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Protein SUMOylation modification and its associations with disease

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SUMOylation, as a post-translational modification, plays essential roles in various biological functions including cell growth, migration, cellular responses to stress and tumorigenesis. The imbalance of SUMOylation and deSUMOylation has been associated with the occurrence and progression of various diseases. Herein, we summarize and discuss the signal crosstalk between SUMOylation and ubiquitination of proteins, protein SUMOylation relations with several diseases, and the identification approaches for SUMOylation site. With the continuous development of bioinformatics and mass spectrometry, several accurate and high-throughput methods have been implemented to explore small ubiquitin-like modifier-modified substrates and sites, which is helpful for deciphering protein SUMOylation-mediated molecular mechanisms of disease.

1. Introduction

Protein post-translational modifications (PTMs) include phosphorylation, glycosylation, acetylation, ubiquitination, SUMOylation and many others [1]. As a competitor of ubiquitination, protein SUMOylation has become one of research hotspots in recent years. SUMOylation is one of PTMs, in which a member of the small ubiquitin-like modifier (SUMO) family of proteins is conjugated to lysine (Lys) residues in target proteins. SUMOylation modification is reversible and dynamic process, in which the modified proteins can be deSUMOylated by sentrin/SUMO-specific proteases (SENPs) [2]. The reversible attachment of a SUMO to a protein is controlled by an enzymatic pathway that is analogous to the ubiquitination pathway [3].

More and more researches have realized the importance of SUMOylation in the normal function of the body [4]. Along with the accumulating knowledge on its biological functions, SUMOylation has been reported to regulate protein subcellular localization, protein–DNA binding, protein–protein interactions, transcriptional regulation, DNA repair and genome organization [5]. Moreover, there is abundant evidence to show the aberrance of SUMO regulation is highly associated with various diseases, including cardiac disease [6], neurodegenerative disease [7] and cancers [8].

2. Small ubiquitin-like modifier family members

The SUMO family is a highly conserved PTM form in all eukaryotes, which is required for viability of most eukaryotic cells. There is only one SUMO gene SMT3 in budding yeast, while at least eight SUMO paralogues are present in

plants [9]. In mammalian cells, SUMO proteins consist of four components, including SUMO-1, SUMO-2, SUMO-3 and SUMO-4 [3,10]. SUMO-1 is one 101-amino-acid protein with 11.6 kDa. The SUMO-2 shares 95% homology with SUMO-3. The SUMO-2 and SUMO-3 differ from each other by only three N-terminal residues and have yet to be functionally distinguished, but together they share only approximately 45% homology with their paralogue SUMO-1 [11]. Despite the low sequence homologies, SUMO-1 and SUMO-2/3 share very similar three-dimensional structures. SUMO-4 is the least well characterized SUMO isoform. SUMO-4 is probably non-conjugated under physiological conditions. A gene coding for SUMO-4 was identified through analysis of single-nucleotide polymorphisms associated with type 1 diabetes [12]. In addition, the expression of SUMO-4 is increased in pre-eclamptic placentas and in models of oxidative stress and hypoxic injury [13]. Therefore, SUMO-4 may be a potential post-translational mechanism in the stressed pre-eclamptic placenta.

Nevertheless, there are important differences between mammalian SUMO paralogues. First, most of target proteins are modified exclusively by SUMO-1 *in vivo*, which is the dominant SUMO type among the four representative ones in mammalian cells [14]. Some other target proteins are conjugated to SUMO-2/3, or readily conjugated with all SUMO paralogues. SUMO is important for cellular response to stress [15,16], such as heat shock, DNA damage and oxidative stress [5,10]. SUMO-1 and SUMO-2/3 have different dynamics and responses to physiological stresses in mammalian cells. For instance, the nucleoplasmic SUMO-1 is more resistant to bleaching than the SUMO-2 or SUMO-3 in HeLa cells [17]. So cellular SUMO-1 dynamical transitions between SUMOylation and deSUMOylation take more time than the modification dynamical reactions of the SUMO-2 and SUMO-3.

3. Signal crosstalk of SUMOylation with ubiquitination

3.1. Small ubiquitin-like modifier is similar to ubiquitin in structure

In the aspect of the structure between SUMOs and ubiquitin, although the amino acid sequence alignments exist 18% identical between ubiquitin and SUMO-1, they have the same three-dimensional structure, especially β -sheet wraps a spherical folding of α -helix [18]. In addition, the position of the two C-terminal Gly residues required for isopeptide bond formation is conserved between ubiquitin and SUMO-1 [18,19]. The biological effects of ubiquitination and SUMOylation are both largely determined by the binding of proteins bearing specific interaction domains [20,21]. However, SUMO has an N-terminal extension that is not found in ubiquitin [20], which is probably the key point that SUMOylation has a different cell biological function than ubiquitination.

3.2. Biochemical process of SUMOylation and ubiquitination

The biochemical process of protein SUMOylation is related to ubiquitination. Ubiquitin and SUMO, the most prominent members of a conserved protein family of ubiquitin-like proteins (Ubls), can be attached to Lys residues of target proteins

via an isopeptide bond [22]. The ubiquitin-like modifications are carried out in a three-step cascade mechanism requiring the consecutive action of activating enzymes (E1s), conjugating enzymes (E2s) and ligases (E3s). In human cells, ubiquitination is mediated by two E1 ubiquitin activating enzymes, approximately 35 kinds of E2 ubiquitin conjugating enzymes and a variety of E3 ubiquitin ligases. The ubiquitinated proteins are recognized by receptors that contain ubiquitin-binding domains, while the deubiquitinases, a specialized family of proteases, remove ubiquitin modifications [23].

Similarly, SUMOylation, an analogous modification of ubiquitination, is similar to the conjugation pathway of ubiquitin in the biochemical process (figures 1 and 2), which is performed in turn under the E1, E2 and E3 enzyme catalysis [20]. During protein SUMOylation, SUMOs are synthesized as propeptides that require cleavage to reveal C-terminal diglycine motifs by SENPs in mammal cells [24]. SUMOs are then activated by an ATP-dependent heterodimer of SUMO activating enzyme subunit 1 (SAE1) and SAE2 [25], which passes the activated SUMO protein onto the specific and unique conjugating enzyme, a ubiquitin conjugating enzyme 9 (Ubc9), through a *trans*-esterification reaction and forming a high-energy thioester bond [26]. The Ubc9 usually acts in conjunction with an E3 ligating enzyme, then catalyses SUMO conjugation to the substrate [27]. Finally, SUMO conjugation forms an isopeptide bond between the SUMO C-terminus and a ϵ -amino group of a Lys within the target protein [11]. A number of proteins have been discovered to have SUMO E3 activity, including Ran binding protein 2 (RanBP2), the protein inhibitor of activated STAT (PIAS), the polycomb protein Pc2 and others [21], which enhance SUMO conjugation to proteins. While the removal of SUMO modification from a protein is mediated by SENPs [28]. Members involved in SUMO pathway in mammal cells are summarized in table 1. In addition, protein SUMOylation requires a consensus SUMOylation motif in the target protein. For example, although there are several Lys residues in a protein, only a few of them could be true SUMOylation sites. SUMO-1, SUMO-2 and SUMO-3 interact with the same N-terminal region of the E2 conjugating enzyme Ubc9 with similar affinities. In general, many SUMOylation sites follow a consensus motif ψ -K-X-E or ψ -K-X-E/D (ψ is a hydrophobic amino acid, K is the target Lys, X is any amino acid and D/E is Asp or Glu) [21].

