Review

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Role of Post-translational Modification of Silent Mating Type Information Regulator 2 Homolog 1 in Cancer and Other Disorders

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Silent mating type information regulator 2 homolog 1 (SIRT1), an NAD^+-dependent histone/protein deacetylase, has multifarious physiological roles in development, metabolic regulation, and stress response. Thus, its abnormal expression or malfunction is implicated in pathogenesis of various diseases. SIRT1 undergoes post-translational modifications, including phosphorylation, oxidation/reduction, carbonylation, nitrosylation, glycosylation, ubiquitination/deubiquitination, SUMOylation etc. which can modulate its catalytic activity, stability, subcellular localization, and also binding affinity for substrate proteins. This short review highlights the regulation of SIRT1 post-translational modifications and their pathophysiologic implications.

Key Words Post-translational modification, Protein processing, Sirtuin 1

INTRODUCTION

Silent mating type information regulator 2 homolog (sirtuin) proteins are present in all kingdoms of life and are broadly conserved from yeast to humans. Mammalian sirtuins consist of at least seven isoforms (SIRT1-7) and possess either NAD^+-dependent histone/non-histone deacetylase activity or ADP-ribosyltransferase activity. Structurally, SIRT proteins contain a highly conserved catalytic core domain and variable N- and C-terminal domains as illustrated in Figure 1. Table 1 summarizes differences in subcellular localization, catalytic activity, and functions of representative SIRT isoforms.

SIRT1 has multifaceted roles in physiologic and pathologic processes, such as cellular senescence [1,2], apoptosis [3,4], inflammation [5,6], and energy metabolism [7-9]. SIRT1 contains two nuclear localization signal sequences and two nuclear export signal sequences, so it freely resides in both nuclear and cytosolic compartments in the cells. Notably, it has been reported that abnormally overexpressed SIRT1 in cytoplasm could promote the malignancy of several tumors including those derived from colon [10], prostate [11] and ovary [12]. Likewise, SIRT2 can shuttle between nucleus and cytoplasm to regulate the cardiac homeostasis [13,14], glucose uptake [15,16], and differentiation [17,18]. SIRT3, 4, and 5 are predominantly localized in mitochondrial matrix and involved mainly in the regulation of cellular metabolism such as fatty acid oxidation [19], lipid anabolism [20], and urea cycle [21], respectively. SIRT6 and 7 are the nuclear proteins that are known to play a role in telomere maintenance [22,23] and pre-rRNA processing [24,25], respectively.

Of the aforementioned SIRT isoforms, SIRT1 has been most extensively investigated, and there is a growing number of its substrates, binding partners, and target molecules identified. It influences the protein acetylation dynamics by deacetylating the substrate proteins. Considering its multifarious effects on various cellular events, expression or activity of SIRT1 needs to be properly and precisely regulated. Because SIRT1 is classified as an NAD^+-dependent deacetylase, NAD^+ is primarily regarded as a factor that controls the catalytic activity of SIRT1. In addition, some endogenous proteins can also modulate the catalytic activity of SIRT, independently of biosynthesis, concentrations, and availability of intracellular NAD^+. One such molecule is active regulator of SIRT1 (AROS) that binds to the allosteric site located in the

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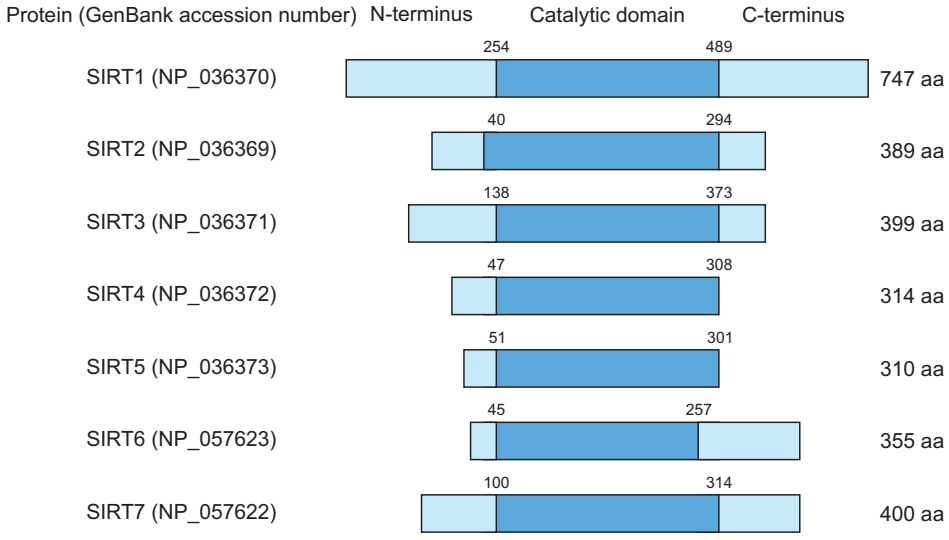

Figure 1. The SIRT family. SIRT proteins contain a highly conserved catalytic core domain, variable N- and C-terminal domains. Genbank is the publicly available genetic sequence database distributed by the National Center for Biotechnology Information (NCBI).

Table 1. Differences of the SIRT family proteins in their subcellular localization, enzymatic activity, and functions

Sirtuin family	Subcellular localization	Enzymatic activity	Targets	Functions	Reference no.
SIRT1	Nuclear/cytoplasmic	Deacetylase	p53, H3K9, H4K16	Cellular senescence	[1,2]
			p53	Apoptosis	[3,4]
			p65	Inflammation	[5,6]
			PGC-1α, PPARα, c-Myc	Energy metabolism	[7-9]
SIRT2	Nuclear/cytoplasmic	Deacetylase	NFAT, LKB1	Cardiac homeostasis	[13,14]
			GKRP, G6PD	Glucose uptake	[15,16]
			FoxO1, Slug	Basal differentiation	[17,18]
SIRT3	Mitochondrial	Deacetylase	LCAD	Fatty acid oxidation	[19]
SIRT4	Mitochondrial	ADP-ribosyltransferase	MCD	Lipid anabolism	[20]
SIRT5	Mitochondrial	Deacetylase	CPS1	Urea Cycle	[21]
SIRT6	Nuclear	ADP-ribosyltransferase	H3K9	Telomere maintenance	[22,23]
SIRT7	Nuclear	Deacetylase	Fibrillarin (24), U3-55k (25)	Pre-rRNA processing	[24,25]

CPS1, carbamoyl phosphate synthetase 1; FoxO1, forkhead box O1; G6PD, glucose-6-phosphate dehydrogenase; GKRP, glucokinase regulatory protein; H3, histone H3; H4, histone H4; LCAD, long-chain acyl CoA dehydrogenase; NFAT, nuclear factor of activated T-cells; PGC-1α, peroxisome proliferator-activated receptor gamma coactivator 1-α; MCD, malonyl CoA decarboxylase; LKB, liver kinase B1.

