Functions of mammalian SIRT4 in cellular metabolism and research progress in human cancer (Review)

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Abstract. Sirtuins are mammalian homologs of yeast silent information regulator two (SIRT) and are a highly conserved family of proteins, which act as nicotinamide adenine dinucleotide (NAD^+)-dependent histone deacetylases. The seven sirtuins (SIRT1-7) share a conserved catalytic core domain; however, they have different enzyme activities, biological functions, and subcellular localizations. Among them, mitochondrial SIRT4 possesses ADP-ribosyltransferase, NAD^+-dependent deacetylase, lipoamidase, and long-chain deacylase activities and can modulate the function of substrate proteins via ADP-ribosylation, delipoylation, deacetylation and long-chain deacylation. SIRT4 has been shown to play a crucial role in insulin secretion, fatty acid oxidation, amino acid metabolism, ATP homeostasis, apoptosis, neurodegeneration, and cardiovascular diseases. In addition, recent studies have demonstrated that SIRT4 acts as a tumor suppressor. Here, the present review summarizes the enzymatic activities and biological functions of SIRT4, as well as its roles in cellular metabolism and human cancer, which are described in the current literature.

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1. Introduction

The acylation of proteins is one of the modifications, which occurs following translation and can modulate the function of the proteins by changing the surface charge and regulating protein conformations or protein-protein interactions, which is similar to phosphorylation (1). Histone deacetylases (HDACs) can remove the acetylated group from the N-acetyl-lysine of histones or non-histones. To date, four types of histone deacetylases have been discovered, class I include HDAC1-3 and HDAC8, class II include HDAC4-7 and HDAC9-10, and HDAC11 belongs to class IV (1,2). Sirtuins are defined as class III HDACs and are dependent on NAD^+, which distinguishes them from the classes I, II and IV, which are zinc-dependent (3). In the 1970s, the silent information regulator 2 gene (SIRT) was first discovered in budding yeast, as a regulator of chromatin structure (4). This group of proteins are now known to be also distributed in a wide range of mammal species, such as drosophila, murine and cattle (4,5). In humans, the SIRT family consists of seven members, SIRT1-7, which are divided into four classes with respect to their sequence information. SIRT-1, -2, and -3 are class I proteins, SIRT4 is class II, SIRT5 is class III, and SIRT-6 and -7 are class IV (6,7). All of the sirtuins share a highly conserved NAD^+-binding catalytic core composed of ~270 amino acids but vary in their N- and C-terminal sequences. The different N- and C-terminal sequences are important for subcellular localization and enzyme activities (8,9).

These seven mammalian sirtuins have different protein structures, subcellular localizations, functional properties, enzymatic activities, and substrate specificities (Table I) (10). SIRT-1, -6, and -7 are primarily located in the nucleus, SIRT2 is primarily located in the cytoplasm, and SIRT3-5 are predominately found in the mitochondria (11). However, some of these proteins are known to translocate from their primary location in different tissues or under various physiological conditions (12). For example, in myoblast cell line, SIRT1 moves to the cytosol under the action of some kinases and PI3K signal cascade (8). and SIRT2 moves into the nucleus during the G_2/M cell cycle transition (13). Each sirtuin also has unique catalytic activities, which enable the sirtuins to exhibit broad and important regulatory functions in numerous

biological processes, such as life span, gene transcription, cell proliferation, differentiation, apoptosis, genome stability, cellular metabolism, tumorigenesis, energy homeostasis, DNA damage, and stress responses (7,14-17).

In contrast to other sirtuins, research on mitochondrial SIRT4 is relatively limited. However, it is well-known that SIRT4 is widely expressed in human organs and tissues, particularly in the heart, liver, kidney, spleen, prostate, testis, and ovaries (18). Unlike other sirtuins, SIRT4 possesses a conserved deacetylase domain, but earlier reports suggested it lacked detectable histone deacetylase activity (19). In 2006, Haigis *et al* (20) first discovered that SIRT4 possesses ADP-ribosyltransferase activity. Subsequently, it was found to have substrate-specific deacetylase, lipoamidase, and long-chain deacylase activities (21). These catalytic activities provide SIRT4 with the ability to play a vital role in insulin secretion, fatty oxidation, leucine catabolism, ATP homeostasis, lipid catabolism, tumorigenesis, neurological disorders and cardiovascular diseases (7,21). However, SIRT4 is still the least well-known sirtuin, as these activities are weak. It has been suggested that SIRT4 will become a novel therapeutic target and molecules which modulate its activity will be developed to treat various diseases in the future (22,23). In the present review, SIRT4 will be discussed and its functions in cellular metabolism and human cancer.

