Sumoylation in gene regulation and cardiac disease: potential for drug discovery

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Abstract: Small ubiquitin-related modifier (SUMO) proteins are members of ubiquitin-like super-family proteins that can be covalently conjugated to their targets through multistep enzymatic reactions. Sumoylation has caught much attention due to its versatility, wide involvement in cellular events, and disease association. Sumoylation has been well studied at cellular and molecular levels. A newly emerging role that SUMO conjugation plays is in cardiac pathophysiology. In this review we will update new advances in the study of implications of the sumoylation pathway in the pathogenesis of cardiac diseases, discuss promise of the SUMO pathway as a potential therapeutic target, and conclude with future directions for SUMO research in the heart field.

Keywords: posttranslational modification, SUMO, SENP, heart

Introduction
SUMO proteins and conjugation
Small ubiquitin-related modifier (SUMO), a family of ubiquitin-like proteins, has been studied for over 15 years.1 Sumoylation is a posttranslational modification, in which SUMO proteins are covalently conjugated to target proteins via a series of enzymatic reactions. Conjugatable SUMO proteins (SUMO-1, SUMO-2, and SUMO-3) are synthesized as precursors, and are activated by the cleavage performed by sentrin-/SUMO-specific proteases (SENP1, 2, 3, 5, 6, and 7 in humans) to expose the C-terminal diglycine needed for covalent attachment to target substrates.2–4 SUMO-1 shares ∼50% sequence similarity with SUMO-2/3, but the active SUMO-2 and -3 exhibit ∼95% homology. The role of SUMO-1 in mouse embryogenesis remains in debate (see “Emerging roles of SUMO in cardiovascular disorders”). However, despite the high similarity at the amino acid sequence level between SUMO-2 and -3, a recent study suggested a differential functional importance of SUMO-2 and -3 in mouse embryonic development. First, SUMO-2 was the most abundantly expressed SUMO isoform during mouse embryogenesis.5 Second, knockout of SUMO-2, but not SUMO-3, caused embryonic lethality.5 Similarly to ubiquitination, SUMO conjugation to targets occurs through an adenosine triphosphate (ATP)-dependent mode by an enzymatic cascade of activating enzyme (E1), a heterodimeric complex of SAE1/SAE2, conjugating enzyme (E2) Ubc9, and/or an E3-ligase (Figure 1).6,7 When only a single SUMO E1 and SUMO E2 exist in the SUMO conjugation pathway, multiple SUMO E3 ligase enzymes have been defined, including RanBP2/Nup358,8 PIAS family,9 polycomb 2,10 tumor necrosis factor associated protein 7,11 TOPORS,12,13 mitochondrial-anchored protein ligase,14 and Nse2/Mms21.15 SUMO conjugation occurs on the lysine residue(s) that are mainly
localized on the consensus sequence ψKXE/D (ψ is a bulky hydrophobic amino acid and X is any residue) existent on many SUMO substrates.16,17

SUMO deconjugation
SENP proteins that function for SUMO precursors’ maturation also demodify the conjugated form of SUMO proteins from substrates. The six SENPs identified in humans differ in their isopeptidase activities, subcellular localizations, and SUMO paralogue preferences.2 SENP proteins may have nonredundant roles in mouse embryogenesis, as evidenced by the findings that knockout of either SENP1 or SENP2 caused embryonic lethality.18,19 For more details about structures and functions of SENP proteins, the readers are referred to some previously published comprehensive reviews.3,4,20

SUMO conjugation and cellular activities
Through targeting many factors, SUMO conjugation is involved in a variety of cellular activities including but not limited to signal transduction, subcellular translocalization, stress regulation, DNA damage and repair.21–24 However, the functional consequence of SUMO conjugation to the target is context-dependent; it may lead to inhibition or activation of the activity of a particular promoter/substrate in a particular pathophysiological setting once conjugated. For instance, serum response factor is sumoylated on lysine 147.25 This SUMO-site mutation enhanced the ability of serum response factor to activate CArG box located in the c-fos promoter,26 but impaired its ability to activate cardiac target gene promoter.27 Given its wide implication in regulation of cellular events, it is conceivable that SUMO conjugation pathway is potentially linked to diseases. Indeed, sumoylation activity is altered in a number of human diseases such as cancer and neurodegenerative diseases.27,28 Recent evidence points to the implication of the SUMO conjugation pathway in cardiac gene regulation as well as in the pathophysiology of cardiovascular disease,29,30 which is the main focus of this updated review.

