Sumoylation in gene regulation, human disease, and therapeutic action
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Abstract

Similar to ubiquitination, sumoylation covalently attaches a small ubiquitin-like modifier (SUMO) protein (92–97 amino acids) to the ε-amino group of a lysine residue. This is quite different from the classically defined post-translational modifications, such as phosphorylation, acetylation, and methylation, which typically add a small chemical group to the targeted residue. Sumoylation has been well studied at the molecular and cellular levels, focusing mostly on site-specific conjugation of human SUMO1, SUMO2, and SUMO3, as well as their homologues in various species. In this short review, we will discuss some recent examples to highlight (a) emerging trends about the coordinated regulation of sumoylation and other post-translational modifications in modulating the function of some transcription factors and pathway-specific regulators, (b) diverse roles of sumoylation in gene regulation implicated in stem cells and different pathogenic conditions, and (c) potential therapeutic strategies related to some of the diseases stated above.

Introduction

First reported by two research groups in late 1996 and early 1997 [1,2], sumoylation is now known to modify various eukaryotic proteins in organisms ranging from yeast to humans. A recent proteomic analysis of sumoylated proteins identified over 580 endogenous targets in human cervical carcinoma-derived HeLa cells, corresponding to approximately 2% of the entire proteome [3]. This modification adds an approximately 10 kDa small ubiquitin-like modifier (SUMO) polypeptide to the ε-amino group of certain lysine residues. SUMO polypeptides are approximately 18% identical to ubiquitin at the amino acid sequence level and their three-dimensional (3D) structural folds are highly similar to that of ubiquitin [4-6]. Like ubiquitination, sumoylation is governed by a conserved cascade consisting of a heterodimeric E1-activating enzyme complex, an E2-conjugating enzyme, and an E3 ligase [4,7]. While there is only one E1 or E2 enzyme, multiple E3 ligases have been identified. The conjugated SUMO moieties are synthesized as precursors, which are cleaved by Sentrin/SUMO-specific proteases ([SENP] up to seven in humans) to expose the C-terminal diglycine required for covalent attachment to target proteins [8]. In addition, SENPs control desumoylation, rendering the modification dynamic and reversible [9].

Both ubiquitination and sumoylation attach small proteins to targets, and these small proteins serve as platforms for interaction with other proteins. This is quite different from other post-translational modifications (PTMs), such as phosphorylation, acetylation, and methylation, which add small chemical groups and thus use mechanisms distinct from ubiquitination and sumoylation to exert their functional impacts. Another aspect of difference is that attached ubiquitin and SUMO moieties form domains for recognizing short peptide motifs on binding partners, whereas phosphorylation, acetylation, and methylation are recognized by modification-specific
protein domains [10]. Short motifs recognized by SUMO polypeptides are known as SUMO-interaction motifs, which conform to a well-conserved consensus sequence and can thus be used to predict and identify SUMO interaction sites on binding partners [4-6].

Sumoylation has been well studied at the molecular and cellular levels (for reviews, see [4,5,7,11,12]). In what follows, we will describe some recent examples to highlight emerging trends about the coordinated regulation of transcription factors and other proteins by sumoylation and to discuss the diverse roles of this PTM in gene regulation, stem cell biology, and human diseases. Particular attention is given to related studies that have been published over the past four to five years. For this, we will start with a brief description about the classification of different sumoylation sites.

**Different types of sumoylation motifs**

Classical sumoylation sites conform to the consensus sequence \( \psi KxE \), where \( \psi \) is a bulky hydrophobic amino acid (such as I, L, and V) and \( x \) can be any residue (Figure 1) [6,7,13,14]. A subgroup of known sumoylation sites contains one or a few acidic residues located two or three residues downstream from the core motif \( \psi KxE \) to form a negatively charged amino acid-dependent sumoylation motif (NDSM) (Figure 1) [15]. The negative charge enhances the binding affinity for the E2-conjugating enzyme Ubc9 (ubiquitin carrier protein 9) that contains a positively charged \( \psi KxE \)-binding pocket (Figure 2a) [15,16]. Similar to the negatively charged motif is the phosphorylation-dependent sumoylation motif (PDSM), \( \psi KxExxxS/T \) (Figure 1), conserved among a number of transcription factors, including HSF1 (heat shock factor 1), PPAR\( \gamma \) (peroxisome proliferator-activated receptor \( \gamma \)), MEF2 (myocyte enhancer factor 2), and estrogen-related receptors (ERRs) [17-20]. Signal-dependent phosphorylation of the serine or threonine residues promotes sumoylation in response to different signaling cues [21].

