez-Jimenez et al., 2013).

Activation of Sirt1 signaling during cerebral ischemia by curcumin (CCM), a compound mainly extracted from *Curcuma longa*, leads to the decreased expression of Ac-p53 and Bax, increased expression of B-cell lymphoma 2 (Bcl-2), and finally attenuated the inflammation (Miao et al., 2016). Similarly, interventions targeting IRF9 inhibition may help to mediate Sirt1-related ischemic neuron survival through decreased expression of p53 (Chen et al., 2014).

Arctigenin (ARC), a phenylpropanoid dibenzylbutyrolactone lignan derived from *Arctium lappa* L., was reported to provide neuroprotection against ischemic stroke by inhibiting NLRP3 inflammasome activation through the activation of Sirt1 signaling pathway in the middle cerebral artery occlusion (MCAO) model with decreased infarct volume, neurological scores, and brain water content (Zhang et al., 2017). Another study also used MCAO model to demonstrate that LKE (lanthionine ketimine-5-ethyl ester) protected ischemic brain tissue partly through CRMP2 and Sirt1 upregulation and PARP1 inhibition (Nada et al., 2012).

Hyperbaric oxygen (HBO) therapy is considered to be one of the safe and feasible methods to provide neuroprotective benefits for patients with ischemic stroke. It was demonstrated that Sirt1 was involved in the HBO therapy induced ischemic tolerance and Nrf2 might be the downstream regulator of Sirt1 (Xue et al., 2016). Hyperbaric oxygen-preconditioning was found to upregulate the expression of Sirt1 protein and mRNA after focal cerebral ischemic injury, leading to the suppression of apoptosis. Upregulation of Sirt1 caused an increased expression of anti-apopotic Bcl-2 and a decreased pro-apoptotic cleaved caspase-3 in oxygen–glucose deprivation (OGD) injury models (Yan et al., 2013).

ANTIOXIDATION

Cerebral ischemia was found to produce a large number of free radicals and cause neurotoxicity in I/R injury (Crack and Taylor, 2005). Increased generation of intracellular reactive oxygen species (ROS) such as the hydroxyl radical is demonstrated to induce oxidative stress and mitochondrial enzyme dysfunction, leading to the pathophysiology of damage of cerebral ischemia, and this damage, in turn, aggravates cerebral injury (Mattiasson et al., 2003; Slemmer et al., 2008). Therefore, to prevent intracellular calcium accumulation and further cell apoptosis followed by oxidative stress in ischemic stroke, it is critical to find targets to inhibit such cellular signal transduction pathways (Chan, 2004; Mehta et al., 2007; Doyle et al., 2008; Thompson et al., 2015).

Several studies suggested that Sirt1 plays an important role in oxidative stress in ischemic stroke. Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) is a potent stimulator of mitochondrial respiration and gene transcription in the liver, heart, skeletal muscle, and neurons (Wareski et al., 2009). Hypoxia increases mRNA levels and the protein expression of PGC-1α in wild type mice (Gutsaeva et al., 2008). Increased expression of PGC-1α could reduce neuronal death mediated by oxidative stress (St-Pierre et al., 2006). Sirt1 could directly affect PGC-1α activity through phosphorylation and deacetylation (Canto and Auwerx, 2009), thereby protecting against ischemia stroke.

Resveratrol (3,5,4′-trihydroxystilbene), a polyphenol found in red wine, can ameliorate neuronal damages caused by cerebral ischemia and neurodegenerative diseases such as Alzheimer's and Parkinson's disease (Sun et al., 2010; Wang et al., 2014; Pasinetti et al., 2015). Resveratrol was demonstrated to protect ischemic stroke by upregulating the Sirt1-PGC-1α signaling pathways and exert an antioxidative effect under ischemic stress (Shin et al., 2012). Furthermore, resveratrol preconditioning was showed to upregulate BDNF and downregulate uncoupling protein 2 by mediating Sirt1, tolerance in brain (Koronowski et al., 2015). In addition, alpha-lipoic acid (ALA, 1,2-dithiolane-3-pentanoic acid), a free radical scavenger in its oxidized state functions as an essential co-factor in the mitochondrial dehydrogenase complexes. Icariin (ICA), one of the major active flavonoids extracted from the Chinese medicinal herb, *Epimedium brevicornum* Maxim, were also proved to protect against ischemic stroke by increasing Sirt1 and PGC-1α expression (Zhu et al., 2010; Fu et al., 2014).

Another study found that Sirt1 was upregulated chronically at 14 days after a single resveratrol preconditioning treatment. This phenomenon was associated with negative regulation of

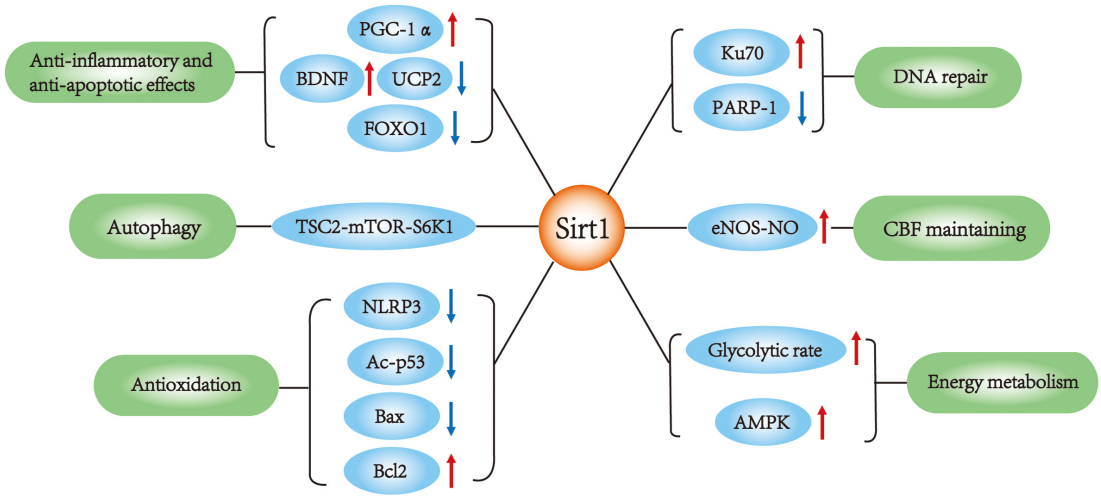

FIGURE 1 | Sirt1 main functions in ischemic stroke. SIRT1 interacts with multiple targets in ischemic stroke, which is involved in the regulation of apoptosis, autophagy, DNA repair, inflammation, metabolism and oxidative stress, cerebral blood flow.

mitochondrial uncoupling protein-2 (UCP2), a proton channel found in the inner mitochondrial membrane that uncouples oxidative phosphorylation (Della-Morte et al., 2009), by binding directly to its promoter (Bordone et al., 2006) and upregulation of brain-derived neurotrophic factor (BDNF), which is an important growth factor that promotes the survival and growth of neurons (Bramham and Messaoudi, 2005).

In addition, salvianolic acid B (Sal B) (Lv et al., 2015), which is the most abundant and bioactive compound of danshen (*Salvia miltiorrhiza*), and Magnolol (Kou et al., 2017), an organic compound found in the bark of Houpu magnolia (*Magnolia officinalis*), were demonstrated to activate Sirt1 signaling, accompanied by reduced expression of ac- FoxO1 whose function is to synthesize antioxidants and further help neurons provide resistance against oxidative stress (Brunet et al., 2004).

DNA REPAIR

As a result of ischemic stroke, oxidative stress will further cause damage to the DNA including oxidative base modifications and strand breaks. The accumulation of oxidative DNA lesions is attributed to the death of neurons. Therefore, the capacity for DNA repair plays an important role in the destiny of neurons in the condition of ischemic stress. Base excision repair (BER) is one of the most crucial parts in the DNA repair systems, which is the endogenous defense mechanism to rescue oxidative DNA damage (Srivastava et al., 1998).

Ku70 is one of the multifunctional DNA repair proteins, which triggers a DNA repair pathway by binding to broken DNA ends including double-strand breaks (Kim et al., 2001). With the decrease of Ku70 after focal cerebral ischemic injury, the repair process was found to be hindered (Kim et al., 2001). Sirt1 is found to regulate the acetylation of Ku70, thereby regulating the DNA repair pathways (Jeong et al., 2007).

Sirt1 also increases the activity of several other DNA repair pathways. The activation of poly [ADP-ribose] polymerase-1 (PARP-1), a key mediator of cell death in excitotoxicity, ischemia, and oxidative stress, contributes to the depletion of NAD^+ and the release of apoptosis-inducing factor (AIF) from mitochondria, leading to cell death (Alano et al., 2010). The replenishment of cellular NAD^+ was demonstrated to confer marked neuroprotection effects by enhancing the DNA repair process against ischemic cell death (Wang et al., 2008). Kolthur-Seetharam et al. (2006) reported that SIRT1 modulates PARP-1 activity upon DNA damage. Sirt1 upregulation by resveratrol can reduce PARP-1 activity, however, there is a drastic increase in PAR synthesis leading to AIF-mediated cell death in the sirt1-null cells. This study indicated the protection mechanism of Sirt1 in ischemic stress. Further research is still needed to elucidate the full picture of the role of Sirt1 in DNA repair under ischemic stroke conditions.

CEREBRAL BLOOD FLOW (CBF) MAINTAINING

Sirt1 can deacetylate endothelial nitric oxide synthase (eNOS) and then maintain CBF (Hattori et al., 2014). In one particular study, mice overexpressing Sirt1 were found to provide resistance to global ischemia by retaining cerebral perfusion up to 45%–50% of baseline data (Hattori et al., 2015). Moreover, Hattori et al. (2014) found that endothelial Sirt1 deacetylates and activates eNOS, thus normalizing CBF. They further suggested that Sirt1-eNOS-NO system is responsible for the suppression of Sirt1-induced cerebral hypoperfusion. Sirt1 overexpression significantly attenuated blood–brain barrier (BBB) disruption, and an interaction of Sirt1 with eNOS facilitated NO-dependent vascular relaxation (Hattori et al., 2014).

FUNCTION ON ENERGY METABOLISM

The brain consumes more energy per gram of tissue than any other organ (Taxin et al., 2014). The state of ischemia in the brain confers onto energy exhaustion. In turn, this metabolic stress aggravates the ischemic injury. Therefore, to promote cell survival and improve ischemic injury in the brain, it is important to find defense mechanisms in energy exhaustion (Guo et al., 2017). AMP-activated protein kinase (AMPK) is considered to be a major metabolic energy sensor. Decreased cellular energy charge (increased AMP/ATP ratio) activates AMPK and then regulates energy metabolic homeostasis (Briski et al., 2014; Zainal Abidin et al., 2015). Pharmacologic inhibition of AMPK was reported to alleviate ischemic damage in an ischemia model (Li et al., 2007). Sirt1 is suggested to be associated with the regulation of energy metabolism (Boutant and Canto, 2014). Sirt1-related AMPK pathway was reported to protect against ischemia stroke in some studies (Wang et al., 2011).

Estrogen was proved to have beneficial effects under cerebral ischemic stress through Sirt1-AMPK signaling pathway. Estrogen deficiency is considered as a risk factor for ischemic stroke in females after menopause (Ahnstedt et al., 2016), which partly explains the stroke-related gender differences (Persky et al., 2010; Sohrabji et al., 2013). Guo et al. (2017) showed that upregulating Sirt1 expression and then promoting AMPK activation can further regulate energy exhaustion, finally contributing to neuron survival under ischemic stress. In addition to estrogen, the adipokynin hormone, leptin, plays a role in severe energy depletion. Avraham et al. (2010) found that the expression of Sirt1 gene increased in the cortex after leptin administration, which was in line with the reduction of infarct volume. This finding suggested that Sirt1 expression protects cortical neurons by modulating energetic status (Avraham et al., 2010).

Nicotinamide phosphoribosyltransferase (Nampt, also known as visfatin), the rate-limiting enzyme in mammalian NAD^+ biosynthesis (Wang et al., 2012), plays several roles in protecting against ischemia, one of which was associated with the Nampt-Sirt1-AMPK neuroprotective signaling pathway. Wang et al. (2012) reported that Nampt was significantly upregulated in the penumbra and infarct core of MCAO models. Inhibition and overexpression of Nampt augmented and reduced the infarction in MCAO rats, respectively. The upregulation of Nampt positively modulated NAD^+ levels and then upregulated Sirt1, contributing to LKB1 deacetylation, and thereby activating AMPK. This finding indicated that Nampt is an important protective factor in ischemic stroke (Wang et al., 2012).

Emerging studies showed that resveratrol could activate AMPK (Dasgupta and Milbrandt, 2007). Resveratrol was reported to activate a PDE-mediated signaling pathway that activates p-AMPK and Sirt1, thereby conferring cerebral ischemic tolerance (Wan et al., 2016).

The brain relies heavily on glucose for energy production (Koronowski et al., 2017). Production of ATP from glycolysis is crucial for fast axonal transport of vesicles (Zala et al., 2013), the energetic demand of action potential firing (Ashrafi et al., 2017), and the maintenance of synaptic ATP levels under energetic stress (Jang et al., 2016). Under ischemic conditions, it is important to utilize glucose more efficiently. Glycolysis can produce a significant amount of ATP to maintain ion gradients and delay depolarization. This effect is associated with neuronal Sirt1. One particular study demonstrated that resveratrol preconditioning increased glycolytic rate in a Sirt1-dependent manner in neurons, thereby combating energetic stress in ischemic conditions (Koronowski et al., 2017).

AUTOPHAGY

Autophagic processes have been implicated as cell death mechanisms in the degradation and recycling of subcellular organelles (Kundu and Thompson, 2008). Generally, in the neuronal system, moderate autophagy is neuroprotective while inadequate or excess autophagy may lead to neuronal death (Shacka et al., 2008; Yue et al., 2008). Recently, autophagy has been recognized as a key process in ischemic stroke in addition to neurodegenerative diseases such as Alzheimer's and Parkinson's disease (Yue et al., 2008; Puyal et al., 2009). Sirt1 was first reported to regulate autophagy in Lee et al. (2008).

In addition to the Nampt-Sirt1-AMPK signaling pathway mentioned above, Nampt was demonstrated to have regulatory effects on autophagy under cerebral ischemic conditions (Wang et al., 2012). Wang et al. (2012) reported that Nampt promotes neuronal survival through inducing autophagy via regulating the TSC2-mTOR-S6K1 signaling pathway in a Sirt1-dependent manner during cerebral ischemia. Further studies are needed to find out more about the relationship between autophagy and ischemic stroke.

IMPACT ON BLOOD–BRAIN BARRIER (BBB)

Although most studies suggest that Sirt1 is a protective mediator in ischemic stroke, there are still a few studies that contradict these findings. Chen et al. (2018) reported that the activation of Sirt1 was associated with increased BBB permeability through AMPK-PGC1. Disruption of BBB and the cerebral edema that follows are the key pathogenic events contributing to neurological dysfunction and cerebral infarct after ischemic stroke (Chen et al., 2018). It is important to investigate further to find out the detailed mechanism and explain the contradiction between such studies.

OTHER MECHANISMS

Some studies reported that Sirt1 is important in the protection against ischemic stroke. Caloric restriction (CR) is defined as approximately 30% reduction in caloric intake, without compromising the maintenance of all essential nutrients (Redman et al., 2007). Short-term food restriction (40% less food over a 3-month period) was reported to attenuate ischemia-induced damage and improve functional recovery following global ischemia (Roberge et al., 2008). Proper period of CR can

protect neurons from focal ischemic injury (Duan et al., 2001). Caloric restriction was found to increase the synthesis of Sirt1 and reduce the downregulation of Sirt1 expression in MCAO (Ran et al., 2015). Another study found that CDP-choline (citicoline), an intermediate in the biosynthesis of phosphatidylcholine, acted as Sirt1 activator by upregulating its expression and thereby reducing infarct volume in ischemic models (Hurtado et al., 2013). Further studies are still needed to elucidate the specific mechanisms.

CONCLUSION AND PROSPECTS FOR FUTURE RESEARCH

Since Sirt1 could exacerbate energy depletion and is associated with increased BBB permeability, there is still a controversy whether choosing Sirt1 as a treatment target and increasing the Sirt1 level would benefit the cerebral tissue under ischemic stress. Therefore, additional studies investigating the role of Sirt1 in ischemic stroke involving different cerebral cell types and animal models are necessary to arrive at more convincing conclusions. Besides, the relationship between Sirt1 genetic polymorphism and ischemic stroke has not been researched extensively, which also needs further exploration. Altogether, Sirt1 is a promising therapeutic target for ischemic stroke for attenuating ischemic stress and improving stroke outcome.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

FUNDING

This work was partially supported by the grant of Clinical Research Innovation Plan of Shanghai General Hospital (Grant No. CTCCR-2018B03) and National Natural Science Foundation of China (Grant Nos. 81671251 and 81371410).

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Endocrinology

REVIEW
published: 19 November 2018
doi: 10.3389/fendo.2018.00652

Advances in Cellular Characterization of the Sirtuin Isoform, SIRT7

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SIRT7 is one of seven mammalian sirtuins that functions as an NAD^+-dependent histone/protein deacetylase. SIRT7 is the least well-known member of the sirtuin family, but recent efforts have identified its involvement in various cellular processes, such as ribosome biogenesis, gene expression, cellular metabolism and cancer. Here we provide an update on the functions and mechanisms of SIRT7 in cellular regulation and disease.

Keywords: SIRT7, rDNA transcription, ribosome biogenesis, cellular stress, metabolism, aging, genome stability, cancer

OPEN ACCESS

Edited by:
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Specialty section:
This article was submitted to Cellular Endocrinology, a section of the journal Frontiers in Endocrinology

Received: *23 August 2018*
Accepted: *17 October 2018*
Published: *19 November 2018*

Citation:
Wu D, Li Y, Zhu KS, Wang H and Zhu W-G (2018) Advances in Cellular Characterization of the Sirtuin Isoform, SIRT7. Front. Endocrinol. 9:652. doi: 10.3389/fendo.2018.00652

INTRODUCTION

In the year 2000, researchers identified that silent information regulator 2 (Sir2)—a nicotinamide adenine dinucleotide (NAD)-dependent histone deacetylase—extends the lifespan of yeast (1, 2). Since then, the Sir2 homologs in mammals, known as sirtuins, have received increasing attention. There are seven sirtuin homologs in mammals (SIRT1 to SIRT7) that share a conserved NAD^+ binding domain, but their cellular localization, activities and functions differ (**Figure 1**). SIRT1, SIRT6, and SIRT7 predominantly localize to the nucleus (SIRT7 primarily resides in nucleolus), SIRT2 localizes to the cytoplasm and SIRT3, SIRT4, and SIRT5 localize to the mitochondria (3). Sirtuins mediate various cellular functions that regulate a wide range of physiological processes, including the cell cycle, proliferation, apoptosis, aging, genomic stability, stress resistance and metabolism (4). SIRT7 is the least well-studied of the sirtuins, but recent breakthroughs have shown that it is also involved in numerous cellular processes and its biological function is gradually becoming clear. In this review, we outline the current data regarding SIRT7 function and highlight the areas where SIRT7 may have a possible therapeutic role in disease.

SIRT7 ENZYMATIC ACTIVITY

SIRT7 encodes a 400 amino acid protein that functions as a class III histone deacetylase in *Homo sapiens*. (5) Compared to other nuclear-localized Sirtuins (SIRT1 and SIRT6), SIRT7 exhibits weak deacetylase activity (3). Within the SIRT7 catalytic domain, serine residue 111 (S111) and histidine residue 187 (H187) are responsible for the deacetylation activity (6, 7), but to date, only a few SIRT7 substrates have been identified. In 2012, Barber et al. (6) reported that SIRT7 is a highly selective histone H3 lysine 18 (H3K18) deacetylase, and that H3K18Ac deacetylation is associated with high-grade tumors and poor patient prognosis. Non-histone substrates have also been identified, including p53, PAF53, U3-55k, GABPβ1, NPM1, PGK1, CDK9, DDB1, FKBP51, FOXO3, SMAD4, and DDX21 (**Table 1**). We discuss the data for each of these substrates in turn below.

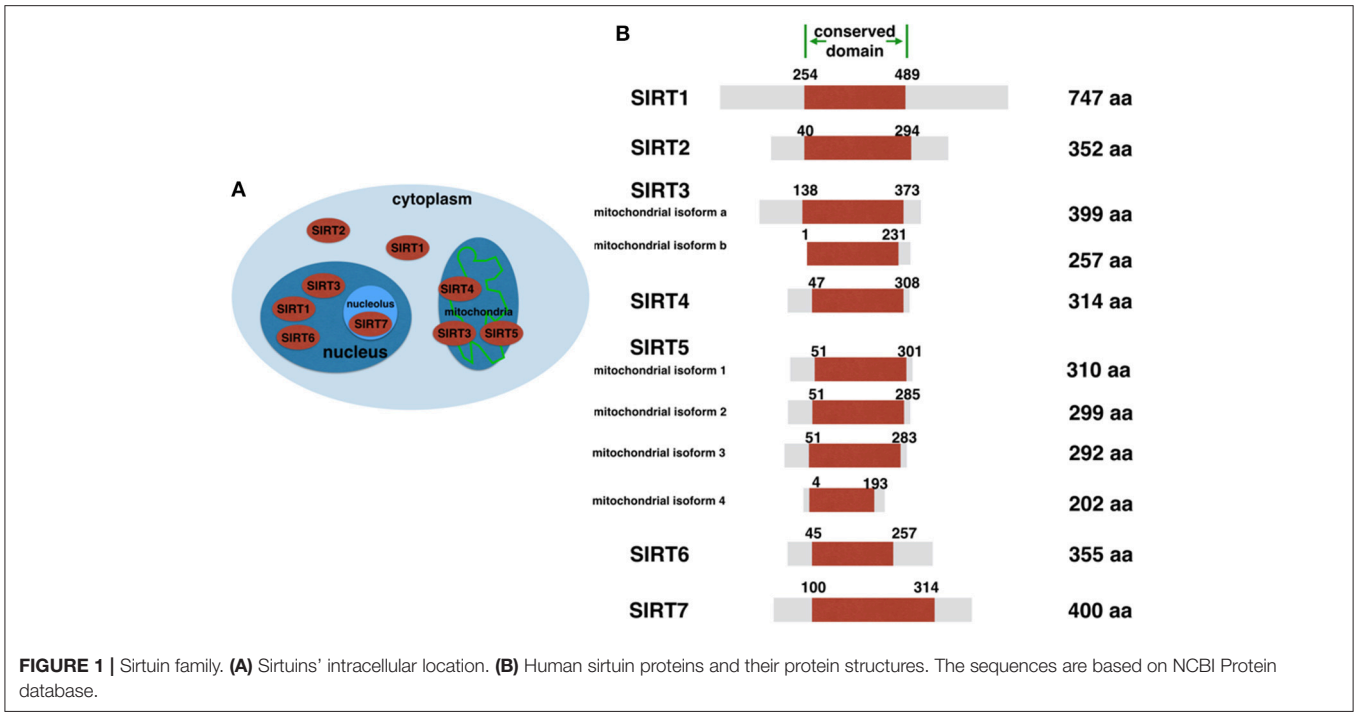

FIGURE 1 | Sirtuin family. **(A)** Sirtuins' intracellular location. **(B)** Human sirtuin proteins and their protein structures. The sequences are based on NCBI Protein database.

Vakhrusheva et al. found that p53 peptides were deacetylated *in vitro* by SIRT7 as efficiently as SIRT1, and mutant SIRT7 nearly abolished p53 decetylation (8). Conversely, Barber et al. did not detect SIRT7 deacetylase activity toward p53 both *in vivo* and *in vitro*, which questioned the ability of SIRT7 to deacetylate p53 (6). Kim et al. found an inverse correlation between p53 acetylation levels and SIRT7 expression in Hep3B cells (9). Finally, Nahalkova et al. identified an interaction between SIRT7 and p53, but did not assess SIRT7 deacetylase activity (10). p53 is debatable to be a SIRT7 substrate, for SIRT7 is found only effective toward p53 peptide, but not p53 protein both *in vivo* and *in vitro*. More studies are required, therefore, to resolve whether p53 is truly a SIRT7 substrate.

The wide variety of non-histone SIRT7 substrates suggests that SIRT7 participates in a diverse range of cellular processes. For example, SIRT7 can deacetylate nucleolar organizer polymerase-associated factor 53 (PAF53) and U3-specific protein U3-55k to regulate the synthesis and ripening of precursor ribose RNA (11, 12). By bioinformatic analysis, Ryu et al. found that GA-binding protein β1 (GABPβ1) is another substrate of SIRT7 (13). Here, SIRT7 interacts with and deacetylates GABPβ1 to regulate mitochondrion function (13). Lee et al. reported that SIRT7 deacetylates nucleophosmin (NPM1), which is involved in regulating aging processes (14), while Hu et al. found that SIRT7 deacetylates phosphoglycerate kinase 1 (PGK1) and participates in glycolysis regulation (15). Yu et al. showed that SIRT7 specifically interacts with and deacetylates FK506-binding protein 51 (FKBP51) at lysine residues 28 and 155 (K28 and K155), which enhances FKBP51–Akt–PHLPP complex formation, resulting in AKT inactivation and enhanced chemo-sensitivity in breast cancer cells (16). Blank et al. showed that SIRT7-dependent deacetylation of Cyclin-dependent kinase 9 (CDK9) activates its kinase activity; CDK9 subsequently phosphorylates the Pol II C-terminal domain (CTD) and facilitates transcription elongation (17). Li et al. observed that SIRT1/SIRT7 deacetylate FOXO3 *in vitro* and *in vivo* to prevent its phosphorylation and block apoptosis in response to lipopolysaccharide stimulation (18). Mo et al. found that SIRT7 is a major deacetylase of DNA damage-binding protein 1 (DDB1): DDB1 acetylation negatively regulates the DDB1–CUL4 interaction and CRL4 activity (19, 20). Tang et al. found that SIRT7 inhibits breast cancer lung metastasis by deacetylating and promoting SMAD4 degradation (21). Finally, Song et al. showed that deacetylation of the nucleolar DEAD-box helicase DDX21 by SIRT7 increases R-loop-unwinding activity and safeguards genome stability (22).

To widen the range of SIRT7 targets, our laboratory conducted the first proteomic screen of SIRT7 substrates. Using stable isotope labeling with amino acids in cell culture coupled with quantitative mass spectrometry, we produced a comprehensive list of candidates that are involved in a variety of functions, ranging from chromatin architecture homeostasis to gene silencing and metabolism. Some selected candidates, such as SIRT2, histone-lysine N-methyltransferase (EZH2), mitogen-activated protein kinase 2 (MAPK2) and glycogen synthase kinase-3 beta (GSK3β) have been validated by *in vitro* co-immunoprecipitation and deacetylation experiments. By combining this approach with predictive tools, we have started to greatly expand the list of SIRT7 candidate substrates. Such studies have enhanced our understanding of the physiological role of SIRT7 and raised awareness as to the global impact of sirtuins in cell homeostasis (23).

SIRT7 exhibits other enzymatic activities in addition to NAD^+-dependent deacetylation. In 2016, Li et al. found

TABLE 1 | SIRT7 catalytic substrates.

Substrate	Modification	Function
H3K18	Deacetylation	Tumorigenesis
p53	Deacetylation	Apoptosis
PAF53 and U3-55k	Deacetylation	Synthesis and ripening of precursor ribose RNA
GABPβ1	Deacetylation	Mitochondrial function
NPM1	Deacetylation	Aging
PGK1	Deacetylation	Glycolysis
FKBP51	Deacetylation	AKT inactivation and chemo-sensitivity in breast cancer cells
CDK9	Deacetylation	Transcription elongation
FOXO3	Deacetylation	Blocks apoptosis in response to LPS
DDB1	Deacetylation	Regulates DDB1-CUL4 interaction and CRL4 activity
SMAD4	Deacetylation	Inhibits breast cancer lung metastasis
DDX21	Deacetylation	Genome stability
H3K122	Desuccinylation	Chromatin remodeling during DNA repair
H3K36/K37	Deacetylation/ debutyrylation	Unknown

that SIRT7 is an NAD^+-dependent histone desuccinylase, and desuccinylates H3K122 to regulate chromatin remodeling during DNA repair (24). However, no non-histone substrates for SIRT7 desuccinylase have been identified. SIRT7 also interacts with some proteins without deacetylating them, such as mTOR, Myc, and TFIIIC2 (25); it is possible that SIRT7 regulates these proteins via its desuccinylase activity. Future investigations should investigate the spectrum of non-histone desuccinylation-mediated regulation by SIRT7. A recent report also showed that SIRT7 has robust deacetylase or debutyrylase activity on acetylated or butyrylated nucleosomes, mainly toward H3K36/K37 (26).

SIRT7 deacetylase activity is markedly enhanced by chromatin compositional DNA and RNA. SIRT7 also exhibits defatty-acylase activity, which can be effectively activated by RNA (27, 28). Conversely, Myb-binding protein 1a (Mybbp1a), a component of the chromatin remodeling complex B-WICH, inhibits SIRT7 deacetylation activity and increases H3K18 levels in cells (29), although the mechanistic details remain to be understood.

SIRT7 EXPRESSION AND REGULATION

SIRT7 is widely expressed in different organs and tissues of the human body: the highest expression is found in hyperplastic tissues, such as the spleen, liver and testis, and the lowest expression is found in the skeletal muscle, heart and brain (30). SIRT7 expression levels are associated with cellular proliferation, differentiation and the stress response, acting as a positive or negative regulator in different organs and tissues (31). Recent findings from the TCGA database suggest that SIRT7 expression is tightly correlated with the development of various types of cancer (32–34). The diverse roles of SIRT7 suggest it is tightly regulated by multiple mechanisms, as evidenced by SIRT1 (35, 36).

Previous reports have indicated that SIRT7 is regulated at the transcriptional, post-transcriptional and translational levels (**Figure 2**). At the transcriptional level, SIRT7 is regulated by upstream molecules, such as X-box binding protein 1 (XBP1), CCAAT-enhancer-binding protein α (C/EBPα), and histone deacetylase 3 (HDAC3) (37, 38). At the post-transcriptional level, SIRT7 is negatively regulated by several microRNAs, such as hsa-miR-125b, miR-125a-5p, miR-125b, miR-93, miR-3666, and miR-340 (9, 39–42). There are only few reports, however, describing how SIRT7 is regulated by post-translational modifications. Grob et al. found that SIRT7 is phosphorylated during mitosis by the cyclin-dependent kinase 1 (CDK1)-cyclin B pathway, although the phosphorylation sites and function have not been defined (43). Sun et al. found that SIRT7 is phosphorylated during cellular energy stress by 5′ AMP-activated protein kinase (AMPK), which has a crucial role in determining SIRT7 subcellular distribution and degradation (44). The researchers also showed that SIRT7 is modified by polyubiquitination (44), which is consistent with our study that showed that SIRT7 is modified by Lys-63-linked polyubiquitination (45). We also found that SIRT7 enzymatic activity is negatively regulated by ubiquitin-specific-processing protease 7 (USP7)-mediated deubiquitination, which helps control gluconeogenesis by regulating *G6PC* expression (45). Post-translational modifications have a crucial role in regulating protein stability, activity, structure and function; thus, further studies into SIRT7 post-translational modifications are warranted.

SIRT7 BIOLOGICAL FUNCTIONS

Ribosome Biogenesis and Protein Translation

Global proteomic studies have identified numerous SIRT7-associated proteins, with most of them having important roles in transcription, ribosome biogenesis and translation (7, 14, 25). Subsequent functional studies have confirmed that SIRT7 is involved in multiple pathways regulating ribosome biogenesis and protein translation. First, SIRT7 can regulate rDNA transcription. Ford et al. found that SIRT7 forms part of the RNA Polymerase I (RNA Pol I) complex, and activation of the RNA Pol I transcription *in vivo* is dependent on its enzymatic activity (30). Specifically, the researchers showed that SIRT7 over-expression increased Pol I-mediated transcription, whereas SIRT7 knockdown or catalytic inhibition decreased Pol I transcription (30). Grob et al. also found that SIRT7 activates Pol I transcription by interacting with upstream binding factor (UBF), which is a component of the RNA polymerase complex (43). Tsai et al. extended the mechanisms of SIRT7-mediated rDNA transcriptional regulation by showing that SIRT7 associates with the B-WICH complex, a chromatin remodeling complex involved in rDNA transcriptional regulation and chromatin structure changes. In addition, the researchers found that down-regulated SIRT7 reduced levels of RNA Pol I protein by decreasing the expression levels of RPA194, the

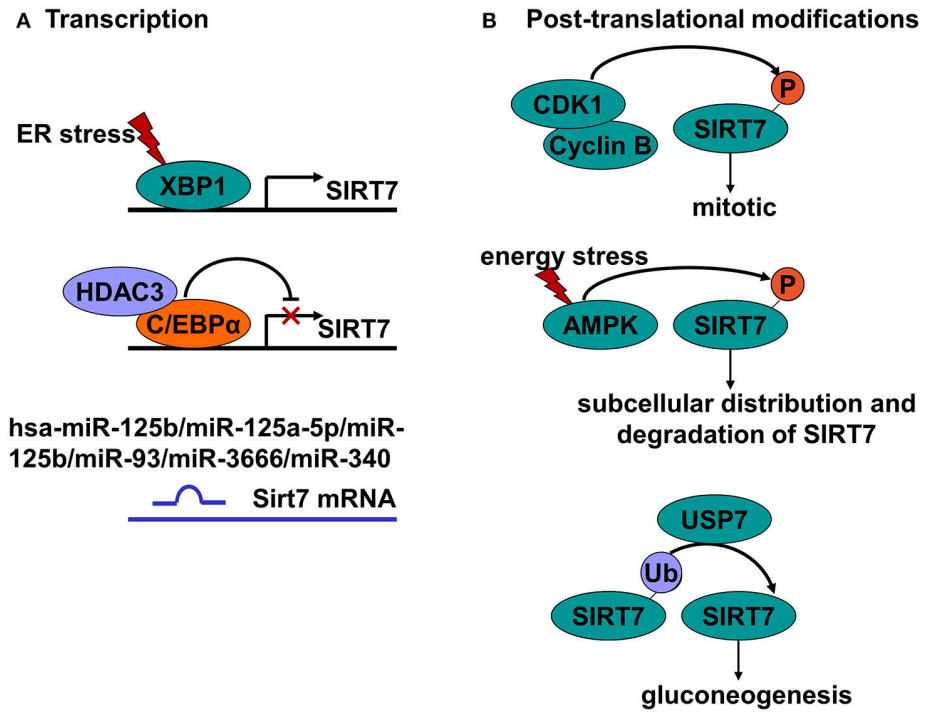

FIGURE 2 | Summary of regulation of SIRT7. **(A)** Various transcription factors regulate SIRT7 expression. The X-box binding protein 1 (XBP1) enhances SIRT7 expression, whereas Histone Deacetylase 3 (HDAC3) and CCAAT/enhancer-binding protein alpha (C/EBP alpha) repress SIRT7 expression. In addition, SIRT7 expression is also repressed by the microRNAs, such as hsa-miR-125b, miR-125a-5p, miR-125b, miR-93, miR-3666, and miR-340. **(B)** Some post-translational modifications affect SIRT7 activity. The cyclin-dependent kinase 1 (CDK1)–cyclin B pathway phosphorylates SIRT7 during mitosis. Under energy stress, SIRT7 can be phosphorylated by Adenosine 5′-monophosphate (AMP)-activated protein kinase (AMPK), thereby determining the subcellular distribution and degradation of SIRT7. Moreover, Ubiquitin-specific protease 7 (USP7) negatively regulates the enzymatic activity of SIRT7 through deubiquitination to control gluconeogenesis.

largest RNA polymerase I subunit (7). Others have shown that SIRT7 regulates Pol I transcription by deacetylating PAF53, another component of RNA polymerase I complex: PAF53 hypo-acetylation correlates with increased Pol I occupancy on rDNA and transcriptional activation (12).