SUMO-targeted cardiac proteins and roles in cardiac gene regulation
Cardiac transcription factors are a group of proteins that regulates cardiac development/function, and are linked to cardiac disease as well. The work from our lab and others has demonstrated that SUMO targets a multitude of cardiac enriched transcription factors that are important for maintaining normal cardiac development and function (Table 1). For more details, the reader is referred to the previous two reviews written by the author.29,30

Emerging roles of SUMO in cardiovascular disorders
SUMO and murine models of cardiovascular disease
In the animal models, knockout of SUMO-1 may cause cardiac structural defects and premature death,31,32 although the genetic background of mice probably also contributed to the development of this phenotype. This finding indicates the importance of SUMO-1 conjugation in cardiac structural morphogenesis. This importance was further corroborated by a gain-of-function model in which SENP2, a SUMO isopeptidase, was restrictedly activated in cardiomyocytes.33 Overexpressed SENP2 promoted deconjugation of SUMO-1

Figure 1 Reversible sumoylation cycle.
Notes: SUMO proteins are produced in an inactive precursor form and are cleaved by SENPs to expose carboxy-terminal diglycine motif for activation. Activated mature SUMO proteins are transferred by E1 heterodimer SAE1/SAE2 in an ATP-consuming step to E2 conjugating enzyme Ubc9. Ubc9 forms an isopeptide bond between carboxy-terminal glycine of a SUMO protein and lysine (K) residue in the target with/without a specific E3 ligase. SENPs cleave the isopeptide bond and release the SUMO protein for the coming cycle.
Abbreviations: ATP, adenosine triphosphate; SENPs, sentrin-/SUMO-specific proteases; SUMO, small ubiquitin-related modifier.
and caused congenital heart defects (CHDs), which was rescued by simultaneously overexpressed SUMO-1.33 The mice with increased SENP2 expression in cardiomyocytes also developed cardiac hypertrophy and fibrosis with aging, which was probably associated with decreased SUMO-2/3 conjugation, because it was not rescued by improved SUMO-1 conjugation.35 A more recent finding showed that SUMO-1 overexpression was protective against phenylephrine- and pressure-overload-induced cardiac hypertrophy, as well as decreased oxidative stress during cardiac hypertrophy and heart failure.34

Compared with the CHDs caused by activated SENP2, overexpression of SENP5, another SUMO isopeptidase, in cardiomyocytes induced cell death and dilated cardiomyopathy.35 In this gain-of-function murine model, mice with restricted SENP5 overexpression in cardiomyocytes showed decreased conjugation of SUMO-2/3 but not SUMO-1, and exhibited increased cell death that led to cardiomyopathy, fibrosis, and heart failure with aging. Mechanistically, overexpressed SENP5 damaged mitochondrial function, and activated apoptotic factors such as cleaved caspase3 and PARP-1.35 Correspondingly, increased expression of Bcl2, an antiapoptotic factor, rescued cardiac dysfunction triggered by overexpressed SENP5. In the SENP5-linked cardiomyopathic phenotype, at least one mitochondrial SUMO substrate, Drp1, a critical factor for mitochondrial fission, is involved. Desumoylation of Drp1 was observed in the cardiomyocytes with overexpressed SENP5, which showed also enlarged mitochondria, and knockdown of Drp1 by lentiviral-mediated short hairpin RNA reduced activation of apoptotic factors triggered by SENP5.35 These observations are in line with the previous report that SENP5 mainly targeted mitochondria in cultured cells.36,37

An earlier study showed a globally increased SUMO-1 conjugation in response to hypoxic insult in heart, resulting in elevated levels of SUMO-1-attached HIF1α.39 However, how sumoylation affects HIF1α’s stability and function remains in debate. Although several lines of evidence indicate that SUMO conjugation to HIF1α increased its stability and activity,39,40 HIF1α activity and stability may also be decreased by SUMO conjugation.18,41 Very intriguingly, a more recent study suggested that SUMO E3 ligases CBX4 and PIASy promoted HIF1α sumoylation on different lysine residues.42 As a result, while CBX4-enhanced sumoylation stabilized HIF1α and increased its activity, PIASy-stimulated sumoylation did the opposite.43 Thus, it seems likely that different sumoylation sites of HIF1α may mediate different functional outcomes once they are SUMO-conjugated.

Another study has provided evidence that extracellular signal-regulated kinase 5 (Erk5), an important mediator of ischemic/reperfused injury and inhibitor of apoptosis, is inhibited by sumoylation.44 Sumoylation of Erk5 is elevated in myocardial infarction, in the H2O2-induced inflammation, and in the diabetic aortas of mice.43,44 An increase in the level of SUMO-1-conjugated Erk5 appears to promote the inflammation, therefore worsening the injury. Consistent with this, SENP2 was shown to protect endothelial dysfunction at least partially via desumoylation of Erk5.45 Given that a multitude of SUMO substrates are involved in regulating ischemic/reperfused heart injury, the net functional outcome of the increased SUMO-1 conjugation in this particular pathophysiological setting in vivo merits further examination.

