Sumoylation and Regulation of Cardiac Gene Expression

Jun Wang, Robert J. Schwartz

Abstract: Sumoylation is a posttranslational modification process in which SUMO proteins are covalently and reversibly conjugated to their targets via enzymatic cascade reactions. Since the discovery of SUMO-1 in 1996, the SUMO pathway has garnered increased attention due to its role in a number of important biological activities such as cell cycle progression, epigenetic modulation, signal transduction, and DNA replication/repair, as well as its potential implication in human pathogenesis such as in cancer development and metastasis, neurodegenerative disorders and craniofacial defects. The role of the SUMO pathway in regulating cardiogenic gene activity, development and/or disorders is just emerging. Our review is based on recent advances that highlight the regulation of cardiac gene activity in cardiac development and disease by the SUMO conjugation pathway. (Circ Res. 2010;107:19-29.)

Key Words: sumoylation ■ cardiac gene expression ■ cardiac development ■ transcription factor ■ review

SUMO proteins Small ubiquitin-like modifier (SUMO) proteins constitute a special group of ubiquitin-like proteins (ULPs). SUMO modification, or sumoylation, is a process in which SUMO proteins are covalently attached to targets via a series of enzymatic reactions, as presented schematically in the Figure. So far, 3 SUMO proteins that are conjugated to target proteins, which are known as SUMO-1, -2, and -3, have been identified in vertebrates, whereas SUMO-4 is believed to participate only in protein-protein interactions but not in covalent conjugation. SUMO-2 and SUMO-3 are nearly identical (≈97% identity) but share only ≈50% sequence similarity with SUMO-1. SUMO-4 is closer to SUMO-2 than to SUMO-3. Under physiological conditions, SUMO-1 appears to be the favored isoform among the SUMO family members. SUMO-1 conjugation is also significantly increased under pathological conditions, such as in hypoxic hearts. SUMO isoforms may have overlapping targets but exhibit preferred substrate specificities. For instance, RanGAP1 and Nkx2.5 are favored by SUMO-1 but not by SUMO-2/3, whereas nuclear actin is preferred by...
SUMO-2/3,7 suggesting that SUMO proteins may play redundant as well as distinct roles in cellular activities.

**Enzymatic Sumoylation Cascade**

The enzymatic cascade of reactions that accomplish SUMO conjugation involves a multitude of enzymes.Sentrin-specific proteases (SENP, see below) convert SUMO proteins to the active form by cleaving the carboxyl-terminal tails of SUMO precursors via hydrolase activity, thereby exposing a diglycine motif essential for covalent conjugation. However, the presence of proline residue 90 in SUMO-4 impedes this cleavage, disabling SUMO-4 for conjugation.2 Heterodimeric activating enzyme E1 (SAE1/SAE2, heterodimer) SUMO E2 (Ubc9), the only E2 identified so far in vertebrates, delivers a conserved catalytic cysteine in the conjugation enzyme E2. Ubc9, the only E2 identified so far in vertebrates, delivers SUMO protein directly to the substrates. In vitro sumoylation studies reveal that Ubc9 alone is sufficient for conjugation and ligation of the SUMO moiety to the substrates.5,9 However, the presence of sumoylation E3 ligases, including the PIAS family,10 RanBP2,11 polycomb 2,12 TOPORS,13,14 TRAF7,15 and mitochondrial-anchored protein ligase (MPAL),3 stimulates the conjugation efficiency by promoting poly-SUMO chain formation and/or introducing additional SUMO acceptor site(s) (Figure). Among these E3 ligases, the PIAS family is the largest SUMO E3 group and contains a large repertoire of SUMO targets. The PIAS family consists of 5 isoforms (PIASxα, PIASxβ, PIAS1, PIAS3, and PIASy).10,16 Although sumoylation, enhanced by PIAS proteins, links SUMO E3 activity to the RING domain, PIASy may also promote SUMO linkage to YY1 independent of its RING finger.17 Interestingly, TOPORS also possesses ubiquitination E3 ligase activity attributable to its RING finger, but its SUMO E3 ligase function is RING domain independent.11 MPAL is a mitochondria-localized SUMO E3 ligase with enzymatic activity attributable to its C-terminal RING finger, and it also harbors ubiquitination E3 ligase activity.3 TRAF7 also contains the RING domain with ubiquitin ligase activity for self-ubiquitination.18 However, TRAF7 promotes sumoylation of c-Myb but not ubiquitination,15 thus pointing to the presence of distinct RING domains specified for ubiquitination versus sumoylation. Among these SUMO E3 ligases, PIAS proteins have been under active investigation and show specificity for both SUMO isoforms and substrates. For instance, PIAS1 and xα, but not PIASy and xβ, catalyze SUMO modification of the androgen receptor.19 Only PIASxα and xβ stimulate SUMO-2 conjugation to Nkx2.5, a cardiac-enriched homeodomain transcription factor.6 These findings suggest that the SUMO E3 ligases may contribute to the substrate specificity of SUMO isoforms and regulate the intensity of the sumoylation reaction.

