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Sumoylation and Human Disease Pathogenesis

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Abstract

Covalent modification by SUMO polypeptides, or sumoylation, is an important regulator of the functional properties of many proteins. Among these are a number of proteins implicated in human diseases, including cancer, Huntington's, Alzheimer's, and Parkinson's Diseases, as well as spinocerebellar ataxia 1 and amyotrophic lateral sclerosis. Recent reports reveal two new examples of human disease-associated proteins that are SUMO modified: amyloid precursor protein and lamin A. These findings point to a function for sumoylation in modulating A β peptide levels, suggesting a potential role in Alzheimer's Disease, and for decreased lamin A sumoylation as a causative factor in familial dilated cardiomyopathy.

Keywords

Sumoylation; SUMO; SUMO-1; cancer; DJ-1; tau; a-synuclein; SOD1; amyotrophic lateral sclerosis; ALS; Parkinson's; Alzheimer's; APP; lamin; laminopathy; heart

The sumoylation cycle

Sumoylation, the covalent attachment of Small Ubiqutin-like Modifier (SUMO) proteins to specific lysine residues in target proteins, regulates many aspects of normal protein function, including subcellular localization, protein partnering, and transcription factor transactivation [1–6]. Cells express three major SUMO paralogs, SUMO-1, SUMO-2, and SUMO-3, with SUMO-2 and SUMO-3 being much more similar to each other than to SUMO-1. A gene encoding SUMO-4 has been described, but it is not yet clear if endogenous SUMO-4 protein is expressed in cells [7]. Recently, a mechanism for the preferential modification of a protein by SUMO-2 and SUMO-3 vs. SUMO-1, which involves the interaction between the substrate protein and SUMO-2 or SUMO-3, was discovered. This finding provides a mode for the increased modification of substrates by UBC9 proteins carrying particular SUMO polypeptides [8].

Like ubiquitin, SUMO attachment to proteins involves a series of enzymatic steps (Figure 1). In the first step, the SUMO protein is cleaved by the SUMO-specific carboxyl-terminal hydrolase activity of a SENP (Sentrin-specific Protease) enzyme to produce a carboxyl-terminal diglycine motif. The mature SUMO protein is covalently attached via a thioester bond to a cysteine in the SAE2 (SUMO-Activating Enzyme subunit 2) subunit of the heterodimeric SUMO E1 activating enzyme in an ATP-dependent reaction [9–12]. The SUMO moiety is then transferred from the E1 to UBC9, the SUMO E2 enzyme, which then attaches the SUMO to a lysine in the target protein that is typically, but not always, found within the consensus

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sequence ΨKXE (Ψ represents hydrophobic amino acids) [13–16]. SUMO E3 proteins stimulate protein sumoylation by associating with both UBC9 and substrates to increase the efficiency of the modification reaction [4,6]. The SUMO E3 proteins identified to date include members of the PIAS (Protein Inhibitor of Activated STAT) family of proteins (PIAS1, PIAS3, PIASx, and PIASy), the polycomb protein Pc2 (Polycomb protein 2), and RANBP2 (Ran binding protein 2) [17–21].

SUMO groups are removed from proteins by the action of enzymes called SENPs (Sentrin-specific Proteases), of which there are 6 in human cells [1,6]. SENP1 and SENP2 are able to remove all 3 SUMO polypeptides from modified proteins, whereas the other SENPs appear to be selective for SUMO-2 and SUMO-3. SENP1 and SENP2 also function as the C-terminal hydrolases described above that catalyze the removal of short stretches of C-terminal residues from the SUMO proteins (Figure 1) that must occur before they can be utilized for conjugation to proteins.

Sumoylation and human disease states

As described above, SUMOylation is important for the normal functions of proteins in the cell. However, in the last several years a number of studies have suggested that sumoylation also plays a role in human disease pathogenesis. Indeed, proteins that play key roles in a number of human disease states, including huntingtin, ataxin-1, tau, α-synuclein, DJ-1 (also called PARK7 (Parkinson's disease 7)), and superoxide dismutase 1 (SOD1), are targets of SUMO modification. For further information on the sumoylation of proteins involved in neurodegenerative diseases, readers are referred to reference [22]. The goal of this review is to provide an overview of how sumoylation could be involved in pathogenesis of human diseases, including cancer, neurodegenerative diseases, and heart disease, culminating with recent results revealing that APP (amyloid precursor protein) and lamin A undergo sumoylation. These new results suggest that loss of APP and lamin A SUMO modification could lead to elevated Aβ levels and cardiomyopathies, respectively.

