

Review

The Ubiquitin-Like SUMO System and Heart Function From Development to Disease

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Abstract: SUMOylation is a ubiquitin-related transient posttranslational modification pathway catalyzing the conjugation of small ubiquitin-like modifier (SUMO) proteins (SUMO1, SUMO2, and SUMO3) to lysine residues of proteins. SUMOylation targets a wide variety of cellular regulators and thereby affects a multitude of different cellular processes. SUMO/sentrin-specific proteases are able to remove SUMOs from targets, contributing to a tight control of SUMOylated proteins. Genetic and cell biological experiments indicate a critical role of balanced SUMOylation/deSUMOylation for proper cardiac development, metabolism, and stress adaptation. Here, we review the current knowledge about SUMOylation/deSUMOylation in the heart and provide an integrated picture of cardiac functions of the SUMO system under physiologic or pathologic conditions. We also describe potential therapeutic approaches targeting the SUMO machinery to combat heart disease. (*Circ Res.* 2016;118:132-144. DOI: 10.1161/CIRCRESAHA.115.307730.)

Key Words: cardiac development ■ heart failure ■ protein quality control ■ stress adaptation ■ SUMO

Numerous signaling pathways and cardiac-specific transcription factors regulate heart development and the function of the adult heart.^{1,2} The complexity of the pathways involved, their multiple interactions requiring a finely tuned balance, and the requirement to form a complex anatomical structure probably explain the rather high incidence of congenital heart defects (CHD) mounting up to ≈1% of all newborns.³ Surprisingly, the primary causes of most common abnormalities, such as atrial septal defect and ventricular septal defect, remain enigmatic also because only a limited number of CHDs follow Mendelian traits.^{4,5} The deficits in our understanding of pathogenetic events leading to CHDs illustrate the need to obtain a comprehensive and integrated view of the complex mechanisms involved in cardiac morphogenesis.

Much progress has been made to identify the molecular events associated with pathological conditions of the heart, including valve abnormalities, hypertension, diabetes mellitus, coronary artery disease, or idiopathic cardiomyopathies. Researchers have characterized changes in gene activity and posttranslational modifications (PTMs) of specific cardiac proteins, contributing to impaired cardiac contractility and promoting pathological mitochondrial or metabolic alterations.^{6,7} However, very few new drugs have emerged from such efforts, despite a rising number of patients suffering from heart failure. It seems likely that the prevalence of heart insufficiency will further increase in the future because of an aging population and a prolonged lifespan of cardiac patients, which represents a pressing need to develop new therapeutic

approaches. On the other hand, numerous signaling pathways, which hold an enormous potential for therapeutic interventions, have barely been investigated in the heart. It seems likely that this terra incognita contains novel targets, which allow modulation of diseases-relevant processes to counteract cardiac dysfunctions.

In the last decade, the PTM of proteins with the small ubiquitin-like modifier (SUMO) has emerged as a central regulatory mechanism for the control of cellular functions, including developmental and differentiation processes. Detailed general reviews that cover mechanistic and functional aspects of SUMO signaling have been published.^{8,9} However, our understanding of tissue-specific functions of the SUMO system is still incomplete. Based on recent findings, we will give an overview of the heart-specific functions of the SUMO system. In particular, we discuss the role of SUMO conjugation and deconjugation in regulating heart development, metabolism, contractility, and protein quality control. We will also examine the potential involvement of the SUMO system in stress adaptation of the adult heart and critically evaluate the physiological and pathological relevance.