**Desumoylation**

SUMO conjugation is also a reversible process in which the isopeptide linkage between the SUMO and substrate is cleaved by a family of isopeptidases known as SENPs. So far, 6 members of the SENP family that show evidence of desumoylation activity have been identified in humans (SENP1, SENP2, SENP3, SENP5, SENP6, SENP7); among them, SENP1, SENP2, and SENP5 also possess hydrolase activity.20–22 However, the isopeptidase capacity of SENP2 and SENP5 overpowers their endopeptidase activity,22 suggesting that they may serve as sumoylation repressors in vivo. SENPs display some specificity for SUMO family members.23,24 For instance, SENP1 and SENP2 generally target all SUMO isoforms for deconjugation,20 whereas SENP3, 5, 6

<table>
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<th>Non-standard Abbreviations and Acronyms</th>
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<td>ASDs</td>
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<td>CHDs</td>
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<td>VSDs</td>
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<td>YY1</td>
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![Figure. A model of the SUMO conjugation/deconjugation pathway and its associated cellular activities. SUMO proteins can be covalently conjugated to targets in the presence of SUMO E1 (SAE1/SAE2, heterodimer), SUMO E2 (Ubc9). The addition of SUMO E3 (PIAS proteins as an example) promotes the formation of poly-SUMO chains/activates additional SUMO site(s). SUMO proteins are released by SENPs from their conjugated state with the associated target and are thereby ready for a new round of conjugation (deconjugation). Regulation of cellular activities: The dynamic sumoylation process is implicated in a number of cellular activities, including cell cycle, DNA repair, transcription, and chromatin remodeling. The factors shown under each category of cellular activity are SUMO substrates and described in this review.](https://circres.ahajournals.org/doi/figure-pdf/10.1161/CIRCRESAHA.110.230487)
Sumoylation and Ubiquitination

The sumoylation and ubiquitination process/mechanism share similarities in terms of the 3-dimensional structures of SUMO and ubiquitin proteins, the requirement of cleavage of precursor into a mature form before conjugation, and the requirement of enzymes for covalent linkage. Nonetheless, other aspects of the sumoylation and ubiquitination pathways are easily differentiated. For instance, the E1 and E2 involved in sumoylation are unique; so far, there is no evidence that these enzymes participate in the ubiquitination pathway. Unlike the well-documented proteasome-mediated degradation of targeted proteins after poly-ubiquitination, the functional consequences of sumoylation is substrate-dependent; the functional performance of substrates is typically regulated by altering 1 or more of the following activities: nuclear-cytoplasmic trafficking, DNA binding, protein turnover, protein-protein interaction, or by interplay between sumoylation and other posttranslational interactions such as acetylation and phosphorylation. Although a recent study suggests that SUMO modification serves as a signal for hypoxia-inducible factor α (HIFα) degradation, in certain cases, sumoylation does not exert any discernable effects on the activity of the targets. In addition, the consensus SUMO targeting sequence has been identified. The most frequent lysine residue subject to sumoylation is localized in the SUMO-recognizable canonical sequence ψKXE, where ψ stands for a large hydrophobic amino acid and X represents any residue, although the lysine residue harbored in ψKXG/D or in other nonconsensus sequences may also be SUMO-targeted. So far, no such equivalent sequence has been identified in ubiquitination, although some ubiquitination ligases such as SCFWDU6-TCI recognize particular consensus sequence DSGδXS motifs for protein binding.