Sumoylation and cancer

Several lines of evidence point to a role for the SUMO modification pathway in tumorigenesis (reviewed in [23,24]). For example, increased UBC9 levels are found in a number of human cancers, and UBC9 overexpression can increase cancer cell growth [25,26]. The SUMO E3 protein PIAS3 (protein inhibitor of activated STAT3) is up-regulated in a number of different cancer types [27], and elevated levels of the SUMO E1 enzyme are associated with lower survival rates in patients with hepatocellular carcinoma [28]. In addition, sumoylation can regulate the activities of important tumor suppressor proteins, including p53, pRB (retinoblastoma protein), p63, p73, and Mdm2 (murine double minute 2) [23]. Other studies indicated that levels of the SUMO protease SENP1 are up-regulated in prostate and thyroid cancer, and that its overexpression promotes neoplasia development in the prostate [29,30]. These findings suggest that the relationship between cancer and sumoylation vs. desumoylation is likely to be complex.

Sumoylation of proteins involved in neurodegenerative diseases

A number of proteins that play important roles in neurodegenerative diseases are known to be sumoylated. These include proteins involved in Huntington's disease (huntingtin), spinocerebellar ataxia type 1 (ataxin-1), Parkinson's disease (tau, α-synuclein, DJ-1), amyotrophic lateral sclerosis (SOD1), and Alzheimer's disease (tau, APP).

Huntingtin

The neurodegenerative disorder Huntington's disease is caused by an increase in the size of the polyglutamine repeat located in the N-terminal region of the huntingtin protein, owing to an expansion of the trinucleotide CAG in the huntingtin gene (reviewed in [31,32]). Expansion of the huntingtin polyglutamine repeat leads to a number of neurological effects, including decreased control of motor functions and deficits in cognitive abilities. Sumoylation has been observed at lysines 6, 9, and 15 of an N-terminal fragment of the huntingtin protein with an expanded (mutant) polyglutamine tract [33]. Sumoylation of these lysine residues was suggested to be associated with increased stability and reduced aggregation of this mutant huntingtin, thereby possibly increasing the levels of toxic intermediate poly-Q oligomers, as well as with an increase in its ability to repress transcription [33]. However, these same lysines are also ubiquitylated, rendering it difficult to be certain that the effects observed in these lysine mutants, except perhaps the alteration in stability, stem from a lack of sumoylation at these sites as opposed to a lack of ubiquitylation. In a *Drosophila melanogaster* model of neurodegeneration, in which all neurons express the huntingtin fragment containing a polyglutamine expansion, *smt3* (SUMO) heterozygotes exhibit reduced neurodegeneration, suggesting that sumoylation of huntingtin promotes the neurodegenerative process [33]. However, it is difficult to rule out the possibility of indirect effects as reduced SMT3 levels would presumably also affect sumoylation of other cellular proteins.

Ataxin-1

Ataxin-1 is another polyglutamine-containing protein that can be sumoylated; its mutant polyglutamine expansion is the cause of spinocerebellar ataxia type 1 (SCA1), a disease that leads to progressive loss of motor control in patients. Ataxin-1 is sumoylated at 5 different lysine residues, lysines 16, 194, 610, 697, and 746 [34]. Sumoylation is decreased in mutant proteins containing the expanded polyglutamine repeat compared to those containing the wildtype polyglutamine repeats, but the mechanism responsible for this effect is not known. Evidence suggests that ataxin-1 phosphorylation negatively regulates its sumoylation, as SUMO modification is greater in the S776A phospho-site mutant. In addition, ataxin-1 sumoylation is significantly decreased in the nuclear localization sequence mutant, K772T, suggesting that the ability of ataxin-1 to enter the nucleus is important for its ability to be modified by SUMO, or that nuclear localization prevents its de-sumoylation, or both. This finding is consistent with previous studies demonstrating that a portion of the sumoylation machinery enzymes are localized to the nuclear pore complex [20,35,36]. Substitution of the sumoylation site lysines to non-sumoylatable arginines did not affect ataxin-1 nuclear localization or its ability to form inclusions. Thus the function of ataxin-1 sumoylation awaits discovery.

Tau

Tau is expressed at high levels in the brain and is associated with a number of neurodegenerative diseases including Parkinson's and Alzheimer's (reviewed in [37]). This protein is sumoylated, preferentially by SUMO-1, at lysine 340, [38]. An increase in tau ubiquitylation, following treatment with the proteasome inhibitor MG132, is associated with a significant decrease in tau sumoylation, suggesting that there could be competition between ubiquitylation and sumoylation for tau modification.