SIRT7 also regulates the conversion between rDNA transcriptional activation and inhibition during the cell cycle. Here, SIRT7 is phosphorylated by the CDK1–cyclin B pathway during mitosis, rendering SIRT7 inactive such that rDNA transcription is inhibited. Upon exiting mitosis, SIRT7 is dephosphorylated by a phosphatase sensitive to okadaic acid such that rDNA transcription can resume (43).

SIRT7 is also involved in pre-rRNA processing and rRNA maturation. SIRT7 can deacetylate U3-55k, a component of the U3 small nucleolar RNP (snRNP) complex, which increases the association between U3-55k and U3 snRNP. This association is essential for processing pre-rRNA. Under stress conditions however, SIRT7 is released from the nucleoli, resulting in U3-55k hyper-acetylation and reduced pre-rRNA processing (11).

A role for SIRT7 in protein synthesis has also been suggested, as supported by Tsai et al. who found that SIRT7 knockdown suppresses both RNA and protein synthesis (25). They showed that SIRT7 associates with mTOR and TFIIIC2 to modulate Pol III-dependent tRNA transcription, and co-localizes with Pol III target genes. Supporting these observations, SIRT7 knockdown decreased tRNA levels and amino acid incorporation rates; however, SIRT7 over-expression did not increase the rate of protein synthesis, indicating that the observed reduction in protein synthesis may be an indirect effect of SIRT7 knockdown. In addition, the reduced amino acid incorporation rates in SIRT7 knockdown cells were due to reduced abundance of tRNAs for different amino acids (25).

Shin et al. found that Myc depletion significantly reduces SIRT7 occupancy at the promoters of ribosomal proteins. SIRT7 is targeted to the promoters of ribosomal proteins by interacting with Myc and repressing Myc-dependent expression (38).

Finally, SIRT7 may have a role in Pol II activation, as data indicate that SIRT7 associates with Pol II and regulates transcription of snoRNAs and other Pol II genes (17). Here, SIRT7 promotes the release of the elongation factor P-TEFb from the inactive 7SK snRNP complex and deacetylates CDK9, a component of the P-TEFb complex. CDK9 deacetylation promotes Pol II C-terminal domain (CTD) phosphorylation and transcription elongation (17).

Regulator of Cellular Stress

SIRT7 is resistant to various cellular stressors, such as endoplasmic reticulum (ER) stress, oxidative stress, mitochondrial protein folding stress, nutrition stress and

genotoxic stress (8, 12, 38, 46). As such, it can be inferred that SIRT7 has an important role in regulating cell survival. The function of SIRT7 in regulating rRNA and protein synthesis also supports a role for SIRT7 in cellular stress, because both processes are reduced under stressed conditions.

ER Stress

The ER is an important intracellular organelle where protein synthesis, folding, modification and trafficking occur. The accumulation of unfolded proteins or depletion of calcium stores triggers the ER stress response (also known as the unfolded protein response, UPR), to restore protein homeostasis by increasing the expression of molecular chaperones, decreasing protein translation and degrading unfolded proteins (47).

SIRT7 can relieve ER stress in two ways. First, under stress conditions, XBP1 induces SIRT7 expression, which in turn reduces ER stress response protein expression, such as CHOP, XBP1s, and GRP78 (38). Second, SIRT7 can interact with Myc to facilitate its recruitment to the promoters of ribosomal proteins, such as RPS20 and RPS14 to repress their gene expression (38).

Mitochondrial Stress

The mitochondrion is an important organelle for regulating cellular energy homeostasis, and is thus sensitive to many stresses. Cellular stress leads to an accumulation of unfolded mitochondrial proteins resulting in mitochondrial protein folding stress (PFS^{mt}) and the unfolded protein response in mitochondria (UPR^{mt}) (48). Mohrin et al. found that SIRT7 alleviates PFS^{mt} by repressing NRF1 activity and reducing the expression of the mitochondrial translation machinery (46). NRF1 is a master regulator of mitochondria, and by interacting with SIRT7, targets it to the promoters of mitochondrial ribosomal proteins (mRPs) and mitochondrial translation factors (mTFs) to repress their expression. This mechanism helps alleviate PFS^{mt} and improve cellular survival under conditions of nutrient deprivation (46).

Oxidative Stress

Many diseases and disorders have been linked with a cellular oxidant–antioxidant imbalance. Data suggest that sirtuins are important in the homeostasis of cellular oxidation–reduction systems. Hypoxia-inducible factors HIF-1 and HIF-2 are essential transcription factors that mediate adaptation to hypoxia (49). Hubbi et al. found that SIRT7 interacts with HIF-1α and HIF-2α proteins and negatively regulates their expression (50). Over-expression of SIRT7 reduced the levels of HIF proteins as well as their transcriptional targets, independent of SIRT7 deacetylase activity and hydroxylation-mediated ubiquitinylation and the proteasomal and lysosomal-mediated degradation pathways. Vakhrusheva et al. found that SIRT7-deficient primary cardiomyocytes exhibit a drastic increase in basal apoptosis compared to wild-type primary cardiomyocytes upon exposure to hydrogen peroxide, suggesting a critical role for SIRT7 in regulating the oxidative stress response and cell death in the heart (8). The researchers speculated that this susceptibility of SIRT7 mutant cells to apoptosis may be due to hyperactive p53, as SIRT7 deacetylates p53 (8). Lewinska et al. also reported that vascular smooth muscle cells exposed to curcumin to induce oxidative damage, exhibited down-regulated SIRT7 and p53 stability (51). SIRT7 down-regulation also decreased Pol I mediated transcription, and the stabilized p53 activated its target protein p21, resulting in cell-cycle arrest. Thus, SIRT7 has a potential role in the resistance to different conditions of oxidative stress.

Cardiac Injury

SIRT7 has a role in cardiac homeostasis as illustrated by SIRT7 knockout mice that suffer from degenerative heart hypertrophy, as evidenced by cardiac cell fibrosis and inflammation, resulting in inflammatory cardiomyopathy (8). SIRT7 knockout mice also show increased blood lactate levels and decreased endurance to physical activity, due to oxygen insufficiency and decreased oxygen consumption by cardiac muscles stemming from mitochondrial respiratory dysfunction (13). A possible pathway by which SIRT7 maintains cardiac health may be through GABP. SIRT7 promotes GABP complex formation and activation by deacetylating GABPβ1 to enhance the expression of mitochondrial genes and promote mitochondrial respiration (13).

Araki et al. described another SIRT7 pathway that may regulate cardiac health (52). The researchers noted that SIRT7 expression increases at active wound healing sites upon acute cardiovascular injury and thus speculated that SIRT7 may be involved in tissue repair. Consistently, SIRT7 depletion led to reduced collagen production and insufficient angiogenic and inflammatory responses, resulting in impaired wound healing (52). TGF-β is essential to wound healing as it regulates fibroblast chemotaxis, differentiation and the epithelial-to-mesenchymal transition (EMT). TGF-β receptor protein 1 (TβR1) is an important component of the TGF-β signaling pathway (53). Researchers showed that SIRT7 depletion decreases TβR1 levels and reduces downstream signaling. The effect on TβR1, however, was indirect as SIRT7 did not interact with TβR1, but the mediator PICK1 (protein interacting with protein kinase C, alpha), which interacts with TβR1 and SIRT7 together. This study also showed that loss of SIRT7 activates autophagy and PICK1, again affecting TβRI status. Thus, the researchers concluded that SIRT7 maintains TβRI protein levels by modulating autophagy and PICK1 to regulate the TGF-β signaling pathway. In this way, SIRT7 can participate in scar formation, angiogenesis, inflammation and wound healing in response to acute cardiovascular injury (52). Based on these data, SIRT7 may be considered as a good predictor or therapeutic target for cardiac diseases.

Genome Stability

Sirtuins, including SIRT7, maintain genomic stability under stress conditions through a variety of mechanisms (24, 31, 54, 55). SIRT7 protects the genome largely by influencing chromatin structure, cell-cycle progression and DNA damage signaling and repair (**Figure 3**). SIRT7 knockout mice show an aging-like phenotype, associated with an increased sensitivity to oxidative

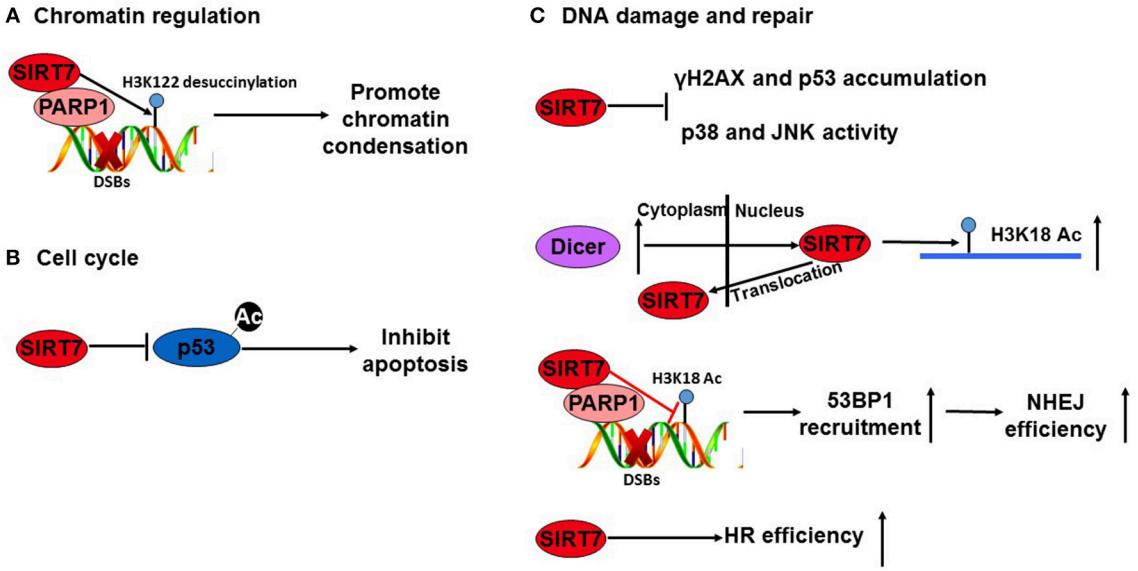

FIGURE 3 | SIRT7 maintains genome stability through multiple pathways. Under stress conditions, SIRT7 participates in the maintenance of genome stability via multiple mechanisms. **(A)** SIRT7 is recruited to DSBs in a PARP1-dependent manner to catalyze desuccinylation of H3K122 at DSBs to promote chromatin condensation. **(B)** SIRT7 can deacetylate p53 to inhibit apoptosis. **(C)** SIRT7 exerts a protective role during genomic insults by attenuating the DNA damage response (preventing γH2AX and p53 accumulation) and the stress-activated MAPK pathway (p38 and JNK activity inhibition). The upregulation of Dicer in cytoplasm promotes SIRT7 translocation from the nucleus to the cytoplasm, thereby causing a decrease in chromatin-associated SIRT7 and elevated H3K18Ac levels. Besides, SIRT7 overexpression increases the efficiency of non-homologous end joining (NHEJ) and homologous recombination (HR). The recruitment of SIRT7 to sites of DNA damage by a PARP1-dependent manner also modulates H3K18Ac levels, thereby influencing 53BP1 recruitment to DNA double-strand breaks (DSBs) to increase the efficiency of NHEJ.

and genotoxic stress, suggesting a link between SIRT7 and genomic protection (8).

SIRT7 can maintain genomic stability by regulating p53 or DNA damage repair. With regards to p53, Vakhrusheva et al. found that SIRT7 knockout mouse embryonic fibroblasts (MEFs) undergo higher levels of apoptosis than control MEFs upon adriamycin treatment. This process is as a result of p53 hyperacetylation in the absence of SIRT7 (8). Kiran et al. found that SIRT7 exerts a protective role during genomic insults by attenuating the DNA damage response (preventing γH2AX and p53 accumulation) and the stress-activated MAPK pathway (p38 and JNK activity inhibition) (31). Moreover, they found that SIRT7 transfers from the nucleolus to the nuclear matrix after doxorubicin chemotherapy (31). Similarly, Zhang et al. found that SIRT7 translocates from the nucleus to the cytoplasm in response to DNA damage. This translocation occurs through an interaction with Dicer, causing a decrease in chromatin-associated SIRT7 and elevated H3K18Ac levels (56).

Regarding DNA damage repair, Mao et al. found that SIRT7 overexpression increases the efficiency of non-homologous end joining (NHEJ) by 1.5-fold and homologous recombination (HR) by 2.8-fold in paraquat toxin-treated human fibroblast cells (57). A more recent study by Vazquez et al. elucidated a mechanism by which SIRT7 contributes to NHEJ. They demonstrated that SIRT7 is recruited to sites of DNA damage in a PARP1-dependent manner, where it modulates H3K18Ac levels, thereby influencing 53BP1 recruitment to DNA double-strand breaks (DSBs) to increase the efficiency of NHEJ (55). These data provide direct evidence for a role of SIRT7-mediated H3K18 deacetylation in maintaining genome integrity by DSB repair. At the same time, Li et al. also found that SIRT7 is recruited to DSBs in a PARP1-dependent manner, but showed that it catalyzes desuccinylation of H3K122 at DSBs to promote chromatin condensation and efficient DSB repair (24). These latest findings extend our understanding as to how SIRT7 helps maintain genome stability.

Metabolic Regulation

Glucose Metabolism

The enzymatic activity of SIRT7 depends on its metabolic co-substrate NAD^+, thus connecting its role to cellular metabolic status. SIRT7 is a low glucose stress sensor that, as discussed, modulates rDNA transcription to preserve energy and resist nutritional stress. Chen et al. identified that SIRT7 redistributes from the nucleoli to the nucleoplasm upon glucose deprivation or treatment with AICAR (a low energy mimic) (12). At the mechanistic level, PAF53 interacts with Pol I and recruits it to rDNA promoters (58). SIRT7 deacetylates PAF53, which acts as a signal to recruit RNA polymerase I to rDNA promoters and activate RNA Pol I-mediated transcription. Under low glucose conditions, the redistribution of SIRT7 from the nucleoli to the nucleoplasm permits PAF53 hyper-acetylation. This hyper-acetylation impairs the interaction between PAF53 and Pol I and decreases Pol I activity, thus leading to rDNA transcription inhibition (12). Sun et al. also showed that glucose

starvation induces SIRT7 redistribution via AMPK-directed SIRT7 phosphorylation. This effect causes REGγ-proteasome-dependent degradation, thereby reducing rDNA transcription to avoid cell death (44).

Recent studies have provided insight on the role of SIRT7 in glycolysis. SIRT7 deacylates phosphoglycerate kinase 1 (PGK1), a key enzyme in glycolysis pathway, and suppresses PGK1 enzymatic activity in liver cancer cells (15). We also reported that SIRT7 regulates gluconeogenesis by modulating G6PC expression via USP7-mediated deubiquitination (45). SIRT7 also suppresses HIF1 and HIF2, which repress glucose oxidation through the tricarboxylic acid cycle (59). Taken together, these findings implicate SIRT7 in glucose metabolism.

Lipid Metabolism

The evidence supporting a role for SIRT7 in lipid metabolism in the liver is conflicting (60). Yoshizawa et al. found that SIRT7 knockout mice (generated by deleting exons 4–9), are resistant to high-fat diet (HFD)-induced fatty liver, glucose intolerance and obesity (61). They also showed that liver-specific SIRT7 knockout mice have reduced hepatic triglyceride accumulation (61). SIRT7 activates Cd36 expression, which is vital for fatty-acid uptake, as well as Mogat that incorporates fatty acids into triglycerides, and Cidea and Cidec that are involved in lipid storage and lipid droplet formation. The expression levels of these four genes were all reduced in the livers of the SIRT7 knockout mice fed a HFD (61). Mechanistically, an interaction between the E3 ubiquitin ligase complex (DDB1-CUL4-associated factor 1 (DCAF1)/damage-specific DNA binding protein 1 (DDB1)/cullin 4B (CUL4B) complex) and TR4 (a nuclear receptor involved in lipid metabolism) promotes TR4 degradation. However, SIRT7 binding to the DCAF1/DDB1/CUL4B complex inhibits TR4 degradation and activates TR4 target genes to increase fatty-acid uptake and triglyceride synthesis and storage. Consequently, the expression level of TR4 and its target genes are reduced in liver-specific SIRT7 knockout mice and lipid synthesis and storage is decreased to resist hepatic steatosis (61).

Two other groups have reported opposing results to Yoshizawa et al. finding that SIRT7 knockout mice instead suffer hepatic steatosis (13, 38). Shin et al. found that SIRT7 knockout mice (generated by replacing exons 4–11 with a LacZ gene), have a fatty liver without obesity (38). They showed that loss of SIRT7 increases lipogenic gene expression, liver triglyceride levels and inflammatory markers, indicating progression to steatohepatitis. These mice also exhibited low levels of plasma triglycerides compared to wild-type controls, due to reduced very-low-density lipoprotein (VLDL) secretion. Importantly, liver steatosis was reversed in these animals by reintroducing SIRT7 specifically in the liver (38). At the mechanistic level, it seems that SIRT7 prevents the development of fatty liver disease by suppressing ER stress. Consequently, SIRT7 knockout mice fail to relieve ER stress such that the UPR pathway is activated causing apoptosis, inflammation, increased lipogenesis and reduced VLDL secretion specifically in the liver (38).

Ryu et al. also described that SIRT7 knockout mice (generated by deleting exons 6–9) exhibited hepatic microvesicular steatosis, and increased plasma levels of triglycerides and free fatty acids. These mice also showed signs of multi-systemic mitochondrial dysfunction due to GABPβ1 hyperacetylation in the absence of SIRT7, including increased blood lactate levels and reduced exercise performance. As discussed, SIRT7 mediates mitochondrial function by deacetylating GABPβ1 (a regulator of multiple nuclear-encoded mitochondrial genes) to promote the formation and activation of the GABP complex that induces mitochondrial gene expression and contributes to mitochondrial homeostasis (13).

Although they used the same mice strain (C57BL/6J), the contradiction of the roles of SIRT7 in lipid metabolism discussed in these three studies may be due to the differences in the genetic background of and construction of SIRT7 knockout mice (60). Further studies with more sample size and parallel experiments are required to clarify this picture.

Cioffi et al. found that SIRT7 induces differentiation and maturation of early adipocyte precursors to promote adipogenesis (39). Consistently, SIRT7 knockdown resulted in reduced Oil Red O staining and adipogenesis marker (FABP4, PPARγ, C/ebpα, adipoq) expression. The researchers also found that miR-93 prevents adipogenesis by inhibiting SIRT7: adipogenesis was enhanced in mir-25-93-106b$^{-/-}$ mice but repressed in miR-93-reintroduced mice. Interestingly, SIRT7 expression was enhanced in the mir-25-93-106b$^{-/-}$ mice while nuclear SIRT7 expression was reduced upon injection of miR-93 mimics into the visceral fat pads of leptin-deficient (ob/ob) mice (39). Despite these preliminary findings, the definitive mechanism underlying how miR-93 regulates SIRT7 and how SIRT7 promotes adipogenesis remains unclear. Fang et al. demonstrated that SIRT7 can promote adipogenesis by binding to SIRT1 and inhibiting its activity by preventing its auto-deacetylation. SIRT1 is reported to repress PPARγ through interaction with nuclear receptor corepressor 1 (NCoR1, PPARγ corepressor) to inhibit adipogenesis (62). As such, SIRT7 knockout mice possess a notably diminished proportion of white fat due to enhanced SIRT1 activity, which blocks PPARγ and adipocyte differentiation (63). SIRT1 activity depletion restores adipogenesis in Sirt7 knockout mice (63). These data implicate a potential cross-regulatory network within the sirtuin family.

SIRT7 in Mitochondrial Metabolism

As discussed, the mitochondrion is a crucial organelle involved in regulating cellular energy homeostasis and thus cell survival. Mitochondria uptake energy from nutrients and then convert it into ATP by oxidative phosphorylation (OXPHOS) (64). In response to cellular stress, the mitochondrion has an armamentarium of quality-control mechanisms based on mitochondrial biosynthesis, mitophagy and mitochondrial unfolded protein responses, to maintain proper mitochondrial function. SIRT7 is an important regulator of mitochondrial homeostasis. As stated above, Ryu et al. found that SIRT7 knockout mice show multi-systemic mitochondrial dysfunction, including lactate accumulation in the blood and age-related hearing loss (13). Mechanistically, SIRT7 impacts on mitochondria function by deacetylating GABPβ1, which forms a hetero-tetramer complex with GABPα, occupies

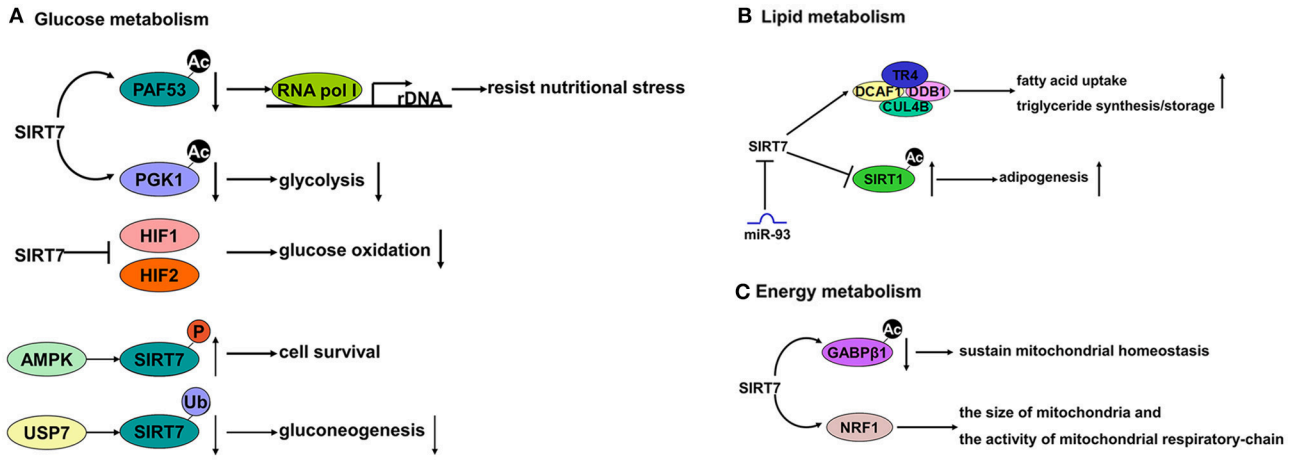

FIGURE 4 | The roles of SIRT7 in metabolism. **(A)** SIRT7 can deacetylate RNA polymerase I (Pol I)-associated factor PAF53 to resist nutritional stress and deacetylate Phosphoglycerate kinase-1 (PGK1) to affect glycolysis pathway. SIRT7 also suppresses the transcription factor Hypoxia-inducible factor-1 (HIF1) and HIF2 to influence glucose oxidation. In addition, SIRT7 can be modified by AMPK or USP7 to regulate glucose metabolism. **(B)** The interaction between SIRT7 and DCAF1/DDB1/CUL4B complex inhibits degradation of Testicular orphan nuclear receptor 4 (TR4) and activates TR4 target genes subsequently increasing fatty acid uptake and triglyceride synthesis/storage. SIRT7 also interacts with SIRT1 to promote adipogenesis. Besides, inhibition of SIRT7 by miR-93 represses adipogenesis. **(C)** SIRT7 can impact the mitochondria function by deacetylating GA-binding protein β1 (GABPβ1). Besides, the interaction of SIRT7 with Nuclear respiratory factor 1 (NRF1) regulates the size of mitochondria and the activity of mitochondrial respiratory-chain.

nuclear-encoded mitochondrial target genes and promotes their transcription. Further studies also found that SIRT7 deficiency affects OXPHOS in the heart and liver (13). Mohrin et al. also reported that SIRT7 interacts with NRF1 (a master regulator of mitochondria) to regulate mitochondria size and activity of the mitochondrial respiratory-chain (46).

Overall, SIRT7 has a vital role in metabolic homeostasis (**Figure 4**), but there remain many unresolved questions. The contradictory effect of SIRT7 on lipid metabolism also needs clarification. Future studies need to address these issues and determine whether SIRT7 may be a suitable therapeutic target in metabolic disorders.

Aging and Senescence

Consistent with the known functions of sirtuins in senescence and lifespan-extension, numerous studies have established a close relationship between SIRT7 and age-related processes (36). SIRT7-knockout mice have a shorter lifespan than control mice, and succumb to premature aging phenotypes around 1 year, with symptoms, such as kyphosis, decreased gonadal fat pad content, reduced IGF-1 plasma levels, hepatic steatosis, degenerative heart hypertrophy, reduced hearing and reduced hematopoietic stem cell–regenerative potential (13, 46, 65). SIRT7 expression gradually declines with age in mice, rat and several cells, including endothelial cells, fibroblasts, hepatocytes and HEK293FT cells (13, 14, 66–68). Kiran et al. found that SIRT7 over-expressing cells show a mostly normal morphology, with very few enlarged cells upon treatment with a low dose of doxorubicin (a cellular senescence inducing agent) (69). By contrast, control GFP expressing cells exhibit cell enlargement and multi-nucleation (typical features of cellular senescence) following doxorubicin exposure. The researchers also found that p53 and p21 senescence marker expression decreases in SIRT7 overexpressing cells upon doxorubicin treatment compared to control cells.

Lee et al. reported that the acetylation levels of nucleophosmin (NPM1) are increased while SIRT6 and SIRT7 levels are decreased in senescent cells. SIRT7 can deacetylate NPM1, which results in up-regulated p53 transcriptional activity in MEFs to induce cellular senescence (14, 70).

Telomeres have the potential to serve as the biomarker of biological cell age (71). Conomos et al. found that TR4 recruitment to the telomere can attribute to the ALT (alternative lengthening of telomeres) phenotype (72). SIRT7 positively regulates the protein level of TR4 (19), suggesting that SIRT7 might play an upstream role in DNA repair and telomere maintenance pathways.

A role for SIRT7 in aging and senescence has also been attributed to SIRT7-mediated regulation of mitochondrial ribosomal proteins (mRPs). SIRT7 interacts with NRF1 to repress mRPs, resulting in hematopoietic stem-cell longevity (46). Conversely, SIRT7-mediated GABPβ1 deacetylation promotes the formation and activation of the GABP complex to increase mRPs, resulting in hematopoietic stem-cell aging (13).

One study reported an interaction between SIRT7 with Tripeptidyl peptidase II (TPPII) that permits SIRT7 cytoplasmic localization (52). TPPII has regulatory effects on apoptosis and senescence, as observed in TPPII knockout mice that exhibit early immuno-senescence and have a shorter lifespan than controls (73). SIRT7 may, therefore, regulate aging and senescence by interacting with TPPII, but the underlying mechanisms need further study.

rDNA instability is common in premature aging syndromes. A recent study by Paredes et al. uncovered a critical role for

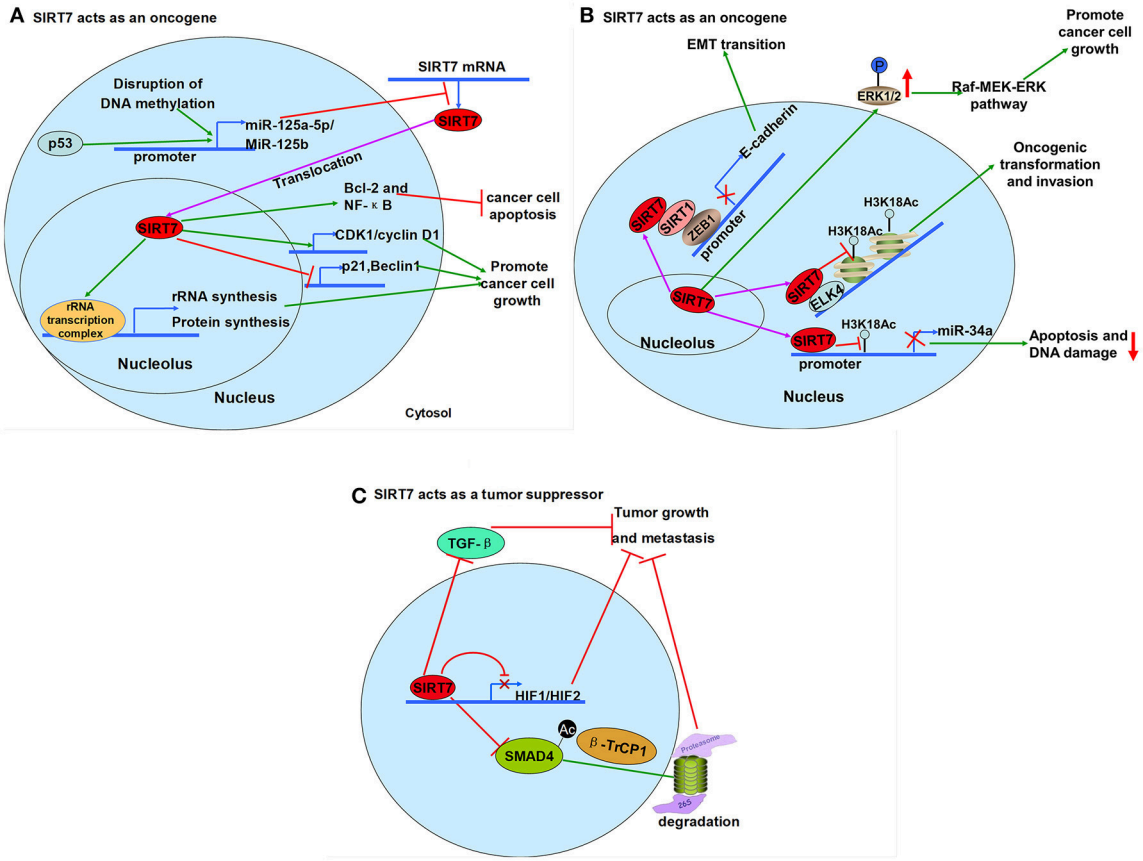

FIGURE 5 | The roles of SIRT7 in cancer. **(A,B)** SIRT7 acts as an oncogene. **(A)** The disruption of DNA methylation and p53 activates miR-125b and miR-125a-5p to inhibit the expression of SIRT7. Overexpression of SIRT7 upregulates the expression of CDK1/cyclin D1 while downregulates the level of p21 and Beclin1 as well as promotes rRNA and protein synthesis by interaction with rRNA transcription complex to promote cancer cell growth. In addition, overexpression of SIRT7 can also upregulate Bcl-2 and NF-κB to repress cancer cell apoptosis. **(B)** ETS-like transcription factor 4 (ELK4) targets SIRT7 to promoters of many tumor suppressor genes for H3K18 deacetylation and leads to cancer cell growth and metastasis eventually. SIRT7 induces ERK1/2 phosphorylation and activated the Raf-MEK-ERK pathway to promote cancer cell growth. SIRT7 also reduces miR-34a expression by deacetylating H3K18Ac to decrease cancer cell apoptosis and DNA damage. Besides, SIRT7 reduces expression of E-cadherin by interaction with SIRT1 to promote EMT transition. **(C)** SIRT7 acts as a tumor suppressor. SIRT7 inactivates TGF-β signaling and represses epithelial-to-mesenchymal transition. SIRT7 also negatively regulates HIF1 and HIF2 transcription. Moreover, SIRT7 deacetylates Mothers against decapentaplegic homolog 4 (SMAD4) and promotes *β*-transducin repeat–containing protein 1 (β-TrCP1)-dependent degradation to inhibit tumor growth and metastasis.

SIRT7 in protecting against cellular senescence by maintaining heterochromatin silencing and rDNA stability. Mechanistically, they found that SIRT7 acts as a scaffold to stabilize SNF2H at rDNA promoters for chromatin silencing (74). Taken together, SIRT7 might prevent aging-induced physiological changes and possibly extend lifespan via numerous pathways.

Cancer

Although ample evidence supports the involvement of SIRT7 in carcinogenesis, SIRT7 exhibits opposing roles in different cancer types (**Figure 5**). SIRT7 is up- regulated in the majority of cancers, including colorectal, gastric, thyroid, node-positive breast, bladder, ovarian and cervical cancers, hepatocellular and epithelial prostate carcinoma, where it acts as an oncogene (9, 40, 75–82). The exception is seen in pancreatic cancer, where SIRT7 is down-regulated and seems to act as a tumor suppressor (83) (**Table 2**).

As an oncogene, SIRT7 inhibition reduces cancer-cell growth, represses colony formation and cancer-cell metastasis and increases cancer-cell apoptosis. High SIRT7 expression is also considered a predictor of poor survival in various cancers (9, 40, 75–82). Many studies have reported mechanisms of action by which SIRT7 promotes cancer-cell growth (**Figure 5**). Yu et al. found that SIRT7 induces ERK1/2 phosphorylation and activates the Raf–MEK–ERK pathway to promote cancer-cell growth (81). Hypoacetylation of H3K18 is associated with oncogenic transformation, aggressive tumor phenotypes and poor prognosis and maintenance of essential human cancer-cell features, including anchorage-independent growth and escape from contact inhibition (84). As shown in prostate cancer, SIRT7 binds and maintains the deacetylated state of H3K18 at

TABLE 2 | SIRT7 related cancers.

Cancer	SIRT7 expression	Role/Impact
Colorectal cancer	Overexpression	Oncogene
Colorectal cancer	Downregulated	Induce radio-sensitivity; Enhance therapeutic effects
Gastric cancer	Overexpression	Oncogene
Thyroid cancer	Overexpression	Oncogene
Node-positive breast cancer	Overexpression	Oncogene
Breast cancer	Deficiency	Increase metastasis
Bladder cancer	Overexpression	Oncogene; promote cancer-cell growth
Ovarian cancer	Overexpression	Oncogene
Cervical cancer	Overexpression	Oncogene
Hepatocellular carcinoma	Overexpression	Oncogene; promote cancer-cell growth
Epithelial prostate cacinoma	Overexpression	Oncogene; maintaining cancer phenotypes; increase metastasis
Pancreatic cancer	Downregulated	Might be tumor suppressor
Liver cancer	Knockdown	Promote cancer-cell growth

the promoters of many tumor suppressor genes by interacting with the ELK4 transcription factor (85, 86). Thus, SIRT7 has a fundamental role in maintaining cancer phenotypes (6).