Sumoylation and Biological Significance

SUMO targets a variety of substrates that have been implicated in a number of cellular functions including cell cycle, DNA repair, transcription, and epigenetic regulation.

Cell Cycle

Cell proliferation is an indispensable process for cell propagation and organogenesis, and accumulating evidence implicates sumoylation in the control of mitotic chromosome structure, cell cycle progression, kinetochore function and cytokinesis. RanGAP1 and DNA Topoisomerase II (Topo II), which are centrally important for cell division, are SUMO substrates. SUMO modification of Topo II, enhanced by E3 RanBP2 during mitosis, is required for proper localization to centromeres, which is essential for normal cell division, and RanBP2-potentiated sumoylation of RanGAP1 is a prerequisite for k-fiber assembly in mitosis and when suppressed, causes missegregation of chromosomes. In addition, a number of SUMO pathway components are directly involved in regulating mitosis. For instance, PIAS E3 ligases such as PIAS-3 and -γ are required for faithful chromosome segregation via sumoylation. Knockdown of SENP5 by RNAi impairs cell proliferation. Under pathophysiological conditions such as oxidative stress, SENP3 levels increase, resulting in deconjugation of SUMO-2/3 from PML, and ultimately, leading to increased cell proliferation. These studies demonstrate the critical involvement of SUMO pathway in vertebrate cell proliferation.

DNA Repair

DNA repair in response to DNA damage is an essential means to maintain genomic integrity. Nucleotide excision repair (NER) and homologous recombination are two major DNA repair processes. Two key factors involved in the NER process, XPC and Centrin-2, are SUMO substrates. Sumoylation of XPC occurs after UV exposure, and prevents XPC degradation. Sumoylation of centrin-2 is preferred by SUMO-2/3 and is promoted by polycomb 2 (PC2). Knockdown of the SUMO pathway alters the subcellular localization and subsequent binding of centrin-2 to its partners, thus influencing its activity. Sumoylation of human Rad52, a protein central to homologous recombination, has no discernable impacts on DNA binding, D-loop formation, but affects the cytoplasmic and nuclear shuttling of Rad52. Thus, SUMO conjugation initiates the DNA repair pathway to maintain genomic integrity.

Transcriptional Regulation

A large subset of SUMO targets, such as transcription factors (TFs), coactivators and corepressors, are implicated in the regulation of gene expression. For instance, the repressive function of the corepressor N-CoR is enhanced by SUMO conjugation to K152, K1117 and K1330, whereas P300-dependent transcriptional activation is suppressed by sumoylation. Also, the activities of many TFs are governed by sumoylation, most of which are suppressed by SUMO conjugation. Still, SUMO modification exerts a positive impact on the activity of a number of transcription factors. For instance, maximal activity of the ETS domain transcription factor PEA3 requires sumoylation on mainly three lysine residues, K96/222/256, which is critical for PEA3 recycling via ubiquitination. This sumoylation-dependent activation also occurs for multiple cardiac transcription factors (see below).

Chromatin Remodeling

Epigenetic regulation is essential for gene activation/silencing. A number of chromatin remodeling factors have been identified as substrates of SUMO conjugation. HDAC1 is sumoylated on K444 and K476, mutations of which decrease transcriptional repression mediated by HDAC1. Sumoylation of DNA methyltransferase 3a (Dnmt3a) impairs its physical association with HDAC1/2, resulting in suppression of its repressive function. In addition, some SUMO pathway components such as SENP1 and Ubc9 themselves are part of chromatin remodeling complexes like reptin- and pontin-
containing complexes. Ezh2, a Histone 3 methyltransferase, is also SUMO modified, although the functional consequence and/or physiological relevance of Ezh2 sumoylation remains unknown. Pc2, a critical component of polycomb repressive complex 1 (PRC1) that recognizes H3K27Me3, is a SUMO E3 ligase and, by itself, is also a target for sumoylation. Thus, the SUMO pathway governs transcription activation/silencing via modulating the activity of chromatin remodeling factors/complexes.

It is noteworthy that most SUMO pathway components such as SUMO proteins themselves, Ubc9 and E3 ligases may also exhibit function(s) independent of covalent conjugation capacity or enzymatic activity. Recently, a number of SUMO interacting motifs that mediate protein binding to SUMO have been identified. In certain cases, noncovalent interaction of a substrate with SUMO, mediated by the SUMO interacting motif, modulates the efficiency of covalent linkage of SUMO to that target.