N-terminus of SIRT1, whereby increasing its activity [26,27]. The AROS-SIRT1 complex formation provokes conformational changes in SIRT1. This allows SIRT1 to attain a structure favorable for its catalytic activity without obstructing the interaction between SIRT1 and its substrates [26]. On the other hand, deleted in breast cancer-1 (DBC1) directly interacts with the catalytic domain of SIRT1, resulting in inhibition of SIRT1 activity [28].

Post-translational modifications are also recognized as an important means to control the function of SIRT1 (Fig. 2 and Table 2). Since the discovery of SUMOylation of SIRT1 as the first post-translational modification of SIRT1 to regulate its deacetylase activity [29], many other chemical alterations have been reported so far. Post-translational modifications that occur in various residues of proteins can alter their functional activity, stability, subcellular localization, interaction with other proteins, etc.

Analysis of affinity-purified FLAG-SIRT1 by mass spectrometry identified 13 phosphorylatable serine/threonine residues; tyrosine residues were not found to be phosphorylated in the samples interrogated [30]. Subsequent studies by other investigators proposed additional 4 different serine or threonine residues [31-34].

Furthermore, SIRT1 contains 19 cysteine residues in its full sequence, some of which are susceptible to redox modification. Due to the high reactivity of the cysteine thiol toward reactive oxygen species (ROS) and electrophiles, SIRT1 is prone to be structurally modified through oxidation, *S*-nitrosylation, *S*-glutathionylation and *S*-sulfenation. Like phosphorylation, modifications occurring at cysteine residues modulates intracellular signaling, but it also contributes to maintenance of balanced cellular redox environment.

Glycosylation as a common post-translational modification of proteins in cells involves the covalent attachment of a

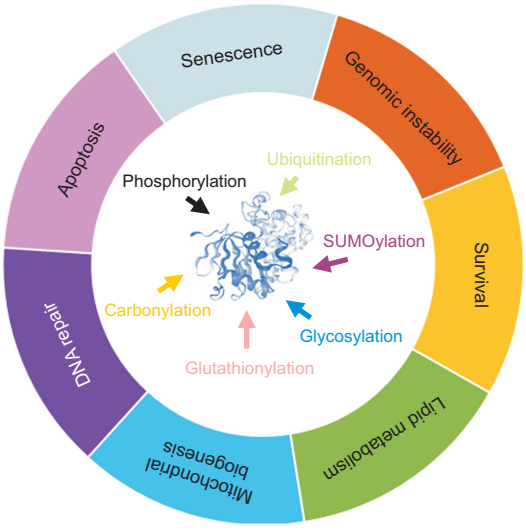

Figure 2. Post-translational modifications of SIRT1 and their functional significance.

carbohydrate moiety to proteins and other macromolecules. It can be classified into *N*-, *O*-, *C*-linked glycosylation depending on the type of glycans added. *N*-linked glycosylation needs glycans to be attached to a nitrogen of asparagine or arginine side chains, whereas *O*-linked glycosylation occurs on hydroxyl groups of serine and/or threonine residues of proteins. Glycosylation has been reported to play a critical role in protein folding [35,36], trafficking [37], and stability [38].

Both ubiquitination and SUMOylation target lysine residues. Ubiquitination is believed to be a prerequisite for proteasomal degradation in order to not only eliminate dysfunctional or misfolded proteins to control protein quality but also maintain a reasonable level of target proteins in cells. Furthermore, non-proteolytic functions of atypical polyubiquitination also exist to regulate such as enzymatic activity, protein interaction, and cellular localization. So far, at least 8 lysine residues have been identified as a SIRT1 ubiquitination site (lysine 238, 311, 335, 377, 499, 523, 601, and 610) [39-41]. Similar to ubiquitination, SUMOylation is catalyzed by a three-step enzymatic process in which target proteins are ultimately tagged with proteins called small ubiquitin-like modifier (SUMO). SUMOylation has been known to participate in a variety of cellular processes including nuclear localization [42,43], transcriptional activation [44], protein stability [45,46], and DNA damage repair [47].

This review highlights the post-translational modifications of specific residues of SIRT1 by intracellular molecules, particularly at serine/threonine, tyrosine, and cysteine, and lysine in diverse biological contexts (Fig. 3).

SIRT1 PHOSPHORYLATION BY

c-Jun N-terminal kinases (JNKs)

Ford et al. [48] reported JNK2-mediated phosphorylation of SIRT1 at the serine 27 residue, which contributed to enhanced protein stability of SIRT1 in human colon cancer (HCT-116) cells. Authors of this study identified serine 47 as an additional phosphorylation site, but phosphorylation at this site showed no correlation with SIRT1 protein stability. Interestingly, JNK1, another isoform of the JNK family, phosphorylates murine SIRT1 at serine 46 that corresponds to serine 47 of human SIRT1, thereby inducing ubiquitination-dependent degradation of SIRT1 in mouse adipocytes (3T3-L1) and human embryonic kidney (HEK293) cells [49]. The half-life of SIRT1 protein was prolonged in JNK1 knockout murine embryonic fibroblasts (MEFs), further corroborating the involvement of JNK1 in the destabilization of SIRT1 [49].

Ca^{2+}/calmodulin-dependent protein kinase kinase β (CaMKKβ)

SIRT1 can also undergo phosphorylation on serine 27 and 47 under pulsatile shear stress mimicking atheroprotective flow in human umbilical vein endothelial cells (HUVECs), which protects these cells from oxidative stress [50]. According to this study, phosphorylation at serine 27 and 47 was associated with an increased protein level of SIRT1 without change in its mRNA transcript level. An in vitro kinase assay and nano-LC-MS/MS analysis revealed that CaMKKβ could directly phosphorylate SIRT1 at aforementioned residues [50]. Thus, the increased SIRT1 protein stability in response to pulsatile shear stress was abrogated in embryo fibroblasts from CaMKKβ-deficient mice. In line with this finding, the expression of anti-oxidative genes such as those encoding superoxide dismutase, catalase, Nrf2, heme oxygenase-1 and thioredoxin 1 was upregulated in $SIRT1^{-/-}$ MEFs expressing phosphomimetic mutant SIRT1 (S27D/S47D) compared with that in control cells [50]. Taken these findings all together, CaMKKβ-dependent phosphorylation of SIRT1 is likely to provoke atheroprotective effects in vascular endothelial cells.