Treatment of cells with the phosphatase inhibitor okadaic acid also resulted in higher levels of tau sumoylation. This finding suggests that tau sumoylation could be regulated by phosphorylation, although it is also possible that indirect effects, involving phosphorylation of other proteins, could be responsible for this result. On a related note, SUMO-1 immunoreactivity co-localizes with phospho-tau in aggregates of neuritic plaques in *App* transgenic mice; however, again it is not clear whether this effect is indicative of phospho-tau

sumoylation or the modification of other proteins present in these aggregates [39]. Tau phosphorylation also negatively regulates its ability to interact with microtubules [40], and thus it is possible that the observed increase in tau sumoylation upon phosphatase inhibition indicates that the free soluble pool of tau is the primary sumoylation substrate. Consistent with this hypothesis, treatment with the microtubule depolymerizing drug colchicine is also associated with increased tau sumoylation [38].

α -synuclein

α -synuclein aggregation has been implicated in the pathogenesis of Parkinson's Disease (PD), and mutations in its gene are associated with PD, a condition that leads to difficulties in controlling motor function and speech, dementia, and mood disturbances (reviewed in [41]). This protein can be sumoylated and, like tau, it appears to be preferentially sumoylated by SUMO-1 compared to SUMO-2 or SUMO-3 [38]. Sumoylation occurs on lysine 102 of α -synuclein, but it appears that this is not the only site because changing this residue to arginine decreased, but did not eliminate, the modification. Unlike what was found for tau, α -synuclein sumoylation is not affected by treatment with the proteasome inhibitor MG132, suggesting that SUMO modification of these two proteins is differentially regulated [38]. The role of sumoylation in regulating α -synuclein function is currently unknown.

DJ-1

DJ-1 functions include acting as an anti-oxidant, transcriptional co-activator, and molecular chaperone. *PARK7 (DJ-1)* mutations account for 1–2% of early onset cases of Parkinson's Disease (reviewed in [41,42]). DJ-1 can be sumoylated at lysine 130, and preventing sumoylation at this site by converting this lysine to arginine is associated with a decrease in DJ-1-mediated Ras-dependent transforming and cell growth-promoting activities [43]. DJ-1 sumoylation increases upon UV irradiation and loss of this modification decreases the anti-apoptotic function of DJ-1, suggesting that DJ-1 sumoylation is important for its ability to protect cells from UV-induced apoptosis [43]. Members of the PIA family of proteins, which function as SUMO E3 proteins, have been shown to interact with DJ-1 and stimulate its sumoylation [43,44].

The results from additional studies suggest that DJ-1 can also regulate the sumoylation of other proteins, thereby modulating their activities [45,46]. In particular, DJ-1 appears to inhibit the sumoylation of PSF (pyrimidine tract-binding protein-associated splicing factor), a transcriptional co-repressor; this inhibition has been suggested to be involved in the DJ-1 mediated transcriptional up-regulation of the tyrosine hydroxylase and MnSOD (Manganese superoxide dismutase, *SOD2*) genes. Thus, the role of SUMO modification with respect to DJ-1 activity could be complex, involving both sumoylation of DJ-1 itself as well as DJ-1 mediated regulation of the SUMO modification of other factors.

SOD1

Amyotrophic Lateral Sclerosis, or ALS, is a disease that affects motor neurons in the brain and spinal cord, resulting in symptoms of muscle atrophy and paralysis (reviewed in [47,48]). A large number of mutations have been identified in the copper-zinc superoxide dismutase 1 (*SOD1*) gene which are associated with at least 20% of the cases of familial ALS. The aggregation of mutant SOD1 is believed to be a primary factor in causing disease in these cases [47–49].

A number of proteomic studies have indicated that the yeast Sod1 protein is sumoylated on multiple lysine residues, under both non-stress and stress conditions [50–53]. Human SOD1 is also sumoylated, but only at one site, lysine 75, and this modification is observed for both wildtype and ALS-associated mutant forms of SOD1 [54]. In addition, SUMO-1 co-localizes

with SOD1 in inclusion bodies. The substitution of lysine 75 to a non-sumoylatable arginine is associated with increased SOD1 protein levels and aggregation, suggesting that SOD1 sumoylation could negatively regulate its ability to aggregate, and therefore its contribution to the pathogenesis of ALS [54].

APP

Alzheimer's disease is a debilitating disease that impairs cognitive function; it is the most common aging-related human neurodegenerative disease [55–57]. It is widely believed that amyloid- β (A β) protein, produced by APP processing via the amyloidogenic proteolytic pathway, is a likely causative factor in this disease.