**SUMO Targets Factors Critical for Normal Cardiac Development/Function**

The SUMO pathway has roles in a number of important biological activities such as cell cycle progression, epigenetic modulation, signal transduction, and DNA replication/repair, as well as its potential implication in human pathogenesis such as cancer development and metastasis, neurodegenerative disorders and craniofacial defects. In addition, the SUMO conjugation pathway has a central role in regulation of cardiac gene activity and heart development. It is well known that expression of cardiac specific genes in the heart is governed by a group of transcription factors whose function is integrated with a variety of signal transduction pathways and/or cofactors. Thus, to understand the regulation of cardiac gene activity by the SUMO conjugation pathway, it is important to first determine whether SUMO targets transcription factors that are critical for differentiation of cardiomyocytes and/or normal heart development. Indeed, our laboratory and others have identified a number of SUMO targeted transcription factors that contribute significantly to the modulation of cardiac gene activity and normal cardiogenesis, as summarized in the Table.

Serum Response Factor (SRF) is a highly conserved transcription factor containing the MADS box, which recognizes the sequence motif CArG box in the *cis*-regulatory region of target genes. SRF is absolutely required for mesoderm formation and plays the earliest role in the emergence of cardiac sarcomere formation. The conditional SRF knockout (*Srf<sup>−/−</sup>* in the cardiac progenitors blocks the appearance of rhythmic beating of myocytes, one of the earliest cardiac defects caused by the ablation of a cardiac-enriched transcription factor. The ability of SRF to be the universal “myogenic driver” is totally abrogated in the *Srf<sup>−/−</sup>* cells and supports the concept that SRF resides at a high point in the regulatory hierarchy governing sarcomerosogenesis from worms to mammals. Like many other transcription factors, the transcriptional activity of SRF is regulated by protein-protein interactions with other factors such as GATA4, Nkx2.5, and myocardin and by posttranscriptional modification. For example, phosphorylation on serine 162 in the MADS box plays an important role in switching SRF function to direct expression of genes associated with proliferation or differentiation. Similar to SRF, Myocardin is sufficient to induce cardiac gene expression in Xenopus, it alone does not possess the ability to activate cardiac genes in pluripotent 10T1/2 fibroblast cells. Like SRF, myocardin is also a SUMO target on the principal sumoylation site, K445. Wild type SUMO-1, but not the conjugation-deficient SUMO-1 ΔGG mutant, greatly

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**Table. Major SUMO-Targeted Transcription Factors Critical for Normal Cardiac Development**

<table>
<thead>
<tr>
<th>Transcription Factors</th>
<th>Primary SUMO Acceptor Sites</th>
<th>Effects on Cardiac Gene Activity</th>
<th>After Sumoylation</th>
<th>References</th>
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<tbody>
<tr>
<td>SRF</td>
<td>K&lt;sub&gt;147&lt;/sub&gt;ME</td>
<td>Activation</td>
<td>74, 81</td>
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<tr>
<td>Myocardin</td>
<td>K&lt;sub&gt;640&lt;/sub&gt;OE</td>
<td>Activation</td>
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<tr>
<td>GATA4</td>
<td>K&lt;sub&gt;151&lt;/sub&gt; TE</td>
<td>Activation</td>
<td>9, 87</td>
<td></td>
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<tr>
<td>Nkx2.5</td>
<td>F&lt;sub&gt;51&lt;/sub&gt;P</td>
<td>Activation</td>
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<tr>
<td>Mef2</td>
<td>K&lt;sub&gt;190&lt;/sub&gt;SE</td>
<td>*</td>
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<tr>
<td>YY1</td>
<td>K&lt;sub&gt;590&lt;/sub&gt;E</td>
<td>*</td>
<td>17</td>
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<tr>
<td>Prox1</td>
<td>K&lt;sub&gt;560&lt;/sub&gt;SE</td>
<td>*</td>
<td>34, 112</td>
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*Undetermined.*
potentiates capability of myocardin to induce transcripts of cardiac specified genes such as cardiac α-actin and cardiac α-myosin heavy chain (MHC) in 10T1/2 fibroblast cells without significantly affecting its ability to direct smooth muscle differentiation.\textsuperscript{81} The myocardin mutant K445R fails to activate these genes in the presence of SUMO-1.\textsuperscript{81} These observations suggest the importance of sumoylation in switching the role of myocardin from a smooth muscle gene enhancer to a cardiogenic gene potentiator. Also, the direct physical interaction between myocardin and E3 ligase PIA1 plays a role in triggering cardiac gene expression because K445R together with PIA1 is still capable of activating cardiac α-actin.\textsuperscript{81} The exact mechanisms underlying the functional enhancement of myocardin by SUMO modification remains enigmatic because SUMO modification does not significantly influence myocardin subcellular localization and its physical association with SRF. It is noteworthy that the presence of either Ubc9 or PIA1 also activates a nontypical sumoylation site on myocardin.\textsuperscript{81}