Cyclin-dependent kinase 5 (CDK5)

One of the most prominent functions of SIRT1 is an anti-aging property. In the vascular aging process, SIRT1 phosphorylation at serine 47 suppressed its catalytic activity, thus mitigating the anti-senescent action of SIRT1 while promoting development of atherosclerosis [51]. Repetitive passages or treatment with agents promoting senescence of porcine aortic endothelial cells (PAECs) displayed the increased phosphorylation of SIRT1 at serine 47, which was accompanied by a senescence phenotype such as intensive β-galactosidase (β-gal) activity [51].

Ectopic expression of mammalian wild type (SIRT1-WT) and non-phosphorylatable mutant SIRT1 (SIRT1-S47A) decreased the number of senescent cell population in PAECs

Table 2. Post-translational modification of SIRT1

Type of PTM	Residue	Inducer /Modifier	Type of Cells/Animal	Biological effects/ responses	Reference no.
Phosphorylation	Ser27	JNK2	HCT-116 cells	Enhanced protein stability	[48]
		CaMKKβ	HUVECs	Enhanced protein stability	[50]
Phosphorylation	Ser47	JNK1	3T3-L1, HEK293 cells	Degradation	[49]
		CaMKKβ	HUVECs	Enhanced protein stability	[50]
		CDK5	PAECs	Decreased catalytic activity	[51]
S-Glutathionylation	Cys67	GSNO	HEK293T cells	Did not affect the basal activity of SIRT1, it dampened the responsiveness of SIRT1 to resveratrol, resulting in decreased catalytic activity of SIRT1	[63]
S-Glutathionylation	Cys67 Cys326 Cys623	GSH	HepG2 cells	Decreased catalytic activity	[64]
O-GlcNAcylation	Thr160/Ser161 (corresponds to the human Ser169)	OGT	C57B16 mice	Degradation	[74]
Phosphorylation	Ser164 (corresponds to the human Ser 172)	CK2	HFD-fed C57BL/6J mice	Decreased catalytic activity	[52]
Phosphorylation	Tyr280 Tyr301	JAK1	MCF-10 cells	Enhanced interaction between SIRT1 and STAT3	[55]
Ubiquitination	Lys311	MDM2	HEK293T cells HeLa cells	Had a little effect on SIRT1 stability at steady state but facilitates SIRT1 degradation In response to DNA damaging agents	[40]
Phosphorylation	Thr344	AMPK	U2OS cells	Enhanced catalytic activity	[58]
			HepG2 cells	Decreased catalytic activity	[59]
Reduction	Cys371 Cys374	Ref-1	HUVEC cells	Enhanced catalytic activity	[65]
S-Nitrosylation	Cys387 Cys390	SNO–GAPDH	HEK293 cells	Decreased catalytic activity	[69]
S-Nitrosation	Cys395	GSNO	In vitro assay	Decreased catalytic activity	[66,67]
	Cys398	$ONOO^-$	Nicotine-treated SIRT1 overexpressing mice	Decreased catalytic activity	[68]
Phosphorylation	Ser434	PKA	U2OS cells	Enhanced catalytic activity	[32]
Carbonylation	Cys482	4-HNE	BEAS-2B cells	Inactivation and degradation	[70,71]
Ubiquitination	Lys499 Lys523	SMURF2	HCT-116 cells HEK293T cells	Degradation	[41]
Phosphorylation	Thr522	DYRK1A DYRK3	U2OS cells	Enhanced catalytic activity	[34,60]
Phosphorylation	Thr530	DYRK2	HCT116 and K562 cells	Enhanced interaction between DNA replication-related proteins	[61]
		CyclinB/CDK1	$SIRT1^{-/-}$ and $Sirt1^{+/+}$ ES cells	Enhanced catalytic activity	[30]
Phosphorylation	Ser540	CyclinB/CDK1	$SIRT1^{-/-}$ and $Sirt1^{+/+}$ ES cells	Enhanced catalytic activity	[30]
O-GlcNAcylation	Ser549	OGT	H1299 cells	Enhanced catalytic activity	[72]
Phosphorylation	Ser615 Ser669 Ser732	LKB1	HEK293T cells	Enhanced catalytic activity	[62]
Phosphorylation	Ser659 Ser661	CK2	HeLa cells H1299 cells	Enhanced catalytic activity	[53,54]
SUMOylation	Lys734	Unknown SUMO E3 ligase	DU145 cells H1299 cells	Enhanced catalytic activity	[29]

AMPK, AMP-activated protein kinase; CK2, casein kinase II; DYRK, dual-specificity tyrosine phosphorylation-regulated kinase; ES, embryonic stem; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GSH, glutathione; GSNO, *S*-nitrosoglutathione; 4-HNE, 4-hydroxy-2-nonenal; JNK, c-Jun N-terminal kinase; NO, nitric oxide; OGT, *O*-linked-β-N-acetylglucosamine transferase; PKA, protein kinase A; Ref-1, redox factor-1; CaMKKβ, Ca^{2+}/calmodulin-dependent protein kinase kinase β; CDK5, Cyclin-dependent kinase 5 OGT, *O*-linked-β-N-acetylglucosamine (O-GlcNAc) transferase; JAK1, Janus kinase 1; MDM2, Mouse double minute 2 homolog; 4-HNE, 4-Hydroxy-2-nonenal; SMURF2, Smad ubiquitination regulatory factor 2; LKB, liver kinase; $ONOO^-$, peroxynitrite.

