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

Yeon-Hwa Lee¹, Su-Jung Kim², Young-Joon Surh¹,³

¹Research Institute of Pharmaceutical Sciences, College of Pharmacy, ²Department of Molecular Medicine and Biopharmaceutical Science, Graduate School of Convergence Science and Technology, ³Cancer Research Institute, Seoul National University, Seoul, Korea

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
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 [27]. 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.

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] |
|                |                          |                    | GKR, 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; GKR, 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 [27]. 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
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).
| 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    |               |
| 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 | 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/ Ty301 | JAK1 | MCF-10 cells | Enhanced interaction between SIRT1 and STAT3 | [55] |
| Ubiquitination | Lys311  | MDM2              | HEK293T cells        | Decreased catalytic activity  | [40]          |
|                |         |                   | HeLa cells            |                               |               |
| Phosphorylation | Thr344  | AMPK              | U2OS cells            | Enhanced catalytic activity   | [58]          |
|                |         |                   | HepG2 cells           | Decreased catalytic activity  | [59]          |
|                |         |                   | HUVEC cells           | Enhanced catalytic activity   | [65]          |
| Reduction      | Cys371/Cys374 | Ref-1 | REF-1                 | Decreased catalytic activity  | [69]          |
| S-Nitrosylation | Cys387/Cys390 | SNO–GAPDH | HEK293 cells | Decreased catalytic activity | [69] |
| S-Nitrosation  | Cys395/Cys398 | GSNO | In vitro assay | Decreased catalytic activity  | [66,67]       |
|                |         | ONOO–             | Nicotine-treated SIRT1 overexpressing mice | Decreased catalytic activity | [68] |
| Phosphorylation | Ser434  | PKA               | U2OS cells            | Enhanced catalytic activity   | [32]          |
|                |         |                   | BEAS-2B cells         | Inactivation and degradation  | [70,71]       |
| Carboxylation  | Cys482  | 4-HNE             | HCT-116 cells         | Degradation                   | [41]          |
| Ubiquitination | Lys499/Lys523 | SMURF2 | HEK293T cells | Enhanced catalytic activity   | [34,60]       |
| Phosphorylation | Thr522  | Dyrk1A/Dyrk3      | U2OS cells            | Enhanced catalytic activity   | [32]          |
| Phosphorylation | Thr530  | Dyrk2             | HCT116 and K562 cells | Enhanced interaction between DNA replication-related proteins | [30] |
|                |         | CyclinB/CDK1      | SIRT1<sup>−/−</sup> and Sirt1<sup>+/+</sup> ES cells | Enhanced catalytic activity | [30] |
|                |         |                   | Enhanced catalytic activity | Enhanced catalytic activity | [30] |
| Phosphorylation | Ser540  | CyclinB/CDK1      | SIRT1<sup>−/−</sup> and Sirt1<sup>+/+</sup> ES cells | Enhanced catalytic activity | [30] |
| O-GlcNAcylation | Ser549 | OGT               | H1299 cells           | Enhanced catalytic activity   | [72]          |
| Phosphorylation | Ser615  | LKB1              | HEK293T cells         | Enhanced catalytic activity   | [62]          |
|                |         |                   | H1299 cells           | Enhanced catalytic activity   |               |
| Phosphorylation | Ser669  | CK2               | HELa cells            | Enhanced catalytic activity   | [53,54]       |
|                | Ser732  |                   | H1299 cells           | Enhanced catalytic activity   |               |
| Phosphorylation | Ser659  | Unknown SUMO     | DU145 cells           | Enhanced catalytic activity   | [29]          |
|                | Ser661  | E3 ligase         | H1299 cells           | Enhanced catalytic activity   |               |

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<sup>2+</sup>/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.
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.healthtech.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-T530A/S540A). The proliferative capacity which was defective 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

---

**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, carboxylation, 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-Nitrosoylation or S-Nitrosation; C, carboxylation; G, O-GlcNacylation; Ub, ubiquitination; S, SUMOylation; jNK, c-Jun N-terminal kinase; GSNO, O-N-acetylglucosamine (O-GlcNAC) transferase; CK2, 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.
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 phosphorolyses 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 \( ^{32} \text{P} \)-orthophosphate [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 adenyl cyclase activator (forskolin)-induced increase of cellular cAMP in U2OS cells. According to this study, SIRT1-deficient MEF cells retrovirally transduced with non-phosphorylatable 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 [52].