General Features of SUMO Conjugation/ Deconjugation System

SUMOylation is a transient and reversible PTM in which SUMO proteins are conjugated to lysine residues of target proteins.^{8,9} In human, 4 SUMO isoforms (SUMO1, 2, 3, and 4) have been identified to date, which share a common

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Nonstandard Abbreviations and Acronyms

AMPK	adenosine monophosphate activated kinase
CHD	congenital heart defect
Drp1	dynamain-related protein 1
E1, E2, E3	SUMO activating, conjugating, and ligating enzymes, respectively
GATA4/6	GATA binding protein 4/6
HDAC	histone deacetylase
HIF1α	hypoxia-inducible factor 1 α
IR	ischemia-reperfusion
KO	knockout
MEF2	myocyte enhancer factor 2
Nkx2.5	Nk2 homeobox 5
PGC-1α	peroxisome proliferator-activated receptor coactivator-1alpha
PIAS	protein inhibitors of activated STATs
PPAR	peroxisome proliferator-activator receptor
PTM	posttranslational modification
ROS	reactive oxygen species
SENP	SUMO/sentrin-specific protease
SERCA2a	sarcoplasmic/endoplasmic reticulum calcium ATPase 2a
SIRT1	sirtuin 1
SUMO	small ubiquitin-like modifier
Ubc9	ubiquitin conjugating enzyme 9
UPS	ubiquitin-proteasome system

structure that is characterized by the evolutionary conserved ubiquitin-fold. The primary structure of SUMO1 shows $\approx 50\%$ sequence identity with both SUMO2 and SUMO3, which are very similar (with 97% of identity).¹⁰ The physiological role of SUMO4, which is absent in most cell types and cannot be conjugated because of the lack of C-terminal processing (see below), is still under debate.^{11–13} Genetic and biochemical data indicate that the 3 SUMO variants SUMO 1, 2, and 3 are likely to play partially redundant roles, although distinct effects in cellular processes have been described.^{10,14,15} All SUMOs are expressed as precursor proteins and have to be processed before conjugation. Similar to ubiquitylation, conjugation generally proceeds through 3 reaction steps in which SUMO-activating, -conjugating, and -ligating (E1, E2, and E3, respectively) enzymes act sequentially (Figure 1). Currently, it is not entirely clear how substrate specificity of conjugation is achieved because only 1 dimeric E1 enzyme (SUMO-activating enzyme 1/SUMO-activating enzyme 2), a single E2 enzyme (ubiquitin-conjugating enzyme 9 [Ubc9]), and a handful of E3 ligases are known.⁸ The present view in the field is that the E3 ligases of the PIAS (protein inhibitors of activated STATs) family (PIAS1, PIAS2, PIAS3, PIAS4) and Mms21 (nonstructural maintenance of chromosomes element 2) homolog as well as RanBP2 (ras-related nuclear protein binding protein 2), or the human polycomb protein polycomb 2/chromobox homolog 4 (hPC2/CBX4) stimulate conjugation of SUMO to a whole group rather than to individual target proteins. This concept has been termed group modification.^{16,17}

The general function of SUMO conjugation or SUMO group modification involves regulation of distinct protein/

protein interactions or the organization of higher-order protein complexes.¹⁷ Attachment of SUMO can either promote or prevent the assembly of protein complexes. Steric hindrance or masking of an interaction domain are the main mechanisms for SUMO-mediated disassembly of a complex.¹⁸ The promotion of protein interactions by SUMO conjugation is typically achieved through SUMO-dependent recruitment of binding partners, which contain distinct SUMO-binding modules termed SUMO-interacting motifs (SIMs)^{8,18} (Figure 1).

SUMO modification is reversed by SUMO-specific hydrolases that remove SUMO from the target through cleaving the isopeptide bond of a SUMO conjugate (Figure 1).⁸ This enables a highly dynamic regulation of protein functions, making SUMOylation an ideal regulatory mechanism for fast cellular decisions. In higher vertebrates, 3 classes of SUMO-specific hydrolases have been defined to date. The largest and best-studied class is the ubiquitin-like protein (Ubl)-specific protease/SUMO/sentrin-specific proteases (SENP) family, which consists of 6 members: SENP1, 2, 3, 5, 6, and 7. The deSUMOylating isopeptidase class DeSI (deSUMOylating isopeptidase-1 and deSUMOylating isopeptidase-2) and ubiquitin-specific protease-like 1 (USPL1) also act as SUMO-proteases but their substrate specificity and function is still not clear.⁸ Interestingly, most SENPs also catalyze the processing of SUMO precursors, which will promote conjugation. This dual function of SENPs in both maturation and deconjugation of SUMO adds