A growing number of proteins have been reported to act as substrates for both ubiquitination and SUMOylation. The modified proteins have a wide range of functions, which are mainly found in their modified substrates [8]. These two modifications between ubiquitination and SUMOylation have many communications in biological functions, including the control of signal transduction pathways, the maintenance of chromosome integrity and genomic stability.

3.3. Correlation between SUMOylation and ubiquitination-mediated biological functions

Although protein SUMOylation and ubiquitination both act on the Lys amino acid residue, sometimes they are cooperated, and other times they are competitively modified for a target protein. SUMO modification usually increases protein stability. For instance, the SUMOylation of Oct4 significantly increased Oct4 stability and its DNA binding ability during embryonic and germ cell development [29]. While SUMO regulates the

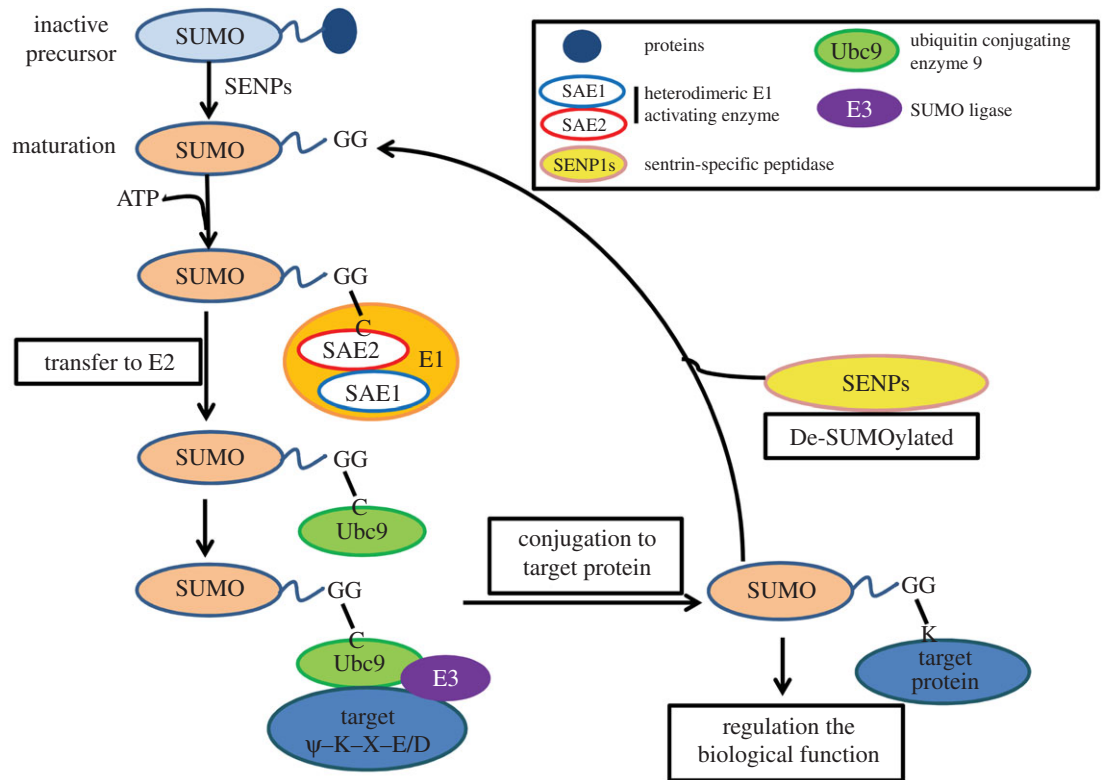


Figure 1. Biochemical process of SUMO modifications in mammal cells. All small ubiquitin-like modifier (SUMO) paralogues are synthesized as pre-proteins that are first cleaved by a SENP to expose a carboxy-terminal diglycine (GG) motif (maturation). An ATP-requiring activation step by the heterodimeric E1 activating enzyme (including SAE1 and SAE2) then generates a SUMO–SAE2 thioester. SUMO is then transferred to the E2 conjugating enzyme Ubc9, again forming a thioester. This last step usually requires a SUMO E3 ligase to bring about an isopeptide bond between the SUMO C-terminus and a lysine within the target protein.

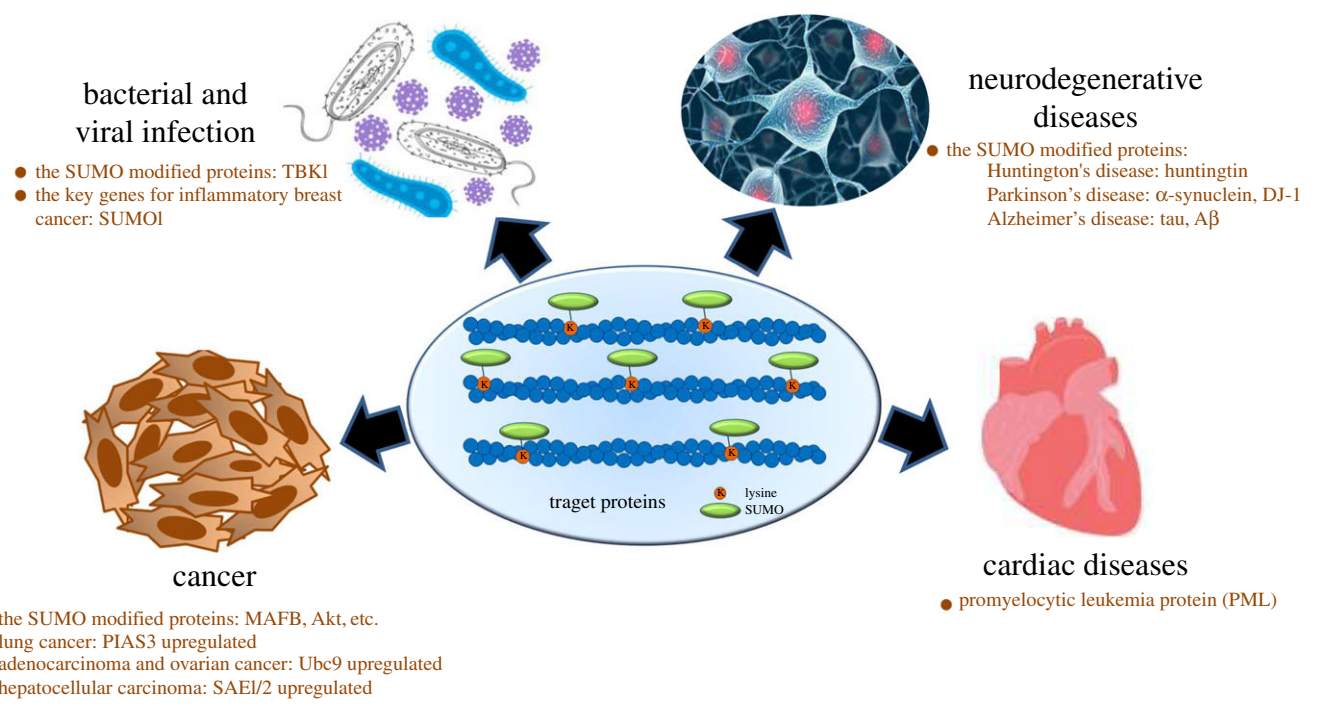


Figure 2. Relationship of SUMO-modified proteins with different diseases, along with some examples of representative proteins and SUMO pathway members.

expression of tripartite motif-containing proteins TRIM21, which functions as the Oct-1 ubiquitin E3 ligase to control Oct-1 degradation. Therefore, a higher TRIM21 expression enhances Oct-1 ubiquitination and reduces Oct-1 stability consequently [30].