APP was identified as a sumoylation target by an *in vitro* expression cloning strategy, in which candidate sumoylation substrate proteins were identified by assaying successive subdivisions of cDNA pools with *in vitro* sumoylation reactions [58]. We recently showed that lysines 587 and 595 of the APP protein, which are immediately N-terminal to the β -secretase cleavage site, are sites of covalent modification by both SUMO-1 and SUMO-2 (Figure 2A) [59]. Both of these lysine residues are surrounded by matches to the sumoylation consensus sequence Ψ KXE/D. The loss of lysine 587 and/or 595 sumoylation increases A β protein aggregation, suggesting that this modification inhibits A β formation (Figure 2B). Further, up-regulating cellular levels of the SUMO E2 enzyme UBC9 resulted in elevated APP sumoylation and decreased A β protein aggregate levels. These results suggest that interventions that alter APP sumoylation could represent a potential new approach for combating Alzheimer's disease. Interestingly, one of the identified sumoylated APP residues, lysine 595, is substituted to asparagine in the previously characterized Swedish (KM-to-NL) APP mutant [60]. Thus, an inability to be sumoylated at lysine 595 could contribute to the increased A β production observed in the Swedish APP mutant.

Two previous studies indicated that SUMO-3 overexpression affects A β levels [61,62]. However, these studies did not examine whether APP was sumoylated; moreover, the meaning of their results is not clear as the two studies observed opposite effects of SUMO protein overexpression on A β levels. Regardless, it appears that these effects of SUMO overexpression on A β are likely mediated by a mechanism different from that of direct APP sumoylation, because the effect was observed following the overexpression of SUMO proteins incapable of covalent substrate attachment [62].

Before these findings, sumoylation of proteins that pass through the endoplasmic reticulum (ER) or other compartments of the secretory pathway had not been reported. However, the identification of SUMO modification of APP suggested that sumoylation could occur in one or more of these membrane-bound compartments. In support of this hypothesis, immunofluorescence analysis revealed co-localization between Calnexin and a portion of the cellular UBC9, suggesting that the SUMO E2 enzyme co-localizes with the ER [59]. This UBC9 staining pattern is consistent with the results of previous studies which showed that in addition to a nuclear-localized population, some UBC9 staining is found outside the nucleus in a pattern reminiscent of ER localization [15,63,64]. This data not only provides an explanation for how lysines 587 and 595 of APP can be sumoylated, but also suggests the possibility that other proteins that enter the ER could also be targets of sumoylation. The mechanism by which UBC9 associates with the ER is unclear: UBC9 does not have an obvious signal sequence, but the prediction program SecretomeP [65] identifies it as a candidate non-classical secretory pathway protein. Thus, it is possible that UBC9 enters a membrane-bound compartment in the cell which then ultimately merges with the ER. The presence of UBC9 within the ER suggests the possibility that sumoylation could play a role in other diseases associated with this cellular compartment.

Sumoylation and heart disease

The lamin A protein plays an important role in nuclear structure and function, and mutations in the lamin A gene cause a large number of different human diseases, including cardiomyopathies, muscular dystrophies, and Hutchinson-Gilford Progeria Syndrome [66–70]. An interaction between lamin A and UBC9, the SUMO E2 enzyme, was detected using a yeast two-hybrid screen [71], thus suggesting that lamin A could be a target of SUMO modification. Analysis of the lamin A amino acid sequence revealed a match to the sumoylation consensus sequenceΨKXE (MKEE) surrounding lysine 201 in the rod-containing domain, suggesting that sumoylation could occur at this site (Figure 3A) [72]. Subsequent experiments found that lamin A is indeed sumoylated at lysine 201 and that it is more efficiently modified by SUMO-2 than by SUMO-1 [72]. Lysine 201 sumoylation appears to be important for the normal subcellular localization of lamin A, as a non-sumoylatable mutant (K201R) exhibits an altered sub-cellular localization pattern of concentrating into foci, in contrast to the relatively continuous nuclear peripheral localization exhibited by the wildtype lamin A [72].