A third subgroup of classical sumoylation motifs possesses an N-terminal cluster of hydrophobic residues to enhance sumoylation. Such motifs are known as hydrophobic cluster-containing sumoylation motifs (HCSMs) (Figure 1) [22]. A recent study further reveals an inverted sumoylation motif (iSM), E/DxK\( \psi \) (Figure 1) [22]. These classical and inverted consensus sequences help predict new sumoylation sites, especially when the putative sites are located at evolutionarily conserved sequence islands. Sumoylation is quite different from ubiquitination and other PTMs, such as acetylation and methylation, for which the surrounding sequences have a rather limited value in predicting the modification sites. It should be noted, however, that according to a proteomic analysis, atypical sites with little sequence similarity to the above motifs are also important for sumoylation [14]; in such cases, the motifs are of no predicting value.

**Selective regulation of NDSM sumoylation by Ubc9 acetylation**

The E2 SUMO-conjugating enzyme Ubc9 is itself subject to regulation by PTMs. A recent study reveals that Ubc9 is acetylated at Lys-65 and the acetylation selectively reduces the sumoylation of NDSM-containing proteins, such as the ETS-domain transcription factor ELK1 (Figure 2a and b) and the acetyltransferase transcriptional coactivator CBP [23]. The acidic patch within an NDSM (Figure 1) forms a negatively charged surface for interaction with a positively charged cluster formed by Lys-65, Lys-74, and Lys-76 of Ubc9 (Figure 2a) [16]. These three residues are in proximity on the 3D structure [16]. By disrupting this charge interaction, Lys-65 acetylation decreases NDSM sumoylation of proteins such as ELK1 (Figure 2b) and CBP but has
Besides acetylation, Ubc9 itself is also sumoylated, which serves as a cofactor to promote efficient SUMO transfer and stimulate polysumoylation of target proteins [24]. It will likewise be interesting to know whether other modifications, such as phosphorylation and ubiquitination, modify Ubc9 and regulate its enzymatic activity. ELK1 sumoylation is inhibited by its own phosphorylation by MAP kinases [25], so it is important to determine how this regulation coordinates with Ubc9 acetylation.

**Crosstalk of sumoylation with other modifications for coordinated regulation**

As described above for PDSMs (Figure 1), sumoylation is often regulated by other PTMs occurring at regions flanking the sumoylated site. The crosstalk between sumoylation and other PTMs is well exemplified by the tumor suppressor p53, whose last 30 amino acids (aa) 363-393 of human p53) are heavily modified by sumoylation, ubiquitination, neddylation, phosphorylation, acetylation, and methylation (Figure 3a) [26-28]. A single sumoylation site identified at Lys-386 of human p53 represents an SM similar to NDSMs and PDSMs (Figure 3a), in that Ser-392 is flanked by two Asp residues (that is, DSD) and becomes acidic upon phosphorylation by cellular kinases, such as

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### Figure 2. Mechanisms by which Ubc9 recognizes the extended sumoylation motifs

A

Human Ubc9 contains three lysine residues forming a positively charged surface at the three-dimensional structure for recognizing an acidic patch just C-terminal to the classical sumoylation motif in ELK1. This recognition stimulates sumoylation at Lys-249 of ELK1. A similar mechanism of regulation operates with CBP sumoylation (not shown). SM, sumoylation motif.

B

Acetylation of Lys-65 in Ubc9 impairs its recognition of an acidic patch situated at the C-terminal region of ELK1. (c) It remains to be determined whether acetylation of Lys-65 in Ubc9 may affect the recognition of the phosphorylated serine residues within the phosphorylation-dependent sumoylation motifs of myocyte enhancer factor 2 (MEF2), peroxisome proliferator-activated receptor γ (PPARγ), and other transcription factors.