2. Structural characteristics of the sirtuin family

As aforementioned, every member of the sirtuin family contains a highly conserved catalytic center comprised of ~270 amino acids and divergent N- or C-terminal sequences (24,25). Therefore, the structure of sirtuins was divided into the non-enzymatic and the enzymatic parts. The non-enzymatic part includes seven specific N- and C-terminal segments, which determines the subcellular localization and catalytic function of the sirtuin (25). The presence of an N-terminal mitochondrial targeting sequence ensures that mitochondrial SIRT3-5 localize within the mitochondrial matrix. When these signal sequences are cleaved, the enzymatic functions of these proteins are activated (26,27).

The enzymatic part of the seven sirtuins is characterized by a high degree of fidelity. In 2001, Finnin *et al* (28) first identified the structure of sirtuins, and SIRT2 was the first reported subtype, which almost represents the structural basis of all sirtuins in the enzymatic part. The catalytic core consists of two main parts: A conserved large Rossmann fold domain and a variable small domain (28). The large inverted Rossmann fold domain consists of 6 β-strands and 6 α-helices. The small domain contains a zinc finger module and a helical module (29). These two modules of the small domain are joined to the large Rossmann fold domain by four polypeptide chains, which form a large groove between the small and large domains. This junctional groove includes the NAD^+-binding site, and most of the residues are highly conserved. The catalytic core of sirtuins are also included in the junctional groove, and mutations within these sites result in the loss of deacetylation catalytic activity (28,30-33). Moreover, when the substrate is close to the center of SIRTs, a conformational rearrangement occurs, and the small domain is rotated by ~25, thereby changing the conformation of the binding site to allow the catalytic reaction to proceed in a continuous process (34,35).

With respect to SIRT4, the non-enzymatic part has no C-terminal sequence and only contains 28 positively charged amino acids in the N-terminal sequence, which serves as a mitochondrial localization signal with low sequence conservation (36,37). In the enzymatic part, the typical sirtuin structure is located, which consists of a large Rossmann fold domain with a conserved His160 catalytic residue and a small domain. There are 2 specific structures, which separate SIRT4 from the other isoforms. The first one is a flexible loop, which contains an additional 12 residues (residues 195-206) in the Zn^{2+}-binding module. This loop is located deep within the catalytic core, which contributes to substrate binding and restrains relevant active site dynamics. The second unusual structure is the channel in the catalytic core, which starts from the acyl-Lys binding tunnel and terminates at the protein surface. This channel has a small positively charged area on the outer entrance and is predominantly hydrophobic. This channel serves as the binding site for longer substrate acyls and regulatory metabolites (37,38). The positive potential of the channel accounts for its weak NAD^+-dependent deacetylase, which is within the 14-600 μM range, and also explains why the positively charged NADH was easier to combine with SIRT4 and the mitochondrial concentration of free NADH, NAD^+/NADH ratio could act as a physiological SIRT4 regulator (39,40).

3. Enzymatic activities, substrates, and cellular functions of SIRT4

The robust enzymatic activities of SIRT4 are not fully understood; however, SIRT4 is associated with numerous cellular metabolic processes, such as insulin secretion, glutamine metabolism, and fatty acid metabolism (21). The principal enzymatic activities of SIRT4, involved in these biological processes, are ADP-ribosyltransferase, NAD^+-dependent deacetylase, lipoamidase, and long-chain deacylase (Table II). These functions will be described in more detail and are also summarized in Fig. 1.

ADP-ribosyltransferase and insulin secretion. Glutamine is the most abundant free cytoplasmic amino acid and has been found to be the staple nitrogen donor for the synthesis of nucleotides and amino acids (41). It can be converted into glutamate and α-ketoglutarate (α-KG) by two consecutive reactions and subsequently enters the TCA cycle. Glutamine is first converted into glutamate by glutaminase, then it is further converted into the TCA cycle intermediate α-KG either by glutamate dehydrogenase (GDH) or transamination-coupled reactions (42,43). GDH was the first mitochondrial protein to be identified to be inactivated through mono-ADP-ribosylation, which was found to be catalyzed by SIRT4. SIRT4 directly and enzymatically transfers the ADP-ribosyl group from NAD^+ to the C172 histone residue of GDH, thereby inhibiting its function and ultimately inhibiting the metabolism of glutamine in the mitochondria and reducing ATP production (20,42,44).