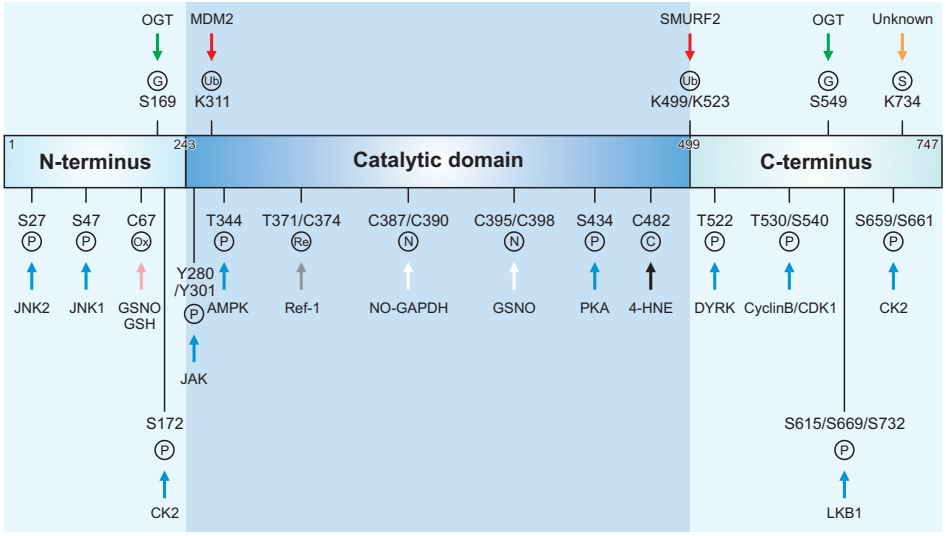

Figure 3. Post-translational modification of SIRT1 by intracellular signaling molecules. SIRT1 protein consists of 747 amino acids and is commonly divided into three functional domains, N-terminal domain, catalytic domain, and C-terminal domain. SIRT1 can be modified by various post-translational modifications, leading to alteration of its catalytic activity or expression. In general, cysteine residues are susceptible to oxidation/reduction, carbonylation, *S*-nitrosylation, and *S*-nitrosation. Likewise, serine/threonine residues are particularly sensitive to phosphorylation and *O*-linked glycosylation. Lysine residues are readily targeted by ubiquitination and SUMOylation. P, Phosphorylation; Ox, oxidation; Re, reduction; N, *S*-Nitrosylation or *S*-Nitrosation; C, carbonylation; G, O-GlcNacylation; Ub, ubiquitination; S, SUMOylation; JNK, c-Jun N-terminal kinase; GSNO, *S*-Nitrosoglutathione; GSH, glutathione; CK, casein kinase; JAK, Janus kinase; OGT, *O*-linked-β-N-acetylglucosamine (O-GlcNAc) transferase; MDM2, mouse double minute 2 homolog; AMPK, AMP-activated protein kinase; Ref-1, redox factor-1; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; PKA, protein kinase A; 4-HNE, 4-Hydroxy-2-nonenal; DYRK, dual-specificity tyrosine phosphorylation-regulated kinase; SMURF2, Smad ubiquitination regulatory Factor 2; CDK, Cyclin-dependent kinase; LKB1, liver kinase 1.

by 42% and 56%, respectively. However, this enhanced anti-senescent activity was lost in the PAECs expressing SIRT1-S47D, a phosphomimetic form of mutant [51]. In silico prediction using the NetPhosK program (http://services.heathtech.dtu.dk/service.php?NetPhos-3.1) proposed CDK5 as a potential kinase responsible for SIRT1 phosphorylation at the serine 47 residue, and the in vitro kinase assay further validated this prediction [51].

Cyclin B/CDK1

Sasaki et al. [30] reported that dephosphorylation of SIRT1 by phosphatases, such as calf intestinal phosphatase or lambda phosphatase, attenuated its deacetylase activity. Comparative analysis of SIRT1 amino acid sequences revealed that threonine 530 and serine 540 are relatively well conserved among the orthologs of SIRT1. Of note, these two residues fit the consensus sequence for a CDK substrate (S/T*-P or S/T*-P-x-K/R, where the asterisk displays the phosphorylation site, and x represents any amino acid). In the above study, the complex formation between endogenous or exogenous SIRT1 and cyclin/CDK, specifically cyclin B/CDK1 was verified by the co-immunoprecipitation assay [30].

Despite cyclin B/CDK1-mediated phosphorylation of SIRT1 at threonine 530 and serine 540 residues, there were no significant differences in NAD^+-dependent in vitro deacetylase activity between wild type and the threonine 530 or serine 540 to alanine mutant (SIRT1-T530/S540A). The proliferative capacity which was defected in SIRT1-deficient cells ($SIRT1^{-/-}$ MEFs or embryonic stem cells) was restored by transfecting cells with SIRT1-WT, but not with the SIRT1-T530A/S540A, suggesting that phosphorylation of SIRT1 is associated with mitotic activity of the cell [30]. It is suggested that phosphorylation of threonine 530 and serine 540 modulates the activity of SIRT1 by altering the accessibility of substrate molecules to the catalytic groove of SIRT1.

Casein kinase 2 (CK2)

Liver extracts from mice adenovirally expressing Flag-mouse SIRT1 fed a normal or a high-fat diet (HFD) were subjected to LC-MS/MS-based proteomic analysis. Of interest, serine 164 (corresponds to the human serine 172) was found to be phosphorylated only in the HFD-induced obese mice [52]. Furthermore, the level of SIRT1 phosphorylation was highly elevated at the corresponding residue in hepatic tissues of patients with non-alcoholic fatty liver disease. Phosphorylation of SIRT1 at serine 164 was associated with its suppressed deacetylase activity as evidenced by increased acetylation of peroxisome proliferator-activated receptor gamma coactivator 1α (PGC-1α) and sterol regulatory element-binding protein 1 (SREBP-1) that are well-defined downstream substrates of SIRT1 and transcriptional regulators of lipid metabolism in liver, in monkey kidney epithelial (Cos-1) cells expressing phosphomimetic SIRT1-S164D [52].

Consistent with in vitro results, the levels of triglyceride and

liver cholesterol were higher in the HFD mice harbouring the phosphomimetic mutant form of SIRT1 (SIRT1-S164D) than those in mice expressing SIRT1-WT or non-phosphorylatable SIRT1-S164A [52]. In addition, the plasma levels of glucose, insulin, and IL-6 were significantly escalated in the HFD-induced obese mice expressing SIRT1-S164D [52]. SIRT1 harbours the CK2 motif (S/TXXE/D) containing serine164; CK2 phosphorylates SIRT1 at this amino acid through direct interaction with its N-terminus [52].

CK2 also participates in phosphorylation of SIRT1 mainly at serine 659 and 661 residues [33]. Recombinant CK2 phosphorylated immunoprecipitated FLAG-tagged SIRT1 in human cervical cancer (HeLa) cells as verified by an in vitro kinase assay. Moreover, knockdown of CK2 using small interfering RNA (siRNA) or pharmacologic inhibition reduced the phosphorylation intensity of immunoprecipitated endogenous SIRT1 from HeLa cells labeled with [γ-^{32}P]ortho-phosphate [33]. Subsequent amino acid sequence analysis identified serine 659, 661 and 684, which are located within the CK2 consensus sequence (S/TxxD/E) as putative CK2 phosphorylation sites. Single mutation of these residues showed reduced radioactive phosphorylation signal compared with SIRT1-WT, whereas the S684A mutant did not exert such an effect on phosphorylation intensity in vitro. These findings suggest that SIRT1 undergoes phosphorylation predominantly at both serine 659 and 661 by CK2.