Zhang et al. found that SIRT7 knockdown promotes gastric cancer-cell apoptosis (82). Mechanically, SIRT7 prevents cellular apoptosis by down-regulating miR-34a via H3K18ac deacetylation. The miR-34 family is associated with cell-cycle arrest, senescence and apoptosis in cancers, and low miR-34a expression is associated with poor prognosis (82).

Another mechanism as to how SIRT7 promotes cancer-cell growth was identified by Kim et al. (9). They found that SIRT7 knockdown increases the number of liver cancer cells in G1/S phase and delays the cell-cycle transition: $p21^{WAF1/Cip1}$ expression was increased and cyclin D1 (a G1/S cell cycle regulator) was suppressed in SIRT7 knockdown cells. In patients with hepatocellular carcinoma, increased SIRT7 expression was attributed to p53 mutation or the endogenous hypermethylation of the microRNAs miR-125a-5p and miR-125b. Over-expression of SIRT7 led to $p21^{WAF1/Cip1}$ suppression and induction of cyclin D1 expression to promote cancer-cell growth (9). Han et al. also found that SIRT7 expression is regulated by hsa-miR-125b in bladder cancer. They observed that in bladder cancer, hsa-miR-125b and SIRT7 are inversely associated with the oncogenic long non-coding RNA MALAT1. Up-regulated hsa-miR-125b resulted in down-regulated SIRT7 and MALAT1; this effect inhibited bladder cancer cell growth, induced apoptosis, and decreased cell motility (40).

With regards to apoptosis, Wang et al. found that shRNA-mediated SIRT7 silencing reduced anti-apoptotic factor B-cell lymphoma 2 (Bcl-2) and nuclear factor kappa B (NF-κB) levels (80). The NF-κB signaling pathway is important in cellular proliferation, apoptosis and migration in malignant diseases (87). The researchers concluded that SIRT7 inhibits cancer-cell apoptosis by up-regulating Bcl-2 and NF-κB levels (80). The precise regulatory mechanisms remain to be identified.

SIRT7 also influences cancer-cell metastasis. High SIRT7 expression is associated with aggressive cancer phenotypes, metastatic diseases and poor patient prognosis; down-regulating SIRT7 reverses the metastatic properties of epithelial and mesenchymal cancer cells. In epithelial carcinomas, SIRT7 is associated with the EMT, a key process in metastatic progression. Malik et al., Yu et al., Zhang et al. focused on classic EMT regulatory factors and discovered that the expression of E-cadherin (an EMT regulatory factor) showed inverse correlation with SIRT7 *in vivo*. In SIRT7-deficient cells, E-cadherin and DAB2 interacting protein (DAB2IP; a tumor suppressor gene, whose loss promotes EMT and metastasis in prostate cancer) are significantly increased in mRNA level. These findings suggest a role for SIRT7 in cancer prevention and as prognostic factor (78, 81, 82). SIRT7 interacts with SIRT1 to enhance SIRT1-dependent prostate cancer cell metastatic properties and promotes E-cadherin transcriptional repression (88).

In non-epithelial cancers, SIRT7 may impact metastasis regulatory pathways to affect cellular metastatic properties. The expression levels of matrix metalloproteinase MMP16 and vascular endothelial growth factor (VEGF-A) are reduced in SIRT7-deficient HT1080 cells compared to control cells (78). Thus, SIRT7 may be of vital importance for cancer-cell metastasis (78).

As a tumor suppressor, low levels of SIRT7 are associated with aggressive tumor phenotypes and poor patient outcomes. One study found that patients with pancreatic cancer and high levels of nuclear SIRT7 had a longer lifespan (succumbed to disease later) than those with low levels of SIRT7. However, the precise mechanism underlying this association is unclear (83). Similar to the other sirtuins, SIRT7 has also been recognized as a tumor suppressor based on its negative regulation of HIF1 and HIF2 transcription (50, 89), as previously discussed.

The contradictory roles of SIRT7 in cancer may be related to its multiple interactions and functions in various cellular processes. First, SIRT7 deacetylates H3K18Ac, whose depletion is associated with highly malignant cancers and poor patient prognosis. Second, SIRT7 influences ribosome biogenesis to meet the high biosynthetic and metabolic needs of cancer cells. At the same time, although SIRT7 inactivates p53 by deacetylation, up to half of all tumors exhibit mutated p53, which may diminish the oncogenic role of SIRT7 and result in tumor suppressive characteristics (83, 90). Even though the definitive function of SIRT7 is uncertain, many consider SIRT7 as a cancer biomarker or a predictor of prognosis (73, 75, 81, 83). In sum, data suggest that SIRT7 may be a potential novel biomarker for prognosis in pancreatic cancer (83), a circulating marker in head and neck squamous cell carcinoma (73), and a predictive biomarker of pancreatic cancer (PCa) aggressiveness (91).

Our lab also found that down-regulation of SIRT7 after 5-FU exposure induces radio-sensitivity in human colorectal cancer and enhances therapeutic effects (92). Shi et al. found the microRNA-3666 induces SIRT7 inhibition, which in turn inhibits non-small cell lung cancer cell growth (41).

Recently, Tang et al. found that SIRT7 deficiency promotes breast cancer cell metastasis, while temporal expression of SIRT7 inhibits metastasis in a polyomavirus middle T antigen breast cancer model. Here, SIRT7 deacetylates SMAD4 and promotes β-TrCP1-dependent degradation. Finally, SIRT7 deficiency activates TGF-β signaling and enhances the EMT (21). Therefore, it can be assumed that SIRT7 is a worthwhile target to explore for cancer therapy.

CONCLUSIONS AND FUTURE PERSPECTIVES

The function of SIRT7 was ignored in the initial years of sirtuin-based research due to its localization in the nucleolus. However, major breakthroughs have been made over the past decade in SIRT7 biology. New substrates for SIRT7 deacetylase activity have been identified, which have highlighted new enzymatic activities that are critical for different cellular processes. SIRT7 has strong potential as a therapeutic target in various cancers, due to the identification of its up-regulation and activation in various cancer cells. SIRT7 is also considered a potential novel biomarker for prognosis in several cancers, such as pancreatic cancer. However, due to its complicated and controversial mechanism in the maintenance of cancers, future studies are needed to understand the precise molecular mechanism and downstream pathways of SIRT7 in the specific cancer types. The therapeutic uses of SIRT7 in cancers will rely on the further clinical trials. A recent report suggests a role for SIRT7 in the adaptive immune system and neurogenesis (93). This finding indicates that the roles and function of SIRT7 are still diversifying, and are wider than previously thought. Further studies are now needed to better understand and elucidate the molecular role of SIRT7, identify its substrate partners/cofactors, and delineate the intracellular pathways that regulate their activity in different disease models.

AUTHOR CONTRIBUTIONS

DW, YL, and KZ wrote the primary manuscript and revised the manuscript. HW and W-GZ conceived and designed the manuscript.

FUNDING

This review was supported by the National Natural Science Foundation of China (grant number 81472627), the Discipline Construction Funding of Shenzhen (2016), and the Shenzhen Municipal Commission of Science and Technology Innovation (grant number JCYJ20160427104855100).

ACKNOWLEDGMENTS

The authors would like to thank Dr. Jessica Tamanini (Shenzhen University and ETediting) for editing the manuscript prior to submission.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Neuroscience

REVIEW
published: 26 October 2018
doi: 10.3389/fnins.2018.00778

Sirtuins in Neuroendocrine Regulation and Neurological Diseases

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Silent information regulator 1 (SIRT1) is a mammalian homolog of the nicotinamide adenine dinucleotide (NAD)-dependent deacetylase sirtuin family. Sirtuin was originally studied as the lifespan-extending gene, silent information regulator 2 (SIRT2) in budding yeast. There are seven mammalian homologs of sirtuin (SIRT1–7), and SIRT1 is the closest homolog to SIRT2. SIRT1 modulates various key targets via deacetylation. In addition to histones, these targets include transcription factors, such as forkhead box O (FOXO), Ku70, p53, NF-κB, PPAR-gamma co-activator 1-alpha (PGC-1α), and peroxisome proliferator-activated receptor γ (PPARγ). SIRT1 has many biological functions, including aging, cell survival, differentiation, and metabolism. Genetic and physiological analyses in animal models have shown beneficial roles for SIRT1 in the brain during both development and adulthood. Evidence from *in vivo* and *in vitro* studies have revealed that SIRT1 regulates the cellular fate of neural progenitors, axon elongation, dendritic branching, synaptic plasticity, and endocrine function. In addition to its importance in physiological processes, SIRT1 has also been implicated in protection of neurons from degeneration in models of neurological diseases, such as traumatic brain injury and Alzheimer's disease. In this review, we focus on the role of SIRT1 in the neuroendocrine system and neurodegenerative diseases. We also discuss the potential therapeutic implications of targeting the sirtuin pathway.

Keywords: sirtuin, SIRT1, central nervous system, axon degeneration, neuronal development

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Specialty section:
This article was submitted to Neuroendocrine Science, a section of the journal Frontiers in Neuroscience

Received: *09 July 2018*
Accepted: *08 October 2018*
Published: *26 October 2018*

Citation:
Fujita Y and Yamashita T (2018) Sirtuins in Neuroendocrine Regulation and Neurological Diseases. Front. Neurosci. 12:778. doi: 10.3389/fnins.2018.00778

INTRODUCTION

The sirtuins are nicotinamide adenine dinucleotide (NAD)-dependent deacetylases, which are widely conserved proteins from bacteria to humans. The sirtuin protein was originally identified in *Saccharomyces cerevisiae* as silent information regulation 2 (SIRT2) (Klar et al., 1979; Rine et al., 1979), which regulates the lifespan by inhibiting genomic instability via chromatin modification. Sirtuins are categorized as class III histone deacetylases (HDACs). In mammals, seven sirtuin homologs (SIRT1–7) are categorized into four classes based on their DNA sequence. Sirtuins are typically composed of a conserved catalytic domain and variable N- and C-terminal domains. For example, the human *sirt1* gene is located on chromosome 10 and encodes a protein that is composed of 746 amino acids, which comprises the NAD-binding catalytic core domain. Sirtuins deacetylate histone lysine residues. This results in chromatin condensation, leading to transcriptional repression (**Figure 1**). However, several sirtuins do not appear to show

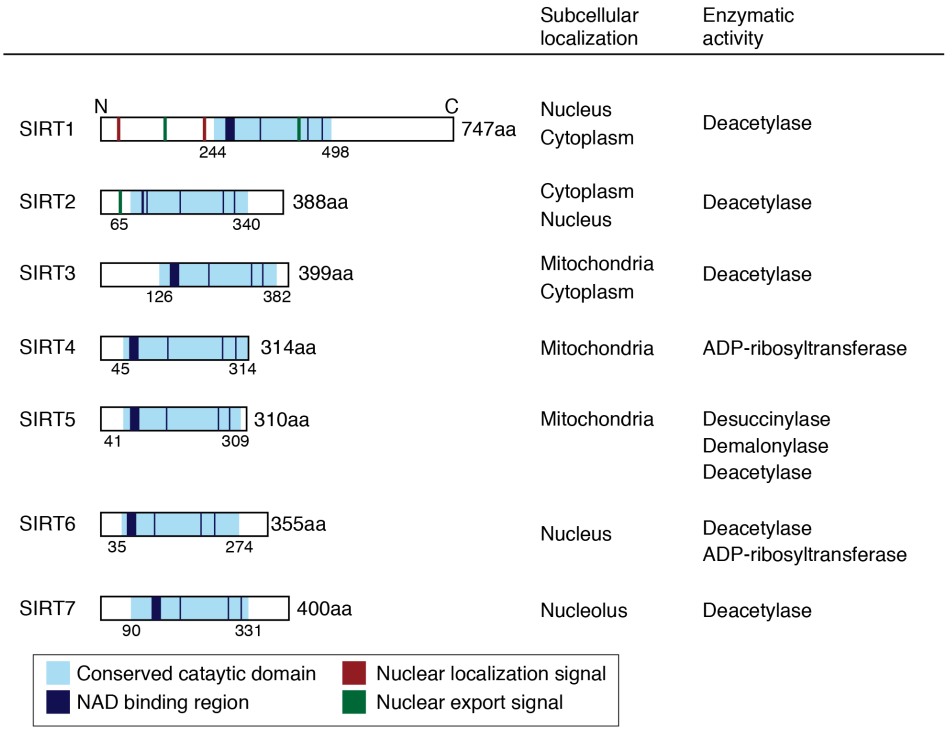

FIGURE 1 | Schematic images and biological activities of human sirtuins. Conserved catalytic domains, NAD binding regions, nuclear localization signals, and nuclear export signals are shown in the schema.

deacetylase activity. Silent information regulator 1 (SIRT1), SIRT2, SIRT3, and SIRT7 have NAD-dependent deacetylase activity; whereas SIRT4, SIRT5, and SIRT6 have weak or no detectable deacetylase activity. SIRT4 has adenosine diphosphate (ADP)-ribosyl transferase activity. SIRT5 shows more activity as an NAD-dependent demalonylase and desuccinylase than as a deacetylase. SIRT6 has both NAD-dependent deacetylase activity and ADP-ribosyl transferase activity (Haigis and Sinclair, 2010; Houtkooper et al., 2012). The crystal structure of the catalytic domain of human SIRT1 was identified, and revealed that SIRT1 activity is regulated by a C-terminal regulatory segment (Davenport et al., 2014). Intrinsically, disorder in the protein structure of SIRT1 might be related to its activity and physiological functions in the CNS (Khan and Lewis, 2005; Autiero et al., 2008; Sharma et al., 2012; Uversky, 2015).

Diversity in the subcellular localization of sirtuins can affect their cellular functions. SIRT1 is predominantly localized in the nucleus and deacetylates transcriptional factors, such as p53, FOXO, and NF-κB. It has been reported that SIRT1 shuttles into the cytoplasm during neuronal differentiation. SIRT2 is detected in the cytosol and colocalizes with microtubules and deacetylate α-tubulin. SIRT3, SIRT4, and SIRT5 are found in the mitochondria. SIRT3 is cleaved by the mitochondrial matrix processing peptidase (MPP) into a short form. The long form of SIRT3 can also localize in the nucleus. SIRT6 is associated with chromosome 19p13.3 in the nucleus. SIRT7 is a nuclear protein and regulates RNA polymerase 1-mediated transcription (Ford et al., 2006).

Silent information regulator 1, the most extensively studied mammalian ortholog of sirtuin, is classified as a class 1 sirtuin. Since the activity of SIRT1 depends on NAD+, the energy status of the cell and nutrient deprivation, such as fasting and caloric restriction, may affect its function (Rodgers et al., 2008). Although there have been some controversial aspects, SIRT1 can be associated with lifespan extension in many organisms. Accumulating studies suggest that SIRT1 plays vital roles in the development of the central nervous system (CNS) and brain functions. SIRT1 has been shown to mediate neuronal survival, neurite outgrowth, fate determination of neural precursor cells, and synaptic plasticity, through the deacetylation of target molecules (Guarente, 2011; Imai and Guarente, 2014). Lack of SIRT1 function impairs brain function, such as endocrine function, cognitive function, learning, and memory formation (Gao et al., 2010; Ramadori et al., 2010). Moreover, SIRT1 can ameliorate neurodegeneration in *in vivo* and *in vitro* models of Alzheimer's disease, amyotrophic lateral sclerosis (ALS), and Wallerian degeneration (Araki et al., 2004; Qin et al., 2006b; Kim et al., 2007), suggesting that SIRT1 is important for neuronal protection against neurotoxic insults. Thus, the activation of SIRT1 may be a therapeutic target to overcome neurodegeneration, and several synthetic SIRT1 activators are attractive as putative drugs.

In this review, we summarize the role of sirtuins, especially SIRT1, in the CNS under physiological and pathological conditions. We also discuss the potential benefits of SIRT1 activators in the animal models of neurological diseases.

DISTRIBUTION OF SIRT1 IN THE CENTRAL NERVOUS SYSTEM

Silent information regulator 1 is ubiquitously expressed and demonstrates high expression in the brain (Sakamoto et al., 2004). SIRT1 is expressed in both neurons and glial cells (Chen J. et al., 2005; Hisahara et al., 2008; Cheng et al., 2014). Histological studies revealed that SIRT1 is prominently expressed in the hippocampus and hypothalamus within the adult mouse brain (Ramadori et al., 2008; Michan et al., 2010; Zakhary et al., 2010). The highest SIRT1 expression is observed in the early embryonic stage, and it gradually decreases during development. The expression levels of sirtuins seem to be affected by aging and pathological changes. SIRT1 deacetylation activity is downregulated in the aged brain and in several neurodegenerative models (Pallas et al., 2008; Quintas et al., 2012; Tang, 2017). In contrast, calorie restriction (CR) induces SIRT1 expression in the brain, as well as fat, kidneys, and liver (Cohen et al., 2004).

SIRT1 FUNCTIONS IN NEUROGENESIS

Silent information regulator 1 is known to regulate pluripotency of embryonic stem cells and fate determination of neural progenitors. Mice carrying null alleles for SIRT1 show impaired embryogenesis (McBurney et al., 2003). The expression and/or acetylation levels of key pluripotency factors, such as Nanog, Oct-4, and Sox-2 are controlled by SIRT1 (Han et al., 2008; Yoon et al., 2014; Zhang et al., 2014). Overexpression of SIRT1 in neural tube of chick embryos decreases neurogenesis (Ichi et al., 2011). SIRT1 increases hairy and enhancer of split homolog-1 (Hes1) expression, which is important for neural stem cell maintenance, and decreases neurogenin2 (Neurog2) expression, which is involved in the promotion of neurogenesis. Treatment of nicotinamide, a SIRT1 inhibitor, enhances the differentiation of neural stem cells (Hu et al., 2014). Furthermore, nuclear translocation of SIRT1 regulates neuronal differentiation (Hisahara et al., 2008). SIRT1 predominantly shows cytoplasmic localization in neural precursor cells (NPCs), whereas its expression is mostly observed in the nucleus in differentiated NeuN-positive neurons. Nuclear SIRT1 interacts with nuclear receptor corepressor N-CoR, and this complex represses the transactivation of Hes1, leading to neuronal differentiation. Inhibition of SIRT1 using the pharmacological SIRT1 inhibitors (splitomicin or nicotinamide) or SIRT1-siRNA lentivirus decreases Tuj1-positive neurite length and neuronal differentiation both in culture and in the mouse brain after in utero electroporation (Hisahara et al., 2008). Furthermore, cytoplasmic SIRT1 promotes nerve growth factor (NGF)-induced neurite outgrowth in PC12 cells (Sugino et al., 2010). These observations suggest that subcellular localization of SIRT1 is essential for neuronal differentiation.

Silent information regulator 1 also modulates neurogenesis not only in the embryonic but also in adult rodent brain. Neurons are produced throughout life mainly in the germinal niches of the subventricular zone (SVZ) and dentate gyrus (DG) in the hippocampus. Reduced adult neurogenesis is known to be important for various brain functions, including learning and memory (Deng et al., 2010; Cameron and Glover, 2015). SIRT1 is expressed in proliferating cells in the SVZ and DG. Lentiviral-mediated knockdown of SIRT1 increases neurogenesis in the SVZ and hippocampus, whereas it does not affect the proliferation of neural precursors (Saharan et al., 2013). Genetic ablation of SIRT1 also promotes adult neurogenesis and activation of SIRT1 signaling by lentiviral-mediated forced expression or administration of resveratrol, which is known as a stimulator of SIRT1 activity, inhibits differentiation of adult neural precursors. These results suggest that SIRT1 is a negative regulator of adult neural precursor differentiation. However, other studies have demonstrated that stem cell-specific knockout of SIRT1 increases the proliferation and self-renewal rates of adult neural stem cells (Ma et al., 2014). Given these results, SIRT1 is likely to have dual functions in regulating both proliferation and differentiation of adult neural stem cells.

In addition, SIRT1 has been shown to enhance neurite and axon length as well as dendrite branching in hippocampal neurons. Upregulation of SIRT1 promotes axonogenesis and axon elongation via Akt deacetylation, which leads to inhibition of glycogen synthase kinase 3 (GSK3) activity (Li et al., 2013). Knockdown of SIRT1 enhances mTOR signaling and impairs neurite outgrowth and neuronal survival (Guo et al., 2011). Overexpression of SIRT1 or treatment with the SIRT1 activator resveratrol increases neuronal dendritic branching, possibly mediated by Rho-kinase (ROCK) activity (Codocedo et al., 2012). The tumor suppressor protein p53 is widely known to regulate neuronal apoptosis in pathological and physiological conditions. SIRT1 is a key regulator of p53 via deacetylation (Brooks and Gu, 2009; Revollo and Li, 2013). SIRT1 deacetylates p53 leading to the reduction of p53 activity and protecting cells from DNA damage (Luo et al., 2001; Vaziri et al., 2001). SIRT1 is associated with the maternally imprinted gene necdin and reduces p53-induced apoptosis in cortical neurons (Hasegawa and Yoshikawa, 2008). Thus, SIRT1 plays an important role during CNS development.

SIRT1 FUNCTION IN THE HYPOTHALAMUS

Since sirtuins are NAD-dependent enzymes, they can be considered metabolic-sensor proteins (Coppari, 2012; Chang and Guarente, 2014; Satoh and Imai, 2014). The hypothalamus monitors endocrine responses and metabolic changes, which regulate food intake, the synthesis and secretion of hormones, and physiological rhythms. Hypothalamic nuclei, including the arcuate nucleus (ARC), ventromedial hypothalamic nucleus (VMH), paraventricular nucleus (PVN), and lateral hypothalamic area (LH) are involved in controlling food intake (Schwartz et al., 2000). There are two opposing types of neurons in the ARC: anorexigenic pro-opiomelanocortin (POMC) neurons, which secrete alpha-melanocyte stimulating hormone (α-MSH) and cocaine and amphetamine-regulated transcript peptide (CART); and orexigenic neuropeptide Y (NPY)/agouti-related peptide (AgRP) neurons, which produce

NPY, AgRP, and gamma-aminobutyric acid (GABA). α-MSH secreted from POMC neurons activates melanocortin 3 and 4 receptors (MC3R and MC4R), promoting satiety. In contrast, AgRP inhibits these receptors and counteracts the function of α-MSH, thus promoting food intake (Dietrich et al., 2010; **Figure 2**).

Silent information regulator 1 is expressed in several hypothalamic nuclei, including anorexigenic POMC neurons and orexigenic AgRP neurons in the ARC, and other nuclei in the hypothalamus, such as LH and VMH. There have been conflicting reports regarding SIRT1 expression changes affected by fasting (**Table 1**). Compared to *ad libitum* feeding, fasting increased the ubiquitination of SIRT1 and decreased SIRT1 expression in the hypothalamus (Sasaki et al., 2010). Conversely, another study reported that fasting or diet restriction increased SIRT1 protein levels in the dorsomedial hypothalamus and LH (Satoh et al., 2010). Since SIRT1 expression changes (increased or decreased) are different in each tissue, tissue-specific analysis would be helpful. There are also varied findings on the role of hypothalamic SIRT1 on feeding behavior. Intracerebroventricular injection of Ex-527, a SIRT1 inhibitor, or small interfering RNA (siRNA)-mediated knockdown of SIRT1 in the ARC inhibits food intake due to the downregulation of AgRP and upregulation of POMC (Cakir et al., 2009). Specific knockout of SIRT1 in AgRP neurons decreases electrical responses of AgRP neurons to ghrelin, a stomach-derived peptide, and decreases ghrelin-induced feeding behavior (Dietrich et al., 2010). In contrast, it has been demonstrated that whole-body SIRT1 knockout mice are hyperphagic (Chen D. et al., 2005). Although specific knockout of SIRT1 in POMC neurons does not affect feeding behavior, mice lacking SIRT1 in POMC neurons demonstrate hypersensitivity to diet-induced obesity by reduced energy expenditure (Ramadori et al., 2010). In mice, adenovirus-mediated forced expression of SIRT1 in the mediobasal hypothalamus reduces food intake when compared to GFP-expressing control mice (Sasaki and Kitamura, 2010; Sasaki et al., 2010). Thus, SIRT1 is widely recognized as an important factor that controls food intake. More specific studies targeting individual neurons will reveal the detailed functions of hypothalamic SIRT1.

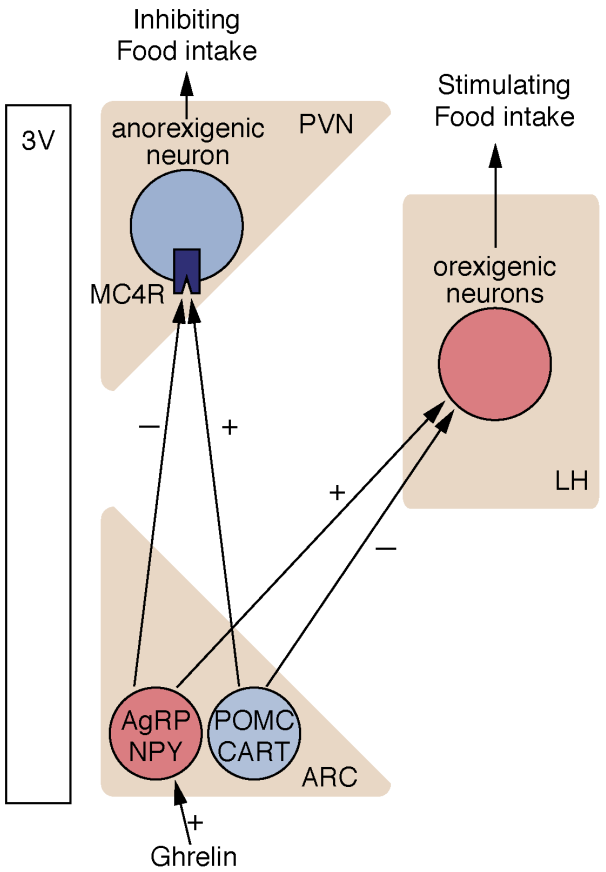

FIGURE 2 | Neural circuits controlling food intake. Nutritional information is integrated into ARC in the hypothalamus. In ARC, orexigenic neurons express NPY, and AgRP, whereas anorexigenic neurons express POMC and CART. SIRT1 is expressed in both AgRP neurons and POMC neurons in the ARC. ARC, arcuate nucleus; PVN, paraventricular nucleus; LH, lateral hypothalamic area; AgRP, agouti-related peptide; NPY, neuropeptide Y; POMC, pro-opiomelanocortin; CART, cocaine- and amphetamine-related transcript; 3V, third ventricle.

Agouti-related peptide neurons also regulate adaptive immune responses. Deletion of SIRT1 in AgRP neurons induces a pro-inflammatory state, which is associated with a decrease in regulatory T cell functions and consequent increase in effector T cell activity, leading to increased autoimmune disease susceptibility in mice (Matarese et al., 2013).

SIRT1 AND SIRT2 IN HIGHER-ORDER BRAIN FUNCTIONS

Sirtuins, especially SIRT1 and SIRT2, also mediate higher-order brain functions, such as learning, memory, and emotions (Donmez and Outeiro, 2013; Herskovits and Guarente, 2014). They regulate various neurological processes involving dendritic arborization, synaptic plasticity, and adult neurogenesis, which underlie these brain functions. Deletion of SIRT1 impairs cognitive functions. SIRT1 null mice exhibit deficits in short- and long-term associative memory and spatial learning (Michan et al., 2010). In SIRT1 null mice, there is less dendritic branching in the hippocampus, a key structure for learning and memory (Michan et al., 2010). Further, genes associated with synaptic function, membrane fusion, myelination, and amino acid and lipid metabolism were altered. Another study reported a direct role for SIRT1 in brain function (Gao et al., 2010). Deletion of SIRT1 in nestin-positive neural progenitor cells impaired memory and synaptic plasticity (Gao et al., 2010). SIRT1 conditional knockout mice have decreased memory performance in fear conditioning and novel object recognition tasks. In these mice, there is reduced expression of brain-derived neurotrophic factor (BDNF) and cAMP response binding protein (CREB), which are critical for synaptic plasticity and modulation of synapse formation, whereas microRNA (miRNA)-134 expression was upregulated. In the normal brain, SIRT1 cooperates with the transcriptional factor Yin

TABLE 1 | The expression changes of SIRT1 in the hypothalamus.

The treatment that evoked the response (fasting/CR, duration)	SIRT1 expression changes (increase/decrease)	RNA or protein	Location of expression changes	Reference
Fasting in mice (Refeeding 24 h after 24 h starvation)	Increase	Protein	Liver	Rodgers et al., 2005, Nature
Fasting in mice (24 h starvation)	Increase	Protein	Brain, heart, muscle, white adipose, kidney	Kanfi et al., 2008, FEBS Letters
Fasting in mice (Refeeding 3 h after 24 h starvation)	Increase	Protein	Hypothalamus	Sasaki, 2010, Endocrinology
Fasting in mice (Refeeding 3 h after 24 h starvation)	Decrease	Protein	Cortex	Sasaki, 2010, Endocrinology
CR in rats (Lifelong restriction, starting immediately after weaning, with 60% of daily food)	Increase	Protein	Brain, fat kidney, liver	Cohen, 2004, Science
CR in mice (3–4 months old animals were subjected to a 30% CR diet)	Increase	Protein	Cortex, hippocampus	Chen et al., 2008, Exp Gerontology
CR in mice (3–4 months old animals were subjected to a 30% CR diet)	Decrease	Protein	Cerebellum, midbrain	Chen et al., 2008, Exp Gerontology
CR in mice (8–12 weeks old animals were subjected to a 60% of daily food for 14 days)	Increase	Protein	DMH, LH, SCN in the hypothalamus	Satoh, 2010, J Neurosci.

Yang1 (YY1) and restricts the expression of miR-134 (Gao et al., 2010). SIRT1 deletion induces miRNA-134 expression, leading to the downregulation of BDNF and CREB, which subsequently impairs synaptic plasticity. Intraventricular injection of resveratrol, a natural compound that activates SIRT1, facilitates memory formation and synaptic plasticity in aged mice (Zhao et al., 2013). miR-124 and miR-134 expression decreased, whereas BDNF and CREB expression increased in resveratrol-treated mice. It has also been shown that miR-34c negatively regulates SIRT1 expression. Increased expression of miR-34c and decreased expression of SIRT1 were detected in mice with age-associated memory impairment and APPPS-21 mice, which are a model of amyloid pathology linked to Alzheimer's disease (AD) (Yamakuchi et al., 2008; Zovoilis et al., 2011). These results suggest the possibility that decreased SIRT1 expression regulated by miRNAs may correlate with memory impairment. Furthermore, dysregulation of SIRT1 mediates obesity-induced memory impairments. High fat diet-induced obesity causes deficits in hippocampal-dependent spatial memory, synaptic plasticity, and altered gene expression. These effects were associated with decreased expression of SIRT1 (Heyward et al., 2012). Neuron-specific knockout of SIRT1 within the forebrain reversed obesity-induced hippocampal-dependent spatial memory deficits (Heyward et al., 2016). Meanwhile, another member of the HDAC family, HDAC2, negatively regulates synaptic plasticity and memory formation (Guan et al., 2009). Collectively, these findings suggest that targeting HDACs may be a key factor for regulating synaptic plasticity.

Silent information regulator 1 also modulates emotional responses, possibly associated with adaptive ability in a changing environment of food availability (Libert et al., 2011). Brain-specific SIRT1-knockout mice have less anxiety-like behaviors and more exploratory drive than their wild-type (WT) littermates. SIRT1 activates monoamine oxidase A (MAO-A) expression through the deacetylation of transcription factor nescient helix-loop-helix 2 (NHLH2), leading to a decrease of serotonin. MAO-A is the enzyme that degrades both serotonin and noradrenaline and is associated with mood disorders. Indeed, MAO inhibitors have been widely used for depression and several anxiety disorders (Preskorn, 2006). SIRT1 polymorphism frequencies were investigated in individuals with psychiatric disorders and controls; both rare and common alleles were associated with a higher risk of anxiety. Consistent with these observations, the mutations found in these individuals were linked to increased SIRT1 activity (Kishi et al., 2010; Jansen et al., 2016; Luo and Zhang, 2016). A genome-wide association study revealed that genetic variations near the *Sirt1* gene are significantly linked to major depressive disorder (Converge consortium, 2015). Later studies demonstrated decreased expression of SIRT1 in subjects with major depressive disorder compared to controls (Jansen et al., 2016; Luo and Zhang, 2016). An independent study of Japanese subjects demonstrated a significant association between SIRT1 SNP and major depressive disorders (Kishi et al., 2010). In a mouse model of depression, SIRT1 expression was increased in the nucleus accumbens (NAc), a brain region associated with reward and motivation. SIRT1 induction in the NAc promoted depression- and anxiety-like behaviors. Intra-NAc bilateral infusion of the SIRT1 agonist resveratrol in mice with viral-mediated overexpression of SIRT1 increased anxiety- and depression-like behavior in the open field, elevated plus maze, and forced swim tests. Intra-NAc infusion of the SIRT1 antagonist EX-527, or viral-mediated knockdown of SIRT1 in the NAc reduced those behavioral effects. Hippocampal SIRT1 is associated with chronic stress-induced depressive behavior (Abe-Higuchi et al., 2016). Chronic stress reduces SIRT1 expression in the hippocampus, and decreases dendrite length and spine density. Depressive behaviors in the mice subjected to chronic stress were reversed by viral-mediated overexpression

of SIRT1 into the DG area of hippocampus (Abe-Higuchi et al., 2016).

Furthermore, sirtuins control behavioral responses to cocaine and morphine in the NAc. Addictive drugs, such as cocaine, induce gene expression changes in the NAc, which affect reward circuitry (Freeman et al., 2001; McClung and Nestler, 2003; Yao et al., 2004; Hyman et al., 2006). It is known that increased acetylation of histone H3 or H4 causes transcriptional activation, whereas increased methylation of histone H3 at Lys9 causes transcriptional repression. Chromatin immunoprecipitation (ChIP) analysis revealed that chronic administration of cocaine increases or decreases histone acetylation in the NAc at the gene promoters encoding the genes known to show drug-induced upregulation or downregulation, respectively (Kumar et al., 2005; Renthal et al., 2008). In a subsequent study, genome-wide ChIP followed by promoter microarray analysis (ChIP-chip) methods demonstrated that SIRT1 and SIRT2 were the targets of increased histone H3 acetylation in the NAc after chronic cocaine administration. Furthermore, the catalytic activity of these sirtuins was increased in the NAc by chronic cocaine administration (Renthal et al., 2009). Overexpression of SIRT1 or SIRT2 in the NAc using adeno-associated viruses (AAV) promotes the rewarding effects of both cocaine and morphine reward, while genetic deletion of SIRT1 in the NAc decreases drug reward (Ferguson et al., 2013).

Collectively, dysregulation of sirtuins are involved in diverse phenomena associated with higher brain dysfunction, including synaptic dysfunction, altered neurotransmitter secretion, and genetic variations.

SIRTUINS AND NEURODEGENERATIVE DISORDERS

Numerous studies have demonstrated that sirtuins mediate a variety of neurodegenerative disorders (Gan and Mucke, 2008; Donmez, 2012; Herskovits and Guarente, 2014). Altered sirtuin expression and/or activation might be associated with disease development and progression (**Table 2**). Beneficial effects of SIRT1 activation using genetic manipulation and pharmacological treatment have been reported in various animal models for neurodegenerative diseases (**Table 3**). In contrast, inhibition of SIRT1 signaling has also been shown to exert neuroprotective effects. Other sirtuins, especially SIRT2, are reported to be involved in neurodegenerative disorders. Thus, the sirtuin family shows diverse effects. The following sections discuss the pleiotropic effects of sirtuins in neurodegenerative disorders.