The inhibitory effect of SIRT4 on GDH represses insulin secretion in response to glucose and amino acids in pancreatic β cells. As insulin secretion is an ATP-dependent process, the

Table I. Characteristics and properties of the sirtuins family.

Name	Class	Length, aa	Location of catalytic domain, aa	Primary location	Catalytic activities
SIRT1	I	747	244-498	Nucleus	Deacetylation Deacylation
SIRT2	I	389	65-340	Cytoplasm	Deacetylation Deacylation Demyristoylation
SIRT3	I	399	126-382	Mitochondria	Deacetylation Decrotonylation
SIRT4	II	314	45-314	Mitochondria	ADP-ribosylation Deacetylation Deacylation Delipoylation
SIRT5	III	310	41-309	Mitochondria	Deacetylation Demalonylation Desuccinylation
SIRT6	IV	355	35-274	Nucleus	Deacetylation ADP-ribosylation Demyristoylation
SIRT7	IV	400	90-331	Nucleus	Deacetylation Desuccinylation

inhibition of GDH will lead to a decrease in ATP production in the mitochondria (36,44). Glucose and amino acid metabolism lead to an increased ATP/ADP ratio. Increased levels of ATP can close ATP-sensitive potassium channels which results in the depolarization of the plasma membrane, thus opening voltage-gated L-type calcium channels, facilitating the fusion of insulin-containing secretory vesicles to the plasma membrane to cause insulin exocytosis (45,46). SIRT4 inhibits the metabolism of glutamate, resulting in decreased production of ATP in mitochondria and inhibition of insulin secretion in pancreatic β cells (20). In addition, SIRT4 can interact with insulin-degrading enzyme (IDE) and ADP/ATP translocase 2/3 (ANT2/3) to synergistically inhibit insulin secretion (36).

NAD^+-dependent deacetylase and lipid metabolism. Reversible acetylation of lysine residues is an important post-translational modification, which changes the charge of lysine residues and potentially alters enzyme activity, structure, specificity, and subcellular localization of the protein (47). Deacetylation is the most important and common activity of sirtuins. They catalyze deacetylation by breaking the bonds between NAD^+ and niacinamide (NAM) ribosomes, transferring the acetylated groups from proteins to ADP-ribose, then releasing the deacetylated products and NAM. 2-O-acetyl-ADP-ribose is generated as a result of the transfer of the acetyl group onto the ADP-ribose residue. NAM is an inhibitor of deacetylation, which can also reverse the reaction to reproduce NAD^+ (25,48).

As a member of the sirtuin family, SIRT4 also possesses typical NAD^+-dependent deacetylation activity, but this activity is weak, has substrate specificity, and is associated with cellular lipid metabolism (49). In 2010, Nasrin *et al* (50) found that downregulating the expression of SIRT4, using adenoviral shRNA, in hepatocytes and myocytes significantly enhanced the expression of the genes, which are associated with fatty acid oxidation (FAO), such as MCAD, PDK4, CTP1, PPARα, PGC1α, ERRα, and CoxV. Meanwhile, the FAO of hepatocytes and myocytes was also significantly increased. During cellular lipid metabolism, malonyl CoA provides the carbon skeleton for lipogenesis and also inhibits fat oxidation. Therefore, the regulation of malonyl CoA levels can control the balance between lipid anabolism and catabolism. Both acetyl CoA carboxylase (ACC) and malonyl CoA decarboxylase (MCD) regulate the cellular malonyl CoA level. ACC converts acetyl CoA to malonyl CoA, and MCD converts it back to acetyl CoA (51-53). ACC activity is regulated by phosphorylation from the AMP-activated protein kinase (AMPK) signaling pathway (51). MCD activity is regulated via deacetylation by SIRT4. For example, the deacetylation of the K471 residue of MCD by SIRT4 inhibited the activity of MCD, which further repressed the oxidative decomposition of intracellular fatty acids and promoted the synthesis of lipids in white adipose tissue (52). *In vivo*, SIRT4-knockout mice had a greater exercise tolerance and protection against diet-induced obesity (52).