Some proteins can be simultaneously modified by SUMO or ubiquitin along with different even opposite roles mediated by each modification. For example, SENP1 plays a key role in the regulation of the hypoxic response through regulation of HIF1 α stability. In this regulation process, HIF1 α SUMOylation

Table 1. The members of SUMO pathway in mammal cells.

members of SUMO pathway	<i>Homo sapiens</i>
SUMO	SUMO-1,-2,-3,-4
activating enzyme E1	SAE1,SAE2
conjugating enzyme E2	Ubc9
ligase E3	RanBP2;PIAS1,-2,-3,-4;Pc2 and etc.
SUMO protease	SEN1,-2,-3,-4,-5,-6,-7

can serve as a direct signal for the ubiquitin-dependent degradation of VHL [31]. Promyelocytic leukaemia protein (PML) could be SUMOylated and ubiquitinated when exposed to arsenic trioxide. A pathogenic fragment of huntingtin (HTT) protein can be modified by both SUMO-1 and ubiquitin at the same Lys residue. The SUMOylation of HTT-fragment increases neurodegeneration, whereas its ubiquitination decreases neurodegeneration in a Huntington's disease model [32]. PES1 is a component of the PeBoW complex; when stimulated by oestrogen, the SUMOylation of PES1 upregulates its stability and function via inhibiting its ubiquitination [33]. Post-translational modification of proliferating cell nuclear antigen PCNA can be modified by ubiquitin and SUMO in response to DNA damage [34].

In conclusion, SUMO modification has been shown to compete with ubiquitination for common Lys residues in most cases. On the other hand, SUMO modification also cooperates with ubiquitination to regulate biochemical function.

4. Protein SUMOylation relates to multiple diseases

4.1. SUMOylation and cancer

Recently, there are many studies have shown that expression of the SUMO E1 activating enzyme (a heterodimer of SAE1 and SAE2), the SUMO E2 conjugating enzyme (Ubc9) or the SUMO E3 ligases appears to be enhanced in numerous cancers [8,35–37]. The expression level of Ubc9 E2 is upregulated in adenocarcinoma and ovarian cancer cells, and PIAS3 is also increased with different degrees in lung cancer, breast cancer, prostate cancer and colorectal cancer [8]. The enzymes involved in SUMO modification is usually increased, which is closely related to the pathogenesis of hepatocellular carcinoma (HCC). For example, the expression of SAE1/2 is significantly upregulated in cancer tissues of HCC patients [38]. Survival rate of patients with liver cancer is related to the expression level of SUMO-2. The only E2 enzyme Ubc9 is overexpressed in HCC during SUMO modification [39], while SENP2, which regulates the process of removing SUMO modification, can inhibit the proliferation of HCC cells [40,41]. Moreover, SUMOylation is important in the development of multidrug resistance in HCC [42].

In addition, human tumorigenesis is closely related to SUMOylation and SUMO-modified substrate proteins. SUMOylation is critical to cancer stem cell maintenance and self-renewal. Knockdown of SUMO activating enzyme E1 or SUMO conjugating enzyme (E2) inhibits maintenance and self-

renewal of colorectal cancer stem cells [30]. The SUMOylated MAFB promotes colorectal cancer tumorigenesis through cell cycle regulation [43]. Similarly, SUMOylation of Akt is required for cell growth and tumorigenesis, and K276 is the major SUMO acceptor site of Akt [44].

4.2. SUMOylation and cardiac disease

Recent studies show that protein SUMOylation plays an important role in cardiac function, and balanced SUMOylation/deSUMOylation is important for proper cardiac development, metabolism and stress adaptation [6,45–47]. SUMOylation is attempted to treat cardiac disease. The increase of Ubc9-mediated SUMOylation may represent a novel strategy for increasing autophagic flux and ameliorating morbidity in proteotoxic cardiac disease [47]. The Ubc9/The PML/RNF4 (a SUMO-targeted ubiquitin ligase) axis plays a critical role as an important SUMO pathway in cardiac fibrosis, which provides an attractive therapeutic target for treatment of cardiac fibrosis and heart failure by modulating the signal axis pathway [45].

4.3. SUMOylation and neurodegenerative disease

Neurodegenerative diseases often involve the formation of abnormal and toxic protein aggregates, which are thought to be the primary factor in neurodegenerative disease occurrence and progression. Accumulating evidences demonstrate perturbations of neuronal SUMOylation contribute to numerous pathological conditions and neurological disorders [7,48,49].

4.3.1. Huntington's disease

It is known abnormality of HTT protein modification is associated with Huntington's disease (HD) [50]. A pathogenic fragment of HTT can be modified by SUMO-1 at the Lys residue, HTT-fragment SUMOylation increases neurodegeneration in HD model. In addition, HTT SUMOylation increases the degradation of ubiquitin–proteasome pathway, resulting in the accumulation of HTT, which finally leads to HD [32]. Other reports show HTT is modified by SUMO-2 to modulate insoluble mutant HTT protein accumulation, and PIAS1 enhances SUMO-2 modification [50,51].

4.3.2. Parkinson's disease

SUMOylation is linked with the development of Parkinson's disease (PD) [52]. The α -synuclein, which highly expressed in the brain and associated with PD, has been verified to be SUMOylated preferentially by SUMO-1 [53]. The SUMOylation of DJ-1 plays an intriguing potential role for PD. The SUMO-modified DJ-1 participates in the transcriptional regulation of genes concerned with the cellular regulation of oxidative stress. Whereas DJ-1 mutation will prevent SUMOylation and abolish all of its known functions [54,55]. The PIAS family members, as SUMO E3 proteins, interact with DJ-1 and stimulate its SUMOylation in the process of eliminating ROS [52,56].

4.3.3. Alzheimer's disease

Alzheimer's disease (AD) is an age-dependent, progressive neurodegenerative disorder that is characterized by