GATA4, a zinc finger-containing transcription factor, has been extensively studied with regards to its role in cardiomyocyte differentiation and in cardiogenesis. GATA4 regulates a number of cardiac gene activities such as α-MHC\textsuperscript{82} and ANF\textsuperscript{83} via binding directly to the sequence motif (A/T)GATA(A/G) present in the regulatory region of the target gene. Like SRF, the transcriptional activity of GATA4 is modulated by protein-protein interaction and posttranslational modification. For instance, phosphorylation by protein kinase C (PKC) on serine 419 and/or serine 420 of GATA4 elevates its transcriptional activity via enhanced DNA binding.\textsuperscript{84} Among the GATA family members, GATA1, GATA2, and GATA4 were reported to be SUMO substrates.\textsuperscript{9,85–87} GATA4 is modified by SUMO-1 on the primary targeting site, K366, in its transactivation domain.\textsuperscript{9,87} Conversion of this lysine to arginine (K366R) blocks sumoylation of GATA4 and alters its nuclear localization.\textsuperscript{9} Remarkably, SUMO modification of GATA4 triggers the expression of cardiac specific genes such as cardiac α-MHC and ANF in pluripotent fibroblast 10T1/2 cells,\textsuperscript{9} indicating that the SUMO pathway substantially potentiates GATA4 transcriptional activity. This is further corroborated by the observation that GATA4 function is strengthened by Ubc9, the E2 in the SUMO conjugation pathway.\textsuperscript{88} The fact that K366R mutant completely blocks sumoylation catalyzed by PIA1\textsuperscript{9} suggests that E3 ligase PIA1 promotes SUMO attachment to the primary sumoylation lysine residue, but does not introduce an additional SUMO acceptor site. Approximately 20% of endogenous GATA4 extracted from cultured cardiomyocytes is SUMO-1 conjugated,\textsuperscript{9} pointing to the physiological relevance of GATA4 sumoylation.

Nkx2.5, the cardiac specific homeobox gene, is a member of the nk-2 class of homeodomain (HD)-harboring factors recognizing the NKE targeting sequence, 5'-TNNAGTG-3', required for normal cardiac development.\textsuperscript{89} Nkx2.5 genes are evolutionarily conserved across vertebrate species and are expressed in early cardiac progenitor cells before cardiogenic differentiation, with expression continued into adulthood in mice.\textsuperscript{90,91} Like its cofactors SRF and GATA4, posttranscriptional modification, such as phosphorylation on serine 163 is one of the mechanisms augmenting the transcriptional activity of Nkx2.5 via increased DNA binding.\textsuperscript{92} Nkx2.5 is also targeted by SUMO on the primary site, K51, which is conserved across different species, but not present in other NK-2 class members examined.\textsuperscript{6} Indeed, mutation of K51 to arginine (K51R) reduces its activity via altered protein-protein interaction and diminished DNA binding.\textsuperscript{6} Also, K51R is poly-ubiquitinated, suggesting that the natural presence of K51 may prevent Nkx2.5 from being a ubiquitin target, although this poly-ubiquitination does not cause degradation of Nkx2.5; thus, its physiological relevance remains unclear. As expected, SUMO-1 is the most potent of the three SUMO isoforms to modify Nkx2.5. While all other PIAS isoforms except PIAS3 facilitate SUMO-1 conjugation to Nkx2.5, PIASx (-xα and -xβ), but not by the other PIAS proteins, promotes covalent attachment of SUMO-2 to Nkx2.5.\textsuperscript{6} Although SUMO attachment elevates Nkx2.5 transcriptional activity, unlike sumoylation of GATA4 and myocardin, the combination of Nkx2.5 with SUMO pathway components such as SUMO-1 and PIAS-1 is not sufficient to trigger the expression of cardiogenic gene in fibroblast cells.\textsuperscript{6} SUMO modification enhances the physical association of Nkx2.5 with its binding partners,\textsuperscript{6} which likely contributes to cofactor interactions, thereby elevating Nkx2.5 function.