With the progress of research, it has been found that the regulation of lipid metabolism by SIRT4 involves several mechanisms. Carnitine O-palmitoyltransferase 1, liver isoform (CPT1) is the rate-limiting enzyme to transfer fatty acids into both the inner and outer mitochondrial membranes. The inhibition of SIRT4 by MCD increased the concentration of malonyl CoA, which downregulated CPT1 activity and inhibited fatty acid oxidation (FAO) (53). Peroxisome proliferator-activated receptor-α (PPARα) is one of the mediators of the hepatic response to fasting and a ligand-activated transcription factor, which promotes the transcription of genes involved in fatty acid catabolism, such as Lipg, Acot3, Pdk4, Acox1, Cpt1a and Acadm (54,55). Accompanied by the high expression of SIRT1

Table II. Enzymatic activities, substrates and metabolic function of SIRT4.

Enzymatic activities	Substrates	Metabolic functions	(Refs.)
ADP-ribosyltransferase	GDH	Insulin secretion Tumor suppressor	(20)
NAD^+-dependent deacetylase	MCD MTPα	Fatty acids oxidation	(52,57)
Lipoamidase	PDH	Glycolysis, Cellular metabolism	(59)
Long-chain deacylase	MCCC ANT2	Leucine metabolism, ATP homeostasis	(62,66)
Debiotinylase	No native substrate has been identified		(59)

GDH, glutamate dehydrogenase; MCD, malonyl CoA decarboxylase; MTPα, mitochondrial trifunctional protein α; PDH, pyruvate dehydrogenase; MCCC, methylcrotonyl-CoA carboxylase; ANT2, ADP/ATP translocase 2.

both at mRNA and protein levels, SIRT4 decreased the rate of FAO by inhibiting the expression of PPARα and downstream genes associated with fatty acid catabolism (55). In addition, the deacetylase activity of SIRT4 also acts on the mitochondrial trifunctional protein α (MTPα) in hepatocytes, which is a critical enzyme for fatty acid β-oxidation (56). Deacetylation by SIRT4 increased the activity of MTP-α, which inhibited the oxidation of fatty acids and eventually led to hepatic steatosis. In contrast, low expression of SIRT4 promoted the acetylation of MTP-α, increased cellular FAO, and prevented the development of non-alcoholic fatty liver disease. In addition, SIRT4 might inhibit the activity of enoyl-CoA hydratase α-subunit (ECHA), which is an enzyme involved in branched-chain amino acid (BCAA) catabolism, to suppress FAO (57).

Lipoamidase and glycolysis. The pyruvate dehydrogenase (PDH) complex is a mitochondrial complex, and is the rate-limiting enzyme that catalyzes the decarboxylation of pyruvate to produce acetyl CoA. It also links glycolysis to the TCA cycle and consists of three catalytic subunits: E1, a pyruvate decarboxylase; E2, a dihydrolipoyllysine acetyltransferase (DLAT), and E3, a dihydrolipoyl dehydrogenase (58). Mathias *et al* (59) demonstrated that SIRT4 had a higher NAD^+-dependent lipoamidase activity compared with the catalytic efficiency for deacetylation, and exhibited a greater enzymatic activity for lipoyl- and biotinyl-lysine modifications. The E2 component of the PDH is a biological substrate of SIRT4 lipoamidase activity. SIRT4 enzymatically hydrolyzes lipoamide cofactors from DLAT, which results in a reduction in PDH lipoyl levels and inhibition of its function (59). PDH controls pyruvate decarboxylation, fueling multiple downstream pathways, such as aerobic oxidation of glucose to the tricarboxylic acid cycle and oxidative phosphorylation (58). These findings suggest that SIRT4 is a critical modulator of mitochondrial function and cellular metabolism by regulating glycolysis via the inhibition of PDH.