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 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 siRNAs 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 deacetylase 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 larger extent than SIRT1-WT or phospo-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 (Uqrc1), COX5b (cytochrome c oxidase subunit 5B), and ATP synthase F1 subunit alpha (ATP5F1a).

**SIRT1 CYSTEINE THIOL MODIFICATION BY S-Nitrosothiol**

S-Nitrosothiol (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 activated 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 Zn²⁺-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 tetraethiolate motif were mutated to serine, Ref-1 could not prevent the loss of SIRT1 activity caused by hydrogen peroxide (H₂O₂)-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 Zn²⁺-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, Zn²⁺ was released from Zn²⁺-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 Zn²⁺ 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 supers) 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 supers 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

http://www.jcpjournal.org
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-l-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 carboxylated 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 more 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 invasive-
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 normal samples, 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-overexpressing 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, H₂O₂, 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 H₂O₂ underwent cell death in the presence of SIRT1-WT upon H₂O₂ 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 debiquitination 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-mediated deubiquitination controls the turnover and the abundance of target proteins. USP7 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.
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 (TM) (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 is 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 H2O2-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 intermediate 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-translational 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.

FUNDING

This work was supported by the Basic Science Research Grant (2021R1A2C2014186 to Y.-J. S.) Basic Science Research Program grant (No. 2021R1I1A1A046540 to S.-J. K.) and the BK21 FOUR Program (5120200513755) from the National Research Foundation, Republic of Korea.

CONFLICTS OF INTEREST

No potential conflicts of interest were disclosed.

ORCID

Yeon-Hwa Lee, https://orcid.org/0000-0001-8310-1795
Su-Jung Kim, https://orcid.org/0000-0002-3636-0644
Young-Joon Surh, https://orcid.org/0000-0001-9288-1789

REFERENCES

1. Langley E, Pearson M, Farella M, Bauer UM, Frye RA, Minucci S, et al. Human SIR2 deacetylates p53 and antagonizes PML/p53-induced cellular senescence. EMBO J 2002;21:2383-96.
2. Hayakawa T, Iwai M, Aoki S, Takimoto K, Maruyama M, Maruyama W, et al. SIRT1 suppresses the senescence-associated secretory phenotype through epigenetic gene regulation. PLoS One 2015;10:e0116480.
3. Han MK, Song EK, Guo Y, Ou X, Mantel C, Brockmeyer HE. SIRT1 regulates apoptosis and Nanog expression in mouse embryonic stem cells by controlling p53 subcellular localization. Cell Stem Cell 2008;2:241-51.
4. Vaziri H, Dessain SK, Ng Eaton E, Imai SI, Frye RA, Pandita TK, et al. hSIR2(SIRT1) functions as an NAD-dependent p53 deacetylase. Cell 2001;107:149-59.
5. Yoshizaki T, Schenk S, Imamura T, Babendure JL, Sonoda N, Bae EJ, et al. SIRT1 inhibits inflammatory pathways in macrophages and modulates insulin sensitivity. Am J Physiol

166 J Cancer Prev 27(3):157-169, September 30, 2022
Endocrinol Metab 2010;298:E419-28.

6. Yang H, Zhang W, Pan H, Feldser HG, Lainez E, Miller C, et al. SIRT1 activators suppress inflammatory responses through promotion of p65 deacetylation and inhibition of NF-κB activity. PLoS One 2012;7:e46364.

7. Purushotham A, Schug TT, Xu Q, Surapureddi S, Guo X, Li X. Hepatocyte-specific deletion of SIRT1 alters fatty acid metabolism and results in hepatic steatosis and inflammation. Cell Metab 2009;9:327-38.

8. Ren NSX, Ji M, Tokar EJ, Busch EL, Xu X, Lewis D, et al. Haploinsufficiency of SIRT1 enhances glutamine metabolism and promotes cancer development. Curr Biol 2017;27:483-94.