Myocyte Enhancer Factor-2 (MEF2) family proteins are MADS box-containing transcription factors that act as homodimers or heterodimers, binding to the consensus sequence YTA(A/T)\textsubscript{4}TAR,\textsuperscript{93} and governing muscle development and differentiation.\textsuperscript{94} For instance, MEF2B promotes myogenic lineage commitment and is involved in regulation of activity of the smooth muscle myosin heavy chain gene.\textsuperscript{95,96} The functions of MEF2 proteins rely on physical association with cofactors and on posttranslational modification. Acetylation of lysine 4 in the MADS box of MEF2 C by p300 promotes its DNA binding and mediates MEF2C-dependent gene activation programs such as skeletal muscle differentiation.\textsuperscript{97} MEF2 proteins have also been identified as SUMO substrates.\textsuperscript{98,99} The primary SUMO moiety acceptor site is localized in the consensus sequence IKSE found in the transactivation domain of MEF2 proteins and is conserved among various species, as well as among all 4 isoforms of the MEF2 family. Intriguingly, some HDAC proteins such as HDAC4, 5, 7, and 9 stimulate sumoylation of MEF2 via unknown mechanisms.\textsuperscript{99} When tested using an artificial promoter fused to a luciferase reporter, SUMO modification suppresses the activity of MEF2A, C, and D.\textsuperscript{98,99} Although the MEF2 activity may regulate a multitude of cardiogenic genes, it remains to be determined how sumoylation of MEF2 proteins affects cardiac target gene expression during heart development. No functional consequence of sumoylation on MEF2B has yet to be reported.

Yin Yang 1 (YY1) is a ubiquitously expressed and evolutionarily conserved transcription factor that recognizes the consensus sequence NNN(C/A)CATNTNNN with embedded core sequences, ACAT and CCAT.\textsuperscript{100,101} YY1 appears to be a component of a polycomb chromatin remodeling complex that mainly mediates transcriptional repression.\textsuperscript{102} YY1 can be a transcriptional activator or repressor, depending on the target gene. YY1 is more highly expressed during heart development than its homolog YY2\textsuperscript{103} and YY1 suppresses the target gene cardiac, α-actin, by competition with Nkx2.5.
SUMO Conjugation May Regulate Cardiomycocyte Proliferation

SUMO Conjugation also modulates cardiac ion channel activity. For instance, voltage-gated potassium (kv) channels, Kv2.1 and Kv1.5, are SUMO substrates.\(^{119,120}\) Although the activity of K\(^+\) channel Kv2.1 is inhibited by SUMO conjugation, no SUMO acceptor site has been revealed, and the effect of sumoylation of Kv2.1 in cardiomyocytes awaits further investigation. Kv1.5 is targeted by SUMO on two consensus sequences, IK\(_{22} \)EE and LK\(_{536} \)EE.\(^{120}\) The first motif is conserved in Kv1.2, Kv2.1, and Kv1.5, whereas the second motif is only contained in Kv1.5, suggestive of differential regulation of these family members by the SUMO pathway. The sumoylation of Kv1.5 can only be abolished by simultaneous double mutations K221/S36R. Suppressing sumoylation of Kv1.5 causes substantial hyperpolarizing shift in the voltage dependence of inactivation, indicating a regulatory role for sumoylation in K1.5 activity.\(^{120}\) However, whether this modulation is implicated in human heart disease remains to be elucidated. In addition, potassium channel K2P1 is also a SUMO substrate;\(^{121}\) however, it is unclear whether sumoylation affects the potassium current directed by K2P1.\(^{122}\)

In addition to the above-mentioned proteins whose activities are modulated by the SUMO pathway, sumoylation is also extensively involved in modulation of a number of signal transduction pathways contributing to normal heart development and function,\(^{123}\) including peroxisome proliferator-activated receptor (PPAR) \(\alpha\),\(^{124}\) \(\alpha\)2\(^{125,126}\) and PGC-1\(\alpha\).\(^{127}\) Taken together, these findings demonstrate that the SUMO pathway is important for normal cardiac gene expression and function in heart development.