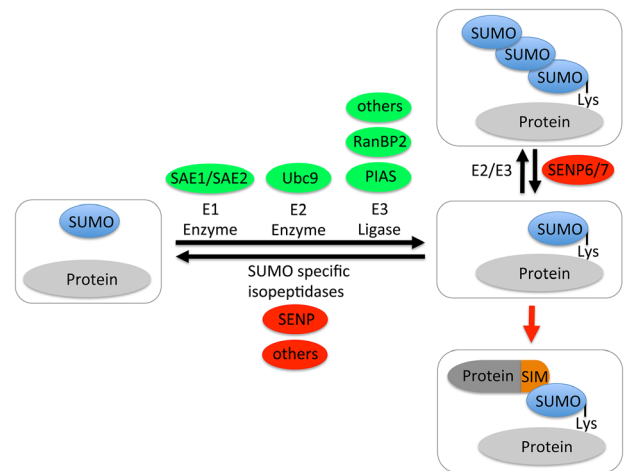


Figure 1 . Pathway of small ubiquitin-like modifier (SUMO) conjugation/deconjugation and the SUMO-dependent recruitment of SUMO interacting motif (SIM)-containing interaction partners. SUMO is covalently attached to specific lysine (Lys) residues of target proteins in an enzymatic process that involves an E1 activating enzyme (SAE1/SAE2), a single E2 conjugating enzyme (Ubc9), and different SUMO E3 ligases, such as Protein inhibitors of activated STATs (PIAS) proteins, ras-related nuclear protein (Ran)-binding protein 2 (RanBP2), and possibly others. As a result, an isopeptide bond is formed between the C-terminal glycine residues of SUMO and an ϵ -amino group of a Lys residue of the target protein. Attachment of additional SUMO moieties to internal Lys residues of SUMO induces polymeric chains (mainly by SUMO2/3). SUMO is deconjugated from target proteins by SUMO-specific isopeptidases (SUMO/sentrin-specific protease [SENP]). SUMO conjugates are specifically recognized by interaction partners that possess a specific binding module termed SIM (SUMO interaction motif). SAE1/2 indicates SUMO activating enzyme 1 and 2.

an additional layer to the complexity of the SUMO conjugation/deconjugation system. How these two functions are balanced and how target specificity of distinct SENPs is achieved has largely remained elusive. Moreover, their specificity toward distinct SUMO forms is not yet fully explored. The current view is that SENP1 possesses both processing and deconjugating activities, mainly removing SUMO1 from substrates,^{19–22} whereas SENP2 has a lower processing activity, but is more active in deconjugation of both SUMO1 and SUMO2.^{19,23–25} SENP3 and SENP5 act preferentially on SUMO2/3 in both processing and deconjugation,^{26–28} whereas SENP6 and SENP7 are not effective in removing monomeric SUMOs but mainly cleave di- or polymeric chains of SUMO2/3, thereby editing lysine-linked SUMO–SUMO chains.^{27,29–31}

Balanced SUMO conjugation/deconjugation plays a pivotal role in the control of many cellular processes.^{8,17,18,32–35} One clear hotspot for SUMO function is nuclear DNA transactions, such as chromatin organization, transcriptional processes, or DNA repair. Additionally, the SUMO pathway has a critical function in the nucleolus by regulating ribosome biogenesis. In addition to these well-established nuclear or nucleolar functions of the SUMO system, the significance of SUMO modifications outside the nucleus has recently been reported, revealing a role in the regulation of ion channel activity, plasma membrane receptors, or G-protein signaling, exocytosis, autophagy, cytoskeletal function, dynamics of mitochondria, mitosis, and apoptosis.³² Considering this wide spectrum of regulatory events, it seems likely that SUMOylation participates in pathogenic events, such as cancerogenesis.⁸ Indeed, increased expression of Ubc9 was found in different tumors,³⁶ and SENPs are differentially expressed in various cancer types. Because mitotically active cells are profoundly dependent on active SUMOylation/deSUMOylation cycles, the SUMO system has been proposed as possible target in cancer therapy.³⁷ On the other hand, post-mitotic cells, for example, neurons are also sensitive to abnormalities in SUMO modification, possibly leading to neurodegeneration.⁸ Furthermore,