Long-chain deacylase and leucine metabolism, ATP homeostasis. Leucine is a BCAA and an effective stimulator of insulin secretion, as it can allosterically activate GDH, thereby promoting glutamine metabolism and providing energy (60). 3-Methylglutaconyl (MGc)-CoA, 3-methylglutaryl (MG)-CoA, and 3-hydroxy-3-methylglutaryl (HMG)-CoA are intermediates of leucine catabolism catalyzed by methylcrotonyl-CoA carboxylase (MCCC) (60,61). MGc-CoA, MG-CoA, and HMG-CoA function as reactive acyl species to covalently modify and inhibit MCCC using a negative feedback loop. The highly conserved α-helical region of SIRT4 removes this modification and reduces insulin secretion by promoting leucine catabolism (62). This effect on insulin secretion in SIRT4-knock-out mice led to elevated glucose- and leucine-stimulated basal insulin levels, which resulted in the development of accelerated age-induced glucose intolerance and insulin resistance. Notably, this is varied with genetic backgrounds, SIRT4KO mice on a C57BL/6NJ genetic background have elevated leucine-stimulated insulin levels; however, SIRT4KO mice on a C57BL/6J background do not (62,63). The repression of SIRT4 activity promoted insulin secretion partly via GDH activation. On the other hand, the loss of SIRT4 function resulted in the accumulation of acyl modified MCCC, which repressed leucine catabolism and allowed for leucine to function as an allosteric activator of GDH. In general, SIRT4 regulates insulin secretion in pancreatic β cells through ADP-ribosylation of GDH and deacylation of MCCC in a coordinated way (20,64).

The deacyl function of SIRT4 plays a crucial role in ATP homeostasis. ANT2 is a transmembrane protein located in the inner mitochondrial membrane, and the acylation of ANT2 is known to uncouple mitochondria and reduce the efficiency of oxidative phosphorylation (65). SIRT4 regulates oxygen consumption via modulating the coupling efficiency in an ANT2-dependent manner. In the absence of SIRT4, ANT2-dependent uncoupling led to a decrease in cellular ATP levels and activation of a feedback loop, which involved a reverse signaling response from the mitochondria to the nucleus through AMPK, CPT1, PGC1α, and ACC (66).

4. Functions of SIRT4 in human cancer

Metabolic reprogramming is an important aspect of cancer cell metabolism, in which glucose and glutamine metabolic reprogramming are two primary forms. In 1927, Warburg *et al* (67) found that the rate of glycolysis was much faster compared with that in normal cells, even in the presence of sufficient

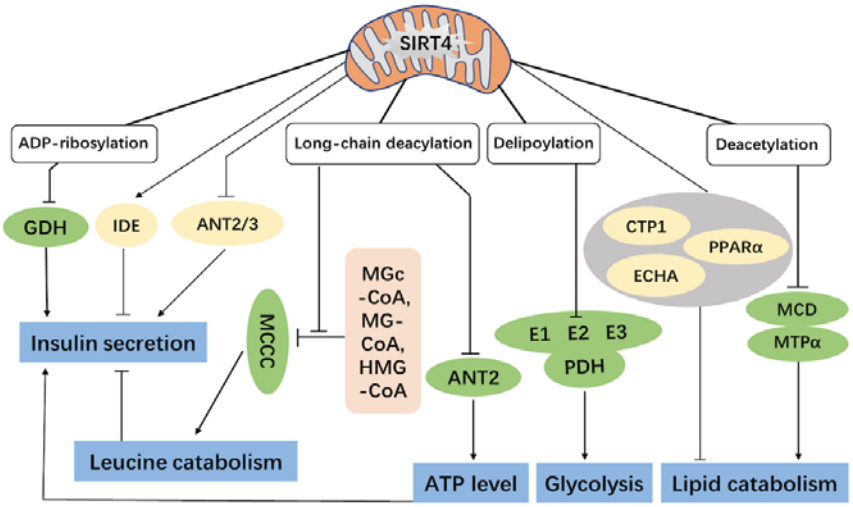

Figure 1. The functions of mitochondrial SIRT4 in cellular metabolism. The ADP-ribosylation on GDH and interactions with IDE and ANT2/3 can regulate insulin secretion in pancreatic β cells. The long-chain deacylation on MCCC can regulate leucine catabolism and affect insulin secretion indirectly. The delipoylation on the E2 component of the PDH complex further impacts cellular energy metabolism. The NAD+-dependent deacetylation on MCD and MTPα can inhibit fatty acid β-oxidation. In addition, SIRT4 also modulates some proteins associated with lipid metabolism (CTP1, PPARα and ECHA) and finally represses lipid catabolism collectively. GDH, glutamate dehydrogenase; IDE, insulin degrading enzyme; ANT2/3, ADP/ATP translocase 2/3; MCCC, methylcrotonyl-CoA carboxylase; PDH, pyruvate dehydrogenase; MCD, malonyl CoA decarboxylase; MTPα, mitochondrial trifunctional protein α; MGc, 3-methylglutaconyl; MG, 3-methylglutaryl; HMG, 3-hydroxy-3-methylglutaryl.