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In addition to the above-mentioned proteins whose activities are modulated by the SUMO pathway, sumoylation is also extensively involved in modulation of a number of signal transduction pathways contributing to normal heart development and function,\(^{123}\) including peroxisome proliferator-activated receptor (PPAR) \(\alpha\),\(^{124}\) \(\alpha\)2\(^{125,126}\) and PGC-1\(\alpha\).\(^{127}\) Taken together, these findings demonstrate that the SUMO pathway is important for normal cardiac gene expression and function in heart development.

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causes septal defects.\textsuperscript{131,132} We discovered that expression of the SENP2 transgene (SENP2-Tg), a desumoylation enzyme in murine cardiomyocytes under the control of cardiac α-myosin heavy chain (α-MHC) promoter, caused profound death in over 50% of the transgenic pups in the first week after birth. Virtually, all of the dead pups exhibited intracardiac defects—atrial septal defects (ASDs) and/or ventricular septal defects (VSDs)—resembling the most common forms of human congenital heart disease (manuscript in preparation). We observed substantial reduction in cell replication in SENP2-Tg hearts versus controls, which was accompanied by the dysregulation of a number of cell cycle regulators, such as cyclin and cyclin-dependent kinase inhibitors. Further investigation is required to determine whether overexpressed SENP2 decreases SUMO modification of targets associated with cell division such as Topo II and/or RanGAP1. Nevertheless, cardiomyocyte cell replication appears to be compromised and a normal balance of SUMO conjugation and deconjugation during cardiogenesis may be crucial for the normal closure of atrioventricular septa.

**SUMO Targets Chromatin Remodeling Complexes Involved in Cardiogenesis**

The establishment and maintenance of epigenetic activation and silencing of genes is essential for cell fate determination. As discussed above, several lines of evidence suggest that a number of chromatin remodeling-involved factors are modulated by the SUMO pathway,\textsuperscript{133} some of which have been shown to be important for normal cardiac development. For instance, HDAC1 and 2, cardiac ablation of which leads to cardiac structural defects,\textsuperscript{134} are regulated by SUMO conjugation,\textsuperscript{61} although the functional consequence of SUMO modification of these two factors on cardiomyocyte differentiation and/or proliferation has yet to be determined. Recently, the murine SENP2 gene knockout caused defects in the embryonic heart and reduced the expression of Gata4 and Gata6, which are essential for cardiac development.\textsuperscript{135,136} SENP2 regulates transcription of Gata4 and Gata6, mainly through alteration of occupancy of Pc2/CBX4, a PRC1 subunit, on their promoters. Pc2/CBX4 is shown as a target of SENP2 in vivo. In SENP2 null embryos, sumoylated Pc2/CBX4 accumulates and Pc2/CBX4 occupancy on the promoters of target genes is markedly increased, leading to repression of Gata4 and Gata6 transcription. Thus, it is likely that altered sumoylation states in the heart, during either embryonic cardiac development or the maintenance of postnatal heart function, will promote abnormal gene expression leading to cardiac structural malformation and/or dysfunction of chromatin remodeling.