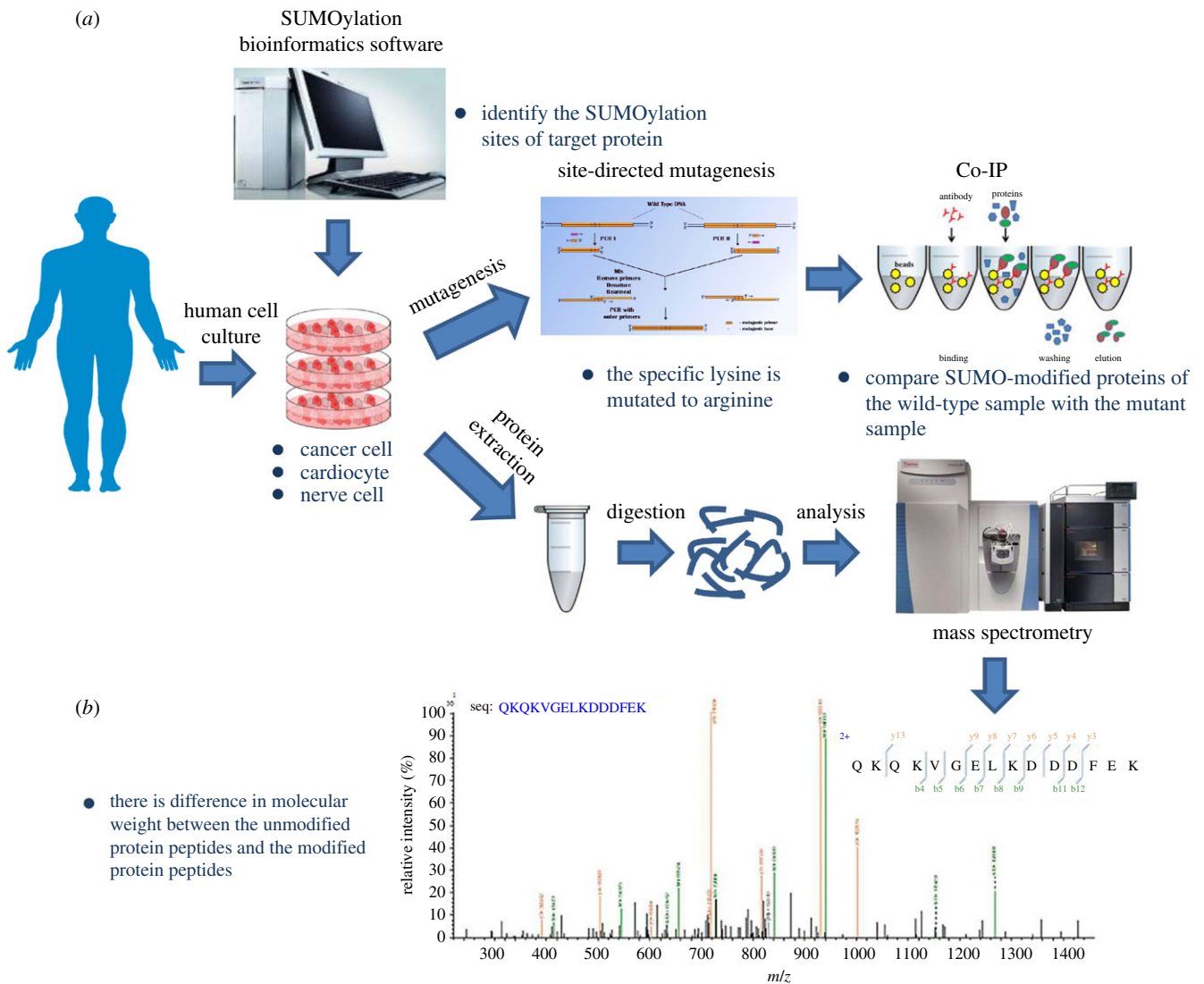


Figure 3. Several methods to identify SUMOylation site. SUMO modification sites are predicted using bioinformatics software analysis. The prediction site is verified using the following methods. (a) The SUMOylation site is analysed through site-directed mutagenesis and Co-IP. (b) SUMO modification site is identified by mass spectrometry.

amyloid- β ($A\beta$) plaque formation [7] and the presence of neurofibrillary tangles composed of hyperphosphorylated tau protein. Previous studies indicated that SUMO-3 overexpression affects $A\beta$ levels [57]. SUMO-1 also modulates $A\beta$ generation via accumulation of the Alzheimer's β -secretase BACE1 [58]. The SUMOylation of tau protein is also associated with the development of AD [59,60]. Tau protein can be both SUMOylated and ubiquitylated [61]. Inhibition of the proteasomal degradation pathway increases the tau ubiquitination and decreases its SUMOylation, suggesting that SUMO and ubiquitin might compete to regulate tau stability [53].

4.4. SUMOylation and innate immunity

SUMOylation also involves in the replication of a large number of viruses, either through the direct modification of viral proteins or through the modulation of cellular proteins implicated in antiviral defense. There is growing evidence that SUMO regulates several host proteins involved in intrinsic and innate immunity, thereby contributing to the process governing interferon production during viral infection [62–65]. SUMOylation of proteins have been implicated in the resistance to RNA viral infection. For DNA viruses, SUMOylation promotes the stability of the DNA sensor

cGAS and the adaptor STING to regulate the kinetics of response to DNA virus [66,67].

SUMOylation is a novel post-translational modification for TANK-binding kinase 1 (TBK1) [63]. TBK1 kinase activity is required to allow the attachment of SUMO-1 or SUMO-2/3 proteins, and a SUMO modification at K694 contributes to the antiviral function of TBK1, while the viral protein Gam1 antagonizes this post-translational modification. Another study identified SUMO1 was the key gene for inflammatory breast cancer [63]. TRIM38 acts as an E3 ubiquitin or SUMO ligase, which targets key cellular signalling components, regulating the innate immune and inflammatory responses [68]. SUMOylation of NF- κ B essential molecule NEMO augments NF- κ B activity, NF- κ B-dependent cytokine production and pancreatic inflammation [69]. In summary, SUMOylation has been deeply studied recently, and its understanding could be vital for developing potential therapeutic strategies.

5. Approaches to identify SUMOylation site

Nowadays, the identification of SUMO modification has faced several challenges due to low abundance of most SUMOylated proteins. The approaches for SUMOylation identification

Table 2. The methods of predicting SUMO modification sites. GPS, group-based prediction system; MotifX, statistical phosphorylation sites prediction method; PSSM, position-specific scoring matrix; SVMa, support vector machines; MS, mass spectrometry. WEKA, Waikato environment of knowledge analysis.

bioinformatic tools	characteristic	year	website	free or not free	refs
SUMOSP	including SUMOSP 1.0 and 2.0 based on two algorithms applied GPS and motifX in SUMOSP	2006	http://sumosp.biocuckoo.org/	free	[70,71]
SUMOPLOT	a commercially available SUMOylation site predictor based on a SUMO-modified conserved sequence and hydrophobicity analysis	2006	http://www.abgent.com/tools/sumoplot/	free	[72]
SUMOPRE	using a probabilistic model for prediction	2008	http://spg.biosci.tsinghua.edu.cn/service/sumoprd/predict.cgi (unable to access)	unknown	[73]
FINDSUMO	based on PSSM	2008	http://findingsumo.com.cutestat.com/	not free	[74]
SUMMON	based on the sequence information automated pattern recognition tool detects PTM fragment ion series within complex MS/MS spectra calculating two independent scores, one for the modification and one for the target peptide	2008	http://summon.sourceforge.net/	free	[75]
SUMOTR	using structure and sequence information higher in correlation coefficient and sensitivity	2010		unknown	[76]
SEESUMO	using the domain-specific knowledge in terms of relevant biological features for input vector encoding	2011	http://bioinfo.ggc.org/seesumo/ (unable to access)	unknown	[77]
SUMOHYDRO	based on hydrophobic properties using SVM for classification	2012	http://protein.cau.edu.cn/others/SUMOhydro/	free	[78]
SUMOHUNT	using random forest-based classifier provided in WEKA needing sequence and several physico-chemical properties	2013		unknown	[79]

mainly include the bioinformatics coupled with the amino acid site-directed mutagenesis and mass spectrometry (MS)-based proteomics analysis. We can predict protein SUMOylation sites by the analogue computation bioinformatics, which is further verified by amino acid site-directed mutagenesis. In addition, the variable SUMO modification sites of target proteins are identified by MS-based techniques and the biochemical validation (figure 3).

The identification of SUMOylation sites and SUMO-interaction motifs in proteins is fundamental for understanding biological functions and regulatory mechanisms of SUMOs. Recently several bioinformatics software tools have been developed to predict SUMOylation modification (table 2), including SUMOSP [70,71], SUMOPLOT [72], SUMOPRE [73], FINDSUMO [74], SUMMON [75], SUMOTR [76], SEESUMO [77], SUMOHYDRO [78] and SUMOHUNT [79]. The SUMOSP and SUMOPLOT approaches predict SUMO modification sites mainly based on the conserved sequence ψ -K-X-E/D. Generally these bioinformatics methods focus on the characteristics of a rigorous algorithm of the existing SUMO-specific sites on the substrate proteins, and some even consider the protein space structure and hydrophobicity. These bioinformatics predictions usually need experimental data validation.