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### Figure 3. Crosstalk among post-translational modifications at the p53 C-terminal regulatory region

A

(a) The last 30 amino acids in human p53 (aa 363-393) showing the sites specifically modified by ubiquitination (Ub), acetylation (Ac), methylation (Me), neddylation (Ne), sumoylation (SUMO), and phosphorylation (P). (b) Sumoylation of p53 at Lys-386 enhances Ser-392 phosphorylation by PKR but inhibits Lys-382 acetylation by p300/CBP.
CDK9, CK2, and PKR [29,30]. Although it remains unclear whether Ser-392 phosphorylation stimulates Lys-386 sumoylation, as shown for other PDSMs, this sumoylation is known to enhance Ser-392 phosphorylation by PKR (Figure 3b) [30]. In contrast, sumoylation of Lys-386 inhibits Lys-382 acetylation by p300/CBP, presumably due to sterically hindrance of the SUMO moiety preventing p300/CBP access to the adjacent lysine residue (Figure 3b) [31]. Additional examples of crosstalk among PTMs include sumoylation-dependent ubiquitination of PML (promyelocytic leukemia protein) [32,33]; sequential phosphorylation, sumoylation, and ubiquitination in governing the fate of FEN1 (flap structure-specific endonuclease 1) during cell cycle progression [34]; coordinated redox-dependent sumoylation and acetylation in regulating the activity of HIPK2 (homeodomain-interacting protein kinase 2) in response to oxidative stress [35]; and phosphorylation-dependent sumoylation of the phosphatase PTEN (phosphatase and tensin homolog) [36,37]. Clearly, PTMs occurring at the flanking regions of a SUMO-conjugation site dictate the efficiency of sumoylation and its functional outcomes.

**Impact of sumoylation on cancer and other diseases**

The basic helix-loop-helix leucine zipper (bHLH-LZ) transcription factor MITF (microphthalmia-associated transcription factor) plays an important role in melanocyte and osteoclast development [38]. Loss-of-function mutations have been linked to microphthalmia, a developmental disorder that causes small eyes [38]. MITF possesses two classical sumoylation motifs, Lys-182 and Lys-316, flanking its DNA-binding domain, with the latter being the major sumoylation site (Figure 4) [39,40]. Lys-316 is conserved in another family member, TFEB, which functions as a master regulator of lysosomal biogenesis [40]. Two recent studies have identified a recurrent heterozygous mutation in the MITF gene leading to the substitution E318K and predisposing fair-skinned individuals to familial and sporadic melanoma (Figure 4) [41,42]. This mutation is also associated with renal cell carcinoma [42]. Interestingly, the functional impact of this mutation is different from the total loss of MITF due to microphthalmia-causing mutations, which have no known links to cancer. As E318 is a crucial residue of the sumoylation motif (Figures 1 and 4), the E318K substitution inhibits MITF sumoylation and affects transcription of its target genes. In support of a causal role in oncogenesis, this mutant promotes migration, invasion, and clonogenicity of melanoma and renal cancer cells. In addition, two more recent studies identified the same mutation in melanoma patients from Italy and Queensland [43,44]. Thus, the loss of MITF sumoylation is positively linked to oncogenesis.

In contrast, sumoylation is required for Myc-dependent tumorigenesis. Both MITF and Myc are bHLH-LZ transcription factors. A genome-wide RNA interference (RNAi) screen identified the heteromeric SUMO-activating enzyme complex, SAE1/2 (SUMO-1-activating enzyme subunits 1 and 2), as a synthetic lethal gene product with Myc [45]. Inactivation of SAE2, expected to disable the sumoylation machinery, leads to mitotic catastrophe and cell death upon Myc hyperactivation. SAE2 inhibition activates a repressed transcriptional subprogram, although the underlying mechanism for this switch remains to be elucidated. Unlike MITF sumoylation, SUMO conjugation is required for Myc-dependent tumorigenesis. Therefore, sumoylation of two transcription factors with similar DNA-binding domains exerts completely opposite effects on cancer development. Along with the aforementioned roles of sumoylation in regulating the functions of the tumor suppressors p53, PTEN, and PML, it is clear that this modification is intimately linked to tumor initiation and progression.