**Potential Involvement of SUMO Pathway in Human Cardiovascular Disorders**

The first study that directly implicates the sumoylation pathway in cardiovascular diseases focuses on human lamin A gene mutations.\textsuperscript{137} Lamin A protein is a nuclear structural protein that plays a critical role in the maintenance and function of the cell nucleus.\textsuperscript{138} A number of naturally occurring lamin A mutations cause human familial dilated cardiomyopathy.\textsuperscript{138} Lamin A is a SUMO-2-favored substrate with an acceptor site, K201, embedded in the sumoylation target sequence, MKEE.\textsuperscript{139} Conversion of lysine 201 to arginine (K201R) resulted in altered subcellular localization and decreased sumoylation activity.\textsuperscript{139} Furthermore, 2 naturally occurring mutations identified in human familial dilated cardiomyopathy, E203G and E203K, also alter cellular localization similar to the pattern of the K201R mutant.\textsuperscript{139} The E203G and E203K mutations of the glutamic acid amino acids are known to be important for SUMO modification and are likely to be involved with defective lamin A protein sumoylation. Indeed, sumoylation assays conducted on a patient bearing E203K associated with cardiomyopathy showed decreased level of SUMO-2-conjugated lamin A.\textsuperscript{139} It will be interesting to see whether any of other human cardiomyopathy-associated lamin A mutations impacts sumoylation, even if those mutations are not in the sumoylation consensus sequence. The finding of the involvement of sumoylation of lamin A in cardiomyopathy also raises several other important questions. For example, do SUMO family members perform distinct functions in vivo, or does a level of functional redundancy exists between family members? Since lamin A is a preferential target for SUMO-2/3, does repression of either SUMO-2 and/or SUMO-3 in the mouse heart cause specific phenotype(s) such as a lamin A-dependent cardiomyopathy? If so, is this due to a decrease in the level of lamin A conjugation?

Another study reported on a 0.8-megabase deletion at the karyotype band location in 19q13.32 in a patient who had severe developmental defects, including cleft lip and cardiac malformation.\textsuperscript{140} The deleted domain contains three identified genes, one of which is SAE1, a subunit of a sumoylation activating enzyme. Given the importance of the SUMO pathway in the cell cycle, loss of SAE1 may be part of mechanisms contributing to the cardiac structural anomaly observed in that patient.

**Challenges and Perspectives**

The association of the SUMO conjugation pathway with cardiac gene regulation is a relatively new area and knowledge about the importance of the SUMO pathway for the development and maintenance of a normal cardiovascular system is just beginning to emerge. We believe that to understand the role of sumoylation in heart development and disease, the heart field will need to address a number of fundamental questions based on recent research.

For example, do the components and targets of the SUMO-1 conjugation pathway, including Nkx2.5, underlie the vast majority of human congenital heart birth defects? Congenital heart defects (CHDs) are the most common of all congenital birth defects, occurring in 0.5% to 1.2% of newborns. The 2 most common CHDs are the ASDs and VSDs, defined as the presence of a communication between the right and left atria and right and left ventricles, respectively. Although a variety of proteins and transcription factors, such as SMAD4, GATA4, Nkx2.5, Tbx proteins, as well as several signaling pathways, such as Notch signaling and Wnt signaling, have been reported to be involved in septogenesis, the unifying molecular mechanism(s) underlying the development of ASD and VSD which ties many of
these factors together has been elusive. We believe that the SUMO conjugation may contribute to normal cardiac morphogenesis by maximizing the activity of cardiac muscle–enriched factors via SUMO modification for activation of cardiogenic genes, as well as repression of noncardiogenic tissue activity.

Another important question is whether the balance of reversible SUMO conjugation and deconjugation regulate cardiac morphogenesis and development. Ubc9 is shown to be required for myotube formation in C2C12 cells and for pharyngeal muscle development in C elegans,\textsuperscript{118,128} thus implicating the sumoylation pathway in muscle development. Recently, knockout of sumoylation pathway components, such as the murine Ubc9, was shown to result in early embryonic lethality during the postimplantation stage.\textsuperscript{128}

Genetic defects of the individual SUMO conjugation pathway components and even the cardiac targets may provide similar CHD phenotypes accounting for the exceedingly high number of congenital birth defects observed in the USA and around the world. It will be important to determine whether mutations in key components of the sumoylation pathway are prevalent in CHD patients and then to determine their biological roles and effects on downstream gene activity.

Finally, do environmental toxins, metabolites, and pharmaceuticals that modulate sumoylation gene activity cause heart disease? Currently, we have little information on gene regulation of the sumoylation pathway, a wide gap that needs to be filled. The perturbation of signaling components and gene activity of pathway components through the intersection of toxins, nutrients and drugs are unknown intangibles that could tilt the balance of the SUMO conjugation pathway and lead to cardiogenic dysregulation. We feel that screens of compound libraries to identify chemicals that stimulate and/or repress SUMO pathway gene activity will become an important future endeavor.

We trust that our short review will further the field’s understanding of posttranslational sumoylation as a key factor in human congenital cardiac birth defects and heart disease and may provide novel insights leading to the development of diagnostic and therapeutic avenues.

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None.

References


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