5.1. Small ubiquitin-like modifier modification site is identified by mass spectrometry

Despite the powerful SUMO-modified prediction software providing a theoretical basis for the prediction of SUMO modification sites, the precise identification of SUMO modification sites is very important for investigating the target protein functions. Recent advances in MS-based proteomics have greatly facilitated the robust identification and quantification of PTMs [80,81], including SUMO modification. The most common approach is to isolate the target SUMOylated protein by affinity chromatography and to identify by MS [61,82–84]. It is noted that this approach requires the expression of a mutant form of SUMO, in which the residue preceding the C-terminal Gly–Gly (diGly) is replaced with a Lys (SUMO (KGG)) [85]. Digestion of SUMO (KGG) protein conjugates with endoproteinase Lys-C yields a diGly motif attached to target lysines. Peptides containing this adduct are enriched using a diGly-Lys (K-ε-GG)-specific antibody and identified by MS. This diGly signature is characteristic of SUMO(KGG) conjugation alone, as no other Ubl yields this adduct upon Lys-C digestion [85].

MS-based identification of SUMOylated sites is hampered by the large peptide remnant of SUMO proteins that are left

on the modified Lys residue upon tryptic digestion. Regarding this problem, tandem affinity purification can carry out a more efficient enrichment of SUMOylated proteins by allowing the use of strong denaturing conditions generally to remove most of the contaminant proteins [86].

5.2. SUMOylation site is confirmed by site-directed mutagenesis

The Lys site on a protein, possibly modified by the SUMO molecule, is usually mutated to the Arg residue by site-directed mutagenesis to check biological function changes. This classic biochemical method is very efficient to confirm the protein SUMOylation site, but the throughput is not high as MS. For instance, the SUMOylation of TARBP2 at K52 is found to require for regulating miRNA/siRNA efficiency by this biochemical method [87].

5.3. Proximity ligation assays for detection protein SUMOylation *in vivo*

Detection of protein SUMOylation *in situ* by proximity ligation assays (PLA) allows easy visualization of endogenous protein–protein interactions at the single molecule level [88–91]. PLA relies on the use of combinations of antibodies coupled to complementary oligonucleotides that are amplified and revealed with a fluorescent probe, with each spot representing a single protein–protein interaction. In PLA, one antibody is directed against the substrate ‘protein X’, while another targets SUMO-1, SUMO-2/3 or ubiquitin. PLA could detect a ‘SUMOylated protein X’ fraction, but also ‘protein X’ interacting with other SUMOylated proteins. PLA offers a quick, cheap and ultrasensitive way for initial testing of ubiquitin-like modifications [92].

5.4. *In situ* SUMOylation assay

Another method is *in situ* SUMOylation assay [93,94], which is based on the fluorescence detection of SUMOylation and deSUMOylation in cultured cells. The recombinant green fluorescence protein fused to the SUMO-1 (GFP-tagging SUMO1) is used to visualize the nuclear rim, nucleolus and nuclear bodies. These GFP signals represent cellular regions where SUMOylation efficiently takes place. The recombinant SUMO-specific protease SENP1 catalytic domain is added to erase GFP signals when deSUMOylation happens. Some novel integrative technologies have been developed according to the above principles.

A semi-intact cell system, in combination with siRNA-based knockdown of nucleoporin RanBP2 [93], reveals a modulatory role of RanBP2 in the nuclear rim and PML bodies.

6. Prospective

SUMO modification has been in existence more than a decade. SUMOs have been established as essential regulators of many cellular functions. It is considered to be one of the important factors regulating the function of the intracellular protein, and abnormal protein SUMOylation will lead to the occurrence of disease.

Recently, the relationship of protein SUMOylation and autophagy has been studied. Autophagy is a catabolic process that facilitates nutrient recycling via degradation of damaged organelles and proteins through lysosomal-mediated degradation [46,95–97]. Autophagy is one of the main mechanisms in the pathophysiology of neurodegenerative disease. The accumulation of autophagic vacuoles (AVs) in affected neurons is responsible for A β production. Previous investigation has proved that SUMOylation is associated with autophagy. Overexpression of SUMO1 increased autophagic activation, inducing the formation of LC3-II-positive AVs in neuroglioma H4 cells [98]. Ubc9 overexpression induced relatively high levels of autophagy and led to an increase in autophagic flux, while Ubc9 depletion led to decreased LC3-II expression. This may represent a novel strategy for increasing autophagic flux and ameliorating morbidity in proteotoxic cardiac disease [6]. Conversely, autophagy can regulate Ubc9 levels during viral-mediated tumorigenesis. Ubc9 and autophagy are important co-factors to prime early stages of human papillomavirus-mediated tumorigenesis [99].

With the continuous development of bioinformatics and MS, several accurate and high-throughput methods have been implemented to explore SUMO-modified substrates and sites, which is helpful for deciphering protein SUMOylation-mediated molecular mechanisms of disease.

Data accessibility. This article has no additional data.

Authors' contributions. All authors participated in the preparation of the manuscript, and read and approved the final manuscript.

Competing interests. All authors declare no conflict of interest.

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References

- Kessler BM, Edelman MJ. 2011 PTMs in conversation: activity and function of deubiquitinating enzymes regulated via post-translational modifications. *Cell Biochem Biophys.* **60**, 21–38. (doi:10.1007/s12013-011-9176-6)
- Guo C, Henley JM. 2014 Wrestling with stress: roles of protein SUMOylation and deSUMOylation in cell stress response. *IUBMB Life* **66**, 71–77. (doi:10.1002/iub.1244)
- Johnson ES. 2004 Protein modification by SUMO. *Annu. Rev. Biochem.* **73**, 355–382. (doi:10.1146/annurev.biochem.73.011303.074118)
- Princz A, Tavernarakis N. 2017 The role of SUMOylation in ageing and senescent decline. *Mech. Ageing Dev.* **162**, 85–90. (doi:10.1016/j.mad.2017.01.002)
- Hickey CM, Wilson NR, Hochstrasser M. 2012 Function and regulation of SUMO proteases. *Nat. Rev. Mol. Cell Biol.* **13**, 755–766. (doi:10.1038/nrm3478)
- Da Silva-Ferrada E, Ribeiro-Rodrigues TM, Rodriguez MS, Girao H. 2016 Proteostasis and SUMO in the heart. *Int. J. Biochem. Cell Biol.* **79**, 443–450. (doi:10.1016/j.biocel.2016.09.015)
- Mun MJ *et al.* 2016 Polymorphisms of small ubiquitin-related modifier genes are associated with risk of Alzheimer's disease in Korean: a case-control study. *J. Neurol. Sci.* **364**, 122–127. (doi:10.1016/j.jns.2016.03.023)