![Figure 4. Deregulation of microphthalmia-associated transcription factor (MITF) sumoylation is associated with predisposition to melanoma and renal cell carcinoma](image-url)

MITF possesses two sumoylation sites, Lys-182 and Lys-316, flanking the DNA-binding domain, Lys-182 and Lys-316, with the latter being the major one. The E318K substitution impairs Lys-316 sumoylation and leads to increased risk of melanoma and renal carcinoma. aa, amino acids.
Sumoylation has been linked to human diseases other than cancer. First, impaired calcium uptake due to decreased expression and reduced activity of the calcium-transporting ATPase SERCA2a are hallmarks of heart failure [46]. SERCA2a is sumoylated at two classical sumoylation motifs and their modification correlates with beneficial health effects [46]. Second, infection by *Listeria monocytogenes*, a facultative intracellular pathogen responsible for human listeriosis, induces degradation of Ubc9, leading to subsequent decreases in the sumoylation of cellular proteins [47]. Enhanced activity of the sumoylation machinery is thus linked to reduced infection. Third, various viruses harness the sumoylation machinery to modulate virus-host interaction [48]. Finally, as discussed in the next section, sumoylation of the nuclear receptor PPARγ modulates adipocyte differentiation and the development of diabetes.

**PPARγ sumoylation in antidiabetic therapy**

PPARγ is a ligand-activated transcription factor highly expressed in white fat tissue. Like other nuclear receptors, PPARγ contains a DNA-binding domain and a ligand-binding domain (Figure 5) [49]. A major sumoylation site, Lys-107 of PPARγ2 (corresponding to Lys-77 in the γ1 isoform), is located at the N-terminal transcriptional activation domain, and another site, Lys-395, is situated in the ligand-binding domain [50]. Downstream from Lys-107 is an SP motif, which is subject to phosphorylation and is important for sumoylation at Lys-107 (Figure 5). Lys-107 and Ser-112 thus form a typical PDSM (Figure 1) [51], in which Lys-107 sumoylation inhibits PPARγ activity [51-53].

PPARγ regulates the expression of many target genes, including the one coding for fibroblast growth factor-21 (FGF21) [49]. To activate the signaling, the circulating hepatokine binds to a heteromeric tyrosine kinase complex composed of β-Klotho and an FGF receptor (FGFR). When administered to obese, insulin-resistant rodents and monkeys, FGF21 beneficially affects carbohydrate and lipid metabolisms by inducing energy expenditure, insulin sensitivity, and weight loss [49]. A recent study of FGF21-knockout mice reveals that this hepatokine inhibits PPARγ sumoylation at Lys-107 and triggers feed-forward regulation [54]. This modification regulates the size of adipocytes. The antidiabetic drug rosiglitazone, a PPARγ agonist, decreases sumoylation and enhances insulin sensitivity. Therefore, inhibition of PPARγ sumoylation exerts beneficial health effects. However, this inhibition also correlates with the side effects of PPARγ agonists in treating type 2 diabetes.

**Sumoylation in pluripotent and intestinal stem cells: insights from studies of Ubc9-null mice**

As mentioned in the Introduction section, the sumoylation pathway involves the sole E2-conjugating enzyme Ubc9, which provides a unique opportunity for genetic analysis of sumoylation functions in vivo. A complete disruption of the mouse Ubc9 gene leads to early embryonic lethality, even though the heterozygous mice are fertile and display no overt phenotypes [55]. Although removal of one allele does not affect sumoylation levels, inactivation of both alleles is expected to block sumoylation. *Ubc9*−/− blastocysts recovered at E3.5 appear normal under a light microscope, but the inner cell mass, which contains the endogenous pluripotent embryonic stem cells, fails to expand and displays apoptosis [55], indicating that Ubc9 is required for the survival of the pluripotent stem cell population during early development of mouse embryos. Moreover, nuclear

![Figure 5. Regulation of peroxisome proliferator-activated receptor γ (PPARγ) sumoylation by cellular signaling](image-url)

PPARγ possesses two sumoylation sites, Lys-107 and Lys-395 (corresponding, respectively, to Lys-77 and Lys-365 of the shorter γ1 isoform). While Lys-395 sumoylation is ligand-dependent in macrophages, Ser112 phosphorylation promotes sumoylation of Lys-107. In contrast, fibroblast growth factor-21 (FGF21) antagonizes Lys-107 sumoylation, although the underlying mechanism has not been defined.
organization and chromosomal segregation are abnormal in cells from E3.5 mutant blastocysts [55]. Consistent with this observation, a dominant-negative inhibition of Ubc9 expression in zebrafish leads to embryonic cell death [56].