The physiological relevance of CK2-mediated phosphorylation of SIRT1 was later elucidated [53]; phosphorylation of SIRT1 conferred resistance to etoposide-induced apoptosis in human non-small cell lung cancer cells, and this effect was mediated through deacetylation of p53.

Janus kinase 1 (JAK1)

Wang et al. [54] performed a mass spectrum-based systemic kinome analysis and discovered that JAK1 potentiates the interaction between SIRT1 and STAT3 by phosphorylating SIRT1 at tyrosine 280 and 301. Both tyrosine residues are present in the catalytic domain of SIRT1, but their phosphorylation did not affect the baseline activity of SIRT1 [54]. SIRT1 has been reported to suppress the transcriptional activity of STAT3 through deacetylation at the lysine 685 residue [55,56].

In human breast epithelial (MCF-10) cells, the mRNA levels of anti-apoptotic Bcl-2 and Mcl-1 declined by the forced expression of SIRT1-WT, but not by ectopic overexpression of double mutant SIRT1 containing phenylalanine in place of tyrosine 280 and 301 (SIRT1-Y280/301F) [54]. In this study, the apoptotic cell death was observed upon treatment with cisplatin. IL-6 treatment hampered this through upregulation of STAT3 target gene expression. Transfection of cells with SIRT1-WT counteracted the anti-apoptotic function of IL-6, whereas SIRT1-Y280/301F double mutant failed to restore the anti-cancer action of cisplatin [54].

AMP-activated protein kinase (AMPK)

It has been shown that AMPK directly phosphorylates SIRT1 at the threonine 344 residue, leading to its release from the endogenous inhibitor DBC1 in human osteosarcoma (U2OS) cells, thereby inactivating p53 through deacetylation [57]. Although DBC1 can also be a substrate of AMPK, the fate of the SIRT1-DBC1 complex depends on the phosphorylation status of SIRT1 at threonine 344. Contrary to this finding, Lee et al. [31] reported that SIRT1 phosphorylated by AMPK at the same residue was subsequently inactivated in human hepatocellular carcinoma (HepG2) cells. In line with this notion, a phosphomimetic mutant of SIRT1 in which threonine 344 is substituted by glutamic acid (T344E) failed to inhibit the transcriptional activity of p53 compared with SIRT-WT or non-phosphorylatable mutant of SIRT1 (T344A). Thus, phosphorylation of SIRT1 at threonine 344 is considered to repress its catalytic activity, resulting in enhancement of p53 acetylation as well as apoptosis in HepG2 cells.

Protein kinase A (PKA)

SIRT1 is known to take part in fatty acid oxidation to protect cells from metabolic stress. In this process, the catalytic activity of SIRT1 toward PGC-1α is positively regulated by the cyclic adenosine monophosphate (cAMP)-dependent PKA [32]. Mass spectral analysis of immunoprecipitated SIRT1 protein revealed that the serine 434 residue was exclusively phosphorylated as a consequence of an adenylyl cyclase activator (forskolin)-induced increase of cellular cAMP in U2OS cells. According to this study, SIRT1-deficient MEF cells retrovirally transduced with non-phsphorylatable SIRT1 mutant in which serine 434 was replaced by alanine failed to induce deacetylation-dependent activation of PGC-1α and subsequent transcription of genes involved in lipid metabolism [32].

Dual-specificity tyrosine phosphorylation-regulated kinase 1A and 3 (DYRK1A and DYRK3)

Two anti-apoptotic DYRK members, DYRK1A and DYRK3, activate SIRT1 through phosphorylation at threonine 522 to promote the cell survival [34]. Thus, pro-survival activity of SIRT1 was associated with its phosphorylation, especially at the threonine 522 residue. Dephosphorylation of SIRT1 in cells in which threonine 522 is replaced by valine prevented DYRK/SIRT1-induced deacetylation of p53 and cell survival [34]. Additionally, hypophosphorylation of SIRT1 by knockdown of genes encoding DYRK1A and DYRK3 using specific siRNA sensitized human osteosarcoma cells to etoposide-induced cell death [34]. Later, it has been suggested that phosphorylation of the threonine 522 residue of SIRT1 is closely related to its conformational stability as well as binding affinity for p53 [58].

To elucidate the relevance of phosphorylation as a regulatory mechanism in controlling the catalytic activity of SIRT1, three recombinant proteins were utilized: WT, non-phosphorylatable (SIRT1-T522A), and phosphorylation mimetic (SIRT1-

T522E) SIRT1. In an in vitro enzymatic assay, SIRT1-T522E exhibited higher ability to deacetylate p53 fusion proteins than did SIRT1-WT or SIRT1-T522A. Enhanced deacetylase activity of SIRT1-T522E appeared to be achieved by maintaining the monomeric state of the SIRT1 protein to block the formation of aggregates.

Dual-specificity tyrosine phosphorylation-regulated kinase (DYRK2)

Another DYRK member, DYRK2, has been reported to participate in preservation of genomic stability by phosphorylating SIRT1 at threonine 530 [59]. An immunoprecipitation assay together with the chromatin immunoprecipitation assay revealed that SIRT1-WT can interact with proteins related to the initiation of DNA replication such as proliferating cell nuclear antigen, replication protein A, and origin recognition complex subunit 2 (ORC2), a member of the pre-replication complex, at the origin of replication to support stable replication of DNA [59]. However, cells expressing non-phosphorylatable mutant SIRT1-T530A not only exhibited decreased interaction with aforementioned proteins but also instigated chromosome breakage, resulting in genomic instability [59].