<p>| Table 1 SUMO targets cardiac transcription factors |</p>
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<tr>
<th>SUMO substrates</th>
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<td>Smad4</td>
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Note: *Dependent upon the context.

Abbreviation: GATA4, GATA binding protein 4; Mef2c, myocyte enhancer factor 2C; Nfetc1, nuclear factor of activated T-cells, cytoplasmic; T-box 5; Nlx2.5, Nkx2.5 homeobox 5; Proxl, prospero homeobox 1; Smad4, SMAD family member 4; SRF, serum response factor; SUMO, small ubiquitin-related modifier; Tbx5, T-box 5.
SUMO and human cardiovascular diseases

The evidence that the SUMO conjugation pathway is implicated in human cardiovascular disorders has been emerging recently. First, human CHD-linked mutations of some transcription factors present deficiency in SUMO conjugation. For instance, several naturally occurring missense mutations of Nkx2.5, particularly those in homeodomain, impaired its sumoylation.\(^4\) Moreover, expression of sumoylation-deficient Nkx2.5 mutant (K51R, conversion of lysine 51 to arginine) in mice with the background of Nkx2.5 haploinsufficiency caused CHDs,\(^4\) further supporting the deficient Nkx2.5 sumoylation underlying Nkx2.5-linked CHDs. Sumoylation of ZIC3 is another example. ZIC3 is an X-linked zinc finger transcription factor that is causally linked to human heterotaxy including CHDs.\(^4\) The nucleocytoplasmic shuttling of ZIC3, which is governed by nuclear export/import signals, is an important mechanism that mediates the activity of ZIC3. A number of naturally occurring missense mutations that are etiologically associated with CHDs negatively affect ZIC3 nuclear occupancy,\(^4\) consequently leading to its decreased activity. Interestingly, our work identified ZIC3 as a novel SUMO substrate on lysine 248. Mutation of lysine 248 to arginine reduced its nuclear localization, although it is not located in any of those nuclear export/import signals identified.\(^4\) Coincidently, a number of human missense mutations that exhibit diminished nuclear occupancy also showed decreased sumoylation, while those which have normal subcellular distribution also showed normal levels of sumoylation compared with wild type ZIC3.\(^4\) Moreover, recovery of SUMO-1 conjugation to these sumoylation-defective ZIC3 mutants by Pias1, a SUMO E3 ligase, promoted their nuclear occupancy.\(^4\) These findings clearly demonstrate that sumoylation deficiency underpins the ZIC3-linked human congenital defects.

A subset of familial cardiomyopathy is also etiologically associated with the SUMO conjugation pathway. Mutations of lamin A, a nuclear structural protein, are associated with inherited dilated cardiomyopathy.\(^4\) Interestingly, SUMO targets lamin A on the lysine residue 201 in the consensus sequence MKEE, and the mutation of glutamic acid 203 to either glycine (E203G)\(^3\) or lysine (E203K),\(^3\) which are causally linked to familial dilated cardiomyopathy and conduction disease, negatively influences the lamin A’s sumoylation, consequently altering its nuclear distribution.\(^3\) which resembles the molecular phenotype exhibited by the sumoylation-resistant mutant E201R.\(^5\) These data support the argument that a defective SUMO conjugation of its substrate lamin A is directly implicated in the initiation/development of dilated cardiomyopathy.

Deficient SUMO conjugation may be involved in another cardiac conduction disease, the progressive familial cardiac conduction block I. This conduction disease exhibits autosomal-dominant inheritance, and the gene TRPM4, which encodes a Ca\(^{2+}\)-activated nonselective cation channel, is associated with its pathogenesis.\(^6\),\(^7\) TRPM4 is a SUMO substrate,\(^8\) although the SUMO attachment site or sites remain elusive. A missense mutation in TRPM4, E7K (mutation of glutamic acid 7 to lysine), displays a resistance to the desumoylation by SENP1, subsequently protecting it from proteasomal degradation and leading to a gain of function.\(^9\)

In human failing hearts, SUMO-1 conjugation was decreased and so was its conjugation to SERCA2a,\(^6\) indicating the implication of both in human heart failure. In the large animal model, SUMO-1 gene transfer greatly improved cardiac function in porcine models with ischemic-induced heart failure.\(^\)\(^0\) SERCA2a as a SUMO substrate is partially involved in mediating SUMO-1 gene transfer-achieved cardiac protection; the protection obtained by the high levels of SUMO-1 expression in this large animal heart failure model may be independent of SERCA2a,\(^9\) indicating the involvement of other substrates/mechanisms.