oxygen. This phenomenon of aerobic glycolysis, to achieve rapid cell proliferation and energy supply, was termed the Warburg Effect. Glutamine is the most abundant non-essential amino acid in cell plasma (68). Studies have found that the majority of cancer cells can utilize glutamine at a high rate, and some types of cancer cells, such as breast cancer cells, HeLa cervical carcinoma cells and hepatocellular cells cannot survive without an exogenous glutamine supply (69). Glutamine metabolic reprogramming in tumor cells primarily occurs by increased glutamine uptake and glutamine catabolism (68,70). In proliferating cells, the Krebs cycle metabolite, citrate is exported to the cytoplasm for the generation of acetyl CoA. Glutamine serves as an important factor for the replenishment of TCA intermediates which are required due to the continual loss of citrate. In addition, it can also promote the production of intracellular NADH and glutathione to stabilize the intracellular redox balance and regulate the activity of some signal transduction systems, including mTORC1 signaling, the ERK pathway and signaling associated with regulation of mitochondrial ROS production (71-73).

Every member of the sirtuin family has been associated with tumorigenesis (74). SIRT4 serves as a tumor suppressor as it inhibits glutamine metabolism in the mitochondria of cancer cells. Early studies found that SIRT4 mRNA levels were reduced in several malignant tumor tissues, such as bladder cancer, T-cell leukemia, lung cancer, ovarian cancer, and thyroid cancer (75-77). In 2013, Jeong *et al* (78) discovered that SIRT4 acted as a tumor suppressor by regulating glutamine metabolism in HepG2 cells and PC3 human prostate cancer cells, and SIRT4 knock-out mice spontaneously developed lung cancer. As a result, the tumor suppressor function of SIRT4 had been confirmed in a variety of malignant tumors (Table III). In these studies, the expression level of SIRT4 in normal tissues was significantly higher compared with that in corresponding tumors in the majority of the cancers, and SIRT4 suppressed the biological function of tumor cells *in vitro*. In addition, several studies have shown that the low expression levels of SIRT4 was significantly associated with the advanced stage of cancer and the poor prognosis of patients (79-95).

In terms of the molecular mechanism, SIRT4 notably represses the metabolism of glutamine through the ADP-ribosylation of GDH, which decreases the energy and material supply required for nucleic acid and protein synthesis to the rapidly proliferating tumor cells (78). This has been confirmed in breast cancer, colorectal cancer, esophageal squamous cell carcinoma, myc-induced B cell lymphoma and thyroid cancer (78,88,92-94). Furthermore, stress-induced DNA damage causes genomic instability in carcinogenic genes, such as TP53, ATM and CDKN2A, which induces SIRT4 expression to inhibit glutamine metabolism, and results in cell cycle arrest (96). This provides sufficient time for DNA damage repair (DDR) and protects the stability of the genome in HeLa cells (97). SIRT4 enhances E-cadherin expression and inhibits the expression of N-cadherin and vimentin, thus inhibiting the process of epithelial-mesenchymal transition, and decreasing the migratory and invasive abilities of gastric and colorectal cancer cells (84,88). In addition, aerobic glycolysis leads to the accumulation of intracellular acidic metabolites, such as pyruvate and lactic acid, and ammonia produced by glutamine metabolism can alleviate this pH imbalance to maintain the homeostasis of the intracellular environment. However, SIRT4 disturbs this pH balance and produces an acidic environment in breast cancer (98). This regulatory effect of SIRT4 was modulated by C-terminal binding protein and varied with glucose metabolic levels (98,99). Furthermore, overexpression of SIRT4 blocks cell cycle progression and decreases cancer cell replication by inactivating ERK, p-ERK, cyclin D, and cyclin E in thyroid cancer and gastric cancer cells (87,94).

Table III. Summary of research on tumor suppressor function of SIRT4.