Liver kinase B1 (LKB1)

LC-MS in combination with an in vitro kinase assay has revealed that serine 615, 669, and 732 residues of SIRT1 are susceptible to phosphorylation by LKB1. When all the three serine residues were substituted by aspartic acid to produce a phosphomimetic mutant, PGC-1α, a well-defined substrate of SIRT1, was deacetylated to a lager extent than SIRT1-WT or phospho-defective SIRT1-S615/669/732A in HEK293T cells [60]. Deacetylated and activated PGC-1α drove transcription of target genes responsible for active mitochondrial biogenesis and mitochondrial electron transport such as *Nrf-1*, *Nrf-2*, *NADH:ubiquinone oxidoreductase core subunit S8 (NDUFS8)*, *succinate dehydrogenase subunit B (SDHB)*, *ubiquinol-cytochrome c reductase core protein 1* (*Uqcrc1)*, *COX5b (cytochrome c oxidase subunit 5B)*, and *ATP synthase F1 subunit alpha (ATP5F1A).*

SIRT1 CYSTEINE THIOL MODIFICATION BY

S-Nitrosoglutathione (GSNO) and reduced glutathione (GSH)

GSNO, formed by reaction of nitric oxide (NO) and GSH, affected resveratrol-induced activation of SIRT1 by directly modifying the cysteine 67 residue in vitro [61]. Even though GSNO-induced oxidation of cysteine 67 did not affect the basal activity of SIRT1, it dampened the responsiveness of SIRT1 to resveratrol, resulting in decreased catalytic activity of SIRT1. Meanwhile, cysteine 61 of murine SIRT1, which is equivalent to cysteine 67 of human SIRT1, is prone to be modified by GSH, together with cysteine 318 and 613 that correspond to cysteine 326 and 623 of human SIRT1, respectively [62]. Reduction of the GSH-SIRT1 adduct by glutaredoxin-1 overexpression preserved activity of SIRT1 in HepG2 cells. In addition, C57BL/6J mice fed high fat and high sucrose diet showed increased accumulation of GSH-protein adducts with concomitant reduction in the SIRT1 activity in the fatty liver of mice, suggesting oxidative inactivation of SIRT1 under metabolically stressed conditions.

Redox factor-1 (Ref-1), nicotine, and peroxynitrite ($ONOO^-$)

Ref-1, as a cellular reductant, stimulated SIRT1 activity by maintaining SIRT1 in the reduced form [63]. The catalytic domain of SIRT1 has a Zn^{2+}-tetra-thiolate motif containing cysteine 371, 374, 395 and 398 residues, which is highly conserved from yeast to human. When cysteine 371 and 374 residues in this tetrathiolate motif were mutated to serine, Ref-1 could not prevent the loss of SIRT1 activity caused by hydrogen peroxide (H_2O_2)-induced oxidative stress in HUVEC cells, suggesting that sustained maintenance of SIRT1 in a reduced form is important for retaining its optimal catalytic activity under oxidative stress.

Likewise, *S*-nitrosation of SIRT1, at cysteine 395 and 398 residues that are other constituents of Zn^{2+}-tetra-thiolate, has been reported to be correlated with the inhibition of its catalytic activity [64,65]. These modifications, which result from the attachment of GSNO or NO to cysteine residues of SIRT1, provoked conformational changes of SIRT1. As a result, Zn^{2+} was released from Zn^{2+}-tetra-thiolate of SIRT1, and this hampered the binding of a substrate molecule and NAD^+, lowering the activity of SIRT1. In another study, alteration of Zn^{2+} binding to cysteine 395 and 398 by peroxynitrite ($ONOO^-$) potentially increased by nicotine absorbed from cigarette smoking, which led to reduction both in the protein expression and the catalytic activity of SIRT1 without influencing its mRNA abundance [66]. When SIRT1-overexpressing mice ($SIRT1^{Super}$) were infused with nicotine using osmotic pumps, the decrease in circumferential cyclic strain in both the carotid artery and the abdominal aorta was less than that in nicotine-treated SIRT1-WT mice. Furthermore, the protein levels of fibronectin and matrix metalloprotein (MMP) 2 in aorta from $SIRT1^{Super}$ mice were less induced by nicotine infusion compared to those in nicotine-administered SIRT1-WT mice, suggesting that overexpression of SIRT1 may have an inhibitory effect on nicotine-induced reactive nitrogen species-driven extracellular matrix remodeling, thereby mitigating arterial stiffness.

SNO–glyceraldehyde-3-phosphate dehydrogenase (SNO-GAPDH)

It has been suggested that SNO-GAPDH can donate NO to SIRT1, thereby affecting the activity of SIRT1 [67]. GAPDH was first nitrosylated by NO, and then the resulting SNO–GAPDH complex physiologically transnitrosylated SIRT1 at cysteine 387 and 390. This led to the inhibition of SIRT1

enzymatic activity and consequently, transcriptional activity of PGC-1α [67].

4-Hydroxy-2-nonenal (4-HNE)

It has been suggested that SIRT1 is inactivated prior to protein degradation under certain conditions, such as cigarette smoke-mediated oxidative stress. Pretreatment of human bronchial epithelial (BEAS-2B) cells with a thiol reducing agent *N*-acetyl-lL-cysteine (NAC) prevented cigarette smoke extract (CSE)-induced loss of SIRT1, suggesting involvement of oxidative modifications in CSE-mediated degradation of SIRT1 [68]. Clinical observations of smokers and patients with chronic obstructive pulmonary disease (COPD) showed that 4-HNE, which is a CSE-induced lipid peroxidation product, formed covalent adducts with SIRT1 in lungs [69]. In this study [68], MALDI TOF/TOF mass spectrometry identified SIRT1 carbonylated by 4-HNE, particularly at the cysteine 482 residue.

SIRT1 GLYCOSYLATION BY

O-linked-β-N-acetylglucosamine (O-GlcNAc) transferase (OGT)

Han et al. [70] proposed that *O*-GlcNAcylation of SIRT1 by OGT increases its deacetylase activity. In human non-small cell lung carcinoma (NCI-H1299) cells, endogenous OGT was readily detected in the co-immunoprecipitates obtained with the anti-SIRT1 antibody, but not with the control IgG, indicating that OGT physiologically interacted with SIRT1 [70]. Subsequent analysis using a series of deletion mutants of SIRT1 revealed that OGT bound to the C-terminal domain of SIRT1 (480-747), but not to the N-terminal (1-240) or central domain of SIRT1 (210-500). In accordance with this finding, when SIRT1 was forced to be *O*-GlcNAcylated by co-expression of His-tagged SIRT1 and MBP-tagged OGT and was then subjected to electron transfer dissociation-MS analysis, serine 549 within the 544–561 peptide region of SIRT1 appeared to undergo *O*-GlcNAcylation [70].