More recently, our work has revealed that SENP5 was elevated in the human failing hearts at both transcription and protein levels.\(^3\) In the gain-of-function mouse model, overexpressed SENP5 in cardiomyocytes recapitulated the pathogenesis of dilated cardiomyopathy and heart failure of humans.\(^3\) Our finding demonstrates for the first time that SENP5, a desumoylation enzyme, is implicated in cardiomyopathy and heart failure of both human and murine models, therefore presenting it as a potential therapeutic target for cardiomyopathy and heart failure.

The ubiquitin proteasome pathway (UPP) is the major mechanism for degradation of cytosolic and nuclear proteins. Dysfunctional UPP may cause a number of human diseases, including cardiomyopathy.\(^6\) Ubc9, the sole SUMO E2, was found to be required for normal function of UPP.\(^5\) Overexpression of Ubc9 in cultured cardiomyocytes potentiated the activity of UPP, whereas depletion of Ubc9 inhibited the UPP function, leading to increased mis-folded protein aggregations. Thus, Ubc9 is an important factor for UPP to execute its essential function to eliminate aggregated...
proteins. It will be interesting to pursue if any SUMO targets are involved in mediating UPP function by Ubc9.

The main SUMO conjugation pathway components and substrates that are discussed in this section are summarized in Table 2.

**SUMO as a therapeutic target in cardiac disease**

Increasing evidence has pointed to the important role the SUMO conjugation pathway plays in the pathogenesis of cardiovascular diseases. Thus, the sumoylation pathway may represent a new potential therapeutic target for a number of cardiac diseases including cardiomyopathy and heart failure. Given the existence of multiple sumoylation machinery with less specificity and widely distributed targets in various signaling pathways and gene regulatory networks, the guiding principles to develop the small molecules for therapeutic purpose via altering sumoylation activity should be high selectivity and specificity. For instance, increased SUMO-1 conjugation appears to provide a general protection of reperfusion; however, whether and how enhanced conjugation of SUMO-2/3 affects cardiac function and disease progression remains unclear. Thus, a small molecule that increases pan-SUMO conjugation should be cautiously considered as a practical treatment method. Instead, a chemical that favorably improves conjugation of SUMO-1 but not SUMO-2/3 should be more applicable in clinic. Also, the agents that specifically repress the activity of SENP5 instead of all SENPs are desired, because recent evidence shows that only SENP5 was involved in human failing heart.35 Another challenge will be how to preferably increase/decrease SUMO attachment to a particular target in vivo. The additional challenge also lies in the fact of the ubiquitous expression pattern of SUMO conjugation machinery; the molecules that are to be developed should only exert impacts on sumoylation activity specifically in heart as desired but not in other tissues/organs to treat cardiac-specific diseases and reduce nondesired effects.

**Conclusion and prospects**

Sumoylation is a versatile and fascinating posttranslational modification that underlies a wide spectrum of cellular functions. Recently, much attention has been paid to sumoylation in the heart field due to its importance in cardiac pathology/pathophysiology. Indeed, recent studies from our lab and others have implicated SUMO conjugation in human cardiac diseases. As mentioned above, dysregulated levels of SENP5 and SUMO-1 conjugation are present in the human failing hearts, and overexpression of SENP5 in mouse cardiomyocytes recapitulates the development of human cardiomyopathy and heart failure. Still, many questions remain open. For instance, increased SUMO-1 did not reduce cardiac hypertrophy and improve cardiac function of SENP2 transgenic mice.33 Does that suggest that SUMO-2/3 is also involved in cardiac muscle disorders? Also, do any SUMO E3 ligases such as Pias family members play a role in the pathogenesis of human cardiac diseases? Additionally, in a particular cardiovascular disease setting, does SUMO conjugation to only one substrate, or to a group of substrates...
that work in the same functional network or pathway, make a contribution to the disease development? Furthermore, it remains largely unexplored how the expression of the SUMO conjugation pathway components such as SENP5 or SUMO-1 is regulated in heart, although SENP5 was found to positively mediate SUMO-2/3 transcription.\(^{35}\)

In conclusion, to fully understand the molecular mechanisms by which the SUMO conjugation pathway contributes to cardiovascular diseases, it is necessary to have more systemic studies by employing various genetically modified murine models along with stress stimulation. Given that this field is currently catching more attention, we anticipate that in the foreseeable future the studies will advance our knowledge about the importance of sumoylation in heart disease initiation and/or progression, and will pave the way to develop highly specific small molecules for therapeutic purpose to ameliorate heart afflictions by targeting the SUMO conjugation pathway.

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