Two different substitutions of lamin A glutamic acid residue 203 have been identified, E203G and E203K, which are associated with familial dilated cardiomyopathy and conduction system disease [73,74]. Intriguingly, this glutamic acid is only two residues C-terminal to the lamin A lysine 201 sumoylation site (Figure 3B) [72], and in fact occupies the conserved glutamic acid position of the sumoylation consensus sequenceΨKXE [13–16]. This conserved glutamic acid residue is known to be important for the efficiency of SUMO addition to the lysine in the consensus sequence [15,16]. Consistent with this, the E203G and E203K mutant lamin A proteins both exhibit a significant decrease in sumoylation, compared to wild type, as does lamin A protein in skin fibroblasts from a patient harboring the E203K lamin A substitution [72]. Further, the E203G and E203K lamin A mutant proteins exhibit an altered pattern of lamin A subcellular localization very similar to that of the lamin A K201R SUMO attachment site mutant, and the E203K lamin A patient fibroblast cells exhibit a significant increase in the percentage of cells showing abnormal lamin A localization/nuclear morphology. These results suggest that a defect in lamin A sumoylation could play an important role in the underlying molecular mechanism of the familial cardiomyopathies associated with the E203G and E203K lamin A substitutions. These substitutions appear to provide the first examples of human disease-causing alterations that occur in a crucial residue of a sumoylation consensus sequence and that result in decreased sumoylation of the mutant protein.

Concluding Remarks and Future Perspectives

The results of the studies described above indicate that sumoylation is not only an important regulator of the normal function of many vital cellular proteins, but also that this post-translational modification also plays a role in the pathogenesis of at least some human disease states. These findings suggest that interventions, pharmacological or otherwise, that modulate protein sumoylation could represent potential therapeutic approaches for treating the diseases discussed herein in addition to other conditions yet to be discovered in which sumoylation could play a role. The E3 proteins responsible for regulating the sumoylation efficiency of disease-related proteins, such as those discussed in this review, could be potentially desirable targets of such interventions because they would offer greater selectivity than globally altering sumoylation through targeting the SUMO E2 enzyme (UBC9). Future studies are also warranted in determining the mechanisms by which aberrant sumoylation leads to disease states, because the results of these studies would not only increase the understanding of human disease pathogenesis, but also of the role that sumoylation plays in the normal functions of these proteins. Finally, it is likely that new examples of SUMO-modification of disease-associated proteins will also be discovered and characterized in future studies, and that the

information these studies provide will further increase our understanding of the role of sumoylation in human disease pathogenesis.

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Figure 1.

SUMO modification cycle. Before attachment to proteins, SUMO proteins [pink] must be cleaved into their mature forms by SENPs (Sentrin-specific Proteases; [purple]), which remove 4, 11, and 2 amino acids from the C-terminal ends of SUMO-1, SUMO-2, and SUMO-3, respectively. The processed SUMO proteins are then activated by conjugation to the E1 heterodimer SAE1–SAE2 [light blue], after which the SUMO is transferred to the E2 enzyme Ubc9 [gold]. Finally, SUMO is ligated to substrate proteins by an isopeptide bond between the terminal glycine on SUMO and the ϵ -amino group of a lysine in the substrate [green]. The efficiency of the ligation reaction is aided by SUMO ligase E3 proteins (E3; [orange]) which directly interact with both target proteins and the E2 enzyme, thereby acting as bridging factors to increase the efficiency of this reaction. SUMO polypeptides are removed from target proteins by the action of SENPs, which recovers the SUMO proteins for attachment to other proteins in another cycle of the sumoylation pathway.

Figure 2.

Location and function of APP sumoylation. APP SUMO modification occurs at two lysines immediately adjacent to the site of β -secretase cleavage, and is associated with decreased levels of A β aggregates. (A) This schematic shows the locations of the sumoylated lysine 587 and 595 residues in APP, their matches to the sumoylation site consensus sequence (Ψ KXE/D), and proximity to β -secretase cleavage site and the A β peptide generated from APP processing. (B) Regulation of A β aggregate levels by APP sumoylation. Sumoylation of APP near the site of β -secretase cleavage is associated with a decrease in the amounts of A β aggregates.

Figure 3.

Lamin A sumoylation and familial dilated cardiomyopathy. Lamin A is sumoylated at lysine 201 in the rod domain, and mutations associated with familial dilated cardiomyopathy disrupt the lamin A SUMO consensus sequence. (A) Schematic showing the location of a match (MKEE) to the sumoylation site consensus sequence (Ψ KXE) surrounding lysine 201 in the lamin A rod-containing domain. (B) The E203G and E203K substitutions in lamin A associated with familial dilated cardiomyopathy correspond to the conserved glutamic acid residue of the sumoylation site consensus sequence (Ψ KXE) surrounding lysine 201. This glutamic acid position is important for sumoylation at the preceding lysine residue in the consensus sequence.