Wallerian Degeneration

There are numerous studies reporting the protective effects of SIRT1 against axonal degenerative processes. In this section, we introduce early studies, which suggest that activation of the SIRT1 pathway possibly delayed Wallerian degeneration. Wallerian degeneration is the anterograde degeneration

TABLE 2 | The expression changes of sirtuins in neurological diseases and animal models.

Sirtuins	Sirtuin expression changes (increase/decrease)	RNA or protein	Location of expression changes	Human or mouse	Disease or animal model	Reference
SIRT2	Decrease	Protein	Cultured cerebellar granule cells from Wld^S mice	Mouse	Wld^S	Suzuki and Koike, 2007
SIRT1	Decrease	Protein	Spinal cord	Mouse	SCI	Chen, 2017
SIRT1	Increase	Protein	Injured-side cortex	Mouse	TBI	Zhao, 2012
SIRT1	Increase	Protein	Cortex	Rat	SAH	Zhang, 2016
SIRT1	Increase	Protein	Peri-infarct regions of injured-side cortex	Mouse	MCAO	Hernandez-Jimenez, 2013
SIRT1	Increase	Protein	Acute and chronic active lesion in MS brain CD4+, CD68+, GFAP+, oligodendrocytes	Human	MS	Tegla, 2013
SIRT1	Increase	Protein	GFAP+ cells in typical inflammatory perivascular cuffs in brain	Mouse	EAE	Prozorovski, 2008
SIRT1	Decrease	Protein, mRNA	Parietal cortex	Human	AD	Julien, 2009
SIRT3	Decrease	Protein	Frontal cortex	Human	AD	Lee, 2018
SIRT6	Decrease	Protein	Temporal cortex	Human	AD	Kaluski. 2017
SIRT1	Increase	Protein	Forebrain	Mouse	AD	Kim, 2007
SIRT1	Decrease	Protein	Frontal cortex	Human	PD	Singh, 2017
SIRT1 (80 kDa)	Increase	Protein	Temporal cortex	Human	PD	Singh, 2017
SIRT1	Increase	Protein	Spinal cord	SOD1G37R mouse	ALS (severe neurodegeneration)	Kim, 2007
SIRT3	Decrease	mRNA	Spinal cord, brain stem	SOD1 G93A mouse	ALS (end stage)	Buck, 2017

TABLE 3 | The role of SIRT1 in animal models of neurodegenerative diseases.

Manipulations of SIRT1	Effects on CNS	Human or mouse	Animal model	Reference
Resveratrol	Decreased axonal degeneration	Mouse	Wallerian degeneration (DRG explant culture)	Araki et al., 2004
siRNA-mediated knockdown of SIRT1 or Sirtinol	Decreased NAD-dependent axonal protection	Mouse	Wallerian degeneration (DRG explant culture)	Araki et al., 2004
SRT1720	Improved locomotor recovery, decreased proinflammatory cytokine expression, decreased accumulation of macrophages/microglia	Mouse	SCI	Chen et al., 2017
Resveratrol	Improved motor functional recovery, decreased motor neuron loss	Rat	SCI	Zhao et al., 2017
siRNA-mediated knockdown of SIRT1 or SIRT1 inhibitor	Increased apoptosi in cultured cortical neurons, increased ERK1/2 activation after TBI	Mouse	TBI (Primary cortical neuron culture)	Zhao et al., 2012
Resveratrol	Induced ischemic tolerance	Mouse	Transient MCAO	Koronowski et al., 2017
Homozygous deletion of SIRT1	Increased infarct volume	Mouse	Permanent MCAO	Hernandez-Jimenez et al., 2013; Liu et al., 2013
SIRT1 activator A3	Decreased infarct volume	Mouse	Permanent MCAO	Hernandez-Jimenez et al., 2013
Nicotinamide	Decreased infarct volume	Mouse	Permanent MCAO	Liu et al., 2009
Resveratrol	Increased survival of retinal ganglion cells	Mouse	EAE induced by PLP immunization of SJL/J mouse	Shindler et al., 2007
SIRT1-overexpressing mouse (pCaMKIIα-tTA; pTRE-SIRT1/mito/eYFP)	Decreased EAE clinical symptoms, reduced demyelination and axonal injury	Mouse	EAE induced by MOG immunization of C57BL/6 mouse	Nimmagadda et al., 2013
Virus-mediated expression of SIRT1	Decreased Aβ peptide in primary Tg2576 neurons	Mouse	AD (Primary cortical neuron culture of Tg2576 mouse)	Qin et al., 2006, JBC
Virus-mediated expression of SIRT1	Increased survival neurons in CA1	Mouse	AD (Inducible p25 transgenic mouse)	Kim, 2007
Resveratrol	Decreased thioflavine S-positive plaques in cortex, striatum, and hypothalamus	Mouse	AD (Tg19959 mouse)	Karuppagounder et al., 2009
Resveratrol	Decreased Aβ peptide in primary neurons	Mouse	AD (Primary cortical neuron culture of J20 APP mice)	Vingtdeux et al., 2010
Resveratrol	Decreased neurodegeneration	Mouse	PD (MPTP treatment)	Mudo et al., 2012
SIRTl-overexpressing mouse (NSE-SIRT1 mice)	TH-positive neurons	Mouse	PD (MPTP treatment)	Kakefuda et al., 2009
Resveratrol	Extended lifespan, delayed onset of symptoms, increased survival of motor neurons	Mouse	ALS (SOD1G93A mouse)	Han et al., 2012; Mancuso et al., 2014
SIRT1-overexpressing mice (PrP-SIRT1 mice)	Extended lifespan	Mouse	ALS (SOD1G93A mouse)	Watanabe et al., 2014

of axons and synapses after injury (Coleman, 2005). This process usually occurs about 1.5 days after injury. However, Wallerian degeneration mutant (Wld^s) mice, which carry an autosomal dominant mutation in chromosome 4, show delayed Wallerian degeneration for 2–3 weeks (Lunn et al., 1989; Coleman and Freeman, 2010). The Wld^s mutation is an 85-kb tandem triplication, which causes overexpression of chimeric Wld^s protein. The mutation region comprises two associated genes: an E4-type ubiquitin ligase Ube4b (or Ufd2a), and the protein associated with the NAD salvage pathway in mammals, nicotinamide mononucleotide adenylyltransferase 1 (Nmnat1). It has been suggested that Nmnat1 activity is responsible for the protective effect of Wld^s protein thorough SIRT1 (Araki et al., 2004). Knockdown of SIRT1 or treatment with the SIRT2 inhibitor, sirtinol, inhibits NAD-dependent axonal protection in cultured dorsal root ganglion neurons, which had induced degeneration by the removal of cell bodies. Meanwhile, Nmnat1/NAD-induced neuroprotective effects independent of SIRT1 have also been demonstrated. In addition to Nmnat1, the N-terminal of Wld^s is also required for axonal protection mediated by Wld^s (Avery et al., 2009). The extranuclear translocation and axon localization of NMNAT1 protein may exert neuroprotective potency *in vivo* (Babetto et al., 2010). Transgenic mice carrying axon-targeted Nmnat1 showed robust axonal protection after axotomy. Further, SIRT2 can also modulate resistance to axonal degeneration. Tubulin acetylation is associated with microtubule stability. Increased microtubule acetylation

was observed in cultured cerebellar granule cells from Wld^s mice (Suzuki and Koike, 2007). SIRT2 is an NAD-dependent tubulin deacetylase, and SIRT2 expression was decreased in these cells. Overexpression of SIRT2 abolished microtubule hyperacetylation and resistance to axonal degeneration in the cells of Wld^s mice, whereas lentiviral-mediated knockdown of SIRT2 enhanced microtubule acetylation and resistance to degeneration in wild-type cerebellar granule cells (Suzuki and Koike, 2007).

Spinal Cord Injury (SCI)

Although the direct effect of SIRT1 on axonal regeneration remains obscure, activation of SIRT1 has been shown to exert beneficial effects on motor function in the animal model of spinal cord injury (SCI). In SCI, following the initial damage to the spinal cord, tissues are directly damaged and disrupted. Injured neurons, glia, and vasculature cause oxidative stress, free radical generation, edema, and further inflammatory reactions which induce secondary injury, leading to the expansion of spinal cord damage and sustained impairment of neurological function (Ahuja et al., 2017). Immune responses after SCI, such as infiltration and activation of inflammatory cells and inflammatory cytokine production, mediate the pathogenesis of SCI. Overwhelming immune responses aggravate the injury. In this regard, controlling immune responses contributes to functional recovery after SCI (Courtine et al., 2011; Donnelly et al., 2011). SIRT1 expression at the lesion site, where inflammatory responses must be evident was decreased 4 h after SCI and persisted for 3 days. Treatment with an SIRT1 activator, SRT1720, reduced inflammatory cytokines and inflammatory cells and promoted functional recovery after SCI (Chen et al., 2017). Mx1-Cre-mediated knockout of SIRT1 in mice, which ablated SIRT1 expression in inflammatory cells, such as macrophages, neutrophils, dendritic cells, T cells, and B cells, caused increased levels of inflammatory cytokines and severe inhibition of motor recovery compared to those in wild-type mice. These findings suggest that the anti-inflammatory role of SIRT1 contributes to beneficial effects on motor function after SCI. Consistent with these observations, treating rats with resveratrol had neuroprotective effects after SCI through the enhancement of autophagy and inhibition of apoptosis regulated by SIRT1/AMP-activated protein kinase (AMPK) signaling (Zhao et al., 2017). Resveratrol also promoted motor function recovery after SCI. Thus, in addition to its neuroprotective functions, the anti-inflammatory effects of SIRT1 may also promote motor function recovery after SCI.

Traumatic Brain Injury

Silent information regulator 1 was shown to prevent neuronal apoptosis in a transection model *in vitro* and weight-drop model *in vivo*, which mimics traumatic brain injury (TBI). SIRT1 expression was increased 30 min to 24 h after TBI with a peak level at 6 h after injury in the animal model of TBI. Inhibiting SIRT1 using a pharmacological inhibitor (salermide) or siRNA decreased ERK1/2 activation and enhanced neuronal apoptosis after mechanical traumatic injury *in vivo* (Zhao et al., 2012). Inhibition of ERK reduced apoptosis and decreased SIRT1 upregulation after TBI. ERK1/2 activation has neuroprotective functions and mediates the beneficial effects of neuroprotectants (Fukunaga and Miyamoto, 1998; Guerra et al., 2004; Karmarkar et al., 2011). However, other studies have demonstrated that ERK1/2 activation can have neurotoxic signals *in vitro* and *in vivo* (Alessandrini et al., 1999; Stanciu et al., 2000; Lesuisse and Martin, 2002). In the adult mouse brain, SIRT1 is predominantly expressed in the cytosol, where ERK1/2 is localized (Tanno et al., 2007; Li et al., 2008). Considering these observations, there may be a synergistic relationship between SIRT1 and ERK pathways that regulate neuronal apoptosis following TBI. Furthermore, it has been reported that upregulation of SIRT1 is involved in the neuroprotective effects induced by natural components, such as vitamin E and omega-3 fatty acids in a TBI model (Wu et al., 2006, 2007; Aiguo et al., 2010).

Stroke

Stroke is a cerebrovascular disease that can lead to death or neuronal dysfunction. Vascular occlusion causes a deprivation of oxygen and energy, followed by the collapse of ionic gradients across the cell membrane and neuronal death caused by an excess release of excitatory neurotransmitters. These sequential events result in the formation of reactive oxygen species, gene expression changes, and induction of inflammatory processes, which contribute to irreversible tissue damage (Iadecola and Anrather, 2011). A variety of animal stroke models have been widely used. The transient or permanent middle cerebral artery occlusion (MCAO) model is a well-established model for human ischemic stroke (Koizumi et al., 1986; Longa et al., 1989; Hossmann, 1998). Oxygen and glucose deprivation (OGD) is a useful *in vitro* model alternative to the animal ischemia model (Ogawa et al., 1990). SIRT1 expression is decreased after transient MCAO or prolonged OGD (Yan et al., 2013), whereas its expression is not altered after moderate ischemia and short OGD (Wang et al., 2013). Since both beneficial and detrimental effects of SIRT1 have been reported, the protective effect of SIRT1 in ischemia remains controversial. Initial studies reported that treatment with resveratrol shows neuroprotective effects in OGD in organotypic hippocampal slices and global cerebral ischemia in rats (Raval et al., 2006, 2008). A subsequent study showed that both ischemic preconditioning, which develops brain tolerance to a secondary ischemic damage, and resveratrol treatment had neuroprotective effects, possibly via the downregulation of mitochondrial uncoupling protein 2 (UCP2) (Della-Morte et al., 2009). UCP-2 reduces mitochondrial membrane potential and inhibits ATP production. SIRT1 binds to the UCP-2 promoter and regulates its transcription. Since the SIRT1 inhibitor sirtinol reversed the effect of resveratrol, SIRT1 activity seems to be required for resveratrol-mediated neuroprotective effects in cerebral ischemia. Emerging studies demonstrated that the glycolytic function of SIRT1 mediates resveratrol-induced ischemic tolerance in an animal model of stroke (Koronowski et al., 2017). Neuron-specific knockout of SIRT1 in the adult brain abolishes resveratrol-induced neuroprotection in MCAO. Altered glucose metabolism and impaired glycolytic ATP

production were observed in neuron-specific SIRT1-knockout mice. The neuronal activities of SIRT1 seem to precede resveratrol-induced neuroprotection.

Ischemic brain injury depletes intracellular NAD^+, which is indispensable for the catalytic activity of SIRT1 (Endres et al., 1997). Treatment with NAD ameliorates ischemic injury in a rat model of transient focal ischemia (Ying, 2007; Ying et al., 2007). Moreover, nicotinamide phosphoribosyltransferase (Nampt), which is the rate-limiting enzyme in NAD+ production within the NAD+ salvage pathway, mimics the positive effects of NAD+ against stroke (Rongvaux et al., 2003; Revollo et al., 2007; Yang et al., 2007). Overexpression of Nampt reduces ischemic infarct in experimental cerebral ischemia rats, and SIRT1 global knockout blocks this effect (Wang et al., 2011). LKB1 deacetylation and AMPK activation regulated by SIRT1 contribute to the neuroprotection of Nampt. The results from *in vitro* and *in vivo* studies demonstrate that increased autophagy through SIRT1-dependent TSC2-mTOR-S6K1 signaling is also involved in Nampt-induced neuroprotection in cerebral ischemia (Wang et al., 2012). Furthermore, SIRT1-knockout mice demonstrate greater infarct volumes after MCAO than their wild-type counterparts (Hernandez-Jimenez et al., 2013; Liu et al., 2013). Pharmacological modulation using the SIRT1 activator A3 decreases the infarct volume, while the SIRT1 inhibitor sirtinol increases the infarct volume in the MCAO model. Increased acetylation of p53 and NFκB via inhibition of SIRT1 aggravate ischemic injury.

Neurovascular protection of SIRT1 was also reported in cerebral hypoperfusion induced by bilateral common carotid artery stenosis (Hattori et al., 2014). SIRT1-overexpressing transgenic mice preserved cerebral blood flow after cerebral hypoperfusion. SIRT1 is implicated in the neuroprotective effects of experimental subarachnoid hemorrhage (SAH) in rats (Zhang et al., 2016). SIRT1 expression was increased and peaked 24 h after SAH. Pharmacological inhibition of SIRT1 using sirtinol exacerbates neuroinflammation and neuronal apoptosis after SAH, whereas activation of SIRT1 using A3 reduces SAH-induced early brain injury.

Silent information regulator 1 activation does not always exert neuroprotective functions (Ng and Tang, 2013; Sansone et al., 2013). Overexpression of human SIRT1 in neurons under the control of the neuron-specific enolase promoter does not induce neuroprotection in mice (Kakefuda et al., 2009). An NAD+ precursor and a SIRT1 inhibitor, nicotinamide, reduces infarct size in a permanent focal cerebral ischemic model (Liu et al., 2009). Other sirtuins are also involved in ischemic processes. Mitochondrial NAD + -dependent SIRT3 mediates the beneficial effects of ketone bodies after MCAO (Yin et al., 2015). SIRT6 overexpression decreases cerebral infarction and attenuates neurological deficits after MCAO/reperfusion (Zhang et al., 2017). SIRT6 activates nuclear factor-erythroid 2-related factor-2 (NRF2), which is a basic leucine zipper transcription factor that regulates the expression of antioxidant proteins. NRF2-knockout mice abolish the neuroprotective effects of SIRT6. As such, the activation of NRF2 induced by SIRT6 overexpression may be implicated in its protective effects after stroke.

Multiple Sclerosis (MS)

Multiple sclerosis (MS) is a chronic inflammatory demyelinating disease of the CNS, mostly driven by autoimmune causes. Immune cells infiltrate the CNS and attack myelin sheaths, leading to demyelination, axonal damage, and neurological disability (Hauser and Oksenberg, 2006; Trapp and Nave, 2008). CD4+ T cells are critical effector cells in CNS inflammation (Goverman, 2009). Individuals with MS often show permanent axonal damage and neuronal loss. MS lesions are often located in the brain, spinal cord, cranial nerves, and optic nerve. Experimental autoimmune encephalomyelitis (EAE) is one of the best available models for human MS. Several studies have demonstrated that pharmacological activation of SIRT1 confers protective functions in mouse models of MS. Intravitreal injection of a SIRT1 activator, SRT501 (resveratrol) or SRT647, attenuates retinal ganglion cell death in optic neuritis of a relapsing-remitting EAE model in SJL mice (Shindler et al., 2007). This neuroprotective effect is blocked by the SIRT1 inhibitor, sirtinol. A subsequent study demonstrated that treatment with SRT501 preserves axonal density in the spinal cord when compared to vehicle treatment (Shindler et al., 2010). Similar beneficial effects were also reported in chronic EAE in C57BL/6 mice and in a virus-induced CNS demyelination model (Fonseca-Kelly et al., 2012; Khan et al., 2014). Genetic overexpression of SIRT1 in EAE mice support the beneficial effects of SIRT1. Transgenic mice with neuron-specific overexpression of SIRT1 were induced with chronic EAE by immunization with myelin oligodendrocyte glycoprotein (MOG) peptide, and showed suppressed EAE clinical symptoms when compared to wild-type EAE mice (Nimmagadda et al., 2013). Increased BDNF and NAD could be responsible for the neuroprotective effects observed in this mouse line.

In contrast, other studies have shown that inhibition of SIRT1 contributes to the amelioration of EAE. Adult neural progenitor cells could be a possible regenerative tool to compensate for neuronal loss after CNS damage. However, most NPCs generate more glial cells than neurons, and the compensation for damaged neurons is insufficient (Ridet et al., 1997). SIRT1 expression was increased in GFAP-positive cells around EAE inflammatory lesions. Mild oxidation induced by buthionine sulfoximine or diethyldithiocarbamate, or resveratrol-induced SIRT1 activation, suppressed proliferation of NPCs and increased differentiation toward astrocytes, whereas redox conditions induced by lipoic acid of N-acetylcysteine showed opposite effects (Prozorovski et al., 2008). Upregulation of SIRT1 in oxidative conditions promotes SIRT1 binding to Hes1 and inhibits Mash1, resulting in NPC differentiation toward astrocytes. NPC-specific knockout of SIRT1 also increases the generation of oligodendrocyte progenitor cells (OPCs), which are the origin of the myelin-forming glial cells, oligodendrocytes (Rafalski et al., 2013). SIRT1 inactivation enhances remyelination and delays onset of paralysis in a chronic EAE model. Furthermore, global knockout of SIRT1 inhibits production of pro-inflammatory T helper 17 (Th17) cells and ameliorates EAE clinical scores in Th17 cell-mediated autoimmune diseases (Lim et al., 2015). Pharmacological inhibition of SIRT1 using Ex-527 also attenuates

the infiltration of immune cells into the spinal cord and ameliorates EAE. Th17 cells are involved in the onset and pathogenesis of autoimmune diseases. The transcription factor, RAR-related orphan receptor γ-t (RORγt), regulates Th17 cell differentiation (Ivanov et al., 2006; Kebir et al., 2007). SIRT1 physically interacts with RORγt and promotes Th17 differentiation via deacetylation of RORγt. Therefore, SIRT1 inhibition can exert both beneficial and detrimental effects on EAE. Although specific deletion in particular cells may be challenging, distinctive pharmacological inhibition of SIRT1 in NPC and/or immune cells may serve as a potential treatment for MS.

Studies in Individuals With MS

Studies on the brains of individuals with MS revealed increased SIRT1 expression in acute and chronic lesion sites, whereas its expression is rarely detected in normal brains (Tegla et al., 2014). A high level of SIRT1 expression is observed in MS plaques. Moreover, CD4+ and CD68+ inflammatory cells, oligodendrocytes, and glial fibrillary acidic protein (GFAP)-positive astrocytes in MS plaques co-localize with SIRT1. Furthermore, SIRT1 expression in peripheral blood mononuclear cells (PBMCs) obtained from patients with MS that had relapses was decreased compared to that in controls and stable patients with MS. Responders to glatiramer acetate treatment in relapsing-remitting MS show higher SIRT1 expression (Hewes et al., 2017). These results suggest that low levels of SIRT1 can be used as a putative biomarker for MS patients.

Alzheimer's Disease

Deposition of aggregate amyloid-β (Aβ), as well as tau phosphorylation and neurofibrillary tangles, are well-characterized hallmarks of AD. These abnormal protein aggregations are considered to be related to neurodegeneration that causes neuronal death, brain atrophy, and subsequent memory loss and cognitive deficits in AD (Hardy and Selkoe, 2002; Ballard et al., 2011). Many studies have demonstrated that SIRT1 activation has beneficial effects in diverse animal models of AD, and activation of SIRT1 has therapeutic potential for AD (Bonda et al., 2011). Numerous systematic reviews discussing the beneficial functions of SIRT1 in AD have been published (Bonda et al., 2011; Donmez and Outeiro, 2013; Herskovits and Guarente, 2014; Ng et al., 2015); we therefore focus on recent findings on the role of SIRT1 and other sirtuins in AD in this section.

Silent information regulator 1 activation using resveratrol and SIRT1 overexpression has been shown to reduce amyloid plaque formation and confer protective effects in diverse animal models of AD (Chen J. et al., 2005; Qin et al., 2006b; Kim et al., 2007; Karuppagounder et al., 2009; Vingtdeux et al., 2010). CR, which is known to induce SIRT1 activation, attenuates amyloid toxicity both in murine and primate AD models (Wang et al., 2005; Qin et al., 2006a). SIRT1 has also been shown to reduce neurofibrillary tau pathology (Green et al., 2008; Min et al., 2010). Furthermore, the role of SIRT1 appears to be involved in the association between neuronal energy metabolism and AD. Increased accumulation of sterol was observed in the brains of humans with AD and rodent models of AD. Prevention of the accumulation of ceramides and cholesterol resulted in neuronal protection from cell death induced by Aβ. These findings suggest that perturbed cholesterol metabolism could be responsible for triggering neurodegenerative cascades in AD (Cutler et al., 2004; Xiong et al., 2008; Bandaru et al., 2009; Fernandez et al., 2009; Panchal et al., 2010). SIRT1 and AMPK have been shown to positively regulate each other's activities and mediate various processes, such as cellular metabolism, mitochondrial function, and inflammation (Ruderman et al., 2010). The plant-derived protein osmotin has been shown to ameliorate Aβ-induced synaptic dysfunction and memory impairment in rats (Shah et al., 2014; Teller et al., 2015). Subsequent studies demonstrated that osmotin treatment reduces cholesterol biosynthesis and exerts beneficial effects through the activation of the SIRT1/AMPK pathway in an AD mouse model (Shah et al., 2017a,b). The drugs that inhibit cholesterol biogenesis and/or activation of SIRT1/AMPK axis may serve as candidates for developing therapies against excess cholesterol accumulation in AD.

Possible roles for other sirtuins in AD have been reported. Recent studies revealed that apolipoprotein E4 is a genetic factor in late-onset AD. SIRT3 expression is decreased in the frontal cortex of patients with AD, and dysregulation of SIRT3 induces p53-mediated mitochondrial and neuronal damage in AD (Lee et al., 2018). In addition, individuals with AD show decreased expression of SIRT6 (Kaluski et al., 2017). SIRT6 regulates DNA repair and maintenance of genomic stability via the base excision repair pathway (Giblin et al., 2014; Kugel and Mostoslavsky, 2014). SIRT6-deficient mice show genomic instability, progeroid features, and severe metabolic deficits, such as fatal hypoglycemia (Xiao et al., 2010; Etchegaray et al., 2013; Jung et al., 2016). Brain-specific SIRT6-knockout mice show increased DNA damage, apoptosis, and learning impairments (Kaluski et al., 2017). Lack of SIRT6 induces tau protein stabilization and increases tau phosphorylation via activation of glycogen synthase kinase 3 (GSK3). Individuals with AD show a reduction in SIRT6 expression. Notably, there is a further reduction with increased severity of Braak stages. These findings suggest that SIRT6 is required to keep the brain healthy by preventing naturally occurring DNA damage.

Parkinson's Disease

Recently, accumulating studies have revealed the relationship between sirtuins and Parkinson's disease (PD) *in vitro* and *in vivo*. PD is an age-associated neurodegenerative disease characterized by motor disorders due to the degeneration and dysfunction of dopaminergic neurons in the substantia nigra and striatum. Mitochondrial abnormalities and Lewy bodies, mainly containing misfolded and aggregated α-synuclein protein, are implicated in the pathology of PD. SIRT1 activity is reduced in post-mortem brain tissue obtained from individuals with PD (Singh et al., 2017). SIRT1 activity is also decreased in induced pluripotent stem cell (iPSC)-derived dopaminergic neurons carrying a glycine to serine mutation (G2019S) in leucine-rich repeat kinase 2 (LRRK2), which is causally associated with PD and is involved in the impairment of mitochondrial function

(Schwab et al., 2017). Three heterozygous sequence variants within the promoter regions of *SIRT1* gene in patients with sporadic PD were identified, but these were absent in controls. These variants are associated with the reduced expression of SIRT1 in PD patients (Zhang et al., 2012). Several studies have proposed mechanisms for the protective effects of SIRT1 on PD. The 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated animal model is widely used as an animal model of PD. Administration of resveratrol or genetic overexpression of peroxisome proliferator-activated receptor-gamma coactivator-1α (PGC1α), a transcriptional coactivator that is deacetylated by SIRT1, decreases MPTP-induced neuronal degeneration (Mudo et al., 2012). Cellular models of PD have implicated SIRT1-mediated autophagy and mitophagy under α-synuclein-induced toxicity (Sampaio-Marques et al., 2012). Knockout of SIRT1 worsened movement in an MPTP-treated model, whereas other studies reported that SIRT1 did not prevent neuronal damage to tyrosine hydroxylase (TH)-positive dopaminergic neurons induced by MPTP (Kakefuda et al., 2009; Zhang et al., 2018). Thus, the agents inducing SIRT1 activation and/or expression could be therapeutic drugs for PD. However, a few studies suggested that SIRT1 inhibition does show neuroprotective effects. 1-methyl-4-phenylpyridinium (MPP+) is the active metabolite of MPTP, and has dopaminergic toxicity (Dauer and Przedborski, 2003). Knockdown of SIRT1 using siRNA reduces MPP + -induced apoptosis in SH-SY5Y human neuroblastoma cells (Park et al., 2011). Therefore, the beneficial effects of SIRT1 activating compounds on PD model may need to be assessed in various conditions.

Silent information regulation 2 inhibition contributes to reduced α-synuclein toxicity. Pharmacological inhibition or siRNA-mediated inhibition of SIRT2 decreases the number of α-synuclein inclusions in a cellular model of PD (Outeiro et al., 2007). SIRT2 deacetylates α-synuclein, and knockdown of SIRT2 suppresses α-synuclein aggregation and toxicity in a mouse model of PD (de Oliveira et al., 2017). The acetylation of α-synuclein promotes the clearance of α-synuclein inclusions via autophagy and exerts neuroprotective effects in cultured neurons, whereas blocking the acetylation of α-synuclein causes the loss of nigral dopaminergic neurons. Other studies showed that deletion of SIRT2 reduces MPTP-induced neuronal damage in TH-positive cells through increased acetylation of FOXO3a and reduces expression of a proapoptotic factor, Bim, thus blocking the apoptotic pathway (Liu et al., 2012). These results suggest that SIRT2 inhibition may have beneficial effects for PD.

Amyotrophic Lateral Sclerosis (ALS)

Amyotrophic lateral sclerosis is a progressive neurodegenerative disease that affects motor neurons in the brain and spinal cord. ALS is a uniformly fatal disorder and causes death about 2–5 years after onset, mostly because of respiratory paralysis. Mutations in various genes have been identified in ALS, including superoxide dismutase (SOD1) (Rosen et al., 1993) and TAR DNA-binding protein 43 (TDP43) (Arai et al., 2006; Neumann et al., 2006; Kabashi et al., 2008; Sreedharan et al., 2008; Van Deerlin et al., 2008). It has been reported that sirtuin expression is altered in both mouse models of as well as patients with ALS (Kim et al., 2007; Lee et al., 2012; Korner et al., 2013; Buck et al., 2017). Treating with resveratrol or lentivirus-mediated forced expression of SIRT1 protects against neurodegeneration in the SOD1 mutant mouse model of ALS (Kim et al., 2007; Han et al., 2012; Mancuso et al., 2014). SOD mutant mice that show consistent pan-neural expression of exogenous SIRT1 have extended lifespans compared to those without this expression (Watanabe et al., 2014).

SIRT3 has also received attention for its role in ALS. Expression of SIRT3 decreases in the spinal cord and brain stem during the progression of diseases in $SOD1^{G93A}$ mouse, a widely used mouse model of ALS (Buck et al., 2017). In contrast, the mitochondrial isoform of SIRT3 is increased in the muscle and spinal cords of $SOD1^{G93A}$ mice (Salvatori et al., 2017). SIRT3 expression attenuates mitochondrial fragmentation and cell death in neurons from $SOD1^{G93A}$ mice (Song et al., 2013). Although inhibition of SIRT2 is thought to have beneficial effects on neurodegenerative diseases such as PD, the deletion of SIRT2 did not appear to effect the disease course of $SOD1^{G93A}$ mice (Taes et al., 2013). Additional studies will be invaluable to unravel the role of other sirtuins in ALS.

SIRT1 Activator and Clinical Trials

Since accumulating studies have demonstrated the protective effects of SIRT1 against neurodegenerative diseases, potent SIRT1 activators are currently in clinical trials[1]. Clinical trials that are both underway and have been completed have used various proprietary formulations of resveratrol (i.e., SRT501) to treat AD. In one study, treating AD subjects with resveratrol for 52 weeks stabilized the progressive decline in CSF Aβ40 and plasma Aβ40 compared to placebo (Turner et al., 2015). Resveratrol decreased CSF MMP9 and induced adaptive immunity (Moussa et al., 2017), suggesting that resveratrol is beneficial for AD subjects. In addition, there is currently a clinical trial testing resveratrol in HD subjects[2]. However, since SIRT1 is expressed in various tissues, the risk of adverse effects could be considered. To ensure its safety, additional resveratrol and other SIRT1 activator clinical studies are warranted.

CONCLUSION

Sirtuins mediate diverse functions in the CNS. The evidence of the beneficial effects of SIRT1 obtained from animal models and human studies imply that SIRT1 activation can be a potential therapeutic treatment for neurodegenerative diseases. Neuronal degeneration in various traumatic injury and neurological disorders, such as SCI, stroke, and AD, is often accompanied by inflammation. It has been reported that activation of SIRT1 also contributes to the suppression of inflammatory responses. Therefore, SIRT1 activation would be a unique strategy in that it is able to control both neurons and inflammatory cells.

[1]https://clinicaltrials.gov/ct2/results?cond=&term=resveratrol&cntry=&state=&city=&dist=

[2]https://clinicaltrials.gov/ct2/results?cond=Huntington+Disease&term=resveratrol&cntry=&state=&city=&dist=

However, there have also been reports in several animal models of neurodegenerative diseases that SIRT1 activation does not have neuroprotective effects. These conflicting results may be due to a number of factors, including the cell-type-specific alteration of SIRT1 expression following injury.

Understanding the more detailed molecular mechanisms of sirtuins in regulating neuroprotection and degeneration as well as the precise expression patterns of sirtuins following neuronal pathology will contribute to the development of novel anti-neurodegenerative therapeutics. For example, combination treatment with SIRT1 activators and NAD may provide a synergetic strategy that contributes to neuroprotection.

The observation that SIRT2 activity promotes neurodegeneration in a PD model suggests that sirtuins have family-dependent functions. Further studies linking neurodegenerative diseases and sirtuin members, especially SIRT4, 5, and 7, will be helpful to reveal the tissue or disease-specificity of the role of sirtuins. Highly selective compounds targeting specific sirtuins may therefore serve as attractive candidates for a variety of neurological conditions.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

FUNDING

This work was supported by KAKENHI from the Japan Society for the Promotion of Science (JSPS) (17H05767) to YF.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Endocrinology

MINI REVIEW
published: 23 October 2018
doi: 10.3389/fendo.2018.00605

The Essential Role of SIRT1 in Hypothalamic-Pituitary Axis

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The endocrine system plays an essential role in the physiological adaptation to malnutrition. The adaptive response of various hormones directs the energy utilization toward the survival functions and away from growth and reproduction. Particularly, the hypothalamic pituitary axis plays an integral and a central role in the regulation of endocrine organs. Sirtuin 1 (SIRT1) is a nicotinamide adenine dinucleotide (NAD)-dependent histone deacetylase that is activated in response to calorie restriction (CR). SIRT1 is involved in cellular processes via the deacetylation of histone as well as various transcription factors and signal transduction molecules and thereby modulates the endocrine/metabolic functions. There is much evidence to demonstrate clearly that SIRT1 in the hypothalamus, pituitary gland, and other target organs modifies the synthesis, secretion, and activities of hormones and in turn induces the adaptive responses. In this review, we discussed the role of SIRT1 in the hypothalamic pituitary axis and its pathophysiological significance.

Keywords: SIRT1, adaptation, calorie restriction, hypothalamus, pituitary

OPEN ACCESS

Edited by:
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Specialty section:
This article was submitted to Cellular Endocrinology, a section of the journal Frontiers in Endocrinology

Received: *27 June 2018*
Accepted: *24 September 2018*
Published: *23 October 2018*

Citation:
Yamamoto M and Takahashi Y (2018) The Essential Role of SIRT1 in Hypothalamic-Pituitary Axis. Front. Endocrinol. 9:605. doi: 10.3389/fendo.2018.00605

INTRODUCTION

The Physiological Role of Sirtuin 1 (SIRT1)

SIR2 is the first sirtuin protein to be discovered in *Saccharomyces cerevisae* (yeast) (1). SIR2 orthologs are highly conserved throughout various species including mammals, plants, bacteria, worms, flies, and fishes. In mammals, the seven SIR2 orthologs are termed SIRT1-7 and are presumed to be ubiquitously expressed in all the tissues (2). Sirtuins were initially reported as protein deacetylases and ADP ribosyltransferases (3, 4). However, subsequent analyses revealed that sirtuins exhibit various enzymatic activities. For instance, SIRT5 exhibits lysine-desuccinylase and -demalonylase activities (5, 6). These enzymatic activities of sirtuins are generally activated in response to CR by the utilization of nicotine adenine dinucleotide (NAD+) as the co-substrate.