In light of this early embryonic lethality, a LoxP-based conditional mouse line (Ubc9f/f−) was engineered to study tissue-specific and postnatal functions of mouse Ubc9 [57]. Crosses between this and the above Ubc9f/f− mice yielded a heterozygous line, Ubc9f/f−. Further mating with the ROSA26-CreERT2 strain, in which expression of the CreERT2 fusion protein is under the control of the ubiquitous ROSA26 promoter, enables a tamoxifen-inducible global deletion of the remaining copy of the Ubc9 gene [57]. Tamoxifen administration to 2- to 3-month-old mice led to severe diarrhea on the second day and subsequent death due to the intestinal failure within 6 days [57]. Analysis of different cells in the intestines revealed that Ubc9 is required in regions with active cell division, and deletion of the Ubc9 gene leads to rapid depletion of stem cells. Although this study indicates that Ubc9 is important for adult intestinal stem cells, the acute lethality prevents further analysis of its function in other tissues and organs. Further studies via tissue-specific deletion should help address this issue. Of relevance, antisense-based Ubc9 silencing in zebrafish reveals an important role for sumoylation in chondrocyte development, as well as in the specification and patterning of cranial neural crest and pharyngeal pouch cells [56].

In addition to playing an important role in embryonic stem cells and adult intestinal stem cells, sumoylation also regulates the activity of key transcription factors in induced pluripotent stem (iPS) cells. Ectopic expression of four reprogramming factors, Oct4 (also known as Pou5f1), Sox2 (SRY (sex determining region Y)-box 2), KLF4 (Krüppel-like factor 4), and c-Myc, leads to iPS cell formation [58]. Among these factors [58], Oct4, Sox2, and KLF4 are known to be sumoylated [59-62]. Sumoylation of these transcription factors negatively regulates reprogramming [63]. Similarly, sumoylation of the nuclear receptor NR5A inhibits reprogramming [64]. Moreover, ERRs (estrogen-related receptors) are subject to phosphorylation-dependent sumoylation and play a role in reprogramming [20,65]. The sumoylation inhibits their transcriptional activity [20] and is thus likely to inhibit reprogramming. The negative role of sumoylation in iPS cells is counterintuitive to the positive role of Ubc9 in embryonic stem cells and adult intestinal stem cells, suggesting that sumoylation of additional factors plays a positive role in the stem cells. Because of the enormous potential of stem cell therapy, further studies of Ubc9 and the sumoylation pathway in pluripotent and adult stem cells will provide novel insights for improved therapeutic strategies.

Conclusions
Since its discovery approximately 16 years ago [1,2], sumoylation has emerged as a major post-translational modification for the regulation of transcription factors and other eukaryotic proteins. An emerging trend is that this modification does not act alone but interplays actively with other modifications, such as phosphorylation, acetylation, and ubiquitination, to form integrated modification programs for coordinated regulation of various proteins in response to diverse signaling cues. Different from many other modifications, many sumoylation sites are frequently located within conserved consensus sequences, which greatly facilitate the prediction of uncharacterized sumoylation sites (Figure 1) [6]. Recent studies provide a biological link from the sumoylation machinery to different human diseases, including melanoma, renal carcinoma, heart failure and diabetes, and pathogenic infection by bacteria and viruses. This modification is of relevance to therapeutic actions, as some antidiabetic drugs act directly on PPARγ sumoylation (Figure 5) [49]. Sumoylation is also important in different stem cells [55,57,63] and is highly related to stem cell-based therapy. Therefore, by providing a regulatory switch in modulating protein functions, sumoylation has emerged as a promising target for the development of therapeutics against various human diseases.

Abbreviations
3D, three-dimensional; FGF, fibroblast growth factor; iPS, induced pluripotent stem; KLF4, Krüppel-like factor 4; MEF2, myocyte enhancer factor 2; MITF, microphthalmia-associated transcription factor; NDSM, negatively charged amino acid-dependent sumoylation motif; PDSM, phosphorylation-dependent sumoylation motif; PML, promyelocytic leukemia protein; PPARγ, peroxisome proliferator-activated receptor γ; PTEN, phosphatase and tensin homolog; PTM, post-translational modification; SAE, SUMO-1-activating enzyme subunit; SENP, Sentrin/SUMO-specific protease; SM, sumoylation motif; Sox2, SRY (sex determining region Y)-box 2; SUMO, small ubiquitin-like modifier.

Disclosures
The authors declare that they have no competing interests.

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