Thiamet-G, an *O*-GlcNAcase specific inhibitor, was able to decrease the level of acetylated p53 in SIRT1-WT-transfected but not in the SIRT1-S549A-transfected NCI-H1299 cells, suggesting that *O*-GlcNAcylation of SIRT1 at serine 549 enhances its deacetylase activity. Under the genotoxic stress caused by the topoisomerase inhibitor etoposide, acetylation of p53 at lysine 382 was elevated in NCI-H1299 cells, which was declined by transfection of cells with SIRT1-WT much longer than that of cells with SIRT1-S549A. This indicates that *O*-GlcNAcylation of SIRT1 protects cells from death by deacetylating and inactivating p53. In another study, however, shRNA-mediated OGT knockdown enhanced the protein level of SIRT1 and its catalytic activity as evidenced by the reduced acetylation of p53 at K382 in breast cancer (MDA-MB-231) cells [71].

Notably, silencing of OGT led to the decreased invasiveness of MDA-MB-231 cells, and this was associated with the reduced levels of FOXM1, a transcription factor responsible for expression of MMP2 and MMP9. Even though this study could not reveal the SIRT1-dependent alteration of FOXM1 in its acetylation status, SIRT1 seemed to regulate the ubiquitination-dependent protein degradation of FOXM1 by exploiting E3 ubiquitin ligase Cdh1. The reduced protein stability of FOXM1 by ectopic expression of SIRT1 was rescued by specific shRNA targeting Cdh1 in MDA-MB-231 and HEK293T cells. In addition, when OGT-deleted MDA-MB-231 cells were injected intracardially into 6-week-old female NSG mice, mice displayed not only the decreased metastatic lesions, but the decreased liver macrometastasis and micrometastasis. However, concurrent inhibition of OGT and SIRT1 restored the metastatic activity of injected cells compared with OGT-silenced cells in vivo.

On the other hand, glycosylated SIRT1 seems to be susceptible to protein degradation during a fasted-to-refed transition; hyperglycosylation of SIRT1 has been suggested to be linked to hepatic dysfunctions leading to diabetes-like state [72]. In this study, OGT-mediated glycosylation of SIRT1 increased when primary hepatocytes were cultivated in high-glucose media. Likewise, liver tissues harvested from mice administrated insulin (0.75 IU/kg body weight) displayed a higher level of glycosylated SIRT1 compared with that of control mice. In both cases, total SIRT1 disappeared as glycosylated SIRT1 increased.

In silico prediction using YingOYang 1.2 software (http//serviceshealthtech.dtu.dk/service.php?YinOYang-1.2) revealed that Thr160/Ser161 in mouse SIRT1 (corresponding Ser169 in human) could be a potential glycosylation site [72]. Double mutant (T160A and S161A) cells were less responsive to high glucose conditions than cells harbouring SIRT1-WT; hence, no breakdown of SIRT1 occurred in double mutant cells [72]. Mutation of the known glycosylated residue (SIRT1-S549A), however, showed the same degree of glycosylation as SIRT1-WT exhibited. Adenovirus-based ectopic expression of SIRT1-T160/S161A in the liver of C57B16 mice led to disrupted glucose homeostasis along with a significant increase in fasting blood glucose [72]. Moreover, reduced oil-red staining combined with elevated transcripts of fatty acid oxidation genes such as phosphoenolpyruvate carboxykinase, glucose 6-phosphatase, and medium or long-chain acyl-CoA dehydrogenase in non-glycosylatable SIRT1 indicated an altered fat metabolism together with the increased p-AMPK and the reduced p-AKT. In addition, mice harbouring SIRT1-T160/S161A showed a heightened level of inflammatory genes such as *Caspase1*, *IL6*, and *TNFα,* suggesting that non-glycosylated SIRT1 leads to the increased gluconeogenesis and hepatic inflammation.

SIRT1 UBIQUITINATION BY

Smad ubiquitination regulatory Factor 2 (SMURF2)

In an attempt to clarify E3 ubiquitin ligase that can phosphorylate SIRT1, Yu et al. [41] employed the UbiBrowser (http://ubibrowser.bio-it.cn/ubibrowser_v3/) software, and SMURF was found to be a potential candidate. A pull-down assay revealed that SMURF2 was capable of binding to SIRT1 both at endogenous and exogenous levels in HCT-116 and HEK293T cells. Notably, SIRT1 was readily polyubiquitinated by overexpressed SMURF2 in the presence of recombinant E1 and E2 enzymes as assessed by an in vitro ubiquitination assay. Since another enzyme, E3 ligase RING1 and a catalytically inactive mutant of SMURF2 failed to facilitate SIRT1 ubiquitination in an immunoprecipitation assay, it is likely that SMURF2 specifically promotes SIRT1 ubiquitination by serving as a E3 ubiquitin ligase.

Among the potential lysine residues of SIRT1 to be ubiquitinated (lysine 238, 311, 335, 377, 499, 523, 601, and 610), lysine 499 and 523 appeared to be direct targets of SMURF2. Mutation of these two residues to arginine abolished SMURF2-mediated ubiquitination and degradation of SIRT1 in HEK293T cells. Interestingly, deletion of SMURF2 was associated with the increased migration and growth of HCT-116 cells, which was antagonized by SIRT1 knockdown. In human colorectal cancer cell tissues, SIRT1 was upregulated, while SMURF2 was decreased compared with marched normal tissues, implying a negative correlation between SIRT1 and SMURF2.

Ubiquitin conjugating enzyme E2 V (Ube2v)/ Ubiquitin-conjugating enzyme E2 13 (Ubc13)

Degradation of SIRT1 by Ube2v was linked to metastasis of colon cancer [73]. Overexpression of Ube2v1 led to an increased ubiquitination of SIRT1 and consequently a decline in SIRT1 accumulation in human colon cancer (SW480) cells. To target SIRT1 for ubiquitination, Ubc13 cooperates with Ube2v1 since the latter enzyme does not possess the conserved cysteine residue required for the catalytic activity of ubiquitin-conjugating enzymes (E2s). In accordance with this, co-immunoprecipitation showed that Ube2v1 did not physically interact with SIRT1, but Ubc13 was able to directly bind to SIRT1. Treatment with NSC697923, an inhibitor blocking interaction between Ubc13 and Ube2v1, effectively ablated the SIRT1 ubiquitination in SW480 cells. Degradation of SIRT1 by Ube2v1/Ubc13-mediated ubiquitination led to a reduction in acetylation of histone H4 at lysine 16, one of histone substrates of SIRT1, and this subsequently suppressed the expression of autophagy gene epigenetically, which contributes to lung metastasis of the Ube2v1-overexprsesing xenografts.