9. Cantó C, Gerhart-Hines Z, Feige JN, Lagouge M, Nobile G, Milne JC, et al. AMPK regulates energy expenditure by modulating NAD+ metabolism and SIRT1 activity. Nature 2009;458:69-73.

10. Song NY, Surh YJ. Janus-faced role of SIRT1 in tumorigenesis. Ann N Y Acad Sci 2012;1271:10-9.

11. Byles V, Chmilewski LK, Wang J, Zhu L, Forman LW, Faller DV, et al. Aberrant cytoplasm localization and protein stability of SIRT1 is regulated by PI3K/AKT-IR signaling in human cancer cells. Int J Biochem Cell Biol 2010;42:668-74.

12. Zhang Y, Zhang M, Dong H, Yong S, Li X, Olashaw N, et al. Deacetylation of cortactin by SIRT1 promotes cell migration. Oncogene 2009;28:445-50.

13. Sarikhani M, Maity S, Mishra S, Jain A, Tamta AK, Ravi V, et al. SIRT2 deacetylase represses NFAT transcription factor to maintain cardiac homeostasis. J Biol Chem 2018;293:5281-94.

14. Tang X, Chen XF, Wang NY, Wang XM, Liang ST, Zheng W, et al. SIRT2 acts as a cardioprotective deacetylase in pathological cardiac hypertrophy. Circulation 2017;136:2051-67.

15. Watanabe H, Inaba Y, Kimura K, Matsumoto M, Kaneko S, Kasuga M, et al. Sirt2 facilitates hepatic glucose uptake by deacetylating glucokinase regulatory protein. Nat Commun 2018;9:30.

16. Xu SN, Wang TS, Li X, Wang YP. SIRT2 activators G6PD to enhance NADPH production and promote leukemia cell proliferation. Sci Rep 2016;6:32734.

17. Jing E, Gesta S, Kahn CR. SIRT2 regulates adipocyte differentiation through FoxO1 acetylation/deacetylation. Cell Metab 2007;6:105-14.

18. Zhou W, Ni TK, Wronski P, Glass B, Skibinski A, Beck A, et al. The SIRT2 deacetylase stabilizesSlug to control malignancy of basal-like breast cancer. Cell Rep 2016;17:1302-17.

19. Hirsche MD, Shimazu T, Goetzman E, Jing E, Schwer B, Lombard DB, et al. SIRT3 regulates mitochondrial fatty-acid oxidation by reversible enzyme deacetylation. Nature 2010;464:121-5.

20. Laurent G, German NJ, Saha AK, de Boer VC, Davies M, Koves TR, et al. SIRT4 coordinates the balance between lipid synthesis and catabolism by repressing malonyl CoA decarboxylase. Mol Cell 2013;50:686-98.

21. Nakagawa T, Lomb DJ, Haigis MC, Guarente L. SIRT5 regulates deacetylases carbamoyl phosphate synthetase 1 and regulates the urea cycle. Cell 2009;137:560-70.

22. Tennen RI, Bua DJ, Wright WE, Chua KF. SIRT6 is required for maintenance of telomere position effect in human cells. Nat Commun 2011;2:433.

23. Michishita E, McCord RA, Berber E, Kioi M, Padilla-Nash H, Damian M, et al. SIRT6 is a histone H3 lysine 9 deacetylase that modulates telomeric chromatin. Nature 2008;452:492-6.

24. Sirri V, Grob A, Berthelet J, Jourdan N, Roussel P. Sirtuin 7 promotes 45S pre-rRNA cleavage at site 2 and determines the processing pathway. J Cell Sci 2019;132:jcs228601.

25. Chen S, Blank MF, Iyer A, Huang B, Wang L, Grummt I, et al. SIRT7-dependent deacetylation of the U3-55k protein controls pre-rRNA processing. Nat Commun 2016;7:10734.

26. Autiero I, Costantini S, Colonna G. Human sirt-1: molecular modeling and structure-function relationships of an unordered protein. PLoS One 2008;4:e7350.

27. Kim EJ, Kho JH, Kang MR, Um SJ. Active regulator of SIRT1 cooperates with SIRT1 and facilitates suppression of p53 activity. Mol Cell 2007;28:277-90. Erratum in: Mol Cell 2007;28:513.