Neoplasms	Expression of SIRT4 in the cancer tissues[a]	Role of SIRT4 in the cancer cell *in vitro*	Association of expression with advanced stage and metastasis[b]	Association between expression level and overall survival time[a, b]	(Refs.)
HNSCC	Low	NA	NA	Negative correlation	(79)
HCC	Low	Inhibition	Yes	Negative correlation	(80)
NSCLC	Low	Inhibition	Yes	Negative correlation	(81)
EAC	Low	NA	Yes	NA	(82)
NB	Low	Inhibition	Yes	Negative correlation	(83)
GC	Low	Inhibition	Yes	Negative correlation	(84-87)
CRC	Low	Inhibition	Yes	Negative correlation	(88-90)
IBC	Low	NA	NA	Negative correlation	(91)
ESCC	Low	Inhibition	NA	Negative correlation	(92)
BCL	NA	Inhibition	NA	NA	(93)
TC	Low	Inhibition	NA	NA	(94)
PC	NA	Inhibition	NA	NA	(95)

[a]Compared with that in normal adjacent tissue. [b]Low expression levels are associated with advanced stage and tumor metastasis. HNSCC, head and neck squamous cell carcinoma; HCC, hepatocellular carcinoma; NSCLC, non-small cell lung cancer; EAC, endometrioid adenocarcinoma; NB, neuroblastoma; GC, gastric carcinoma; CRC, colorectal cancer; IBC, invasive breast cancer; ESCC, esophageal squamous cell carcinoma; BCL, B cell lymphoma; TC, thyroid cancer; PC, pancreatic cancer; NA, not available.

In addition, mammalian target of rapamycin complex 1 (mTORC1) was associated with the nutritional status and metabolism of cells, and it repressed the protein level of SIRT4 by destabilizing cAMP-dependent transcription factor ATF-4 (CREB2). On the other hand, low mRNA levels of SIRT4 increased the expression of mTORC downstream genes, such as MYC, CCND1, HIF1A and SREBP1. The mutual inhibition between SIRT4 and mTORC1 was important for the proliferation and survival of colon carcinoma cell line DLD1 and prostate cancer cell line DU145 (100,101). In hepatocellular carcinomas, SIRT4 deletion by shRNA of HL7702 cell line increased mTOR signaling by inhibiting AMPK through the regulation of glutamine catabolism and the AMP/LKB1 pathway (80). In non-small cell lung cancer cell lines, SIRT4 reduced mitochondrial fission by interacting with the Fis-1/Drp1 axis and regulated cell invasive abilities by repressing MEK/ERK activity (81). A study also demonstrated that SIRT4 is a substrate of ubiquitin-like with plant homeodomain and ring finger domains 1 (UHRF1) and negatively regulated aerobic glycolysis, tumor proliferation, and metastasis of pancreatic cancer cells (95). A recent study also suggested that SIRT4 overexpression could heighten the sensitivity of ER-positive breast cancer to tamoxifen via inhibiting the interleukin-6/STAT3 pathway (102).

Notably, SIRT4 plays a dual role in tumorigenesis. SIRT4 plays an important role in DDR and protects the stability of the genome, preventing tumorigenic transformation. In the same way, SIRT4 also protects cancer cells against stresses, such as DNA damage and caloric restriction. For example, the knock-out of SIRT4 in HepG2 cells resulted in decreased cell survival and tumor growth following the DNA damage caused by gamma-radiation, and inversely, the overexpression of SIRT4 in HepG2 cells increased drug and radiation resistance (103). Moreover, SIRT4 promotes cancer cell survival by degrading phosphatase and tensin homolog (PTEN) using the lysosome pathway, which is mediated by IDE (104). PTEN is a lipid phosphatase, which inhibits cancer cell survival and proliferation, and the degradation of PTEN accelerates autophagy during nutritional starvation stresses, such as glucose deprivation. During autophagy, autosomes fuse with lysosomes to form autophagic lysosomes (105,106). It is a salvage pathway to produce proteins, lipids, and carbohydrates for cells to survive in unfavorable conditions (107). Therefore, we hypothesize that SIRT4 is a tumor suppressor prior to the development of cancer; however, once cancer has developed, SIRT4 can still exert a tumor suppressor effect by regulating energy metabolism. Therefore, it has a certain protective effect against cancer cells.