Regarding the intracellular localization of sirtuins, SIRT1, SIRT6, and SIRT7 are localized in the nucleus and mostly regulates protein function by the post-translational modification via histone deacetylation (7). Moreover, SIRT1 localizes in the cytosol and directly deacetylates various transcription factors and cofactors. SIRT2 is localized in the cytosol and nucleus and is involved in the metabolic process and cell cycle regulation (8–11). SIRT3, SIRT4, and SIRT5 are localized in the mitochondria and regulate various metabolic enzyme activities and mitochondrial oxidative stress (9). Therefore, in the organisms, sirtuins coordinate the cellular responses to adapt to CR in their corresponding cellular compartments in which they occur.

Among the seven sirtuins, SIRT1 is the most studied and well-characterized sirtuin with respect to its physiological functions. SIRT1 is involved in the production of various hormones and maintenance of homeostasis by regulating the function of histones and transcription factors, to adapt to malnutrition in the endocrine and metabolic systems. For instance, SIRT1 modulates forkhead box protein O1 (FOXO1), peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α), signal transducer and activator of transcription (STAT3) (12–14). SIRT1 interacts with peroxisome proliferator-activated receptor (PPARγ) and represses its transcriptional activity to reduce adipogenesis in differentiated fat cells and mobilize free fatty acid from the white adipose tissues (15). Additionally, SIRT1 negatively regulates the mitochondrial uncoupling protein 2 (UCP2) expression and enhances the insulin secretion in response to glucose level elevation in the pancreas (16).

Recently, accumulating evidence suggests that SIRT1 plays important role in the homeostasis maintenance in the neuroendocrine system. Particularly, the hypothalamic pituitary axes play a central and an integral role in the neuroendocrine system.

PHYSIOLOGY OF THE HYPOTHALAMUS AND PITUITARY GLAND

The neuroendocrine system, especially the hypothalamus and pituitary gland play an essential role in the homeostasis maintenance via orchestrating the endocrine system as a regulatory machinery. The hypothalamus–pituitary complex connects the nervous system with the endocrine system that regulates the systemic hormone secretion in a direct or indirect manner. The neural signaling of central nervous system modifies the hypothalamus–pituitary axis and results in the regulation of corresponding target hormonal secretion (17).

The hypothalamus is responsible for the homeostasis maintenance by regulating the body temperature, appetite, thirst, energy expenditure, behavior, and circadian rhythm of the organisms (18). Moreover, the hypothalamus synthesizes and secretes various hypothalamic hormones that in turn stimulate or inhibit the secretion of pituitary hormones (19). The medial preoptic nucleus regulates the release of gonadotropic hormones and thermogenesis (20). The putative anterior paraventricular (aPV), the paraventricular nucleus (PVN), and the supraoptic nucleus (SON) consist of parvicellular neurons that release corticotropin-releasing hormone (CRH), thyrotropin-releasing hormone (TRH), growth hormone releasing hormone (GHRH), and somatostatin (SST), and magnocellular neurons that produce vasopressin (AVP) and oxytocin (OXT) (21–24).

The pituitary gland consists of anterior posterior lobes and the anterior lobe is functionally subdivided into six cell types based on the production of a specific hormone by each cell type (25). The growth hormone (GH), prolactin, adrenocorticotropic hormone (ACTH), thyroid stimulating hormone (TSH), and luteinizing hormone (LH) or follicular stimulating hormone (FSH) are secreted by the somatotroph, lactotroph, corticotroph, thyrotroph, and gonadotroph, respectively (26). The anterior pituitary hormones are mainly regulated by the release hormones or inhibitory hormones from the hypothalamus via the portal hypophyseal circulation into the anterior lobe and neural connection to the posterior lobe through the pituitary stalk (a physical and functional connection between the hypothalamus and pituitary gland) (27, 28). The hormones secreted by the pituitary gland circulate in the systemic blood flow and thereby stimulate the hormone secretion by each of their respective target organ. Generally, these pathways initiating from the hypothalamus to target organ via the pituitary gland are mainly classified into four axes depending on the target organ, namely, hypothalamus-pituitary-adrenal (HPA), hypothalamus-pituitary-thyroid (HPT), hypothalamus-pituitary-gonadal (HPG), and somatotropic axes. In this review, we described the SIRT1 involvement in each of the aforementioned axes in the subsequent subsections.

SIRT1 in Hypothalamus

In the hypothalamus, SIRT1 is expressed in the SF1 neuron in the ventromedial hypothalamic nucleus (VMH) and in the POMC and AgRP neuron in the arcuate nucleus (ARH), respectively (29–31). POMC neuron specific SIRT1 knockout mice exhibited energy imbalance due to the altered sympathetic activity (30). Also, deletion of SIRT1 in SF1 neurons caused insulin resistance in skeletal muscle. On the contrary, SIRT1 overexpression in SF1 neurons prevented from diet-induced obesity and insulin resistance (29). Both targeted overexpression of SIRT1 in POMC or AgRP neurons prevented age-associated weight gain. However, SIRT1 overexpression in POMC neurons enhanced the energy expenditure mediated by increment of sympathetic activity in adipose tissue while SIRT1 overexpression in AgRP neurons suppressed food intake, indicating a presence of nuclear specific mechanisms (31). Also, food restriction increases SIRT1 protein levels in the dorsomedial (DMH) and lateral hypothalamic nuclei (LH) (32). Brain specific SIRT1 overexpressing mice exhibited extended life span mediated by enhanced neural activity in DMH and LH, through increased orexin type 2 receptor (Ox2r) expression (32). These data indicate that region specific expression of SIRT1 plays an important role in the regulation of appetite, energy expenditure, general metabolism, and life span.

The HPA Axis

The HPA axis is an important neuroendocrine system that plays an essential role in the survival, stress response, metabolism, appetite, immunoreaction, mood, and behavior of the organisms. Corticotrophin releasing hormone (CRH) secreted by the PVN in the hypothalamus initiates stress response in the HPA axis via the production of proopiomelanocortin (POMC) by the pituitary corticotroph. POMC is a precursor polypeptide of ACTH and alpha-melanocyte stimulating hormone (αMSH) that are cleaved by prohormone convertases 1 and 2 (PC1 and PC2), respectively. ACTH induced by corticotroph stimulates the glucocorticoid production by the adrenal cortex.

The SIRT1 expression in the hypothalamic POMC neuron is enhanced by fasting and is associated with the reduced αMSH levels, although the significance to the HPA axis is

unknown (33). On the other hand, another group reported that POMC neuron specific ablation of SIRT1 did not alter POMC, ACTH, and αMSH levels (30). The precursor pro-CRH is post-translationally processed by the PC1 and PC2. SIRT1 increases the PC2 levels in the PVN that in turn increases the active-CRH production and results in the HPA axis activation (34). In mouse corticotroph cell line (AtT20 cells), treatment with resveratrol (a SIRT1 activating compound) increased the PC1 and PC2 levels. In accordance with this, Ex-527 (a SIRT1 specific inhibitor) treatment decreased the PC1 and PC2 levels in AtT20 cells (33). Although there have been no reports demonstrating the expression of SIRT1 in the corticotrophs, these data suggest that SIRT1 may indirectly modulates the HPA axis by regulating the PC1 and PC2 levels. Additionally, resveratrol increased the expression and prolonged the half-life of P450 side chain cleavage enzyme (P450scc) in the adrenal gland that results in an increase in glucocorticoid secretion by the adrenal cortex (35). Moreover, the hypothalamic SIRT1 plays an important role in the energy homeostasis maintenance associated with the HPA axis. These data clearly indicate that SIRT1 in the hypothalamus, pituitary, and adrenal gland regulates the HPA axis activation that acts as an adaptive response to starvation (**Table 1**A).

The HPT Axis

The HPT axis is activated by the thyrotropin-releasing hormone (TRH) secretion by the hypothalamus when the hypothalamus senses low levels of circulating thyroid hormone. The TRH induces thyroid-stimulating hormone (TSH) secretion by thyrotroph cells in the pituitary that in turn stimulates the thyroid hormone (TH) secretion by the thyroid to maintain normal TH levels (36). SIRT1 is expressed in thyrotroph cells and enhances TSH endocytosis via the deacetylation of phosphatidylinositol-4-phosphate 5-kinase type 1γ (PIP5K1γ), which is a main enzyme that synthesizes phosphatidylinositol 4,5-bisphosphate in thyrotroph cells (37). Tyrotroph-specific SIRT1 knockout mice showed decreased TSH secretion resulting in decreased metabolic rate (37). Additionally, SIRT1 knockout in whole body exhibited hypermetabolism caused by an increased oxygen consumption in the hepatic mitochondria leading to the decreased body weight while the mice manifested hyperphagia, reduced serum thyroxin level, and decreased nocturnal physical activity (38). Interestingly, thyroxin treatment suppressed the fasting-induced SIRT1 expression as thyroxin negatively regulates the SIRT1 level and activity in the liver via TH receptor-β (39). These data indicate that the HPT axis and SIRT1 interact with each other and adaptively regulate metabolism (**Table 1**B).

The HPG Axis

The HPG axis is responsible for the reproduction, life cycle, and sexual dimorphism in the organisms. Gonadotropin-releasing hormone (GnRH) is secreted by GnRH neurons that diffusely localize and form a network named pulse-generator in the hypothalamus. The pulsatile secretion of GnRH stimulates the luteinizing hormone (LH) and follicle-stimulating hormone (FSH) secretion by pituitary gonadotroph cells and subsequently the gonads produce estrogen or testosterone (36, 40).

Although secondary effect of the general condition including body weight loss cannot be ruled out, SIRT1 knockout mice exhibited a diminished hypothalamic GnRH expression and in turn reduced serum LH and FSH levels and spermatogenesis arrest, suggesting an important role of SIRT1 in the HPG axis (41). Additionally, GnRH treatment decreases SIRT1 level via the miR-132/212 induction in the pituitary. This results in the downregulation of SIRT1-dependent FOXO1 deacetylation and a decrease in the FOXO1-mediated inhibition of Fshβ transcription that ultimately increases the Fshβ expression in rat primary pituitary cells and LβT2 cell line (42) (**Table 1**C).

The Somatotropic Axis

In the somatotropic axis, GH releasing hormone (GHRH) that is secreted by the hypothalamus induces the GH secretion by pituitary somatotroph cells. Circulating GH binds to GH receptor on hepatocytes and results in the increased serum insulin like growth factor-I (IGF-I) level (36). Moreover, GH induces local IGF-I production in various tissues including bone, muscle, and fat tissue. In various species, numerous evidences demonstrated that the reduced function of somatotropic axis extends lifespan in

TABLE 1 | The role of SIRT1 in hypothalamic-pituitary axis.

	A HPA axis		B HPT axis		C HPG axis		D Somatotrophic axis	
SIRT1	↑	↓	↑	↓	↑	↓	↑	↓
Hypothalamus	PC2 ↓ CRH ↓	POMC ↓				GnRH ↓		
Pituitary	a-MSH ↓ PC1/ PC2 ↓	PC1/ PC2 ↓	TSH ↑			LH ↓ FSH ↑	GH ↓	GH ↓
Periphery	Adrenal GC ↑			Thyroid T4 ↓		Testis Testosterone ↑	IGF-I ↓	IGF-I ↓

(A) HPA axis, (B) HPT axis, (C) HPG axis, (D) somatotrophic axis.

SIRT1 ↑, Overexpression/activation of SIRT1. SIRT1 ↓, Knockout/inhibition of SIRT1. GC, Glucocorticoid, T4, Thyroxine.

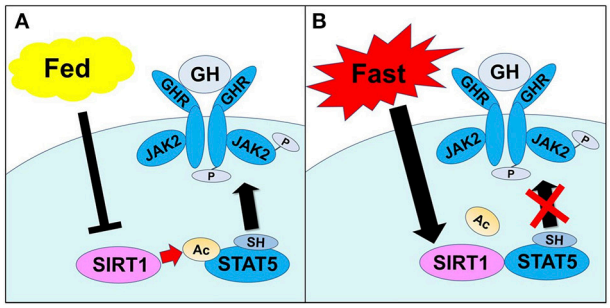

FIGURE 1 | The mechanisms through which SIRT1 regulates STAT5 activation by GH. **(A)** In the fed condition, the SH2 domain of STAT5 recognizes and binds to Tyr-phosphorylated GHR, causing JAK2 to phosphorylate and activate STAT5. **(B)** In the fasting condition, SIRT1 is activated and interacts with STAT5, thereby deacetylating Lys residues adjacent to the SH2 domain of STAT5. This results in an impaired ability to bind Tyr-phosphorylated GHR, which inhibits activation of STAT5. Excerpt from, Yamamoto et al. (60).

animal models (43–48). Regarding the underlying mechanisms, among the species which evolved from C. elegans to mouse, an evolutionarily conserved interplay between SIR2/SIRT1 and the somatotropic axis was reported in which SIR2/SIRT1 modulates the signaling molecules of somatotropic pathway (49–54).

SIRT1 brain-specific knockout (BSKO) mice exhibited dwarfism with small pituitary and reduced GH-IGF-I levels while the other pituitary hormonal levels were unaltered (55). Interestingly, SIRT6 BSKO manifested similar phenotype as that of SIRT1 BSKO. Despite the significant reduction in the number of somatotroph cells and GH content in the pituitary gland of SIRT6 BSKO, the hypothalamic GHRH and somatotropin release–inhibiting factor (SRIF) levels remained unaltered (56). Although the precise mechanism remains unclear in these models, it is speculated that aberrations during the pituitary somatotroph development and GH synthesis were presumably caused by the hypothalamic dysfunction or feedback dysregulation between the hypothalamus and pituitary gland.

Moreover, the direct role of SIRT1 in pituitary somatotroph cells was reported (57). The SIRT1 activation in somatotroph cells suppressed the GHRH-induced GH secretion in *in vivo* and *in vitro* studies. SIRT1 deacetylates glycogen synthase kinase 3 beta (GSK3β) and cAMP response element-binding protein (CREB). Deacetylated-GSK3β gets activated and inactivates CREB via protein phosphatase-1. Deacetylated-CREB exhibits a decrease in its activity and is unable to activate the transcription of POU domain, class 1, transcription factor 1 that results in the GH synthesis impairment (57). These data demonstrate that SIRT1 regulates the somatotropic axis in the hypothalamus and pituitary gland.

During starvation condition, it is well-known that the reduced serum IGF-I level is observed despite the elevated GH level. Moreover, it is reported that exogenous GH treatment did not increase the serum IGF-I level in starving individuals (58). This is considered as GH resistance status in the liver (59). In the organisms, it is considered that GH resistance is an adaptive response to survive during starvation and malnutrition conditions. These responses include the decreased IGF-I level inhibits growth and the elevated GH level causes insulin resistance and free fatty acid mobilization to avoid hypoglycemia. Therefore, we hypothesized that SIRT1 might negatively regulate the GH-dependent IGF-I production during starved condition in the liver. We demonstrated that hepatic SIRT1 directly deacetylates STAT5 and suppresses the tyrosine phosphorylation of STAT5 via GH that results in the impairment of GH signaling during fasting condition (60) (**Figure 1**).

Considering that the somatotropic axis utilizes energy to promote body growth, SIRT1 signaling basically utilizes energy to improve the survival of organisms during CR. Conclusively, SIRT1 plays an important role in switching from growth to survival mode in order to adapt to malnutrition by modulating the somatotropic axis at various steps (**Table 1**D).

CONCLUSION

These studies clearly demonstrated that SIRT1 is involved in the regulatory mechanism of hypothalamus-pituitary axis with respect to the homeostasis maintenance. The determination of a crosstalk between the neuroendocrine system and SIRT1 function is crucial as both of them play an important role in the homeostasis maintenance as well as the regulation of lifespan.

AUTHOR CONTRIBUTIONS

MY drafted the manuscript and MY and YT were responsible for the conception and design of this review.

FUNDING

This work was supported in part by a Grant-in-Aid for Scientific Research from the Japanese Ministry of Education, Culture, Sports, Science and Technology (23591354, 23659477, 26670459 [YT]), and, a grant from the Japan Agency for Medical Research and Development (AMED) (17bm0804012h0001), and by the Uehara Memorial Foundation and the Naito Foundation [YT].

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Endocrinology

REVIEW
published: 17 October 2018
doi: 10.3389/fendo.2018.00624

SIRT1 Is a Potential Drug Target for Treatment of Diabetic Kidney Disease

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Multiple studies have demonstrated a critical role of Sirtuin-1 (SIRT1) deacetylase in protecting kidney cells from cellular stresses. A protective role of SIRT1 has been reported in both podocytes and renal tubular cells in multiple kidney disease settings, including diabetic kidney disease (DKD). We and others have shown that SIRT1 exerts renoprotective effects in DKD in part through the deacetylation of transcription factors involved in the disease pathogenesis, such as p53, FOXO, RelA/p65NF-κB, STAT3, and PGC1α/PPARγ. Recently we showed that the podocyte-specific overexpression of SIRT1 attenuated proteinuria and kidney injury in an experimental model of DKD, further confirming SIRT1 as a potential target to treat kidney disease. Known agonists of SIRT1 such as resveratrol diminished diabetic kidney injury in several animal models. Similarly, we also showed that puerarin, a Chinese herbal medicine compound, activates SIRT1 to provide renoprotection in mouse models of DKD. However, as these are non-specific SIRT1 agonists, we recently developed a more specific and potent SIRT1 agonist (BF175) that significantly attenuated diabetic kidney injury in type 1 diabetic OVE26 mice. We also previously reported that MS417, a bromodomain inhibitor that disrupts the interaction between the acetyl-residues of NF-κB and bromodomain-containing protein 4 (BRD4) also attenuates DKD. These results suggest that SIRT1 agonists and bromodomain inhibitors could be potential new therapeutic treatments against DKD progression.

Keywords: SIRT1, acetylation, diabetic kidney disease, bromodomain inhibitor, podocytes

OPEN ACCESS

Edited by:
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Reviewed by:
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Specialty section:
This article was submitted to Cellular Endocrinology, a section of the journal Frontiers in Endocrinology

Received: *03 July 2018*
Accepted: *01 October 2018*
Published: *17 October 2018*

Citation:
Zhong Y, Lee K and He JC (2018) SIRT1 Is a Potential Drug Target for Treatment of Diabetic Kidney Disease. Front. Endocrinol. 9:624. doi: 10.3389/fendo.2018.00624

INTRODUCTION

Sirtuin family of nicotinamide adenine dinucleotide (NAD^+)-dependent deacetylases, a homolog of yeast Sir2 (silent mating type information regulation 2), has been shown to play an important role in a variety of cellular functions. The mammalian Sir2 ortholog, Sirtuin-1 (SIRT1) is upregulated by caloric restriction and mediates the longevity effect of calorie restriction by regulation of glucose and lipid metabolism (1, 2). At the cellular level, SIRT1 regulates variety of processes including autophagy (3), energetic homeostasis (2), mitochondrial biogenesis (4), and apoptosis (5). A large body of evidence suggests that SIRT1 plays a major role in various kidney diseases by providing protection against cellular stresses associated with kidney injury (6–8). Here, we provide an overview of the role of SIRT1 in kidney cells in the context of diabetic kidney disease (DKD), with a focus on its role on the regulation of transcription factor activation. The review also discusses the potential new therapies by targeting SIRT1 pathway for DKD.

ROLE OF SIRT1 IN REGULATION OF TRANSCRIPTION FACTOR ACETYLATION

Recent evidence suggests that transcription factor activation is regulated not only by protein phosphorylation, but also by acetylation. SIRT1 exerts biological effects not only through deacetylation of histones, but also deacetylation of various transcription factors that include p53, FOXO, RelA/p65, STAT3, PGC1α, and PPAR-γ (9), thereby leading to transcription repression. SIRT1 regulates p53 activity through deacetylation (10–13) and promotes cell survival through suppression of p53-dependent apoptosis in response to DNA damage and oxidative stress (5). SIRT1 was also shown to regulate the activities of FOXO family of transcription factors through deacetylation (14). Deacetylation of FOXO3 by SIRT1 enhances its ability to induce cell cycle arrest and resistance to oxidative stress, while inhibiting its ability to induce cell death (14, 15). We also showed that SIRT1 inhibits podocyte apoptosis through deacetylation of FOXO4 (16, 17). Several studies suggest that the transcriptional activity of signal transducer and activator of transcription 3 (STAT3) is also negatively regulated by SIRT1 (18–20). SIRT1 was found to cause the deacetylation and inactivation of STAT3 during caloric restriction (21). SIRT1 also exerts anti-inflammatory effects through the inhibition of NF-κB pathway. It was shown that the duration of nuclear NF-κB action is highly regulated by reversible acetylation (22, 23), and that SIRT1 inhibits the NF-κB signaling pathway through deacetylation of p65 (24). In addition, SIRT1 modulates cellular response to hypoxia via deacetylation of hypoxia-induced factor 1α (HIF-1α) (25–27). All these highlight an important transcription modulatory function by SIRT1 activity.

SIRT1 PROVIDES RENOPROTECTION AGAINST DKD

Diabetic kidney disease (DKD) is the leading cause of chronic kidney disease and end-stage renal failure in the US (28). Even with optimal therapy, the incidence of DKD remains high, as none of the currently available therapy can reverse or completely forestall the progression of DKD. Therefore, development of more effective treatment for DKD is urgently required. The important role of SIRT1 in DKD has been demonstrated by number of studies (**Table 1**). We have previous shown that SIRT1 expression is significantly reduced in human kidney with DKD and that its reduction is more pronounced in the glomerular compartment than in the tubular compartment (17). An association between single nucleotide polymorphisms within *SIRT1* gene and DKD was observed in Japanese subjects with type 2 diabetes (40). However, the exact mechanism of regulation of SIRT1 expression in DKD remains unclear. On the cellular level, SIRT1 has been shown to regulate autophagy (41, 42) and oxidative stress response in the diabetic kidneys (35). Resveratrol was shown to attenuate DKD through activation of AMPK/SIRT1 pathway (29, 31) and by modulating angiogenesis (43). Studies have demonstrated a clear role of SIRT1 in renal tubular cells in the setting of acute kidney injury (6, 44). In diabetic kidneys, it was shown that reduced proximal renal tubular SIRT1 expression contributes to albuminuria by upregulation of the tight junction protein Claudin-1 in podocytes (32). Interestingly, reduction in *Sirt1* expression in tubular cells induced hypomethylation of the *claudin-1* gene in podocytes to promote its expression, while overexpression of *Sirt1* in tubular cells induced hypermethylation of *claudin-1* and downregulated expression in podocytes, indicating an important cross-talk between the two cell types and epigenetic regulation of Claudin-1 expression by SIRT1. Work from our laboratory also demonstrated a critical role of SIRT1 in podocyte injury in DKD. We showed that either knockdown or knockout of *Sirt1* specifically in podocytes aggravated DKD injury in type 2 diabetic *db/db* mice (33) and in STZ-induced diabetic mice (34). Importantly, our recent study demonstrated that the podocyte-specific overexpression of SIRT1 was sufficient to significantly attenuate podocyte injury and to impede DKD progression in type1 diabetic OVE26 mice. Together, these studies clearly demonstrate a protective role of SIRT1 against DKD in experimental models of both type 1 and type 2 diabetes.

RENOPROTECTIVE MECHANISMS OF SIRT1 IN DKD

As the cellular and molecular mechanisms of SIRT1 has been recently reviewed (42, 45, 46), as well as the role and mechanism of other sirtuins in kidney disease (47), this review is focused primarily on its modulation of transcription factor through deacetylation in the setting of DKD.

Effects of SIRT1 in Inflammation in Diabetic Kidneys Through NF-κB and STAT3 Deacetylation

Many studies suggest that SIRT1 regulates activity of several transcription factors that regulate kidney cell homeostasis and are involved in pathogenesis of DKD through deacetylation. Systems biology analysis of microarray data suggests that JAK-STAT and NF-κB are key inflammatory pathways activated in diabetic kidneys (48, 49). Recently, we showed that the acetylation of STAT3 and RelA/p65 is increased in kidneys from diabetic patients and mouse models (33). More importantly, we demonstrated that the podocyte-specific knockout of *Sirt1* in *db/db* mice led to higher levels of p65 and STAT3 acetylation and resulted in greater degree of proteinuria and kidney injury than in control *db/db* mice, implicating SIRT1 as a key inhibitor of the NF-κB- and STAT3-induced inflammatory responses in DKD (33). In addition, we found that expression of the key pro-inflammatory factors mediated by NF-kB and Stat3 were also increased in the kidney of Sirt1 knockout *db/db* mice, further confirming a key role of Sirt1 in regulation of inflammation in the diabetic kidney.

Effects of SIRT1 in Cell Death in Diabetic Kidneys Through p53 and FOXO4 Deacetylation

Several lines of evidence indicate that p53 mediates apoptosis of both podocytes and tubular epithelial cells in DKD (50–52). SIRT1 has been shown to promote cell survival by suppressing p53-dependent apoptosis in response to DNA damage and

TABLE 1 | Summary of the *in vivo* studies of SIRT1 in DKD.

Approaches	Animal models	Tissue/Cell types	Mechanisms regulated	References
Dietary restriction	Diabetic Wistar fatty (fa/fa) rats	Whole kidney	Inflammatory; autophagy	(29)
Resveratrol	db/db mice	Whole kidney	Oxidative stress	(30)
Resveratrol	db/db mice	Whole kidney	AMPK/PGC1a; Oxidative stress	(31)
nicotinamide mononucleotide	STZ-induced diabetic and db/db mice	Tubule/podocyte crosstalk	Epigenetics, Claudin-1	(32)
Sirt1 knockout	Db/db mice	Podocytes	Inflammation; apoptosis	(33)
Pyridoxamine				
Sirt1 knockdown	STZ-induced diabetic mice	Podocytes	Mitochondria; senescence	(34)
hnRNP F	db/db mice	Tubular cells	Oxidative stress	(35)
Glycyrrhizic Acid	db/db mice	Whole kidney	AMPK/PGC-1a	(36)
Puerarin	STZ-induced diabetic eNOS-null mice	Podocytes	Oxidative stress; inflammation	(37)
Tangshen formula	STZ-induced diabetic rats	Whole kidney	NF-kB/inflammation	(38)
Sirt1 overexpression; agonists	OVE26 mice	Podocytes	Mitochondrial function; apoptosis	(39)

oxidative stress (5). The interplay of SIRT1-p53 pathway also controls cellular senescence (53–55). We reported previously that advanced glycation endproducts (AGEs) induce podocyte apoptosis through FOXO4-mediated Bim expression and that acetylation of FOXO4 is critical for mediating this effect (17). Overexpression of SIRT1 inhibited AGE-induced FOXO4 acetylation and podocyte apoptosis.

Effects of SIRT1 in Mitochondrial Dysfunction and Fibrosis in Diabetic Kidneys Through of PGC-1α and Smad3 Deacetylation

SIRT1 has also been shown to regulate PGC-1α activity and to play an important role for maintenance of mitochondrial function in podocytes (56). The PGC-1α in regulation of mitochondrial function has been well described for neurodegenerative disorders (57). Both mitochondrial injury and cellular senescence are key pathological processes mediating kidney injury (58–60). Consistent with this, we have shown recently SIRT1 deficiency in podocytes aggravates aging-related kidney disease through enhanced cells senescence and mitochondrial dysfunction (61). Although the effects of SIRT1 on Smad3 acetylation remain to be determined, resveratrol was shown to affect acetylation but not phosphorylation of Smad3 to inhibit TGF-β1-induced up-regulation of collagen IV and fibronectin mRNA levels *in vitro* and renal fibrosis in the model of unilateral ureteral obstruction (UUO) *in vivo* (62). Therefore, it is plausible that increased SIRT1 activity may also attenuate renal fibrosis in DKD. Taken together, these studies suggest that SIRT1, as a negative regulator of inflammation, cellular senescence and mitochondrial dysfunction, is a key repressor of DKD pathogenesis.

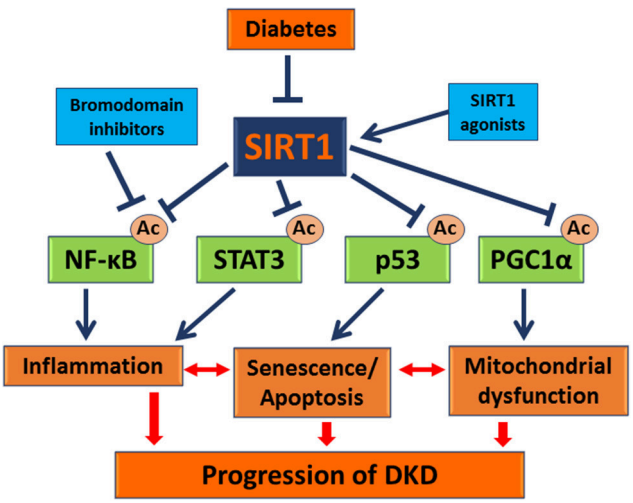

FIGURE 1 | Role of SIRT1 in DKD pathogenesis. This schema summarizes how Sirt1 mediates podocyte injury in DKD. The data suggest that Sirt1 expression is reduced in the diabetic glomeruli including podocytes. Reduced SIRT1 expression leads to increased acetylation and activation of transcription factors, such as NF-κB, STAT3, p53, and PGC1α, leading to exacerbated inflammation, senescence/apoptosis, and mitochondrial dysfunction of kidney cells such as podocytes (shown in blue arrows). All these processes interact each other and contribute to the progression of DKD (shown in red arrows). Therefore, SIRT1 agonists or inhibition of transcription factor acetylation through use of bromodomain inhibitors will reverse these diseased processes and could be developed to treat DKD. (→): stimulation; (⊣): inhibition; (↔): interaction.

SIRT1 IS A POTENTIAL DRUG TARGET FOR TREATMENT OF DKD

Given that SIRT1 is a key mediator in thwarting the progression of DKD and other kidney diseases, development of therapeutic strategies to specifically restore SIRT1 activity is warranted. In support of this, we recently demonstrated that increased SIRT1 expression in podocytes attenuated albuminuria and glomerular injury in OVE26 diabetic mice (39). As SIRT1 expression is reduced in diseased kidneys, identifying the molecular basis of its suppression in diabetic kidneys and to interfere in this process may be an avenue of therapeutic approach. We previously showed that increased advanced glycation endproducts (AGEs)

in in the diabetic milieu contribute to reduced SIRT1 expression in podocytes (17). Inhibition of AGE formation by pyridoxamine *in vivo* restored SIRT1 expression the glomeruli of *db/db* mice and attenuated podocyte injury and progression of DKD (17). Similar observations of SIRT1 reduction by AGEs were made in mesangial cells *in vitro* (63).

Another approach would be to stimulate SIRT1 activity through SIRT1 agonists. Resveratrol is a well-known SIRT1 agonist that has shown to improve DKD in several animal models (30, 31). However, recent reports indicate that resveratrol may not be a SIRT1-specific (64), nor are other purported SIRT1 agonists, such as SRT1720, SRT2183, and SRT1460 (65). We also showed that puerarin, an extract from a Chinese herbal medicine, attenuates diabetic kidney injury through activation of SIRT1 and suppression of NOX4 expression in podocytes in experimental diabetic mouse model (62). Other herbal medicines or compounds have also been reported to improve DKD through activation of SIRT1 (36, 38). Metformin is reported to improve podocyte function by activating SIRT1 (66). However, SIRT1-independent effects on podocytes or in DKD by non-specific SIRT1 agonists or metformin cannot be ruled out. Recently, we developed a new potent and selective SIRT1 agonist, BF175 (39). In cultured podocytes BF175 increased SIRT1-mediated activation of PGC1-α and protected against high glucose-mediated mitochondrial injury. *In vivo*, administration of BF175 for 6 weeks in type 1 diabetic OVE26 mice resulted in a marked reduction in albuminuria and in glomerular injury in a manner similar to podocyte-specific SIRT1 overexpression. BT175 treatment also attenuated diabetes-induced podocyte loss and reduced oxidative stress in glomeruli of OVE26 mice. Therefore, BT175 and its analogs could be developed as novel therapeutic strategy to treat DKD. However, these approaches of targeting SIRT1 are not without limitations. As discussed above, the specificity of the SIRT1 agonists remains a concern. Given the heterogeneity of SIRT1 function, the ever expanding list of its substrates, and the different effects of deacetylation on its target protein functions, it's possible that the beneficial effects of SIRT1 are mixed with potentially pernicious side effects.

As SIRT1 exerts its renoprotective effects through deacetylation of key transcription factors (TFs) involved in DKD, another therapeutic approach may be to directly regulate the transcription factor acetylation through bromodomain inhibitors (BrDi). Acetylated lysines of the key TFs involved in DKD pathogenesis such as p65 NF-κB interact with proteins containing bromodomains (67), and BrDi could suppress their acetylation in a more specific manner. For instance, NF-κB transcriptional activity is dependent upon its acetylation at lysine 310 (Lys310), and Lys310-acetylated p65 NF-κB recruits the BET protein BRD4 in complex with positive transcription elongation factor b (p-TEFb) and RNA polymerase II that together form a productive transcriptional machinery complex (68). We reported that a BET-specific BrDi MS417 suppresses TNF-α-induced acetylation of p65 NF-κB and the expression of NF-κB target genes in kidney cells *in vitro* and attenuates proteinuria and glomerulosclerosis in a mouse model of HIV-associated nephropathy *in vivo*. MS417 also inhibited AGE-induced acetylation of p65 NF-κB in podocytes *in vitro* and mitigated proteinuria in diabetic *db/db* mice. Therefore, MS417 or other BrDi might be another class of potential drug candidates to treat DKD patients.

In summary, SIRT1 has significant renoprotective effects against podocyte injury in DKD (**Figure 1**), and SIRT1 agonists and bromodomain inhibitors are promising candidates as therapeutic approach in treatment of DKD patients.