8. Seeler JS, Dejean A. 2017 SUMO and the robustness of cancer. *Nat. Rev. Cancer* **17**, 184–197. (doi:10.1038/nrc.2016.143)
9. Kurepa J, Walker JM, Smalle J, Gosink MM, Davis SJ, Durham TL, Sung DY, Vierstra RD. 2003 The small ubiquitin-like modifier (SUMO) protein modification system in *Arabidopsis*: accumulation of SUMO1 and -2 conjugates is increased by stress. *J. Biol. Chem.* **278**, 6862–6872. (doi:10.1074/jbc.M209694200)
10. Enserink JM. 2015 Sumo and the cellular stress response. *Cell Div.* **10**, 4. (doi:10.1186/s13008-015-0010-1)
11. Mukhopadhyay D, Dasso M. 2007 Modification in reverse: the SUMO proteases. *Trends Biochem. Sci.* **32**, 286–295. (doi:10.1016/j.tibs.2007.05.002)
12. Guo D *et al.* 2004 A functional variant of SUMO4, a new I kappa B alpha modifier, is associated with type 1 diabetes. *Nat. Genet.* **36**, 837–841. (doi:10.1038/ng1391)
13. Baczyk D, Audette MC, Drewlo S, Levytska K, Kingdom JC. 2017 SUMO-4: a novel functional candidate in the human placental protein SUMOylation machinery. *PLoS ONE* **12**, e0178056. (doi:10.1371/journal.pone.0178056)
14. Saitoh H, Hinchey J. 2000 Functional heterogeneity of small ubiquitin-related protein modifiers SUMO-1 versus SUMO-2/3. *J. Biol. Chem.* **275**, 6252–6258. (doi:10.1074/jbc.275.9.6252)
15. Jongjitwimol J, Baldock RA, Morley SJ, Watts FZ. 2016 Sumoylation of eIF4A2 affects stress granule formation. *J. Cell Sci.* **129**, 2407–2415. (doi:10.1242/jcs.184614)
16. Feligioni M, Nistico R. 2013 SUMO: a (oxidative) stressed protein. *Neuromolecular Med.* **15**, 707–719. (doi:10.1007/s12017-013-8266-6)
17. Ayaydin F, Dasso M. 2004 Distinct *in vivo* dynamics of vertebrate SUMO paralogues. *Mol. Biol. Cell* **15**, 5208–5218. (doi:10.1091/mbc.E04-07-0589)
18. Bayer P, Arndt A, Metzger S, Mahajan R, Melchior F, Jaenicke R, Becker J. 1998 Structure determination of the small ubiquitin-related modifier SUMO-1. *J. Mol. Biol.* **280**, 275–286. (doi:10.1006/jmbi.1998.1839)
19. Su HL, Li SS. 2002 Molecular features of human ubiquitin-like SUMO genes and their encoded proteins. *Gene* **296**, 65–73. (doi:10.1016/S0378-1119(02)00843-0)
20. Gill G. 2004 SUMO and ubiquitin in the nucleus: different functions, similar mechanisms? *Genes Dev.* **18**, 2046–2059. (doi:10.1101/gad.1214604)
21. Martin S, Wilkinson KA, Nishimune A, Henley JM. 2007 Emerging extranuclear roles of protein SUMOylation in neuronal function and dysfunction. *Nat. Rev. Neurosci.* **8**, 948–959. (doi:10.1038/nrn2276)
22. Bergink S, Jentsch S. 2009 Principles of ubiquitin and SUMO modifications in DNA repair. *Nature* **458**, 461–467. (doi:10.1038/nature07963)
23. Swatek KN, Komander D. 2016 Ubiquitin modifications. *Cell Res.* **26**, 399–422. (doi:10.1038/cr.2016.39)
24. Yeh ET, Gong L, Kamitani T. 2000 Ubiquitin-like proteins: new wines in new bottles. *Gene* **248**, 1–14. (doi:10.1016/S0378-1119(00)00139-6)
25. Desterro JM, Rodriguez MS, Kemp GD, Hay RT. 1999 Identification of the enzyme required for activation of the small ubiquitin-like protein SUMO-1. *J. Biol. Chem.* **274**, 10 618–10 624. (doi:10.1074/jbc.274.15.10618)
26. Desterro JM, Thomson J, Hay RT. 1997 Ubch9 conjugates SUMO but not ubiquitin. *FEBS Lett.* **417**, 297–300. (doi:10.1016/S0014-5793(97)01305-7)
27. Sarge KD, Park-Sarge OK. 2009 Sumoylation and human disease pathogenesis. *Trends Biochem. Sci.* **34**, 200–205. (doi:10.1016/j.tibs.2009.01.004)
28. Takahashi Y, Kahyo T, Toh EA, Yasuda H, Kikuchi Y. 2001 Yeast Ull1/Siz1 is a novel SUMO1/Smr3 ligase for septin components and functions as an adaptor between conjugating enzyme and substrates. *J. Biol. Chem.* **276**, 48 973–48 977. (doi:10.1074/jbc.M109295200)
29. Wei F, Scholer HR, Atchison ML. 2007 Sumoylation of Oct4 enhances its stability, DNA binding, and transactivation. *J. Biol. Chem.* **282**, 21 551–21 560. (doi:10.1074/jbc.M611041200)
30. Du L, Li YJ, Fakhri M, Wiatrek RL, Duldulao M, Chen Z, Chu P, Garcia-Aguilar J, Chen Y. 2016 Role of SUMO activating enzyme in cancer stem cell maintenance and self-renewal. *Nat. Commun.* **7**, 12326. (doi:10.1038/ncomms12326)
31. Cheng J, Kang X, Zhang S, Yeh ET. 2007 SUMO-specific protease 1 is essential for stabilization of HIF1alpha during hypoxia. *Cell* **131**, 584–595. (doi:10.1016/j.cell.2007.08.045)
32. Steffan JS *et al.* 2004 SUMO modification of huntingtin and Huntington's disease pathology. *Science* **304**, 100–104. (doi:10.1126/science.1092194)
33. Li S, Wang M, Qu X, Xu Z, Yang Y, Su Q, Wu H. 2016 SUMOylation of PES1 upregulates its stability and function via inhibiting its ubiquitination. *Oncotarget* **7**, 50 522–50 534. (doi:10.18632/oncotarget.10494)
34. Tsutakawa SE *et al.* 2015 Structurally distinct ubiquitin- and sumo-modified PCNA: implications for their distinct roles in the DNA damage response. *Structure* **23**, 724–733. (doi:10.1016/j.str.2015.02.008)
35. Bellail AC, Olson JJ, Hao C. 2014 SUMO1 modification stabilizes CDK6 protein and drives the cell cycle and glioblastoma progression. *Nat. Commun.* **5**, 4234. (doi:10.1038/ncomms5234)
36. Liu X *et al.* 2015 Knockdown of SUMO-activating enzyme subunit 2 (SAE2) suppresses cancer malignancy and enhances chemotherapy sensitivity in small cell lung cancer. *J. Hematol. Oncol.* **8**, 67. (doi:10.1186/s13045-015-0164-y)
37. Coppola D, Parikh V, Boulware D, Blanck G. 2009 Substantially reduced expression of PIAS1 is associated with colon cancer development. *J. Cancer Res. Clin. Oncol.* **135**, 1287–1291. (doi:10.1007/s00432-009-0570-z)
38. Lee JS, Thorgeirsson SS. 2004 Genome-scale profiling of gene expression in hepatocellular carcinoma: classification, survival prediction, and identification of therapeutic targets. *Gastroenterology* **127**, S51–S55. (doi:10.1053/j.gastro.2004.09.015)
39. Tomasi ML, Tomasi I, Ramani K, Pascale RM, Xu J, Giordano P, Mato JM, Lu SC. 2012 S-adenosyl methionine regulates ubiquitin-conjugating enzyme 9 protein expression and sumoylation in murine liver and human cancers. *Hepatology* **56**, 982–993. (doi:10.1002/hep.25701)
40. Jiang QF, Tian YW, Shen Q, Xue HZ, Li K. 2014 SENP2 regulated the stability of beta-catenin through WWOX in hepatocellular carcinoma cell. *Tumour Biol.* **35**, 9677–9682. (doi:10.1007/s13277-014-2239-8)
41. Shen HJ, Zhu HY, Yang C, Ji F. 2012 SENP2 regulates hepatocellular carcinoma cell growth by modulating the stability of beta-catenin. *Asian Pac. J. Cancer Prev.* **13**, 3583–3587. (doi:10.7314/APJCP.2012.13.8.3583)
42. Qin Y, Bao H, Pan Y, Yin M, Liu Y, Wu S, Li H. 2014 SUMOylation alterations are associated with multidrug resistance in hepatocellular carcinoma. *Mol. Med. Rep.* **9**, 877–881. (doi:10.3892/mmr.2014.1882)
43. Yang LS, Zhang XJ, Xie YY, Sun XJ, Zhao R, Huang QH. 2016 SUMOylated MAFB promotes colorectal cancer tumorigenesis. *Oncotarget* **7**, 83 488–83 501. (doi:10.18632/oncotarget.13129)
44. Li R, Wei J, Jiang C, Liu D, Deng L, Zhang K, Wang P. 2013 Akt SUMOylation regulates cell proliferation and tumorigenesis. *Cancer Res.* **73**, 5742–5753. (doi:10.1158/0008-5472.CAN-13-0538)
45. Liu Y *et al.* 2017 Manipulating PML SUMOylation via silencing UBC9 and RNF4 regulates cardiac fibrosis. *Mol. Ther.* **25**, 666–678. (doi:10.1016/j.ymthe.2016.12.021)
46. Gupta MK, Robbins J. 2016 Making the connections: Autophagy and post-translational modifications in cardiomyocytes. *Autophagy* **12**, 2252–2253. (doi:10.1080/15548627.2016.1215384)
47. Gupta MK, McLendon PM, Gulick J, James J, Khalili K, Robbins J. 2016 UBC9-mediated sumoylation favorably impacts cardiac function in compromised hearts. *Circ. Res.* **118**, 1894–1905. (doi:10.1161/CIRCRESAHA.115.308268)
48. Juarez-Vicente F, Luna-Pelaez N, Garcia-Dominguez M. 2016 The Sumo protease Senp7 is required for proper neuronal differentiation. *Biochimica Et Biophysica Acta—Molecular Cell Research* **1863**, 1490–1498. (doi:10.1016/j.bbamcr.2016.03.028)
49. Martins WC, Tasca CI, Cimarosti H. 2016 Battling alzheimer's disease: targeting SUMOylation-mediated pathways. *Neurochem. Res.* **41**, 568–578. (doi:10.1007/s11064-015-1681-3)
50. Ochaba J, Monteys AM, O'Rourke JG, Reidling JC, Steffan JS, Davidson BL, Thompson LM. 2016 PIAS1 regulates mutant huntingtin accumulation and Huntington's disease-associated phenotypes *in vivo*. *Neuron* **90**, 507–520. (doi:10.1016/j.neuron.2016.03.016)
51. O'Rourke JG *et al.* 2013 SUMO-2 and PIAS1 modulate insoluble mutant huntingtin protein accumulation. *Cell Rep.* **4**, 362–375. (doi:10.1016/j.celrep.2013.06.034)