A number of studies have found that SIRT4 is associated with several human diseases. In angiotensin II-induced cardiac hypertrophy in mice, SIRT4 inhibited manganese superoxide dismutase activity and promoted the accumulation of reactive oxygen species in cardiomyocytes, which eventually led to cardiac hypertrophy through the activation of the MAPK/ERK pathway (108,109). Furthermore, the overexpression of SIRT4 protein levels can protect against myocardial ischemia-reperfusion injury by decreasing myocardial infarct size, serum creatine phosphokinase levels, and myocardial apoptosis (110). In the central nervous system, glutamate transport is an energy-dependent process. SIRT4 positively regulated ATP production in neural cells by inhibiting GDH. Specifically, it enhanced the function of the sodium/potassium-ATPase, which led to increased glutamate uptake and glutamate transport to maintain the normal functions of neurons. *In vivo*, SIRT4-knock-out mice have enhanced seizure phenotypes compared with that in wild-type mice following treatment with a potent excitotoxin kainic acid (111,112). With respect to urogenital diseases, a study demonstrated that Leydig cells treated with lipopolysaccharide led to impaired steroidogenesis

and enhanced cellular apoptosis via suppression of SIRT4 by the activation of JNK (113). In addition, SIRT4 controls energy metabolism and meiotic apparatus during oocyte maturation, and mouse oocytes with overexpressed SIRT4 protein levels are unable to undergo meiosis completely (114). These data suggest that the roles of SIRT4 in human diseases are both important and complex. Future studies are required to identify connections and detailed mechanisms between them.

5. Conclusion

Mitochondria are vital for maintaining an energy balance in cellular and organismal physiology, and aberrant mitochondrial function has been associated with cellular dysfunctions and metabolic diseases. Mitochondrial dysfunction diseases are a group of genetically heterogeneous diseases that can involve any organs, onset at any age, and be inherited from an autosome, the X chromosome, or maternally (115). The sirtuin family is evolutionary conserved and exhibits a wide range of biological functions in life span, gene transcription, cell proliferation, differentiation, apoptosis, genome stability, cellular metabolism, tumorigenesis, energy homeostasis, DNA damage and stress responses (14-17). Among them, SIRT4 is predominately located in mitochondria and regulates its functions through some known and unknown mechanisms. Recent studies have unraveled numerous biological processes and human diseases, which are regulated by SIRT4 (116,117). However, there are still two crucial issues that require further investigation to provide a more comprehensive understanding of SIRT4.

The first problem is that SIRT4 is still the least well-known mammalian sirtuin; no convincing enzymatic activity has been verified for SIRT4, and there are limited studies on SIRT4 modulators. Its key roles in metabolism and several catalytic activities have been reported; however, more structural information is required to uncover the robust activity and investigate the regulatory mechanisms of SIRT4. To address this gap in the knowledge, the development of effective chemical modulators of SIRT4 is important. Then, these modulators could be applied to scientific research to elucidate the detailed functions of SIRT4, and eventually, for use in clinical diagnosis, treatment or prognosis prediction (7,21,118).

The second problem relates to all seven members of the sirtuin family. Biologically, each of them has a complicated regulatory mechanism via acting on substrates or being modulated by upstream proteins. In fact, their functions are not independent of each other, and numerous studies have confirmed that there is a tight interaction between the sirtuins family. For example, the mRNA level of SIRT4 in granulocytes and monocytes of patients with type 2 diabetes was lower, while the mRNA level of SIRT1 was higher, compared with that in healthy individuals; however, the interaction between these two SIRTs remains unknown (119). In addition, SIRT1 plays a complicated and important role in cardiovascular metabolic diseases (23). Furthermore, SIRT4 ribosylated GDH and decreased its activity; however, SIRT3 deacetylated GDH and increased its activity (120). Both SIRT-4 and -6 have anti-inflammatory functions (121), whereas the activities of SIRT-4 and -5 partly overlap, and collectively regulate the metabolism of BCAA (122). Therefore, a valid network exists among the seven sirtuins, in which they are involved in similar pathways. However, their specific mechanisms remain unknown, and in the future, an extensive amount of investigation is required to clarify this complex network.

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Authors' contributions

CW drafted the original manuscript. CW and YL created the tables, designed the figures and performed the literature review. YZ was involved in drafting the initial manuscript, read and approved the final manuscript. CK reviewed and revised the manuscript for important intellectual content. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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