Mouse double minute 2 homolog (MDM2)

It has been suggested that SIRT1 at lysine 311 can be modified by ubiquitin, preferentially by MDM2 E3 ubiquitin ligase [40]. An immunoprecipitation assay revealed that ectopically expressed MDM2 and ubiquitin enhanced SIRT1 ubiquitination in HEK293T cells. Ubiquitination barely affected the protein stability of SIRT1 under the normal physiological condition, but dramatically accelerated SIRT1 degradation in response to DNA damaging stimuli such as etoposide, H_2O_2, and ionizing radiation in HeLa cells. The role of SIRT1 as a determinant of cell fate is likely to be dependent on the type and duration of DNA damage. In the rescue experiment, SIRT1-KD-HeLa cells expressing SIRT1-WT were resistant to etoposide-induced cytotoxicity compared to control cells, but cells harboring ubiquitination-defective SIRT1-K311R did not exhibit differential response when compared with control. On the other hand, SIRT1-KD-HeLa cells exposed to H_2O_2 underwent cell death in the presence of SIRT1-WT upon H_2O_2 treatment, whereas SIRT1-KD-HeLa cells expressing SIRT1-K311R showed a similar proportion of the dead cell population as control cells.

Ubiquitin specific peptidase 22 (USP22)

Ubiquitination involving E1/E2/E3 enzymes coordinated with deubiquitinase-mediated deubiquitination controls the turnover and the abundance of target proteins. USP22 has been reported to stabilize SIRT1 by deubiquitinating SIRT1, which suppresses acetylation-dependent transcriptional activity of p53 in HCT-116 cells [74]. In the same context, *usp22* null MEFs exhibited a significant reduction in the level of SIRT1 protein but not its mRNA, which was associated with the increased formation of acetylated p53, a transactive form of p53. USP22 appears to be essential for the early stages of embryonic development as *usp22* knockout mice displayed retardation in embryonic development. Further USP22 enhances SIRT1 stabilization to suppress p53-regulated cell apoptosis gene such as *p21* and *BAX* without causing embryonic lethality in mice.

Ubiquitin specific peptidase 7 (USP7)

Another deubiquitinase, USP7 has been proposed to prolong the half-life of SIRT1 [75]. Affinity purification combined with mass spectrometry using the whole-lysate of HeLa cells stably expressing FLAG-SIRT1 identified USP7 as a binding protein in FLAG-pull downed protein complex. The interaction between SIRT1 and USP7 was confirmed by the co-immunoprecipitation experiment in human breast cancer (HeLa and MCF-7) cells. This physical association led to stabilization of SIRT1 protein as USP7 has deubiquitinase activity toward SIRT1. HeLa cells co-overexpressing USP7 and SIRT1 showed decreased ubiquitination of SIRT1, whereas cells co-transfected with a functionally defective mutant form of USP7 (USP7-C223S) and SIRT1 failed to deubiquitinate SIRT1.

SIRT1 SUMOylation BY

Unknown SUMO E3 ligase

Both SIRT1 and SUMOylation have been reported to participate in cellular response under the genotoxic stress [76-79]. Considering such functional similarity, Yang et al. [29] attempted to investigate whether SIRT1 could be SUMOylated upon genotoxic stress. Direct interaction between SIRT1 and SUMO1 proven by co-precipitation analysis in human prostate cancer (DU145) cells further supports the possibility of SIRT1 SUMOylation.

According to SUMOylation prediction software (SUMOplot™) (http//www.abcepta.com/sumoplot), human SIRT1 possesses two potential SUMOylation sites, lysine 610 and lysine 734 [29]. Substitution of lysine 734 with arginine (SIRT1-K734R) blocked the SUMOylation of SIRT1; however, the introduction of arginine in place of lysine 610 failed to attenuate the SIRT1 SUMOylation, implying that lysine 734 is a dominant site to be SUMOylated by SUMO1 [29]. Notably, SUMOylation of SIRT1 at Lys 734 was closely associated with its deacetylase activity toward acetyl-p53 as SIRT1-WT and SIRT1-K610R mutant reduced abundance of p53 acetylation at Lys 382, whereas SIRT1-K734R rendered SIRT1 incapable of deacetylating p53 in human non-small cell lung carcinoma NCI-H1299 cells. In parallel with this, NCI-H1299 cells expressing SIRT1-WT were resistant to UV- or H_2O_2-induced apoptosis, whereas cells harboring the SUMOylation-deficient mutant (SIRT1-K734R) were not [29]. Thus, SUMOylation of SIRT1 particularly at Lys 734 is likely to play a critical role in cell fate determination as a molecular switch.

Histone deacetylase 4 (HDAC4)

HDAC4, a member of the histone deacetylase family, has SUMO E3 ligase activity which resides in its conserved C-terminal transcription binding domain. HDAC4 has been reported to SUMOylate some proteins such as liver X receptor β and IκBα [80,81]. HDAC4 seemed to directly associate with SIRT1 and stabilize it through SUMOylation in HeLa cells [45]. Forced expression of HDAC4 in young human lung fibroblasts (2BS) displayed a reduced senescence-associated β-gal activity, which is indicative of an alleviated senescence phenotype; thus it is likely that SIRT1 may possess an anti-senescent function as a molecular intermediator connecting HDAC4 to cellular senescence.

CONCLUDING REMARKS

SIRT1 appears to play beneficial roles in normal physiological or moderate inflammatory conditions. While tumor suppressive functions of SIRT were reported [82-84], recent studies have demonstrated that SIRT1 is abnormally overexpressed in various types of human malignancy, including breast [85], prostate [86], liver [87] and colon cancer [88]. The high levels of SIRT1 are associated with the lymph node metastasis and poor prognosis [88-90]. However, the role of SIRT1 in malignant transformation is still uncertain and controversial.

It has also been reported that SIRT1 is highly phosphorylated in colorectal cancer tissues compared to normal tissues and positively associated with Ki-67, a proliferation index, suggesting clinicopathologic significance of post-trnslational modification of SIRT1 [91]. Post-translational modifications, including phosphorylation, oxidation/reduction, carbonylation, nitrosylation, glycosylation, ubiquitination/deubiquitination, SUMOylation etc. have been speculated to modulate the activity, stability, subcellular localization of SIRT1, and also its binding affinity for substrate proteins (Fig. 2). Thus, it is presumable that distinctive post-translational modifications of SIRT1 by different distribution of intracellular molecules in normal and cancer cells, could determine the oncogenic vs. health beneficial function of SIRT1.

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CONFLICTS OF INTEREST

No potential conflicts of interest were disclosed.

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