Clinical Perspectives

Since a large amount of evidence suggest that Sirt1 is a key molecule involving in the pathogenesis of DKD and the expression of Sirt1 is suppressed in human diabetic kidney, enhancing the SIRT1-induced transcription factor deacetylation via SIRT1 agonists or bromodomain inhibitors may serve as potential therapies for human DKD.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

ACKNOWLEDGMENTS

YZ is supported by National Natural Science Foundation of China (2015-81573768), Shanghai Shuguang Scholar (2016-6SG37), Shanghai Three Years' Pilot Project (ZY3-CCCX-2-1002, ZY3-CCCX-3-2001), and Award from Chen Yiping's National Research Studio for Famous Chinese Medicine Practitioners; KL is supported by NIH 1R01DK117913; and JH is supported by NIH 1R01DK078897, NIH 1R01DK088541.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Endocrinology

MINI REVIEW
published: 15 October 2018
doi: 10.3389/fendo.2018.00614

High Levels of SIRT1 Expression as a Protective Mechanism Against Disease-Related Conditions

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SIRT1 protein, a member of Silent Information Regulator 2 (Sir2) protein family, have gained considerable attention as epigenetic regulators for a great area in the human physiology. Changes in sirtuin expression are critical in several diseases, including metabolic syndrome, cardiovascular diseases, cancer and neurodegeneration. Here, we provide an overview of the association of the increasing level of SIRT1 protein for regulating some disease related conditions such as obesity, cardiovascular diseases and neurodegeneration. This review also provides a detailed molecular understanding of the interaction of the some basic molecules with increasing SIRT1 levels rather than reduction of the SIRT1 expression. In this context, the current approaches to enhancing the expression of SIRT1 points the importance of epigenetics in several age-related diseases to provide a healthy aging by developing novel therapies which can prevent or damp the progression of some diseases.

Keywords: SIRT1 expression, oxidative stress, metabolic diseases, cardiovascular diseases, neurodegenerative diseases

OPEN ACCESS

Edited by:
Yang Yang,
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Reviewed by:
Suowen Xu,
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Singapore Institute of Technology, Singapore

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Specialty section:
This article was submitted to Cellular Endocrinology, a section of the journal Frontiers in Endocrinology

Received: *31 July 2018*
Accepted: *27 September 2018*
Published: *15 October 2018*

Citation:
Elibol B and Kilic U (2018) High Levels of SIRT1 Expression as a Protective Mechanism Against Disease-Related Conditions. Front. Endocrinol. 9:614. doi: 10.3389/fendo.2018.00614

INTRODUCTION

Sirtuin 1 (SIRT1) which is encoded by the *SIRT1* gene is the most conserved mammalian nicotinamide adenine dinucleotide (NAD+) dependent histone deacetylase (1). Besides its role being a target for histone and non-histone proteins, SIRT1 functions as a transcription factor for many different physiological processes (2). According to the previous experiments which were performed using yeast, worms and flies as model organisms, sirtuins were accepted as evolutionarily conserved epigenetic mediators of longevity (3–5). In addition to the key role on extending life by regulating the response to some conditions such as fasting, caloric restriction and exercise, SIRT1 regulates many endocrine functions, protects organism from oxidative stress-related cellular events, promotes DNA stability, and decreases various age-related disorders, such as neurodegenerative disease, metabolic abnormalities, and cancer (6–9).

SIRT1 protein is expressed in most of the body parts including brain, heart, kidney, liver, pancreas, spleen, skeletal muscle, endothelial tissue and white adipose tissue. By expression and activation of SIRT1, modulation of its downstream pathways occurs by targeting several cellular proteins, such as nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), peroxisome proliferators-activated receptor-gamma (PPAR-γ) and its coactivator peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α), protein tyrosine phosphatase (PTP), forkhead transcriptional factors (the FoxO subgroup), adenosine monophosphate activated protein kinase (AMPK), CRE-binding protein regulated transcription coactivator 2 (CRTC2),

endothelial nitric oxide synthase (eNOS), p53, myogenic differentiation (MyoD), liver X receptor (LXR), and transcription factor E2F1 (10, 11). Through its deacetylation activity, SIRT1 modulates functions of these critical molecules and shows its critical and multifaceted roles in cellular physiology (**Figure 1**).

Alterations of the level of SIRT1 expression were determined in several diseases including metabolic diseases, neurodegenerative diseases, cancer and aging. Whereas an increase in the expression of the SIRT1 protein was observed in cancer (12, 13), reductions in the SIRT1 level was more common in other diseases such as Alzheimer's Diseases (AD), Parkinson Disease (PD), obesity, diabetes, and cardiovascular diseases (14–18). Recent developments elucidated the relation between downregulation of SIRT1 levels and disease progression as an increase in the oxidative stress and inflammation (16, 17). For example, due to a significant decrease in SIRT1 levels which correlated with an increase in the oxidative stress parameters, accumulation of Tau proteins in AD, enhancement of acetylated p53 expression levels in coronary artery disease and increase in the fatty acid oxidation in obesity were observed in the patients (15, 17, 19, 20).

Previous studies showed that SIRT1 overexpression significantly increased cell viability, decreased cell apoptosis and reduced the release of pro-inflammatory cytokines (21–24). In addition, the regulation of metabolism and longevity by SIRT1 occurs through controlling the maturation of hypothalamic peptide hormones (25, 26). Specificity for SIRT1 increases in the relevant metabolic pathways in the hypothalamic circuitries which is also associated with altered downstream factors of SIRT1 such as FoxO transcription factors (27, 28). In the light of this information, we reviewed recent findings related to the association of the increasing level of SIRT1 protein rather than reduction of the SIRT1 expression and regulation of some disease related conditions such as obesity, cardiovascular diseases and neurodegeneration. The overarching aim of this paper is to provide a basis for hypothesizing that the level of SIRT1 are mechanistically increased to overcome the dysfunction of SIRT1 activity in the diseased conditions.

SIRT1 AND METABOLIC DISEASES

SIRT1 protein protects the functions of adipose tissue and liver in several aspects (29, 30) such as glucose homeostasis and fat metabolism against severe obesity (31, 32). It is also involved in energy balance and stress. Insulin sensitivity is increased in the pancreatic beta cells which have insulin resistance due to overexpression of SIRT1 (30, 33). The activity of PPARγ which have a role in the storage of glucose and fatty acid in adipose tissue is repressed by SIRT1 (34). During short term fasting, the CRTC2 is also depressed by SIRT1 and thus gluconeogenesis is declined in the liver tissue. During long term fasting, SIRT1 expression deacetylates and activates the PGC-1α to decrease adiposity and lipogenesis and to increase fatty acid oxidation (35–37). In addition, SIRT1 deacetlylates sterol regulatory element binding protein (SREBP), farnesoid X receptor (FXR), as well as liver X receptor (LXR) to increase bile acid production and to reverse cholesterol transport (30, 38). Thus, SIRT1 can be called as a "Master Metabolic Regulator" (30). Indeed, the dysregulation of energy sensing may cause inflammation and insulin resistance. Because of prevention of pro-inflammatory responses, SIRT1 behaves as a positive regulator of insulin in the adipose tissue (39). In one of the recent study, after feeding with high dietary fructose, the liver of rats were investigated in response to SIRT1 expression as a main energy sensing protein (40). However, they demonstrated a significant increase in the SIRT1 expression in the fructose-induced inflammation suggesting compensatory rise in the level of SIRT1 to decline the inflammation-related metabolic reactions (40). In addition, overexpression of SIRT1 in obesity which was formed by high-fat diet protects lipid-induced inflammation and hepatic steatosis while providing better glucose tolerance (41). These favorable effects of SIRT1 may be related with the activation of the antioxidant enzymes and stimulation of PGC1α to decrease the level of pro-inflammatory cytokines (41).

In some neurodegenerative diseases, a number of neuropeptide systems in the hypothalamus are affected from activity of SIRT1 which indicate an impact on metabolism. For example, SIRT1 upregulates the level of orexin receptor specifically in the lateral hypothalamic area and the ventromedial nucleus of the hypothalamus, whereas the expression of orexin and melanin-concentrating hormone is reduced in the hypothalamus due to inhibition of the active state of orexin neurons (25, 42, 43). In addition, SIRT1 also regulates the expression of BDNF in the brain. It was found that increased SIRT1 level diminished BDNF signaling which resulted in severe hyperphagia and obesity both in humans and animals (44, 45). These results showed that compensatory increase in the SIRT1 level to cope with the disease outcomes such as oxidative stress brings some additional metabolic dysfunctions in the body due to altered peptides in the endocrine system.

In addition to the obesity, SIRT1 has a role in the hepatic energy metabolism by modulating it nutritionally and hormonally. This modulation is mostly occurred through the deacetylation of metabolic regulators (46). Previous studies also showed that obese patients with non-alcoholic fatty-liver disease (NAFLD), which is the most common liver disease caused by elevated hepatic lipids, inflammation and oxidative stress, had high plasma levels of SIRT1 producing a potential against the physiological mechanisms related to NAFLD (47). In this type of disease, the action mechanism of SIRT1 acted through the modulation of PPARα activity and fatty acid oxidation (48).

On the other hand, it was found that increase in the SIRT1 activity upregulates genes-related metabolic functions, promotes insulin sensitivity and reduces inflammatory gene expressions in the adipose tissue of diet-induced obese animals (49). In addition, we found a polymorphism in the promoter region of *SIRT1* gene in obese children drawing attention to the association between altered SIRT1 activity and the risk of obesity (50). Previous reports also showed the protective role of SIRT1 on the development of osteoarthritis by upregulation of cartilage extracellular matrix genes and downregulation of matrix-degrading enzymes (51, 52). In addition, increase in the SIRT1 activity had a protective effect against osteoarthritis in

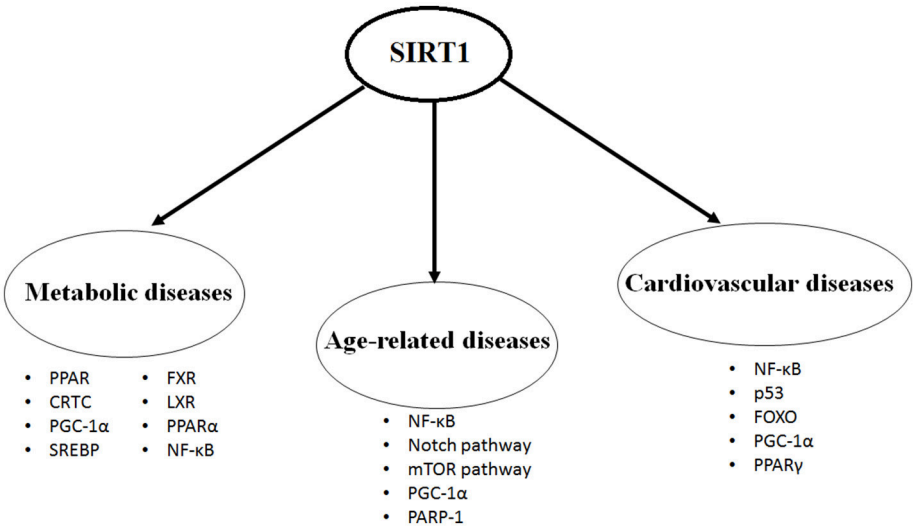

FIGURE 1 | The most studied targets of SIRT1 in the metabolic diseases, age-related diseases and cardiovascular diseases.

animal models (53, 54). Therefore, the investigators suggested that the increase both in the activity and expression of SIRT1 might be a protective strategy for progression of osteoarthritis via the modulation of the NF-κB pathway (55).

SIRT1 AND AGE-RELATED NEURODEGENERATIVE DISEASES

The relationships between SIRT1 and age were investigated in the previous studies related to interaction of lifespan elongation and calorie restriction which is thought as an enhancer for SIRT1 activity (56, 57). Most of these studies showed that calorie intake restriction innervates the extension of life by the inducement of defense of cells against to free radicals and toxins for attenuation of apoptosis or amelioration of cell repair which are desired factors in aging (58–60). That means, altered SIRT1 expression and activity is thought to be a potent way to keep the cells and organs properly functioning for longer times. The positive correlation between age and SIRT1 expression and/or activity may be a compensation against unexpected situations such as oxidative stress (61, 62). For example, in one of our previous study, it was noted higher level of SIRT1 protein in older people compared with the SIRT1 level in the younger people (62). It was thought that increased protein level of SIRT1 in older people may be a compensatory mechanism due to accumulation of oxidative stress-related products and elimination of antioxidant enzyme level in elderly (62). However, increase in the expression does not mean increase in the activity of protein. An oxidative stress-dependent decrease in the SIRT1 activity was noted in aged animals that had high levels of SIRT1 protein (63, 64). The decline in the activity of SIRT1 may not be related directly with SIRT1 protein but also its downstream or upstream molecules such as a decline in NAD+ levels with aging (65).

One of the main risk factor for several neurodegenerative diseases such as Alzheimer's disease (AD) and Parkinson's disease (PD) is age. The common underlying mechanisms of neurodegeneration are increase in the neuroinflammation, mitochondrial damages and oxidative stress (66, 67). In literature, it was shown that sirtuins' hyperactivity could reduce these negative outcomes both *in vivo* and *in vitro* due to its neuroprotective role (68–71). In the AD pathology, SIRT1 deacetylates substrates in favor of the non-amyloidogenic pathway or acts directly on the Aβ and Tau proteins (72). Molecular studies showed that SIRT1 activation prevents the accumulation of Aβ plaques and tau pathology through the NF-κB signaling pathway by upregulation of the *ADAM10* gene, induction of the Notch pathway, and inhibition of the mTOR pathway (20, 73, 74). As shown in previous studies, SIRT1 epigenetically reprograms inflammation taking about AD formation at the earlier stages by altering transcription factors (24, 75, 76). In addition, it was observed that brains of AD patients have consistently reduced NAD+ levels and SIRT1 transcription and/or protein levels involved in chronic inflammation that can also be altered by increased levels of the activated proinflammatory transcription factor NF-κB (77–79). In one of our studies, we found a significant increase in the SIRT1 level of dementia patients (80). Furthermore, in the patients with Huntington's disease (HD), Baldo and his colleagues found higher expression of SIRT1 protein level in the most affected brain regions, especially hypothalamic regions important for metabolic regulation, compared to brain regions which were less affected from the mutant huntingtin protein (28). In PD, SIRT1 inhibits α-synuclein aggregation by deacetylating proteins such as heat shock proteins and PGC-1α and, therefore, it protects dopaminergic neurons against cell death which occur due to the formation of insoluble fibrils called Lewy bodies (81, 82). In the *in vitro* PD model, it was observed that an overexpression of SIRT1 due to application of toxin (rotenone or MPTP) which

causes neurodegeneration was rescued cells from oxidative stress (16, 83). The neuroprotection against to PD occurred by the mechanism of decreasing in the expression of NF-κB and cleaved PARP-1. In a postmortem study, the levels of SIRT1 showed a slight increase in the dementia patients with Lewy bodies (16, 83). However, as seen in the AD, the activity of the SIRT1 protein also decreased in the PD patients producing neurodegeneration in correlation with possible higher oxidative stress, synaptic and cell loss, and neuroinflammation (16).

We thought that the high levels of SIRT1 protein might have a role in alleviating the oxidative stress that is significantly increased in neurodegeneration because an induction of the SIRT1 expression occurs when the organism encounters a biological stress such as aging or an age-related disorder due to the role function of SIRT1 as an important stress sensor molecule.

Also, in literature, it was stated that high levels of SIRT1 may increase the expression of genes related to neuronal protection (84–86). On the other hand, SIRT1 behaves as a double edged sword in response to inflammation which is a cause of neurodegeneration. That means, low levels of SIRT1 cause early acute inflammation-related damages to tissues by increasing NF-kB, and high levels of SIRT1 during late inflammation cause immunosuppression and increased the rate of death (87). In a previous study, investigators observed that increase in the expression level of SIRT1 cannot protect the brain from neurodegeneration without increasing the activity level of SIRT1. For example, Ciriello and his colleagues observed a significant decrease in the level of phosphorylated SIRT1, the active form of SIRT1, in the patients with multiple sclerosis (88). Interestingly, a significant negative correlation between phosphorylated and non-phosphorylated forms of SIRT1 was observed explaining both the SIRT1 overexpression and inactivity of SIRT1 in diseased state. In addition, when a SIRT1-activating molecules were given to the organisms, profound therapeutic benefits and neuroprotective effects were recorded against age-dependent neurodegenerative diseases (89).

SIRT1 AND CARDIOVASCULAR DISEASE

Nowadays, the sirtuin protein family is thought as one of the important target for cardiovascular diseases (CVD). Therefore, the role of SIRT1 protein and its downstream molecules also gains importance in the experimental studies related with CVD development. In cardiomyocytes, during prenatal period, SIRT1 is found in the nucleus, however, it is mostly located in the cytoplasm of myocytes of adult heart of rodents (90). Previously, we found that the level of the SIRT1 expression was significantly higher in the CVD patients compared to the levels of SIRT1 in healthy subjects pointing the cross-talk between SIRT1 protein expression and reactive oxygen species (61). In this previous study, we also found a significant increase in the oxidative stress parameters which may be an inducer for SIRT1 expression. In cardiomyocytes, myoblast gains resistance against to oxidative stress by increasing expression of nuclear SIRT1 protein. To do produce this antioxidative activity, SIRT1 protein enhances the level of MnSOD expression through p53 deacetylation (90). In addition, activation of FoxO1-dependent oxidative pathway by overexpression of SIRT1 protein is another regulatory way of protection of cardiomyocytes from oxidative stress (91). By the help of this pathway, cardiac infarct volume is reduced to ameliorate and recover cardiac function after ischemia/reperfusion in mice (92). It is also thought that transcriptional activity of NF-κB protein, a preconditioner in cardiac ischemia, is inhibited by SIRT1 protein to promote cell protection (93, 94). In literature, it was reported that the activity of SIRT1 protein is directly or indirectly controlled via the JNK1-SIRT1 link by accumulated oxidative stress which is caused by an increase in the ROS level due to aging or age-related diseases enzyme (1). It was demonstrated that ROS inhibited JNK phosphatases which activated the JNK1 to phosphorylate SIRT1 (95, 96). Furthermore, this phosphorylation increased the activity of SIRT1 resulting its translocation into the nuclei (97). Alcendor et al. (91) noted that the rate of SIRT1 overexpression had two-sided action in the cardiovascular system. For example, 2.5- to 7.5-fold increase in the SIRT1 expression attenuated apoptosis, the symptoms of cardiac dysfunction, age-related cardiac hypertrophy and expression of senescence markers. On the other side, 12.5-fold increase in the expression of SIRT1 resulted in increased cardiac hypertrophy due to oxidative stress and apoptosis. This study explained clearly the relation between oxidative stress and overexpression of SIRT1 to the pathological levels in CVD patients. In one of our previous studies (61), we found a positive correlation between total antioxidant level and SIRT1 level in CVD patients. Therefore, we can conclude that the increase in the SIRT1 level may be a compensatory mechanism to increase the antioxidants against oxidative stress in CVD patients.

Contrary to some previous studies (98, 99), the SIRT1 level significantly decreased approaching to control values in the CVD patients receiving statin therapy (100). The decline in the SIRT1 level by statins can be explained by the statins' inducement effect on PPARγ activity to protect patients against the progression of atherosclerosis (101). On the other hand, PPARγ inhibits SIRT1 expression at the transcriptional level which interrupting compensatory action of increased SIRT1 expression (102).

CONCLUSION

Recent studies have shown that age-related diseases or endocrine system dysfunctions are associated with an increase in SIRT1 expression levels, but with a decrease in their activity. The oxidative stress produced during these processes may lead to compensatory or protective increase in the SIRT1 expression to deal with the decline of the SIRT1 activity.

AUTHOR CONTRIBUTIONS

BE wrote the draft of the manuscript and UK finalized the manuscript.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Endocrinology

MINI REVIEW
published: 27 September 2018
doi: 10.3389/fendo.2018.00552

SIRT1 and Estrogen Signaling Cooperation for Breast Cancer Onset and Progression

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Breast cancer remains a significant female mortality cause. It constitutes a multifactorial disease for which research on environmental factors offers little help in predicting onset or progression. The pursuit for its foundations by analyzing hormonal changes as a motive for disease development, indicates that increased exposure to estrogens associates with increased risk. A prevalent number of breast cancer cases show dependence on the increased activity of the classic nuclear estrogen receptor (ER) for cell proliferation and survival. SIRT1 is a Type III histone deacetylase which is receiving increasing attention due to its ability to perform activities over relevant non-histone proteins and transcription factors. Interestingly, concomitant SIRT1 overexpression is commonly found in ER-positive breast cancer cases. Both proteins had been shown to directly interact, in a process related to altered intracellular signaling and aberrant transcription, then promoting tumor progression. Moreover, SIRT1 activities had been also linked to estrogenic effects through interaction with the G-protein coupled membrane bound estrogen receptor (GPER). This work aims to summarize present knowledge on the interplay between SIRT1 and ER/GPER for breast cancer onset and progression. Lastly, evidences on the ability of SIRT1 to interact with TGFß signaling, a concurrent pathway significantly involved in breast cancer progression, are reported. The potential of this research field for the development of innovative strategies in the assessment of orphan breast cancer subtypes, such as triple negative breast cancer (TNBC), is discussed.

Keywords: SIRT1, sex steroids, estrogen receptor, GPER, breast cancer, TNBC

OPEN ACCESS

Edited by:
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Northwest University, China

Reviewed by:
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Specialty section:
This article was submitted to Cellular Endocrinology, a section of the journal Frontiers in Endocrinology

Received: *05 June 2018*
Accepted: *30 August 2018*
Published: *27 September 2018*

Citation:
Liarte S, Alonso-Romero JL and Nicolás FJ (2018) SIRT1 and Estrogen Signaling Cooperation for Breast Cancer Onset and Progression. Front. Endocrinol. 9:552. doi: 10.3389/fendo.2018.00552

INTRODUCTION

Breast cancer (BC) is the most frequent tumor in women and a prevailing cause for female cancer mortality (1). It constitutes a multifactorial disease for which epidemiologic studies over environmental determinants offer little help in predicting disease onset or progression, thus, gender, aging, diagnosed first-degree relatives or previous history of BC remain dominant risk factors (2, 3). The pursuit for BC causes over molecular biology techniques led to the establishment of few genetic markers, such as *BRCA1*, *BRCA2, or DBC-1*, which driver mutation predispose for disease while also explaining cases of familiar clustering; still, the majority of mutations detected account for genes of low penetrance and frequently altered across genomes (4). Whilst, concurrent research efforts tried to find a predictable BC marker or cause based on hormonal changes.Although for most of hormonal hypothesis proposed data is still inconclusive (2, 5), it is accepted

that increased exposure to estrogens associates with higher risk, while reducing exposure is believed to result in protection (6). Therefore, factors such as early menarche, nulliparity or late menopause are associated with enhanced BC likelihood.

Observations on BC incidence are thought to be significantly contributed by the activities of classic nuclear hormone receptors for estrogens and progesterone (ER, PR). This is supported by tumor characterization using microarray techniques, which allowed to discriminate BC subtypes on the expression of key molecular markers including classic receptors. Applying such methods, BC is classified in ascending order indicating for aggressiveness: luminal-A; luminal-B (HER2–); luminal-B (HER2+); HER2-enriched; and basal-like. The first three subtypes share positivity for the expression of ER and/or PR along with the presence or absence of the human epidermal growth factor receptor 2 (Erb-B2; HER2), member of the epidermal growth factor receptor (HER/EGFR/ERBB) family. At last, the basal-like, which displays worsened prognosis and develops predominantly in pre-menopausal women, is characterized for the expression of specific basal-epithelium markers such as keratins (7). However, in clinical practice, cost-effective immunohistochemical methods are preferred to determine receptor presence, as such sub-typing, while providing prognostic information, also allow setting therapies to target specific oncogenic markers (**Table 1**). The anatomopathological absence of ER, PR, and HER2 led to the expression "triple negative" (TNBC), discerning cases devoid for all three markers (15). Worth noting, TNBC is somewhat regarded a surrogate for basal-like, as 70–80% overlap has been described between classifications (8, 16). In any case, while BC triage usually allows for adapted treatments improving prognosis in receptor-positive categories, that does not apply for TNBC cases, for which the lack of targeted approaches frequently restrict options to chemotherapy, with obvious consequences for the prognosis and lethality of the disease (17).

Interest on histone deacetylases (HDACs) is expanding as accumulated findings highlight their impact on regular physiology and pathological condition, staying SIRT1 the most studied (18). Sirtuins comprise a family of proteins (SIRT1–7) described as type III HDACs relying on NAD+ availability to perform a gatekeeping role in the configuration of the cell transcriptome, function that appears highly dependent on the cellular context and has been involved in a variety of biological processes, from modulating energy metabolism to development and cellular senescence (19–21). Intriguingly, Sirtuins in general and SIRT1 in particular display a paradoxical role in cancer, with histological studies showing increased or decreased expression patterns upon cancer origin and/or stage (22–24). In that sense, numerous contributions support the notion that SIRT1 activities influence hormone receptors (HR) actions, expressly those mediating long-term estrogenic effects in the mammary gland, namely the classic ERs, for which a relevant degree of interdependence between these factors has been described with apparent importance for BC onset and development. Moreover, interaction between the anew membrane-bound G-protein coupled estrogen receptor (GPER), which ubiquitously mediates short-term estrogenic effects, and SIRT1 has been also proposed, an interplay which could help fostering BC survivability and progression. Altogether, the conjoint actions of SIRT1 and HRs pose deep implications for BC onset and progression, which turn significantly relevant for the case of drug-resistant cases and conceivably HER2-enriched and TNBC. From that scope, this review synthetizes current knowledge on this emerging field.

KNOWN IMPLICATIONS OF SIRT1 FOR CANCER ONSET AND PROGRESSION

Initially described to deacetylate histones H1, H2, and H4 (18), SIRT1 is supposed to contribute to chromatin remodeling beneficial for tumor progression. However, despite obvious epigenetic capabilities, HDACs and SIRT1 current relevance for cancer strive on their now known ability to act on different substrates. SIRT1 is regarded an established modulator of significant non-histone nuclear proteins, such as p53, E2F1, or NF-kB (25, 26). SIRT1 overexpression has been related to tumor cell survival through the deacetylation and subsequent degradation of the p53 tumor suppressor (27, 28). Additionally, SIRT1 is known to deacetylate the FOXO family of transcription factors, resulting in repression of pro-apoptotic elements (29–31). In this line, using both hormone-responsive and TNBC models, MCF-7 and MDA-MB-231 cell lines respectively, SIRT1 has been found to localize to the promoters of silenced tumor suppressor genes, state reverted upon SIRT1 activity inhibition (32). Additionally, there is open debate on the ability of SIRT1 to act over cytosolic targets, with proposed tumorigenic implications related to PI3K/IGF-1R signaling (33, 34).

Moreover, SIRT1 shows a convoluted role in the regulation of the epithelial-mesenchymal transition (EMT) process, with apparent relevance in the case of reproductive tumors such as prostate and BC (35, 36). Notably, SIRT1 has been found to upregulate the expression of matrix-metalloproteinases in BC cells, condition known to promote invasiveness (37, 38). Moreover, SIRT1 activities seem to coordinate cancer stem cell-EMT changeover through deacetylation of a complex circuitry of transcription factors (39). To this regard, SIRT1 has been recently found to interact with Smad proteins, TGFß signaling canonical transducers (40). Interestingly, TGFß deregulation is regarded a cornerstone for EMT and tumor dispersion, among others markers, affecting the expression of matrix-metalloproteinases (4). While having a demonstrated role on the degradation of the inhibitory-Smad Smad7, SIRT1 has been also shown to relate with receptor-regulated Smads (Smad2 and Smad3), possibly coactivator-Smad Smad4 as well, in a process linked to altered transcriptional output (36, 40–42). Consequently, such interplay prompts for wide implications on cell transformation and tumor progression/dispersion.

ESTROGEN SIGNALING AND BREAST CANCER: THE ER PATHWAY

Among steroids, estrogens comprise a set of hormones involved in the development and maintenance of the female reproductive system, the main representative of which is 17ß-estradiol (E2),

TABLE 1 | Breast cancer anatomopathological surrogate definitions based on immunohistochemical subtyping methods.

Definition marker	Luminal A-like	Luminal B-like (HER2 negative)	Luminal B-like (HER2 positive)	HER2 positive (non-luminal)	Triple negative (ductal)
Erb-B2	–*	–*	+*	+*	–*
ER	+	+	+	–	–
PR	+	–/↓	+	–	–
Other relevant	Ki-67↓	Ki-67↑	Ki-67		Cytokeratins**
Prevalence	30∼70%	10∼20%		10∼20%	15∼25%
Main treatment strategy	Endocrine therapy	Endocrine therapy Cytotoxic		anti-HER2 Cytotoxic	Cytotoxic
Recurrence Risk	↓	↑		↓	↑↑
SIRT1		∼74%		∼55%	∼42%

*Concurrent marker detection is achieved for establishing subtypes. Not mentioned minor special histological types may respond to targeted therapies. Erb-B2, human epidermal growth factor receptor 2 (HER2); *overexpressed or amplified; ER, Estrogen Receptor; PR, Progesterone Receptor; Ki-67, proliferation marker; Cytokeratins, basal-like marker (8–14). **highly overexpressed; ↓low expression/risk; ↑high expression/risk; ↑↑very high expression/risk.*

constituting a major hormonal input along the monthly cycle. For its synthesis, androgens are converted into estrogens on the action of CYP19A1, known as Aromatase, an enzyme being mainly expressed in the ovaries but found in other tissues including the mammary gland (43). ERα and ERß correspond with the classic HRs responsible for E2 long-term effects (44). Both receptors, along with PR, integrate into the nuclear receptor family, which include sex steroids receptors as well as receptors for corticosteroids (45). Its members mostly localize to the nucleoplasm, but a minority of isoforms which help fine-modulating the overall response may also appear at alternate locations, involving a cytoplasmic-nuclear shuttling mechanism (46, 47). Although ERα and ERß share significant sequence homology, both receptors display unique expression patterns depending on either tissue or organ. ERα signaling comes crucial for the regulation of mammary gland development and function (44), also contributing to cancer onset and progression. Elevated ERα levels expressed in benign breast epithelium correlate with enhanced BC risk, whereas estrogen-dependent cancers require of E2 for cell survival and growth (44). Worth noting, PR positivity can be usually regarded a surrogate of ER positivity, as PR expression requires proper ER functioning to occur (48).

The principal ER activation mechanism requires E2 binding, which allows conformational changes promoting receptor dimerization and nuclear translocation to interact with estrogen response elements (ERE) present in the DNA to regulate transcription (44). Additionally, alternate mechanisms for ER activation and modulation had been described through cross-linking with signaling pathways like EGFR/PI3K/ERK, based on changes of ER phosphorylation status (49, 50). Upon landing on EREs, E2-ER complexes further recruit several co-activator proteins such as p300, PPARγ, and PGCα, which leads to histone acetylation and chromatin remodeling necessary for the regulation of transcription (51). Moreover, active ERα also recruit members of the FOXO family into transcriptomic complexes, interplay that has been characterized to greatly influence transcriptomic outcomes, with implications for mammary morphogenesis, BC onset and progression to drug resistant states (52–56).

At the clinic, hormone-responsive BC is usually managed in a straightforward manner due to the multiplicity of pharmacological approaches based in the use of Aromatase inhibitors along with selective ER modulators and downregulators, like Tamoxifen or Fulvestrant, which diminish ER-mediated E2-responses at the breast (57). Consequently, these cases are mostly associated with favorable prognosis. Still, hormone-responsive tumors frequently transform over time into an estrogen-independence status, gaining the ability to proliferate in the absence of hormonal input. This conversion usually becomes a critical step for BC clinical progression, as it is related to increased aggressiveness. In that sense, ER-negative cases at diagnosis are believed to have lost ERα expression over time due to gene silencing, thus reducing therapeutic options targeting HRs and thus associating with less favorable prognosis (58).

ESTROGEN SIGNALING AND BREAST CANCER: THE GPER PATHWAY

Despite the fact that classical HRs may be missing for some BC cases, however, it is now acknowledged that estrogen stimulation would still play a powerful role for the evolution of the disease through the actions of membrane-coupled ERs. Turning into the XXI century, the existence of a membrane-coupled ER was revealed, that would be responsible for most of E2 rapid physiological responses which at the time lacked of proper molecular explanation. This marker corresponded with a G-protein coupled receptor which could be located both at the endoplasmic reticulum and the plasmatic membranes (59, 60). Initial reports indicated that through its binding to E2, GPER actions resulted in cAMP and inositol triphosphate production, triggering intracellular calcium mobilization. However, it was rapidly established that its actions also resulted in the transactivation of diverse intracellular signaling cascades including MAP-Kinases, PI3K, or eNOS (61–63). In this line, further research also found GPER able to attune E2-mediated transcription regulation. This was established on

co-expression experiments showing the existence of a functional cooperation between canonical-ERα-mediated and short-time-GPER-mediated signaling through a mechanism relying on the activation MAP-Kinases, capable of promoting post-translational modifications such as altering ERα phosphorylation status (64). Moreover, additional studies established GPER to be ubiquitously expressed through both reproductive and non-reproductive tissues (65), thus posing new challenges for the understanding of estrogen physiology. In that sense, it was not long before GPER was proposed to contribute for cancer development (61). Still, GPER's specific role for both neoplastic transformation and cancer progression remains unclear, as its actions seem to be highly depend on tissue origin (66). Yet, although anatomopathological studies on GPER expression are not included in routine clinical practices, in the case of estrogen-dependent tumors and especially BC, several works show that its altered expression can be associated with cancer progression, supporting a potential prognostic value (67–69).

At the molecular level, diverse studies advocate for GPER's ability to affect cancer cell survivability and proliferation, by influencing a myriad of signaling pathways responsible for cell cycle regulation or apoptosis control. They also play a role in the regulation of angiogenesis required for tumor nourishment and development [reviewed at (66)]. Moreover, GPER signaling has been proposed to distinctly affect cell migration and cancer invasiveness. Calpains are non-lysosomal proteases that are implicated in the regulation of cell adherence to the extracellular matrix and motility (70). Interestingly, it was reported that incubation of ER-negative BC cells with GPER agonists G-1 promoted Calpain-1 activity and altered adhesion to matrigel (71). Also in this line, GPER has been described to adjust EGFR/PI3K/ERK intracellular signaling, favoring expression changes of migration markers such as SNAIL or ß1-integrin, as well as altering plasticity of cellular adhesions by the activation of the focal adhesion kinase (FAK) (72). Conjointly, these abilities of GPER to promote viability and motility provide a molecular framework for estrogen stimulation of cancer cells despite ER-negativity. Notably, epidemiologic studies would deem clinical functionality to these capacities, as prevalence for GPER overexpression is found in HER2-enriched, basal-like and TNBC cases, also associated with both distant metastasis and recurrence (67, 73).

ESTROGEN SIGNALING AND BREAST CANCER: NOTED SIRT1 INFLUENCE

Numerous contributions support the notion that SIRT1 activities decisively impact HRs actions in relation to disease. With regard to classic ERs, in cellular models, E2 stimulation has been found to promote SIRT1 expression under direct influence of ERα transcriptomic complexes, observation matched by the prevalent detection of elevated levels for SIRT1 manifested for most of ERα-positive BC samples (74, 75). Interestingly, the ability of SIRT1 to interact with p300, PPARγ, and PGCα, ERα transcriptomic co-activators, has been proved to affect chromatin remodeling, disturbing developmental processes (76–78). Following studies found SIRT1 inhibition resulted in suppressed ERα expression, interfering with E2-dependent cell growth in healthy as well as malignant mammary epithelial cells (79, 80). In this line, inhibition of SIRT1 has been found to lessen ERα mediated repression of NRF2-dependent detoxifying enzymes in MCF-7 cells (81).