28. Kim JE, Chen J, Lou Z. DBC1 is a negative regulator of SIRT1. Nature 2008;451:583-6.

29. Yang Y, Fu W, Chen J, Ohashi N, Zhang X, Nicosia SV, et al. SIRT1 sumoylation regulates its deacetylase activity and cellular response to genotoxic stress. Nat Cell Biol 2007;9:1253-62. Erratum in: Nat Cell Biol 2007;9:1442.

30. Sasaki T, Maier B, Koclega KD, Chruszcz M, Gibua W, Stukenberg PT, et al. Phosphorylation regulates SIRT1 function. PLoS One 2008;3:e4020.

31. Lee CW, Wong LL, Tse EY, Liu HF, Leong VY, Lee JM, et al. AMPK promotes p53 acetylation via phosphorylation and inactivation of SIRT1 in liver cancer cells. Cancer Res 2012;72:4394-404.

32. Gerhart-Hines Z, Dominy JE Jr, Blättler SM, Jedrychowski MP, Banks AS, Lim JH, et al. The cAMP/AMPK pathway rapidly activates SIRT1 to promote fatty acid oxidation independently of changes in NAD(+). Mol Cell 2011;44:851-63.

33. Zschoernig B, Mahlknecht U. Carboxy-terminal phosphorylation of SIRT1 by protein kinase CK2. Biochem Biophys Res Commun 2009;381:372-7.

34. Guo X, Williams JG, Schug TT, Li X. Dyrk1A and Dyrk3 promote cell survival through phosphorylation and activation of SIRT1. J Biol Chem 2010;285:13223-32.

35. Li JH, Huang W, Lin P, Wu B, Fu ZG, Shen HM, et al. N-linked glycosylation at Asn152 on CD147 affects protein folding and stability: promoting tumour metastasis in hepatocellular carcinoma. Sci Rep 2016;6:35210.

36. Zhou F, Xu W, Hong M, Pan Z, Sinko PJ, Ma J, et al. SIRT1 activators suppress inflammatory responses through FoxO1 acetylation/deacetylation. Cell Metab 2008;7:9.

37. Zhou W, Ni TK, Wronski P, Glass B, Skibinski A, Beck A, et al. The SIRT2 deacetylase stabilizes Slug to control malignancy of basal-like breast cancer. Cell Rep 2016;17:1302-17.

38. Hirsche MD, Shimazu T, Goetzman E, Jing E, Schwer B, Lombard DB, et al. SIRT3 regulates mitochondrial fatty-acid oxidation by reversible enzyme deacetylation. Nature 2010;464:121-5.

39. Laurent G, German NJ, Saha AK, de Boer VC, Davies M, Koves TR, et al. SIRT4 coordinates the balance between lipid synthesis and catabolism by repressing malonyl CoA decarboxylase. Mol Cell 2013;50:686-98.
the stability and subcellular distribution of human PAT1 protein. FEBS Lett. 2017;591:613-23.

39. Wagner SA, Beli P, Weinert BT, Nielsen ML, Cox J, Mann M, et al. A proteome-wide, quantitative survey of in vivo ubiquitination sites reveals widespread regulatory roles. Mol Cell Proteomics 2011;10:M111.013284.

40. Peng L, Yuan Z, Li Y, Ling H, Izumi V, Fang B, et al. Ubiquinated sirtuin 1 (SIRT1) function is modulated during DNA damage-induced cell death and survival. J Biol Chem 2015;290:8904-12.

41. Yan Q, Gong L, Deng M, Zhang L, Sun S, Liu J, et al. SUMOylation activates the transcriptional activity of PAX-6, an important transcription factor for eye and brain development. Proc Natl Acad USA 2010;107:21034-9.

42. Han X, Niu J, Zhao Y, Kong Q, Tong T, Han L. HDAC4 stabilizes SIRT1 via sumoylation SIRT1 to delay cellular senescence. Clin Exp Pharmacol Physiol 2016;43:41-6.

43. Zhou W, Hannoun Z, Jaffrey E, Medine CN, Black JR, Greenough S, et al. SUMOylation of HNF4α regulates protein stability and hepatocyte function. J Cell Sci 2012;125(Pt 15):3630-5.