52. de Souza ACG, Prediger RD, Cimarosti H. 2016 SUMO-regulated mitochondrial function in Parkinson's disease. *J. Neurochem.* **137**, 673–686. (doi:10.1111/jnc.13599)
53. Dorval V, Fraser PE. 2006 Small ubiquitin-like modifier (SUMO) modification of natively unfolded proteins tau and alpha-synuclein. *J. Biol. Chem.* **281**, 9919–9924. (doi:10.1074/jbc.M510127200)
54. Shinbo Y, Niki T, Taira T, Ooe H, Takahashi-Niki K, Maita C, Seino C, Iguchi-Ariga SMM, Ariga H. 2006 Proper SUMO-1 conjugation is essential to DJ-1 to exert its full activities. *Cell Death Differ.* **13**, 96–108. (doi:10.1038/sj.cdd.4401704)
55. Zhong N, Xu J. 2008 Synergistic activation of the human MnSOD promoter by DJ-1 and PGC-1alpha: regulation by SUMOylation and oxidation. *Hum. Mol. Genet.* **17**, 3357–3367. (doi:10.1093/hmg/ddn230)
56. Takahashi K, Taira T, Niki T, Seino C, Iguchi-Ariga SM, Ariga H. 2001 DJ-1 positively regulates the androgen receptor by impairing the binding of PIASx alpha to the receptor. *J. Biol. Chem.* **276**, 37 556–37 563. (doi:10.1074/jbc.M101730200)
57. Dorval V, Mazzella MJ, Mathews PM, Hay RT, Fraser PE. 2007 Modulation of Abeta generation by small ubiquitin-like modifiers does not require conjugation to target proteins. *Biochem. J.* **404**, 309–316. (doi:10.1042/BJ20061451)
58. Yun SM *et al.* 2013 SUMO1 modulates Abeta generation via BACE1 accumulation. *Neurobiol. Aging* **34**, 650–662. (doi:10.1016/j.neurobiolaging.2012.08.005)
59. Silveirinha V, Stephens GJ, Cimarosti H. 2013 Molecular targets underlying SUMO-mediated neuroprotection in brain ischemia. *J. Neurochem.* **127**, 580–591. (doi:10.1111/jnc.12347)
60. Luo HB *et al.* 2014 SUMOylation at K340 inhibits tau degradation through deregulating its phosphorylation and ubiquitination. *Proc. Natl Acad. Sci. USA* **111**, 16 586–16 591. (doi:10.1073/pnas.1417548111)
61. Thomas SN, Yang AJ. 2017 Mass spectrometry analysis of lysine posttranslational modifications of tau protein from Alzheimer's disease brain. *Methods Mol. Biol.* **1523**, 161–177. (doi:10.1007/978-1-4939-6598-4_10)
62. Hannoun Z, Maarifi G, Chelbi-Alix MK. 2016 The implication of SUMO in intrinsic and innate immunity. *Cytokine Growth Factor Rev.* **29**, 3–16. (doi:10.1016/j.cytogfr.2016.04.003)
63. Saul VV, Niedenthal R, Pich A, Weber F, Schmitz ML. 2015 SUMO modification of TBK1 at the adaptor-binding C-terminal coiled-coil domain contributes to its antiviral activity. *Biochim. Biophys. Acta* **1853**, 136–143. (doi:10.1016/j.bbamcr.2014.10.008)
64. Xia PY, Wang S, Xiong Z, Ye BQ, Huang LY, Han ZG, Fan ZS. 2015 IRTKS negatively regulates antiviral immunity through PCBP2 sumoylation-mediated MAVS degradation. *Nat. Commun.* **6**, Artn 8132. (doi:10.1038/ncomms9132)
65. Liu J, Qian C, Cao X. 2016 Post-translational modification control of innate immunity. *Immunity* **45**, 15–30. (doi:10.1016/j.immuni.2016.06.020)
66. Hu MM, Yang Q, Xie XQ, Liao CY, Lin H, Liu TT, Yin L, Shu HB. 2016 Sumoylation promotes the stability of the DNA Sensor cGAS and the adaptor sting to regulate the kinetics of response to DNA virus. *Immunity* **45**, 555–569. (doi:10.1016/j.immuni.2016.08.014)
67. Cui Y *et al.* 2017 SENP7 potentiates cGAS activation by relieving SUMO-Mediated Inhibition of cytosolic DNA Sensing. *PLoS Pathog.* **13**, e1006156. (doi:10.1371/journal.ppat.1006156)
68. Hu MM, Shu HB. 2017 Multifaceted roles of TRIM38 in innate immune and inflammatory responses. *Cell. Mol. Immunol.* **14**, 331–338. (doi:10.1038/cmi.2016.66)
69. Shao L, Feng B, Zhang Y, Zhou H, Ji W, Min W. 2016 The role of adipose-derived inflammatory cytokines in type 1 diabetes. *Adipocyte* **5**, 270–274. (doi:10.1080/21623945.2016.1162358)
70. Ren J, Gao XJ, Jin CJ, Zhu M, Wang XW, Shaw A, Wen LP, Yao XB, Xue Y. 2009 Systematic study of protein sumoylation: development of a site-specific predictor of SUMOsp 2.0. *Proteomics* **9**, 3409–3412. (doi:10.1002/pmic.200800646)
71. Xue Y, Zhou F, Fu C, Xu Y, Yao X. 2006 SUMOsp: a web server for sumoylation site prediction. *Nucleic Acids Res.* **34**, W254–W257. (doi:10.1093/nar/gkl207)
72. Tankou S *et al.* 2016 SUMOylation of DISC1: a potential role in neural progenitor proliferation in the developing cortex. *MolNeuropsychiatry* **2**, 20–27. (doi:10.1159/000444257)
73. Xu J, He Y, Qiang B, Yuan J, Peng X, Pan XM. 2008 A novel method for high accuracy sumoylation site prediction from protein sequences. *BMC Bioinformatics* **9**, 8. (doi:10.1186/1471-2105-9-8)
74. Friedline CJ, Zhang XP, Zehner ZE, Zhao ZM. 2008 *Advanced intelligent computing theories and applications*, pp. 1004–1011. Berlin, Germany: Springer.
75. Pedrioli PGA, Raught B, Zhang XD, Rogers R, Aitchison J, Matunis M, Aebersold R. 2006 Automated identification of SUMOylation sites using mass spectrometry and SUMMoN pattern recognition software. *Nat. Methods* **3**, 533–539. (doi:10.1038/NMETH891)
76. Yavuz AS, Sezerman U. 2010 Year SUMOtr: SUMOylation site prediction based on 3D structure and hydrophobicity. In *Fifth International Symposium on Health Informatics and Bioinformatics*, IEEE, Antalya, Turkey, pp. 93–97. Piscataway, NJ: IEEE.
77. Teng S, Luo H, Wang L. 2012 Predicting protein sumoylation sites from sequence features. *Amino Acids* **43**, 447–455. (doi:10.1007/s00726-011-1100-2)
78. Chen YZ, Chen Z, Gong YA, Ying GG. 2012 SUMOhydro: A novel method for the prediction of sumoylation sites based on hydrophobic properties. *PLoS ONE* **7**, ARTN e39195. (doi:10.1371/journal.pone.0039195)
79. Ijaz A. 2013 SUMOhunt: combining spatial staging between lysine and sumo with random forests to predict SUMOylation. *ISRN Bioinform.* **2013**, 671269. (doi:10.1155/2013/671269)
80. Liang S, Xu Z, Xu X, Zhao X, Huang C, Wei Y. 2012 Quantitative proteomics for cancer biomarker discovery. *Comb. Chem. High Throughput Screen* **15**, 221–231. (doi:10.2174/138620712799218635)
81. Zeng X, Yang PB, Chen B, Jin XW, Liu YL, Zhao X, Liang SF. 2013 Quantitative secretome analysis reveals the interactions between epithelia and tumor cells by *in vitro* modulating colon cancer microenvironment. *J. Proteomics* **89**, 51–70. (doi:10.1016/j.jprot.2013.05.032)
82. Galisson F, Mahrouche L, Courcelles M, Bonneil E, Meloche S, Chelbi-Alix MK, Thibault P. 2011 A novel proteomics approach to identify SUMOylated proteins and their modification sites in human cells. *Mol. Cell. Proteomics* **10**, Artn M110.004796. (doi:10.1074/Mcp.M110.004796)
83. Hendriks IA, D'Souza RC, Chang JG, Mann M, Vertegaal ACO. 2015 System-wide identification of wild-type SUMO-2 conjugation sites. *Nat. Commun.* **6**, Artn 7289. (doi:10.1038/ncomms8289)
84. Hendriks IA, D'Souza RCJ, Yang B, Verlaan-de Vries M, Mann M, Vertegaal ACO. 2014 Uncovering global SUMOylation signaling networks in a site-specific manner. *Nat. Struct. Mol. Biol.* **21**, 927–936. (doi:10.1038/nsmb.2890)
85. Tammsalu T, Matic I, Jaffray EG, Ibrahim AF, Tatham MH, Hay RT. 2015 Proteome-wide identification of SUMO modification sites by mass spectrometry. *Nat. Protoc.* **10**, 1374–1388. (doi:10.1038/nprot.2015.095)
86. Filosa G, Barabino SM, Bachi A. 2013 Proteomics strategies to identify SUMO targets and acceptor sites: a survey of RNA-binding proteins SUMOylation. *Neuromolecular Med.* **15**, 661–676. (doi:10.1007/s12017-013-8256-8)
87. Chen C *et al.* 2015 SUMOylation of TARBP2 regulates miRNA/siRNA efficiency. *Nat. Commun.* **6**, 8899. (doi:10.1038/ncomms9899)
88. Psakhye I, Jentsch S. 2012 Protein group modification and synergy in the SUMO pathway as exemplified in DNA repair. *Cell* **151**, 807–820. (doi:10.1016/j.cell.2012.10.021)
89. Ristic M, Brockly F, Piechaczyk M, Bossis G. 2016 Detection of protein–protein interactions and posttranslational modifications using the proximity ligation assay: application to the study of the SUMO pathway. *Methods Mol. Biol.* **1449**, 279–290. (doi:10.1007/978-1-4939-3756-1_17)
90. Bedzhov I, Stemmler MP. 2015 Applying the proximity ligation assay (PLA) to mouse preimplantation embryos for identifying protein–protein interactions *in situ*. *Methods Mol. Biol.* **1233**, 57–64. (doi:10.1007/978-1-4939-1789-1_6)
91. Elfineh L, Classon C, Asplund A, Pettersson U, Kamali-Moghaddam M, Lind SB. 2014 Tyrosine phosphorylation profiling via *in situ* proximity ligation assay. *BMC Cancer* **14**, 435. (doi:10.1186/1471-2407-14-435)
92. Bagchi S, Fredriksson R, Wallen-Mackenzie A. 2015 *In situ* proximity ligation assay (PLA). *Methods Mol. Biol.* **1318**, 149–159. (doi:10.1007/978-1-4939-2742-5_15)
93. Saitoh N, Uchimura Y, Tachibana T, Sugahara S, Saitoh H, Nakao M. 2006 *In situ* SUMOylation analysis reveals a modulatory role of RanBP2 in the nuclear rim and PML bodies. *Exp. Cell Res.* **312**, 1418–1430. (doi:10.1016/j.yexcr.2006.01.013)