Simultaneously, significant efforts had been put to apprehend the effects of a less subtle SIRT1-ER interaction mechanism. Interestingly, ERα has been found to relate to and be a target for SIRT1. Upon E2-ERα activation, p300 stabilizes receptor complexes through acetylation in a process that can be reversed by SIRT1 (82). These findings initially suggested a SIRT1 inhibitory role over estrogen signaling. Still, changes in ERα acetylation status are considered to have limited effects (83). On the other hand, as previously mentioned, active ERα recruits FOXO family members into transcriptomic complexes, factors which also fall under SIRT1 spectrum of actions. Within the BC context, a recent report showed FOXN3 to be able to recruit SIRT1 into ERE-dependent transcriptomic complexes, promoting reduced transcriptomic output (84). Interestingly, alterations of FOXM1 and FOXO3a levels had been previously linked to SIRT1 aberrant activity, especially in TNBC (85).

Regarding the interaction between SIRT1 and GPER, knowledge is scarce. Nonetheless, using ER-negative HER2-enriched BC cells, it was recently described that E2 actions via GPER can result in SIRT1 overexpression through activation of the EGFR/ERK/c-fos/AP-1 transduction pathway (86). Interestingly, such induction contributed specifically to enhanced cancer cell survival and proliferation, as it was reversed by the use of SIRT1 inhibitors or GPER silencing as well. Notably, these effects were also observed in cancer associated fibroblast obtained from BC patients (86), posing challenging questions for this interplay in BC progression.

PRESENT AND FUTURE CLINICAL VALUE OF THE ESTROGEN SIGNALING/SIRT1 INTERPLAY

Finding ways to better screen and characterize BC, especially drug-resistant cases and TNBC, constitutes a standing challenge of cancer research. Efforts to establish a SIRT1 prognostic value for BC are increasing, as its overexpression can be commonly detected (9). Recent meta-analysis gathering non-connected data sources, while finding association with higher tumor stage, failed to detect correlation between SIRT1 expression levels and BC overall survival; however, these works addressed statistics letting out BC subtyping (87). Notwithstanding, a single retrospective study incorporating 822 BC patients found SIRT1 expression to correlate with tumor aggressiveness and reduced disease-free-survival (DFS) (88). Interestingly, when BC subtyping is considered, SIRT1 overexpression associates with ER-positivity and likely shortened DFS (9). Moreover, while not associated with HER2-enriched or TNBC status, concomitant SIRT1 overexpression successfully dictates for lymph-node metastasis likelihood (9), observation which has been linked to the ability of SIRT1 to promote an altered expression of key EMT markers such as E-Cadherin, Vimentin, and SNAIL-1 (89). Hence, although data so far may be considered insufficient or poorly curated,

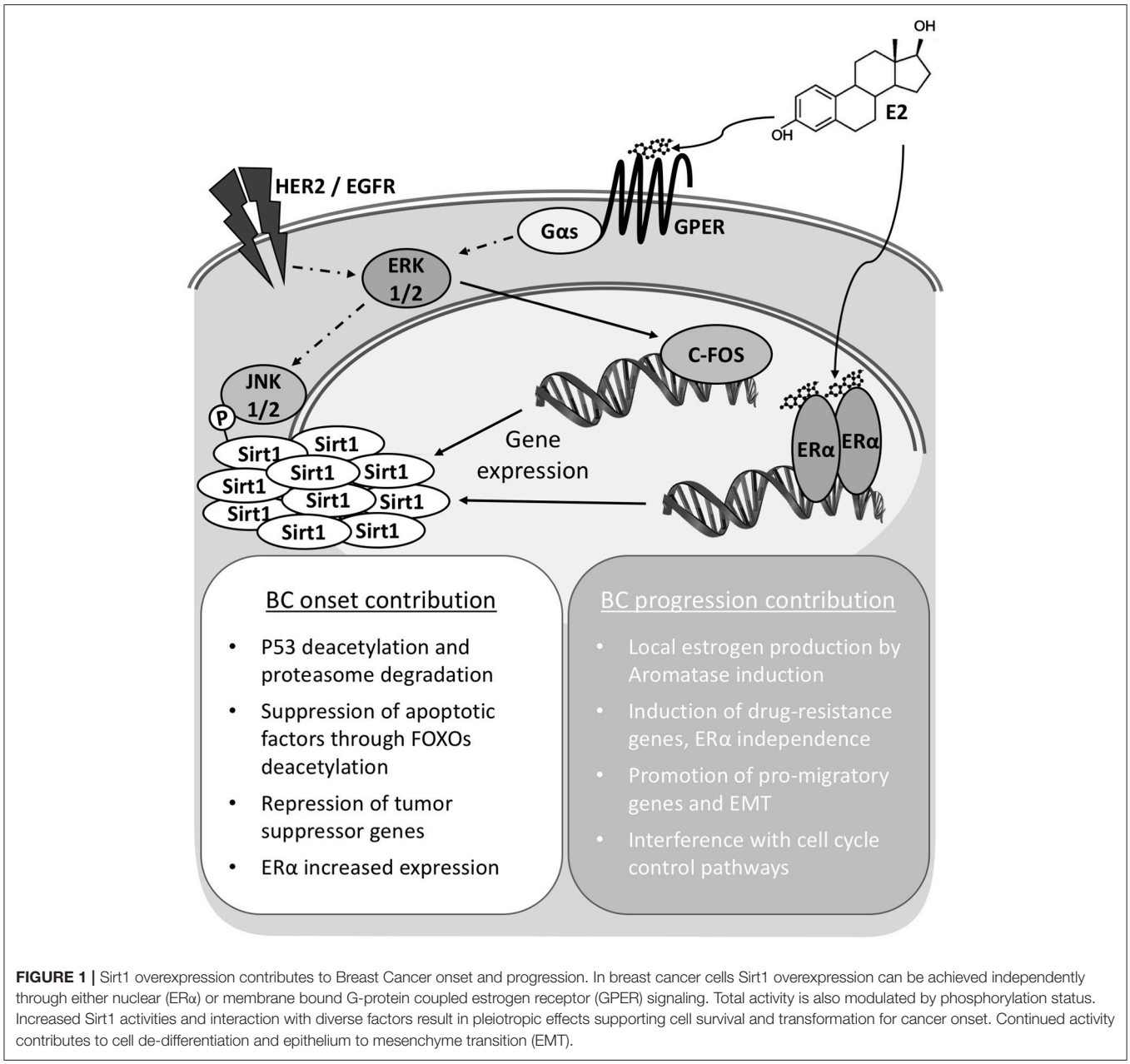

FIGURE 1 | Sirt1 overexpression contributes to Breast Cancer onset and progression. In breast cancer cells Sirt1 overexpression can be achieved independently through either nuclear (ERα) or membrane bound G-protein coupled estrogen receptor (GPER) signaling. Total activity is also modulated by phosphorylation status. Increased Sirt1 activities and interaction with diverse factors result in pleiotropic effects supporting cell survival and transformation for cancer onset. Continued activity contributes to cell de-differentiation and epithelium to mesenchyme transition (EMT).

the accumulated evidence allows to consider SIRT1 histological detection as a valuable marker for assessing BC status, also providing hints on metastasis and relapse odds.

Per BC tumor progression, it is speculated whether SIRT1 has a role in determining tumor conversion to a drug-refractory phenotype. It has been reported that SIRT1 activity helps increasing expression of drug-resistance genes (90), in a process that involves FOXO1 deacetylation and reverts upon SIRT1 suppression (91, 92). Noteworthy, SIRT1 activity is mainly regulated by upholding absolute protein levels with little variation overtime (35), although modulation of its actions depending on MAPK-mediated phosphorylation has been also described (93). To this extent, two considerations should be made. Firstly, it ought to be reminded that estrogen signaling via either HR can augment SIRT1 levels (74, 86). Interestingly, elevated SIRT1 levels has been reported to promote increased Aromatase activity both in ER-positive and TNBC cell lines (94), thereby allowing for local E2 production. Secondly, it should be noted that MAP-kinases signaling can be activated by overexpressed HER2 subunits dimerizing with EGFR and triggering the PI3K/ERK pathway (95). Consequently, a SIRT1 potential role assisting evolution to ER-independence turns reasonable, as deleterious abilities, including aberrant modulation of p53 and FOXO or expression of drug-resistance genes, could be maintained via SIRT1 overexpression depending on GPER signaling, perhaps also

through MAP-kinases dependent activation. In that sense, recent reports showing linkage between SIRT1 overexpression and enhanced SRC and AKT activities in different TNBC cell lines would support the latter notion (96, 97). Worth considering as well, classic ERs and GPER display dissimilar behavior upon exposure to pharmaceutical modulators like Tamoxifen and Fulvestrant, which in the case of GPER appear to have an agonistic function (66). Moreover, in cellular models, GPER activation has been described to negatively affect ERα protein levels (71), perhaps fostering the transition. Hence, considering that solid tumors are composed by a clones plethora subject to selective pressure, it is tempting to propose the monitoring of SIRT1 expression/activity as source of information on the efficiency of treatments related to tumor evolution at the cellular scale. To this regard, procedures based on liquid-biopsy techniques would offer an appropriate framework for such approach (98).

On the aspect of treatments, many efforts had tried to assess the potential of suppressing SIRT1 activities. However, after several years, effects described on the many inhibitors discovered and interference studies do not agree with each other. This is regarded as the result of dissimilar mechanistic involved in knocking-out or knocking-down the enzymatic activity and also on the specificity of inhibitors used (99). Yet, modulators of SIRT1 activity may still retain potential as co-adjuvant treatments, due to sensitizing capabilities useful at specific BC environments (100). In this sense, sirtuins in general, and SIRT1 in particular, have been shown to interact with numerous signaling pathways which decisively affect different aspects of the cell physiology. That is the case with the recently described interaction of SIRT1 with TGFß canonical-signaling-transducers, the Smad proteins. SIRT1 has been found incorporated into Smad-mediated transcriptomic complexes, its activity linked to reduced Smad acetylation and decreased nuclear half-life (36, 40–42). Lack of proper TGFß response is considered a major mechanism for EMT through regulating the expression of key cell adherence and migration markers (4). Interestingly, this TGFß dependent regulation involves the participation of FOXO factors (101, 102). Hence, the overexpression of SIRT1 in BC cells has the potential to facilitate aberrant regulation through these system, thus contributing for the EMT process and cancer progression. Moreover, as signaling via GPER and HER2 are known to trigger MAP-Kinases activation, and activated MAP-Kinases had been related to SIRT1 augmented activity, this interplay provides an additional framework for the promotion of both tumor ER-independence and EMT progression, potentially offering opportunity to develop tailored strategies, which would come particularly useful for the case of TNBC. Perhaps, next-generation inhibitors, with better specificity and increased potency, may provide advancements in this field.

Finally, a brief but necessary mention should be made on non-coding RNAs. Increasing efforts attempt to discriminate the relevance of both long-non-coding and micro-RNAs in BC pathogenesis and progression, due to their dual role as an additional prognostic information source and potential therapeutic targets (103, 104). Not surprisingly, distinct signatures could be found in BC depending ER status (105). In that sense, exposure to either estrogens or selective ER modulators had been shown extensively affect through classic ERs the microRNA profile of different mammary cell lines (106). Interestingly, within the BC context, SIRT1 activity appears to be highly conditioned by the non-coding-RNA environment, with several long- and microRNA species affected by endocrine signaling being able to promote or downregulate its expression (107, 108). Further research in this area may again probe helpful for the development of strategies targeting intrinsic BC subtypes.

CONCLUDING REMARKS

The accumulated evidence prompts to consider SIRT1 as an integrated player into the transduction network activated by estrogens, both through ERs and GPER, in the mammary tissue (**Figure 1**). This tight cooperation conceptualizes and supports a SIRT1 promoting role in mammary tumorigenesis, with meaning for both disease onset and progression. These implications are to be considered both in the case of signaling triggered upon endogenous estrogens exposure, also in the case of exposure to environmental pollutants like xenoestrogens, phytoestrogens, and other synthetic compounds; let aside for the response to BC treatments based on estrogen modulators. Consequently, SIRT1 detection has potential to become a powerful prognostic indicator for tumor evolution and response to chemotherapeutics. Moreover, a better understanding at the molecular level of its cooperation and impact in the signaling through connected pathways may provide opportunity for the development of innovative therapy approaches in the assessment of BC and particularly TNBC cases.

AUTHOR CONTRIBUTIONS

SL conceptualized the work, performed searches in literature databases and prepared, reviewed and edited the original draft of the manuscript. JA-R contributed with formal analysis and the incorporation of clinical aspects, also reviewed the original draft. FN supervised the project, was responsible for funding acquisition and reviewed and edited the original draft. All authors read and approved the final manuscript.

FUNDING

This work was supported by grants (SAF2006–09482; SAF2003–06029) from the Spanish Ministry of Education and Science. Also, this work was supported by a grant (PI13/00794) from the Instituto de Salud Carlos III. And the Fondos FEDER Una manera de hacer Europa (ERDF, A way to build Europe). SL received a fellowship from Roche. FN was supported the Fundación para la Formación e Investigación Sanitarias de la Región de Murcia.

ACKNOWLEDGMENTS

We are indebted to the Hospital Clínico Universitario Virgen de la Arrixaca that strongly supported this research.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Endocrinology

MINI REVIEW
published: 24 September 2018
doi: 10.3389/fendo.2018.00569

SIRT1 in Secretory Organ Cancer

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Mammalian silent information regulator 1 (SIRT1) is reported to play a role in cancers of the secretory organs, including thyroid, pancreatic endocrine, and ovarian tumors (1–4). A recent meta-analysis conducted on 37 selected studies of human cancers analyzed the correlations of overall survival (OS), disease-free survival (DFS) and relapse-free survival (RFS) with SIRT1 expression (5). This study reported that SIRT1 overexpression was associated with a worse OS in liver and lung cancers, while it was not correlated with OS in breast cancer, colorectal cancer, or gastric carcinoma. Collectively, the meta-analysis revealed that an unfavorable OS was associated with SIRT1 expression for solid malignancies. Given the growing importance of this class of lysine/histone deacetylases in human endocrine malignancies, a rational and focused literature assessment is desirable in light of future clinical translations.

Keywords: SIRT1, cancer, secretory organs, acetylation, epigenetic modulation

OPEN ACCESS

Edited by:
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Specialty section:
This article was submitted to Cellular Endocrinology, a section of the journal Frontiers in Endocrinology

Received: *10 May 2018*
Accepted: *05 September 2018*
Published: *24 September 2018*

Citation:
Frazzi R (2018) SIRT1 in Secretory Organ Cancer. Front. Endocrinol. 9:569. doi: 10.3389/fendo.2018.00569

INTRODUCTION

The epigenetic regulation of chromatin structure and gene expression represents a major field of study and intervention for researchers focused on cancer. The class III lysine/histone deacetylases are known as Sirtuins and are represented, in humans, by 7 members (6, 7). Silent information regulator 1 (SIRT1) is the most studied and well characterized among the class III deacetylases and its targets include p53, Ku70, peroxisome proliferator-activated receptor gamma (PPARγ), peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1α), Beclin 1 and β-catenin, among others (8–13). A relevant group of SIRT1 targets is the Forkhead box O (FOXO) family of transcription factors. FOXO3a hyperacetylation mediated by SIRT1 accompanies apoptosis while FOXO4 deacetylation activates this target enhancing its transcriptional and biological activity in the nucleus. FOXO4 activation enhances the cellular defenses against oxidative stress and leads to apoptosis resistance (8, 14, 15). Fibrosis is another aging-related disease involving FOXO1/3, well known modulators of aging and longevity playing a clear inhibitory effects on fibrogenic effector cells and extracellular matrix production (16).

Furthermore, the epigenetic regulation and dysregulation of the hypermethylated in cancer1 (HIC1)/SIRT1/p53 axis is relevant for the development of malignancies (17). SIRT1 is also a target for several miRNAs, small non-coding RNA molecules known to be deregulated in various cancers, whose expression is involved in tumorigenesis (18).

SIRT1, due to its NAD^+ dependency, is also a metabolic sensor and its deacetylating activity toward regulatory target proteins affects cell metabolism (19).

The focus of this review is the role and involvement of SIRT1 in secretory organ cancers since the information recently gathered on this topic should bear new translational benefits. Correlations between SIRT1 and nuclear receptors, transcription factors, master regulators of gene expression, miRNAs and lncRNAs have been reported. The main glands in the body and the most recent literature are taken into consideration.

OVARIAN CANCER

Ovarian cancer is the fifth leading cause of female cancer mortality in the United States (20). This type of cancer is currently treated with a platinum and taxane-based chemotherapy after surgical cytoreduction. However, the rates of progression free survival (PFS) and OS for these patients remain dismal and no benefits seem to arise by the introduction of additional cytotoxic agents (21). Ovarian cancer is usually regarded as a single entity and current treatment protocols for the disease are not subtype specific. Actually, ovarian cancer is a heterogeneous disease that is classified by histopathological examination, including serous, clear cell, endometrioid, and mucinous subtypes. These subtypes develop differently and respond differently to chemotherapy (22).

SIRT1 overexpression is reported by many authors as associated with poor outcome and chemoresistance in ovarian cancer of epithelial origin (23–25). Ovarian cells (both normal and tumoral) express two kinds of estrogen receptors (ER), ERα and ERβ which play different roles in cell proliferation and aggressiveness (4). Specifically, ERα is associated to a poor outcome, while ERβ expression corresponds, on the contrary, to a favorable outcome. ERα levels are closely associated with estrogen-dependent growth and the increased metastatic potential of ovarian epithelial carcinoma (OEC) through the promotion of the epithelial to mesenchymal transition (EMT) (26). ERβ, in contrast, inhibits EMT in presence of 17β-estradiol (E2), thus mediating the opposite effect to ERα (26). SIRT1 is inversely correlated to ERβ mRNA and protein levels, and the specific ERβ activator KB9520 strongly inhibits SIRT1 mRNA expression. These data collectively support the role of SIRT1 as a tumor promoter in OEC (4).

Recently, another association between OEC and SIRT1 was reported (27). These observations are relevant, because OEC patients often acquire resistance to paclitaxel or cisplatin, and the increased expression in *SIRT1* and *TWIST1* (two genes associated with drug resistance) is observed in OVCAR-5 cells that show increased cisplatin resistance, migration and invasion potential (27).

SIRT1 is a downstream target of hypoxia inducible factor 1α (HIF1α) (28). Hypoxia is a condition associated with several types of tumors and, specifically, increased HIF1α expression predicts the poor prognosis of ovarian cancer. Notably it has been demonstrated that SIRT1 expression is induced by hypoxia and that HIF1α silencing indirectly hampers SIRT1 expression. Finally, the NF-κB signaling pathway is involved in hypoxia-induced SIRT1 up-regulation, strengthening the link between this class III lysine deacetylase and ovarian cancer (28).

THYROID CANCER

The incidence of thyroid cancer has increased over the past few decades worldwide. This increase is mainly driven by new cases of papillary thyroid cancer (29). SIRT1 is reported to be oncogenic in thyroid and prostate murine carcinomas initiated by PTEN deficiency (2). The mechanism unveiled by mRNA transcriptional analysis revealed that SIRT1 drives oncogenesis through c-MYC regulation (**Figure 1**). Two pathways upregulated via SIRT1 overexpression are related to translation and ribosomal biogenesis, which are both controlled by c-MYC. The protein product of the c-MYC oncogene lies at the intersection of a transcriptional network regulating cellular proliferation, replicative potential, cell–cell competition, cell size, differentiation, metabolism, and apoptosis (30).

c-MYC increases in response to mitogenic stimuli, and this is accompanied by SIRT1 upregulation. c-MYC activates SIRT1 by promoting NAMPT transcription and then the NAD-salvage pathway. Furthermore, c-MYC can bind directly to DBC1 (that is a SIRT1 inhibitor) thus preventing SIRT1 blockade (31, 32). On the other hand, SIRT1 activation leads to p53 deacetylation and consequent inactivation, decreasing the levels of PUMA and p21 and eventually counteracting the pro-apoptotic effects of p53. Finally, SIRT1 stabilizes c-MYC via lysine-specific deacetylation and increases its transcriptional activity. Altogether, these data demonstrate a positive feedback loop between c-MYC and SIRT1 (31).

The translational significance of these findings is highlighted by the consistent overexpression of SIRT1 in follicular and papillary thyroid carcinomas, positively correlates with c-MYC protein levels and stabilizes c-MYC (2).

The natural phytochemical resveratrol (RSV) is known to affect mammalian cell metabolism and aging by modulating SIRT1 (33, 34). Furthermore, SIRT1 is also a reported molecular target of RSV in cancer cells of both lymphoid and epithelial origin (35). RSV regulates thyroid stimulating hormone (TSH) secretion via SIRT1 activation. RSV also arrests follicular thyroid and papillary thyroid carcinoma growth by activating the mitogen-activated protein kinase (MAPK) signal transduction pathway and p53 phosphorylation (36, 37).

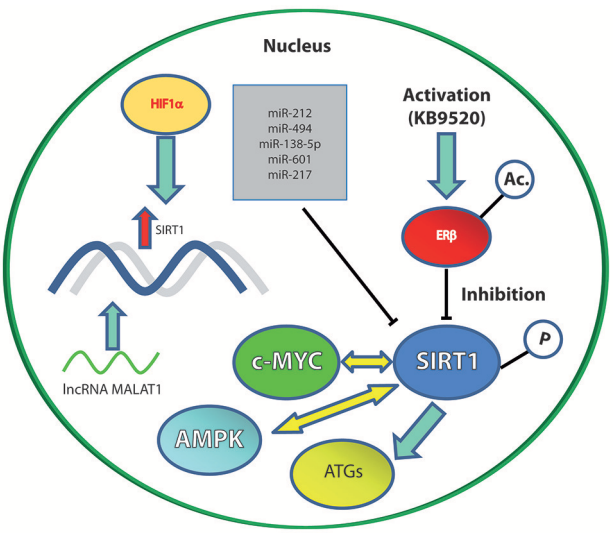

FIGURE 1 | The main SIRT1 molecular interactors (inhibitory or activatory) cited in the text. Solid blue arrows represent an activation; yellow arrows represent an interplay; blunt ended black lines represent an inhibition; red arrow represents upregulation of gene expression.

Among the few studies available, regarding the role of SIRT1 in the thyroid neoplasias, most report involvement as a direct target for miRNAs. miR-212 is a tumor suppressor miRNA that inhibits the proliferation, migration, and invasiveness of thyroid cancer cells. SIRT1 is a direct target of miR-212 and is inversely correlated to miR-212 expression in thyroid cancer tissues (42 samples of human thyroid cancer) (38).

PANCREATIC CANCER

Pancreatic cancer (PC) is one of the most deadly tumors in western countries and the 10th most common cancer in the United States. Pancreatic ductal adenocarcinoma is the most common subtype and is recognized as "incurable" due to its aggressive clinical course and resistance to available therapies (39, 40). miR-494 is specifically involved in PC proliferation (41). The overexpression of miR-494 inhibits PC cell proliferation *in vitro* and *in vivo* in addition to inhibiting invasion. The c-MYC/SIRT1 axis is a direct target of miR-494 and there is an inverse correlation between the two. The simultaneous interference of c-MYC and SIRT1 synergistically reduces the expression of these two targets and inhibits PC proliferation, eventually stimulating miR-494 expression (41). The interplay between c-MYC and SIRT1, therefore, plays a relevant role during PC tumorigenesis and should be taken into account when designing strategies aimed at combating this cancer.

Recent studies on the miRNA-mediated regulation of autophagy in PC have been published (42). Since it is known that hypoxia induces autophagy, PC cells were cultured under normoxic and hypoxic conditions. miR-138-5p emerged among the most down-regulated miRNAs in the hypoxia-grown cells. Targets can analysis further revealed that the *SIRT1* gene contains a classical and conserved domain that binds miR-138-5p. Notably, miR-138-5p specifically binds to the 3′-UTR of *SIRT1* and its overexpression leads to SIRT1 protein downregulation (42). Finally, the authors demonstrated that miR-138-5p also counteracts the hypoxia induction in cancer cells through SIRT1 inhibition in tumor xenografts.

Another player that emerged recently is miR-601, which suppresses PC proliferation and migration (43). The target of this miRNA's activity was identified, once more, as SIRT1. This Sirtuin is downregulated by miR-601 and its overexpression reverses the effect of miR-601 on PC cells (43).

miR-217 also directly targets SIRT1, it is inversely correlated to SIRT1 expression and is down-regulated in chronic pancreatitis and PC (44).

The Ex-527 synthetic inhibitor, which inhibits pancreatic cell proliferation *in vitro* and in combination with the first-line drug for this pathology, gemcitabine, is considered one of the most specific SIRT1 inhibitors currently available (45). The same inhibitor was shown to promote PC xenograft tumors in SCID mice and did not synergize with gemcitabine. Therefore, caution should be used when dealing with Ex-527 inhibitor to target SIRT1 *in vivo*.

The role of histone deacetylases (HDACs) is poorly understood in pancreatic neuroendocrine tumors (pNETs). A comprehensive expression pattern of all the five classes (I, IIa, IIb, III, and IV) of HDACs was recently described (3). The gene expression profiles of a total of $n = 57$ patients revealed a significant upregulation of all the HDAC classes in pNETs over controls with increased levels ranging from 1.5- to 7-fold. The expression of several HDACs, including SIRT1, correlates to the G3 stage and, thus, to pNETs tumor grading (3).

GASTRIC CANCER

Gastric cancer (GC) is among the most prevalent malignancies of the upper gastrointestinal tract. It is a solid aggressive carcinoma where new therapeutic options are warranted (46). Autophagy in GC may have the dual role of promoting the activity of irradiation and anticancer drugs or, on the contrary, may function as a protective mechanism for cancer cells (47–49).

SIRT1 plays a confirmed role in autophagy (50, 51). In GC, it has been demonstrated that SIRT1 deacetylates autophagy-related gene products (ATGs) and a series of histonic and non-histonic targets, eventually interfering with the autophagic process (**Figure 1**) (52).

GC is among the few human tumors where SIRT1 is downregulated, and in one study, the prognosis of low-SIRT1 expressing GC patients was good (52, 53). However, other authors reported a meta-analysis of SIRT1 expression in gastrointestinal cancer in which the results were heterogeneous (54). The overall results from 15 studies showed an association between worse OS and high SIRT1 expression in gastrointestinal cancer. The subgroup analysis revealed that this association was particularly strong in non-colorectal gastrointestinal cancers, especially GC and hepatocellular carcinoma (54). Therefore, SIRT1 is a promising prognostic factor in GC but not in colorectal tumors.

In contrast with the abovementioned results, a study of the clinicopathological parameters in GC patients was analyzed using the Kaplan-Meier plotter (55). Here, the data concerning the seven human Sirtuins were extrapolated from NCBI GEO databases containing mRNA profiles and the corresponding clinical data of a large number of GC patient samples. The Kaplan-Meier plotter was used to investigate the predictive value of mRNA expression of the Sirtuins for the OS of GC patients. Among a total of 631 available cases for SIRT1 expression, the survival curves suggested that a high expression of SIRT1 mRNA was favorable for OS and that different Sirtuins had diverse correlations with OS. Furthermore, the first progression (FP) was positively correlated with SIRT1 expression: in other words, a longer FP time was associated with a higher SIRT1 level (55).

Opposite evidence was reported from the immunohistochemical (IHC) staining of primary GC tissues. High SIRT1 expression, by IHC, was associated with lymphatic invasion ($p = 0.028$), vessel invasion ($p = 0.016$) and lymph node metastasis ($p = 0.014$) and tended to be associated with more advanced disease stages (56).

Clearly, no consistent data on the exact role of SIRT1 in GC are available at present. The evidence available thus far points to a dual role for SIRT1 (that is to say it may act as a tumor

promoter as well as a tumor suppressor), possibly depending on its sub-cellular localization, p53 status, and microenvironmental conditions (57, 58).

ADRENAL GLANDS

The function of SIRT1 in the adrenal glands has been investigated the least. One study reports the researches performed on human adrenal NCI-H295R cells (59) and, in this setting, Sirtuins (including SIRT1) did not seem to be involved in the RSV-mediated lowering of androgen production.

Moreover, steroidogenesis in H295R cells was not affected by Sirtuins, even when SIRT1/3/5 are overexpressed through transgenesis, strengthening the fact that RSV-dependent effects in this setting are SIRT-independent (59).

HEPATIC CANCER

Hepatocellular carcinoma (HCC) is a highly malignant tumor with a higher frequency in East Asia than in Europe and North America (60). HCC is the predominant malignancy in the liver and recent evidence shows a role for SIRT1/PGC-1alpha/mitochondrial biosynthesis pathway in protecting the liver against lipid accumulation and mitochondrial dysfunction (61, 62). Senescence induction is a recently proposed strategy with the aim of counteracting HCC (63). Metformin, for instance, induce senescence via the activation of AMP-activated protein kinase (AMPK) when used at low doses. This effect is accompanied by the phosphorylation of SIRT1 as well as p53-acetylation (**Figure 1**). The prolonged exposure to low doses of metformin leads to human HCC senescence in murine xenografts via modulation of the AMPK-SIRT1 axis (63).

The long non-coding RNA (lncRNA) metastasis associated lung adenocarcinoma transcript 1 (MALAT1) was recently discovered to interfere with SIRT1 activity in hepatocellular cancer (64). MALAT1 is frequently upregulated in several types of cancers and contributes functionally to the development and malignancy of tumor cells (65). MALAT1 is, among lncRNAs, a stable and highly transcribed molecule localized in the nucleus, and it has been reported that, in breast cancer cells expressing ERα, 17β-estradiol treatment negatively regulates MALAT1 transcription, thus inhibiting proliferation, migration and invasion (66). MALAT1 is also regulated during endothelial differentiation in that hypoxia induces its transcription leading to the proliferation of the vasculature (67). In HCC specifically this lncRNA promotes aggressiveness in that releases SIRT1 by sponging miR-204 (64). SIRT1 is a direct downstream target of miR-204 and results inhibited by this interaction. MALAT1 can release the suppression on SIRT1 by base-pairing with miR-204 and leading to its post-transcriptional downregulation (64).

CONCLUSIONS

SIRT1 should be regarded as a multi-faceted enzyme and, as already suggested by several authors, studied in a context-dependent fashion. Secretory organs are no exception to this. **Table 1** summarizes the secretory organ tumors in which SIRT1 has been investigated, with the relevant experimental models. The most recent publications are taken into account.

TABLE 1 | Cancer models of the secretory organs in which SIRT1 has been studied.

	Experimental models and specimens	References
Ovarian cancer of epithelial origin	Normal ovaries, endometriosis with/without carcinoma, OvCa (endometrioid; clear cell; mucinous; serous). OvCa cell lines. Serous EOC.	(4, 23–28)
Thyroid carcinoma	Murine thyroid cancers. Thyroid cell lines. Nude mice models. Human normal and tumor thyroid specimens	(2, 37, 38)
Pancreatic cancer	Confirmed human PC and non-tumor pancreatic specimens. PC cell lines. Normal human pancreatic ductal epithelium. PC xenografts. Human chronic pancreatitis specimens	(41–45)
Pancreatic neuroendocrine tumors	Formalin fixed paraffin-embedded human samples	(3)
Gastric cancer	Human primary GC specimens. GC cell lines. Clinical trials concerning gastrointestinal cancers, overall survival and SIRT1. NCBI GEO databases of mRNA profiles. Formalin fixed paraffin-embedded human samples	(52–56)
Hepatic cancer	Primary liver cancer organoids (hepatocellular carcinoma; cholangiocarcinoma; combined HCC/CC; healthy liver). HCC mouse xenografts. HCC cell lines and primary human tissues	(61, 63, 64)

SIRT1 plays a confirmed role in ovarian, thyroid, and pancreatic cancers, while its role in GC is yet to be definitely established. The evidence available in thyroid cancer confirms that c-MYC and SIRT1 are valuable therapeutic targets, encouraging translational researches aimed at the related pathways. The recent meta-analysis spanning 37 selected studies on human solid cancers demonstrates a correlation between higher SIRT1 expression and worse OS in liver and lung cancers (5). These clinical data are consistent with the experimental ones reported for HCC where SIRT1 activation correlates to malignancy through MALAT1, a lncRNA highly expressed in various cancers including HCC (64, 68).

Furthermore, colorectal and GC do not show the correlation between OS and SIRT1 expression (5). SIRT1 overexpression has been reported either positively associated to FP or negatively associated to OS. Contradictory results also concern SIRT1 IHC and mRNA expression and prognosis of GC, suggesting that this class of lysine-deacetylases is not a reliable biomarker for GC.

In contrast, little evidence is available in the literature concerning the role (if any) of SIRT1 in the pituitary gland. In

the testis, the role of SIRT1 is reported to be associated with spermatogenesis, fertility, and differentiation, but not testicular cancer (7).

In light of the literature presented here, further basic research is warranted in order to establish possible therapeutic strategies for ovarian, thyroid and pancreatic cancer, where a pro-tumorigenic role for SIRT1 has been established. Particular attention should be paid to the role of miRNAs in regulating SIRT1 levels (miR-212 in thyroid cancer; miR-494, miR-138-5p, miR-601, and miR-217 in PC; miR-204 in HCC). Furthermore, given the growing importance of lncRNAs in cancer pathology, the involvement of MALAT1 should be exploited. For instance, by measuring its levels in HCC biopsies and, possibly, by monitoring its expression during therapy administration or disease progression.

AUTHOR CONTRIBUTIONS

RF has been studied SIRT1 in cancer and is currently assessing a possible epigenetic regulation for this enzyme. RF conceived, wrote, and formatted the manuscript.

FUNDING

This work has been funded by Azienda Unità Sanitaria Locale - IRCCS Reggio Emilia, Italy.

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Conflict of Interest Statement: The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Endocrinology

ORIGINAL RESEARCH
published: 23 August 2018
doi: 10.3389/fendo.2018.00476

Long-Term L-Serine Administration Reduces Food Intake and Improves Oxidative Stress and Sirt1/NFκB Signaling in the Hypothalamus of Aging Mice

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Serine has recently been shown to reduce oxidative stress and inflammation, which, when occurring in the hypothalamus, contribute to age-related obesity. To explore whether long-term serine administration reduces oxidative stress and body weight in aging mice, various concentrations of L-serine dissolved in water were administered to 18-month-old C57BL/6J mice for 6 months. The results showed that the administration of 0.5% (w/v) L-serine significantly reduced food intake and body weight gain during the experiment. Moreover, the administration of 0.5% L-serine decreased the concentrations of leptin, malondialdehyde, interleukin-1β, and interleukin-6, while it increased those of superoxide dismutase and glutathione, in both the serum and hypothalamus. Reactive oxygen species and the activity of nicotinamide adenine dinucleotide phosphate oxidase were reduced in the hypothalamus of aging mice treated with L-serine as compared with untreated control mice. Additionally, the expression of the leptin receptor increased while the levels of neuropeptide Y and agouti-related protein decreased in mice that had been treated with 0.5% L-serine. The expression of Sirt1 and phosphorylated signal transducers and activators of transcription 3 (pSTAT3) increased, while that of phosphorylated NFκB decreased in the mice treated with 0.5% L-serine. These results indicated that long-term L-serine administration reduces body weight by decreasing orexigenic peptide expression and reduces oxidative stress and inflammation during aging in mice, possibly by modulating the Sirt1/NFκB pathway. Thus, L-serine has the potential to be used in the prevention of age-related obesity.