44. Zhao W, Zhang X, Zhao J, Fan N, Rong J. SUMOylation of nuclear γ-actin by SUMO2 supports DNA damage repair against myocardial ischemia-reperfusion injury. Int J Biol Sci 2022;18:4595-609.

45. Ford J, Ahmed S, Allison S, Jiang M, Milan J. JNK2-dependent regulation of SIRT1 protein stability. Cell Cycle 2008;7:3091-7.

46. Zhou W, Hannoun Z, Medine CN, Black JR, Greenough S, et al. SUMOylation of HNF4α regulates protein stability and hepatocyte function. J Cell Sci 2012;125(Pt 15):3630-5.

47. Shao D, Fry JL, Han J, Hou X, Pimentel DR, Matsui R, et al. Peroxynitrite-induced cell death and survival. J Biol Chem 2015;290:8904-12.

48. Huang Y, Lu J, Zhan L, Wang M, Shi R, Yuan X, et al. Resveratrol-induced Sirt1 phosphorylation by LKB1 mediates mitochondrial metabolism. J Biol Chem 2021;297:100929.

49. Lee et al. Sirt1 NAD+-dependent protein deacetylase inhibition by cysteine oxidants and other cysteine oxidants. J Biol Chem 2009;284:1333-42.

50. Falko MJ, Sen N, Hara MR, Juluri KR, Nguyen JV, Snowman AE, et al. SIRT1 is a redox-sensitive deacetylase that is post-translationally modified by oxidants and carbonyl stress. FASEB J 2011;25:916-27.

51. Lau AW, Liu P, Inuzuka H, Gao D. SIRT1 phosphorylation by AMP-activated protein kinase regulates p53 acetylation. Am J Cancer Res 2014;4:245-55.

52. Guo X, Kesimer M, Tolun G, Zheng X, Xu Q, Lu J, et al. The NAD(+)-dependent protein deacetylase activity of SIRT1 is regulated by its oligomeric status. Sci Rep 2012;2:640.

53. Liu J, Zhang L, Chen X, Lu Q, Yang Y, Liu J, et al. SIRT1 counteracted the activation of STAT3 and NF-κB to repress the gastric cancer growth. Int J Clin Exp Med 2014;7:5050-8.

54. Wang W, Li F, Xu Y, Wei J, Zhang Y, Yang H, et al. JAK1-mediated Sirt1 phosphorylation functions as a negative feedback of the JAK1-STAT3 pathway. J Biol Chem 2018;293:11067-75.