94. Muramatsu M, Uwada J, Matsumoto N, Saitoh H. 2010 A simple *in situ* cell-based sumoylation assay with potential application to drug screening. *Biosci. Biotechnol. Biochem.* **74**, 1473–1475. (doi:10.1271/bbb.100081)
95. Towers CG, Thorburn A. 2016 Therapeutic targeting of autophagy. *EBioMedicine* **14**, 15–23. (doi:10.1016/j.ebiom.2016.10.034)
96. Jacob JA, Salmani JM, Jiang Z, Feng L, Song J, Jia X, Chen B. 2017 Autophagy: an overview and its roles in cancer and obesity. *Clin. Chim. Acta* **468**, 85–89. (doi:10.1016/j.cca.2017.01.028)
97. Nah J, Yuan J, Jung YK. 2015 Autophagy in neurodegenerative diseases: from mechanism to therapeutic approach. *Mol. Cells* **38**, 381–389. (doi:10.14348/molcells.2015.0034)
98. Cho SJ, Yun SM, Jo C, Lee DH, Choi KJ, Song JC, Park SI, Kim YJ, Koh YH. 2015 SUMO1 promotes Abeta production via the modulation of autophagy. *Autophagy* **11**, 100–112. (doi:10.4161/15548627.2014.984283)
99. Mattosio D *et al.* 2017 Autophagy regulates UBC9 levels during viral-mediated tumorigenesis. *PLoS Pathog.* **13**, e1006262. (doi:10.1371/journal.ppat.1006262)