Keywords: aging, inflammation, oxidative stress, serine, Sirt1

OPEN ACCESS

Edited by:
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Northwest University, China

Reviewed by:
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Specialty section:
This article was submitted to Cellular Endocrinology, a section of the journal Frontiers in Endocrinology

Received: *10 May 2018*
Accepted: *01 August 2018*
Published: *23 August 2018*

Citation:
Zhou X, Zhang H, He L, Wu X and Yin Y (2018) Long-Term L-Serine Administration Reduces Food Intake and Improves Oxidative Stress and Sirt1/NFκB Signaling in the Hypothalamus of Aging Mice. Front. Endocrinol. 9:476. doi: 10.3389/fendo.2018.00476

INTRODUCTION

The hypothalamus is a critical part of the central nervous system that modulates the stress response and senses nutrient-related inputs. However, in aging mice, increased levels of oxidative stress and inflammatory markers are observed in the hypothalamus (1–3). Low levels of antioxidant enzymes and high levels of lipid peroxidation make the hypothalamus vulnerable to reactive oxygen species

(ROS) (4–6). These elevations of oxidative damage and inflammatory responses in the central nervous system are believed to contribute to age-related diseases including obesity and various neurodegenerative disorders (7–9).

The nicotinamide adenine dinucleotide-dependent deacetylase sirtuin 1 (Sirt1) regulates the response to oxidative stress, which correlates with many diseases (10–12). Sirt1 activity in the hypothalamus decreases in association with aging, and its low expression contributes to a low level of antioxidants and increased oxidative damage (10). Importantly, Sirt1 is also a nutrient sensor and plays critical roles in the energy balance in the hypothalamus. Debate is ongoing as to whether hypothalamic Sirt1 has orexigenic or anorexigenic effects, although most of the evidence shows that inhibition of Sirt1 induces a negative energy balance by regulating the activity of forkhead transcription factor FKHR (FOXO1) (13–15) and the expression of agouti-related protein (AGRP) and neuropeptide Y (NPY) (16, 17). Additionally, the transcription factor nuclear factor-κB (NFκB) regulates numerous target genes to exert its biological functions. Importantly, NFκB functions as a central regulator of the immune response and controls the secretion of inflammatory cytokines in many tissues including the hypothalamus (18–20). Moreover, NFκB is also a central mediator of stress responses under conditions of oxidative stress and upon exposure to certain chemicals in the central nervous system (18).

L-Serine is traditionally considered a non-essential amino acid. Recently, dietary supplementation with L-serine has been shown to have antioxidant effects (21–24). L-Serine is a precursor of glycine and cysteine, which can be used for the synthesis of glutathione. In addition, L-serine exerts critical functions in the mammalian central nervous system. It serves as a precursor of the neuroactive substances L-serine and glycine, and mediates neuroprotective effects (25, 26). However, it remains unknown whether L-serine has any effects on age-related oxidative stress in the hypothalamus. Consequently, we conducted the present study to explore the effects of long-term L-serine supplementation on hypothalamic oxidative stress and Sirt1 expression in aging mice.

MATERIALS AND METHODS

Animals

Eighty 18-month-old C57BL/6J male mice were included in the study. The mice were obtained from SLAC Laboratory Animal Center (Changsha, China) and were housed in a temperature-controlled animal facility (lighting cycle: 12 h/d), with free access to food and water. All animals were randomly assigned into four groups: control mice, which were kept untreated, and mice administered with 0.1, 0.2, or 0.5% (w/v) L-serine dissolved in the drinking water. Eighteen-month-old adult mice were used as young controls. The experiment had the duration of 6 months. Food intake and body weight were recorded every week. Upon completion of the experiment, mice were euthanized with isoflurane and the blood was collected from the retro-orbital sinus. The hypothalamus was excised and collected according to the method described in a previous study (7). The hypothalamus samples were either immediately snap-frozen in liquid nitrogen or fixed in formaldehyde solution. This study was performed in accordance with the recommendations of the Guide for Care and Use of Laboratory Animals published by the Animal Welfare Committee of the Institute of Subtropical Agriculture, Chinese Academy of Sciences. The protocol was approved by the Animal Welfare Committee of the Institute of Subtropical Agriculture, Chinese Academy of Sciences.

Biochemical Assays

Superoxide dismutase (SOD), malondialdehyde (MDA), and glutathione (GSH) biochemical assays were performed with commercially available kits from Nanjing Jiancheng Bioengineering Institute (Nanjing, Jiangsu, China). Interleukin (IL)-1β, IL-6, and leptin biochemical assays were performed with kits from Cusabio Biotech (Wuhan, Hubei, China; https://www.cusabio.com/). Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activity was assayed as previously reported (27).

Determination of Reactive Oxygen Species

ROS content was determined as previously described (21). Hypothalamus samples were placed in optimum cutting temperature compound (Sakura Finetek, Tokyo, Japan) and flash-frozen in a methylbutane-chilled bath at $-81 \pm 2°C$, were sliced into 10-μm sections and stained with a solution of 1 μM dihydroethidium (Sigma-Aldrich, St. Louis, MO, USA), for 20 min, at 37°C in a humidified 5% CO_2 incubator. Results were observed and analyzed by fluorescence microscopy and Image Browser software (Leica, Wetzlar, Germany).

Immunohistochemistry Assay

Hypothalamus samples were cut into 4-μm sections and processed for immunohistochemical staining as previously described. Briefly, the samples were incubated with a primary antibody [leptin receptor (LepRb), NPY, or AGRP; Boster, Wuhan, China] overnight at 4°C and then with poly-horseradish peroxidase-conjugated IgG for 60 min at $22 \pm 4°C$. Subsequently, the avidin-biotin-peroxidase complex and the substrate 3,3′-diaminobenzidine were applied for 2 min and the samples were analyzed.

RT-qPCR Assay

Total hypothalamic RNA was isolated using the TRIzol® reagent and reverse-transcribed into cDNA with the PrimeScript™ RT reagent kit (TaKaRa Bio, Otsu, Japan). Quantitative PCR was performed as previously reported (28). Briefly, the reaction was performed with a total volume of 10 μL assay solution containing 5 μL SYBR® Green mix (TaKaRa Bio), 0.2 μL ROX internal reference dye, 1 μL cDNA template, 3 μL deionized H_2O, and 0.4 μL each of the forward and reverse primers. The comparative Ct method was applied to calculate the mRNA expression of the target genes relative to that of β-actin. The primer sequences are presented in **Supplementary Table 1**.

Western Blot Assay

Protein supernatants extracted from hypothalamus samples were run on 10% sodium dodecyl sulfate acrylamide gels and electro-blotted onto nitrocellulose membranes. The membranes were incubated with primary antibodies overnight

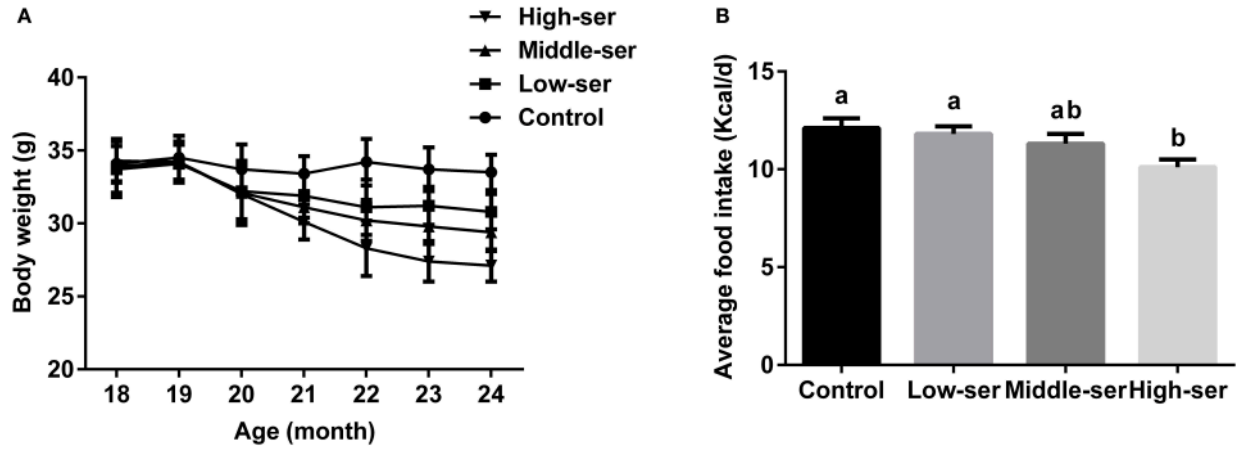

FIGURE 1 | Effects of long-term L-serine administration on body weight and food intake in aging mice. **(A)** Body weight; **(B)** average daily food intake. Control, untreated control mice; Low-ser, mice supplemented with 0.1% (wt/vol) L-serine dissolved in the drinking water; Middle-ser, mice supplemented with 0.2% (wt/vol) L-serine dissolved in the drinking water; High-ser, mice supplemented with 0.5% (wt/vol) L-serine dissolved in the drinking water. Values are expressed as mean ± standard error of the mean (SEM), $n = 20$; ^{a,b}Means of the bars with different letters were significantly different among groups ($P < 0.05$).

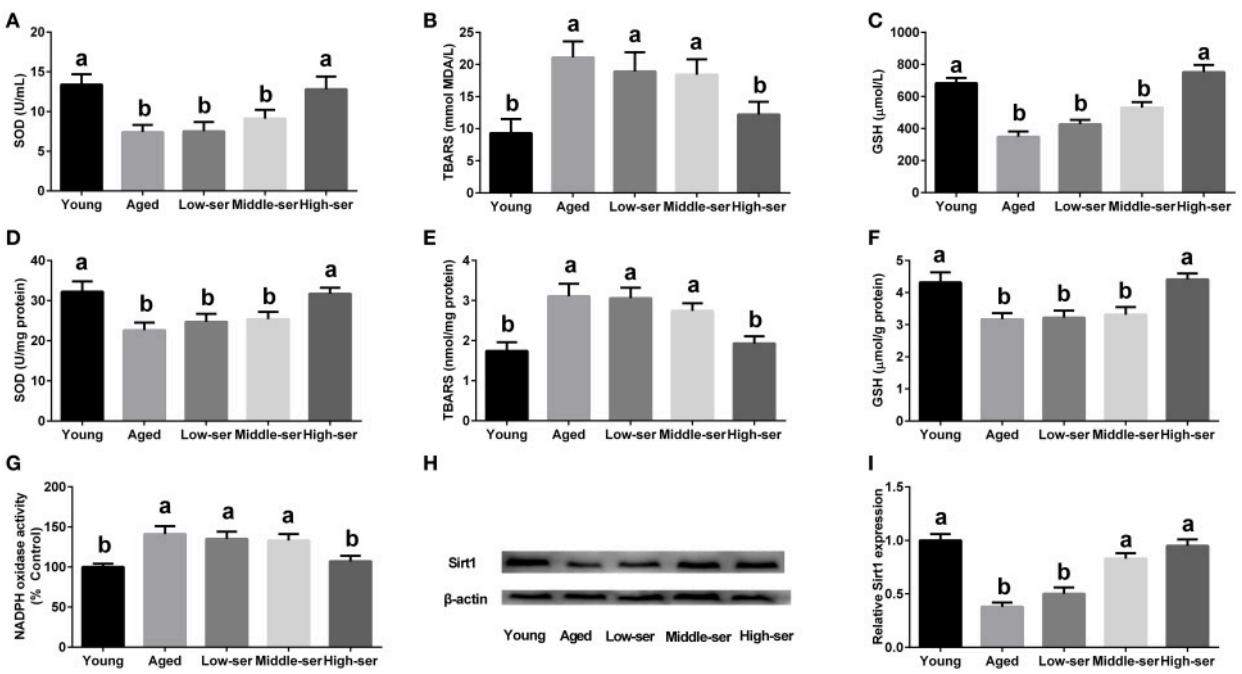

FIGURE 2 | Effects of long-term L-serine administration on oxidative stress in aging mice. **(A–C)** SOD, MDA, and GSH contents in serum; **(D–F)** SOD, MDA, and GSH contents in the hypothalamus; **(G)** NADPH oxidase activity; **(H,I)** Protein expression of Sirt1. Aged, untreated control mice; Low-ser, mice supplemented with 0.1% (wt/vol) L-serine dissolved in the drinking water; Middle-ser, mice supplemented with 0.2% (wt/vol) L-serine dissolved in the drinking water; High-ser, mice supplemented with 0.5% (wt/vol) L-serine dissolved in the drinking water. Young, adult male mice at the age of 18 months. SOD, superoxide dismutase; MDA, malondialdehyde; GSH, glutathione; NADPH, nicotinamide adenine dinucleotide phosphate. Values are expressed as mean ± SEM, $n = 3$ for the statistical analysis of western blotting data and $n = 8$ for the statistical analysis of other data; ^{a,b}Means of the bars with different letters were significantly different among groups ($P < 0.05$).

at 4°C, followed by a second incubation with anti-rabbit or anti-mouse IgG. Primary antibodies specific for Sirt1, STAT3, phospho-STAT3, and total NFκB (Cell Signaling Technology, Beverly, MA, USA) were used. Finally, the EZ-ECL chemiluminescence reagent (Biological Industries, Cromwell, CT, USA) was added onto the membrane and bands were obtained.

Statistical Analysis

All statistical analyses were performed with one-way analysis of variance, using the general linear model and the MIXED procedure (PROC MIXED) from SAS software version 9.2 (SAS Institute Inc., Cary, NC, USA). Data are presented as least squares means ± standard error of the mean. Mean values were considered significantly different when $P < 0.05$.

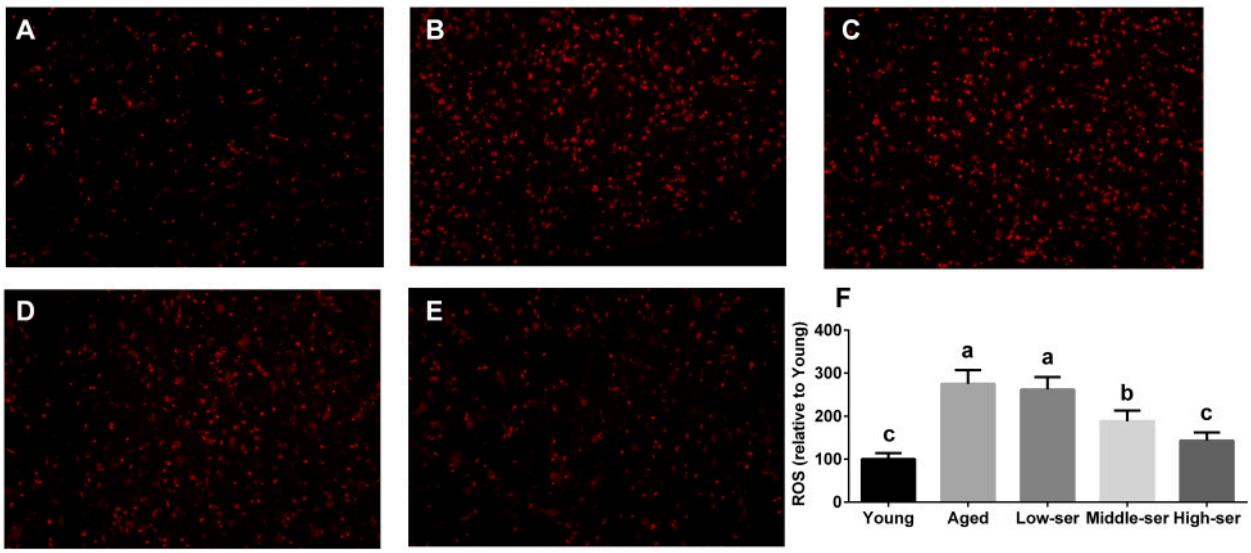

FIGURE 3 | Effects of long-term L-serine administration on the ROS content in aging mice. Representative images of ROS in mice from the Young **(A)**, Aged **(B)**, Low-ser **(C)**, Middle-ser **(D)**, and High-ser **(E)** groups; **(F)** Relative ROS content. Aged, untreated control mice; Low-ser, mice supplemented with 0.1% (wt/vol) L-serine dissolved in the drinking water; Middle-ser, mice supplemented with 0.2% (wt/vol) L-serine dissolved in the drinking water; High-ser, mice supplemented with 0.5% (wt/vol) L-serine dissolved in the drinking water. Young, adult male mice at the age of 18 months. ROS, reactive oxygen species. Values are expressed as mean ± SEM, $n = 4$. ^{a,b,c}Means of the bars with different letters were significantly different among groups ($P < 0.05$).

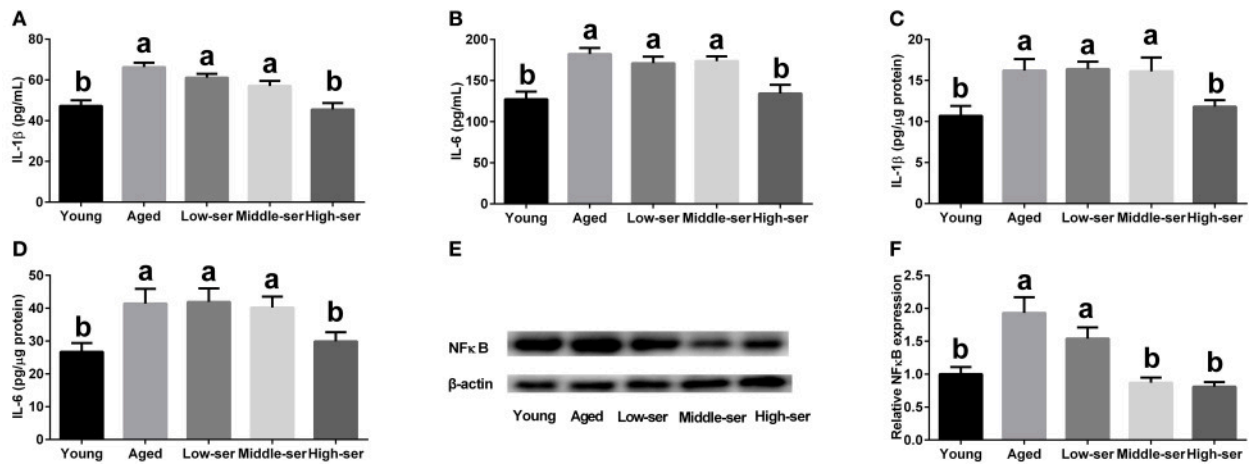

FIGURE 4 | Effects of long-term L-serine administration on the inflammatory response in aging mice. **(A,B)** Concentrations of IL-1β and IL-6 in serum; **(C,D)** Concentrations of IL-1β and IL-6 in the hypothalamus; **(E,F)** Relative NF-κB protein expression. Aged, untreated control mice; Low-ser, mice supplemented with 0.1% (wt/vol) L-serine dissolved in the drinking water; Middle-ser, mice supplemented with 0.2% (wt/vol) L-serine dissolved in the drinking water; High-ser, mice supplemented with 0.5% (wt/vol) L-serine dissolved in the drinking water. Young, adult male mice at the age of 18 months. Values are expressed as mean ± SEM, $n = 3$ for the statistical analysis of western blotting data and $n = 8$ for the statistical analysis of other data; ^{a,b}Means of the bars with different letters were significantly different among groups ($P < 0.05$).

RESULTS

Effects of Long-Term L-Serine Administration on Body Weight and Food Intake in Aging Mice

The long-term administration of 0.5% L-serine significantly decreased body weight and reduced food intake in aging mice when compared with controls (i.e., aging mice that did not receive L-serine), while the administration of 0.1 or 0.2% L-serine did not show such effects (**Figure 1**).

Effects of Long-Term L-Serine Administration on Oxidative Stress in Aging Mice

The long-term administration of 0.5% L-serine significantly increased the SOD and GSH levels while decreasing the

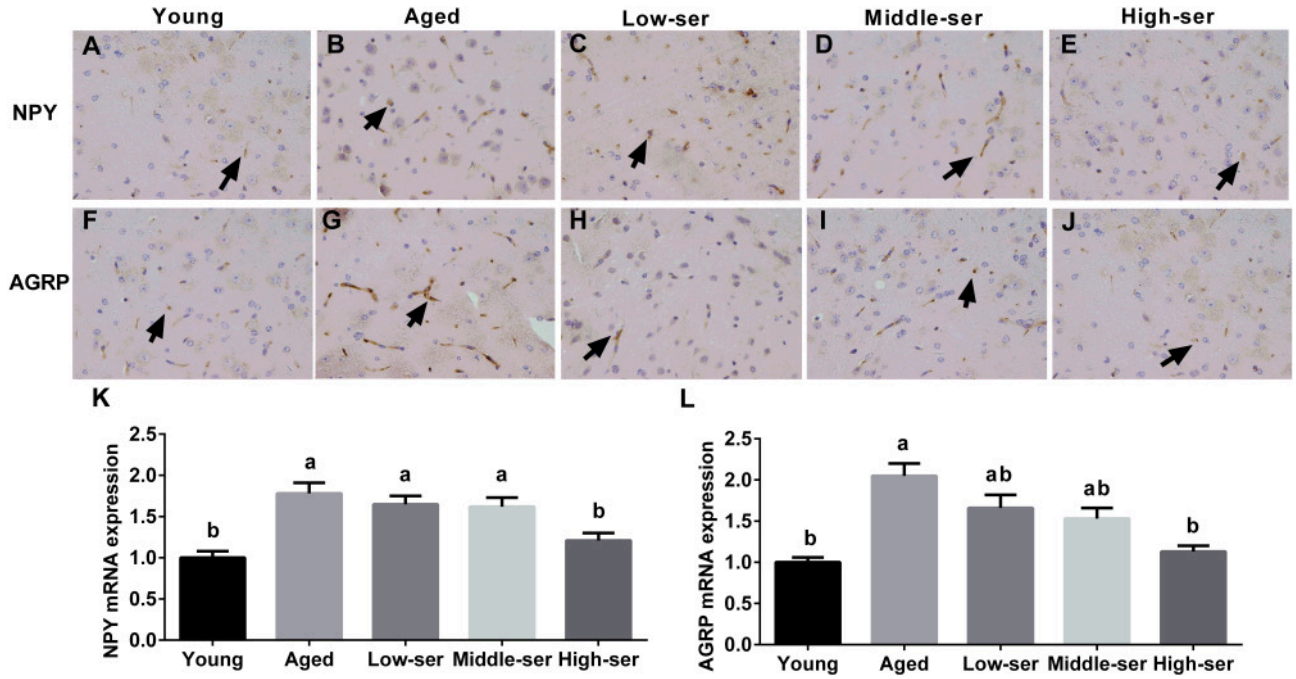

FIGURE 5 | Effects of long-term L-serine administration on orexigenic neuropeptide expression in aging mice. NPY **(A–E)** and AGRP **(F–J)** expression as determined by immunohistochemistry; relative NPY **(K)** and AGRP **(L)** mRNA expression. Aged, untreated control mice; Low-ser, mice supplemented with 0.1% (wt/vol) L-serine dissolved in the drinking water; Middle-ser, mice supplemented with 0.2% (wt/vol) L-serine dissolved in the drinking water; High-ser, mice supplemented with 0.5% (wt/vol) L-serine dissolved in the drinking water. Young, adult male mice at the age of 18 months. Arrow (yellow), expression of target proteins; NPY, neuropeptide Y; AGRP, agouti-related protein. Values are expressed as mean ± SEM, $n = 8$; ^{a,b}Means of the bars with different letters were significantly different among groups ($P < 0.05$).

MDA contents in both the serum and hypothalamus of aging mice in comparison with controls (**Figures 2A–F**). However, the administration of L-serine at 0.1 or 0.2% had no effect. Additionally, the administration of 0.5% L-serine significantly decreased NADPH oxidase activity (**Figure 2G**) and ROS content (**Figure 3**) while it increased Sirt1 expression (**Figures 2H,I**) in the hypothalamus.

Effects of Long-Term L-Serine Administration on the Inflammatory Response in Aging Mice

The long-term administration of 0.5% L-serine significantly decreased the concentrations of IL-1β and IL-6 in the serum and hypothalamus of aging mice when compared with the control animals. By contrast, L-serine at 0.1 or 0.2% had no effect (**Figures 4A–D**). In addition, the long-term administration of 0.5% L-serine significantly decreased NFκB expression in the hypothalamus (**Figures 4E,F**).

Effects of Long-Term L-Serine Administration on Orexigenic Neuropeptide Expression in Aging Mice

Long-term administration of 0.5% L-serine significantly decreased NPY and AGRP expression in the hypothalamus of aging mice in comparison with controls; however, administration of L-serine at 0.1 or 0.2% showed no such effects (**Figure 5**).

Effects of Long-Term L-Serine Administration on Leptin Signal Pathway in Aging Mice

The long-term administration of 0.5% L-serine, but not 0.1 or 0.2% L-serine, significantly decreased the leptin content (**Figure 6F**) in the serum and increased LepRb expression (**Figures 6A–E,G**) in the hypothalamus of aging mice when compared with controls. In addition, the long-term administration of 0.5% L-serine significantly increased the expression of phosphorylated STAT3 in the hypothalamus of aging mice (**Figures 6H,I**).

DISCUSSION

Our previous studies have shown that L-serine could alleviate oxidative stress in various tissues of different rodent models, including diquat- and long-term high-fat-induced oxidative stress in liver (21, 22) and lipopolysaccharides-induced oxidative damage in the small intestine (23). Based on these previous experiments, we chose the dose of 0.1, 0.2, and 0.5% (w/v) L-serine for the current 6-month study, respectively. Additionally, since patients who received the relatively high

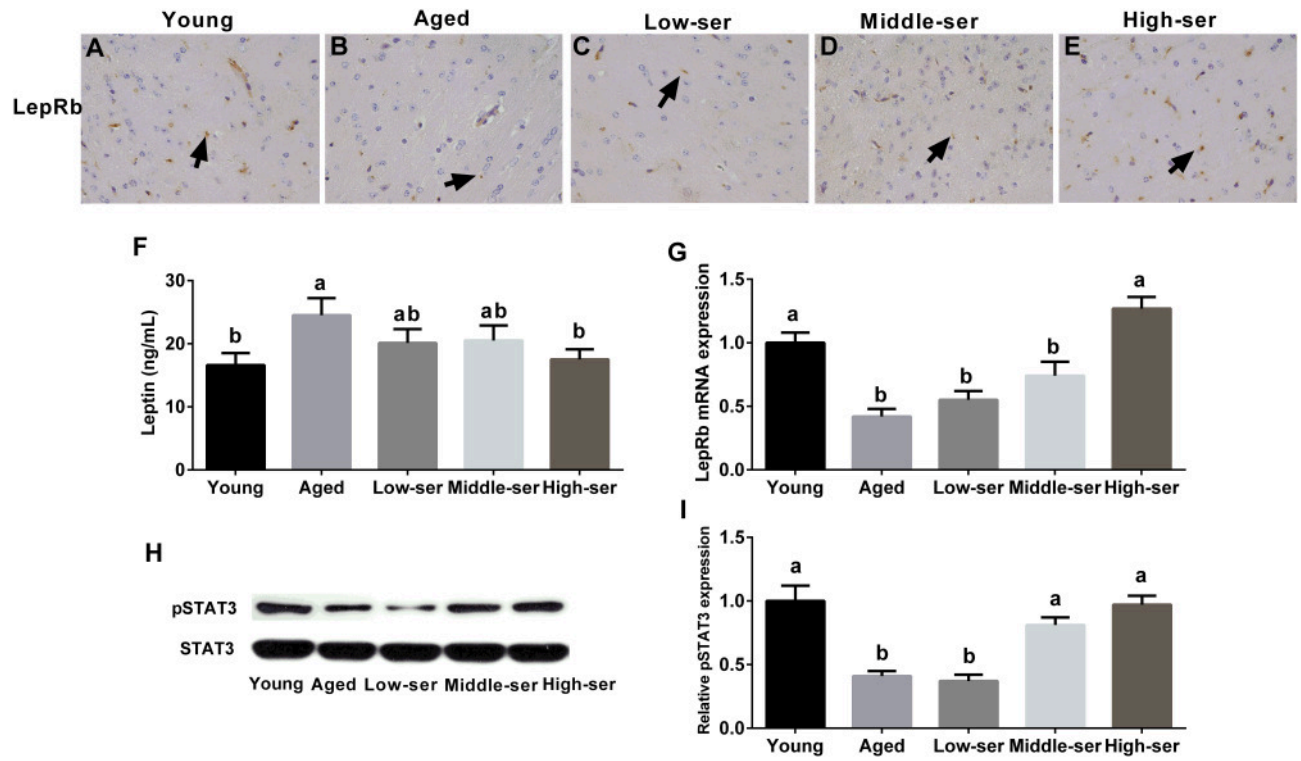

FIGURE 6 | Effects of long-term L-serine administration on the leptin signaling pathway in aging mice. **(A–E)** LepRb expression as determined by immunohistochemistry; **(F)** leptin content in the hypothalamus; **(G)** relative LepRb mRNA expression; **(H,I)** relative pSTAT3 protein expression. Aged, untreated control mice; Low-ser, mice fed with 0.1% (wt/vol) L-serine dissolved in the drinking water; Middle-ser, mice fed with 0.2% (wt/vol) L-serine dissolved in the drinking water; High-ser, mice fed with 0.5% (wt/vol) L-serine dissolved in the drinking water. Young, adult male mice at the age of 18 months. Arrow (yellow), expression of LepRb protein; LepRb, leptin receptor. Values are expressed as mean ± SEM, $n = 3$ for the statistical analysis of western blotting data and $n = 8$ for the statistical analysis of other data; ^{a,b}Means of the bars with different letters were significantly different among groups ($p < 0.05$).

amount of 30 g/day L-serine for 9 months in phase II clinical trials did not show any side effects and L-serine is generally regarded as safe by the United States Food and Drug Administration (25), we employed L-serine as a non-toxic agent. As expected, our results further supported that long-term L-serine supplementation may alleviate age-related oxidative damage, since it is shown herein that L-serine increased the levels of antioxidant enzymes while decreasing those of ROS in the hypothalamus.

The process of aging is associated with a redox imbalance and the accumulation of oxidative damage in many tissues, which is caused by an increase in ROS production and a decrease in antioxidant capacity (29). The hypothalamus is especially oxidatively challenged with increasing age and is vulnerable to oxidative stress (3). Since the hypothalamus plays a critical role in the central nervous system, age-related oxidative stress in this organ usually results in the loss of various physiological functions and the development of diseases such as age-related obesity and neurodegeneration. We found that long-term L-serine supplementation attenuated the decreases in the levels of antioxidants (GSH) and antioxidant enzymes (SOD) and the accumulation of ROS that are observed during aging. Based on these results, we believe that the functionality of the hypothalamus would be improved by long-term L-serine supplementation at an appropriate concentration in aging mice.

In age-related diseases, there is an antagonistic crosstalk between Sirt1 and NFκB. On the one hand, the inhibition of Sirt1 stimulates NFκB-induced inflammation directly through regulating the p65 subunit of the NFκB complex. On the other hand, NFκB inhibits Sirt1 activity mainly by affecting the production of factors such as ROS (30). Sirt1 plays a major role in the regulation of oxidative stress during aging. A reduction in Sirt1 expression with the progression of aging contributes to the upregulation of oxidative damage (31). Consequently, Sirt1-activating dietary supplements such as resveratrol, eicosapentaenoic acid, docosahexaenoic acid, and acetylshikonin show anti-aging effects (32, 33). Moreover, Sirt1 also plays a protective role against neuroinflammation in brain disease (33). Here, we found that L-serine supplementation at the concentration of 0.5% (w/v) restored the expression of Sirt1 after its age-related decrease. Thus, we suggest that the Sirt1 pathway may mediate the antioxidative and anti-inflammatory effects of L-serine in the hypothalamus of aging mice. Additionally, the activity of NFκB signaling was reported to increase in the hypothalamus with aging (34), suggesting that NFκB signaling is a promising target for preventing age-related inflammation.

The inhibition of this pathway by caloric restriction and dietary nutrients such as polyunsaturated fatty acids delays aging and extends the lifespan of rodents (33, 35). Our results also indicate that L-serine attenuated the inflammatory response, which is usually associated with oxidative stress during aging, since the concentrations of inflammatory cytokines and the expression of NFκB decreased in the hypothalamus. These results indicated that NFκB pathway also plays an important role in mediating the preventive effects of L-serine against the increased inflammation in aged mice.

Unexpectedly, we found that long-term L-serine supplementation affected food intake and decreased age-related body weight gain. L-Serine has been used for the treatment of serine deficiency disorders and as a neuroprotective substance for decades (36). A phase I human clinical trial indicated that L-serine could be a generally safe supplement for patients with amyotrophic lateral sclerosis/motor neuron disease (37). However, we noticed that, in these reports, two patients showed a slightly decreased food intake. Supplementation with a high dose of L-serine for a long period might have some effects on food intake. The decreased expression of NPY and AGRP, which are highly conserved neuropeptides with orexigenic actions in the hypothalamus (38), could have contributed to the reduced food intake in aging mice treated with 0.5% L-serine. In addition, the increases in the expression of leptin receptor and the phosphorylation of STAT3, which is involved in the leptin signaling pathway in the hypothalamus, induced by serine would also affect food intake because of the anorectic effects of leptin. However, a high leptin level was found in aged mice; therefore, we suggest that leptin resistance may occur in these aged animals. The preventive effects of long-term L-serine supplementation on age-induced oxidative stress and age-related obesity observed herein suggest that L-serine supplementation might have the same effects as lifelong caloric restriction. Furthermore, L-serine supplementation in aging mice activated *Sirt1*, which is defined as a "longevity gene," suggesting a life-extending effect of serine. Interestingly, researchers found that in the village of Ogimi, which is known as the "Village of Longevity" in Japan, the traditional food items of residents are rich in L-serine (25). Further studies are required to provide more convincing evidence of the life-extending effect of L-serine.

In conclusion, our results suggest that long-term L-serine supplementation at an appropriate concentration attenuates age-related oxidative stress and the inflammatory response in the hypothalamus of aging mice. The Sirt1 and NFκB pathways may mediate these effects of L-serine. However, the molecular mechanisms through which L-serine decreases the expression of NFκB while rescuing that of Sirt1 need to be further elucidated. Additionally, we unexpectedly found that L-serine reduced food intake and age-related body weight gain. L-Serine may exert these effects by regulating the leptin pathway and the orexigenic neuropeptides NPY and AGRP.

AUTHOR CONTRIBUTIONS

XZ, LH, and HZ conducted the experiment. XZ and LH collected and analyzed the data. XW and YY helped with the discussion. XZ and XW designed the experiment and wrote the manuscript. XW and YY revised the manuscript.

FUNDING

This work was financially supported by National Key Research and Development Program of China (2016YFD0501201, 2018YFD0500405), National Natural Science Foundation of China (31702125), Natural Science Foundation of Hunan Province (2017JJ3373) and the earmarked fund for China Agriculture Research System (CARS-35).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2018.00476/full#supplementary-material

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