55. Zee RS, Yoo CB, Pimentel DR, Perlman DH, Burgoyne JR, Zee RS, Yoo CB, Pimentel DR, Perlman DH, Burgoyne JR, et al. Phosphorylated SIRT1 associates with replication origins to prevent excess replication initiation and preserve genomic stability. Nucleic Acids Res 2017;45:7807-24.
patients with chronic obstructive pulmonary disease. Am J Respir Crit Care Med 2008;177:861-70.
70. Han C, Gu Y, Shan H, Mi W, Sun J, Shi M, et al. O-GlcNAcylation of SIRT1 enhances its deacetylase activity and promotes cytoprotection under stress. Nat Commun 2017;8:1491.
71. Ferrer CM, Lu TY, Bacigalupa ZA, Katsetos CD, Sinclair DA, Reginato MJ. O-GlcNAcylation regulates breast cancer metastasis via SIRT1 modulation of FOXM1 pathway. Oncogene 2017;36:559-69.
72. Chattopadhyay T, Maniyadath B, Bagul HP, Chakraborthy A, Shukla N, Budnar S, et al. Spatiotemporal gating of SIRT1 functions by O-GlcNAcylation is essential for liver metabolic switching and prevents hyperglycemia. Proc Natl Acad Sci USA 2020;117:6890-900.
73. Shen T, Cai LD, Liu YH, Li S, Gan WJ, Li XM, et al. Ube2v1-mediated ubiquitination and degradation of Sirt1 promotes metastasis of colorectal cancer by epigenetically suppressing autophagy. J Hematol Oncol 2018;11:95.
74. Lin Z, Yang H, Kong Q, Li J, Lee SM, Gao B, et al. USP22 antagonizes p53 transcriptional activation by deubiquitinating Sirt1 to suppress cell apoptosis and is required for mouse embryonic development. Mol Cell 2012;46:484-94.
75. Song N, Cao C, Tian S, Long M, Liu L. USP7 Deubiquitinates and Stabilizes SIRT1. Anat Rec (Hoboken) 2020;303:1337-45.
76. Brandl A, Wagner T, Uhlig KM, Knauer SK, Stauber RH, Melchior F, et al. Dynamically regulated sumoylation of HDAC2 controls p53 deacetylation and restricts apoptosis following genotoxic stress. J Mol Cell Biol 2012;4:284-93.
77. Luo J, Nikolaev AY, Imai S, Chen D, Su F, Shiloh A, et al. Negative control of p53 by Sir2alpha promotes cell survival under stress. Cell 2001;107:137-48.
78. Sasca D, Hänhel PS, Szybinski J, Khawaja K, Krieger O, Pante SV, et al. SIRT1 prevents genotoxic stress-induced p53 activation in acute myeloid leukemia. Blood 2014;124:121-33.
79. Morris JR, Boutilier C, Keppler M, Densham R, Weekes D, Alamshah A, et al. The SUMO modification pathway is involved in the BRCA1 response to genotoxic stress. Nature 2009;462:886-90.
80. Yang Q, Tang J, Xu C, Zhao H, Zhou Y, Wang Y, et al. Histone deacetylase 4 inhibits NF-κB activation by facilitating IκBα sumoylation. J Mol Cell Biol 2020;12:933-45.
81. Ghisletti S, Huang W, Ogawa S, Pascual G, Lin ME, Willson TM, et al. Parallel SUMOylation-dependent pathways mediate gene- and signal-specific transrepression by LXRs and PPARgamma. Mol Cell 2007;25:57-70.
82. Jung W, Hong KD, Jung WY, Lee E, Shin BK, Kim HK, et al. SIRT1 expression is associated with good prognosis in colorectal cancer. Korean J Pathol 2013;47:332-9.
83. Costa-Machado LF, Martín-Hernández R, Sanchez-Luengo MÁ, Hess K, Vales-Villamarin C, Barradas M, et al. Sirt1 protects from K-Ras-driven lung carcinogenesis. EMBO Rep 2018;19:e43879.
84. Kuo SJ, Lin HY, Chien SY, Chen DR. SIRT1 suppresses breast cancer growth through downregulation of the Bcl-2 protein. Oncol Rep 2013;30:125-30.
85. Wu M, Wei W, Xiao X, Guo J, Xie X, Li L, et al. Expression of SIRT1 is associated with lymph node metastasis and poor prognosis in both operable triple-negative and non-triple-negative breast cancer. Med Oncol 2012;29:3240-9.
86. Jung-Hynes B, Nihal M, Zhong W, Ahmad N. Role of sirtuin histone deacetylase SIRT1 in prostate cancer. A target for prostate cancer management via its inhibition? J Biol Chem 2009;284:3823-32.
87. Hao C, Zhu PX, Yang X, Han ZP, Jiang JH, Zong C, et al. Overexpression of SIRT1 promotes metastasis through epithelial-mesenchymal transition in hepatocellular carcinoma. BMC Cancer 2014;14:978.
88. Lv L, Shen Z, Zhang J, Zhang H, Dong J, Yan Y, et al. Clinicopathological significance of SIRT1 expression in colorectal adenocarcinoma. Med Oncol 2014;31:965.
89. Chen X, Sun K, Jiao S, Cai N, Zhao X, Zou H, et al. High levels of SIRT1 expression enhance tumorigenesis and associate with a poor prognosis of colorectal carcinoma patients. Sci Rep 2014;4:7481.
90. Yu DF, Jiang SJ, Pan ZP, Cheng WD, Zhang WJ, Yao XK, et al. Expression and clinical significance of Sirt1 in colorectal cancer. Oncol Lett 2016;11:1167-72.
91. Zhang X, Chen S, Cheng M, Cao F, Cheng Y. The expression and correlation of SIRT1 and Phospho-SIRT1 in colorectal cancer. Int J Clin Exp Med 2015;8:809-17.