emerging evidence points to an important role of SUMO in both the developing and adult heart.

Heart Development

Heart development is driven by an evolutionarily conserved programme that is governed by diverse signaling molecules and tissue-specific transcription factors.² SUMO proteins have been shown to target and modulate the activity of several critical factors involved in cardiac development, such as serum responsive factor, myocardin, GATA-binding protein (GATA)-4, Nk2 homeobox 5 (Nkx2.5), myocyte enhancer factor-2 (MEF2), Ying Yang 1 (YY1), prospero-related homeobox (Pro1), and T-box transcription factors-2 and -5 (TBX2, TBX5).³⁸ The SUMO-dependent regulation of these specific factors has been described in detail^{39–41} but new findings in mouse models with alterations in the SUMO pathway provide a novel integrated view on SUMO function during embryonic development (Table). Interestingly, both hypo- or hyperSUMOylation because of dysfunction of either the conjugation or deconjugation system result in embryonic or cardiac defects, indicating that a tight control of the degree of SUMOylation is essential for normal cardiac development^{44,54} (Table).

Inhibition of SUMO conjugation by genetic inactivation of the SUMO-conjugating E2 enzyme Ubc9 causes early embryonic lethality in mice between embryonic stage 3.5 and embryonic stage 7.5,⁴² demonstrating that attachment of SUMO is essential for embryonic viability (Table). In heterozygous Ubc9-mutant fibroblasts, mainly the SUMO2/3 conjugation is reduced,⁴² suggesting that SUMO2/3 conjugation is more sensitive than SUMO1 to variations of Ubc9 expression during embryonic development. Indeed, Wang et al reported in a very recent publication that SUMO2 knockout (KO) mice, characterized by compromised cell proliferation and increased apoptosis, die at about embryonic stage 10.5, whereas SUMO3 KOs are viable.⁴⁹ These findings indicate that SUMO2 is indispensable for embryonic development. Despite their high similarity, neither SUMO1 nor SUMO3 can complement SUMO2 most likely because of their significantly lower

Table. Cardiac Phenotype After Genetic Modulation of the SUMO Conjugation/Deconjugation System in Mice

Mouse Line	System Modified		Cardiac Phenotype	References
Ubc9 KO	SUMO conjugation	↓	Embryonic lethality at early post-implantation stage (between E3.5 and E7.5)	42
Ubc9-Tg		↑	Not analyzed (more tolerant to brain ischemia)	43
SUMO1 KO		↓	Embryonic lethality, ASD/VSDs vs normal	44 vs 45, 46
αMHC-SUMO1-Tg		↑	Normal (beneficial in heart failure)	44, 47
rAAV9-SUMO1		↑	Normal (beneficial in cardiac hypertrophy)	48
SUMO2 KO		↓	Embryonic lethality at E10.5	49
αMHC-SUMO2-Tg		↑	Premature death, cardiomyopathy of various severity with enhanced apoptosis	50
SUMO3 KO		↓	Normal	49
SENP1 KO	SUMO deconjugation	↓	Embryonic lethality at E12.5–E14.5	51
rAAV9-SENP1		↑	Dilated cardiomyopathy with mitochondrial abnormalities	52
SENP2 KO		↓	Embryonic lethality at E10, congenital heart defect	53
αMHC-SENP2-Tg		↑	ASD/VSD or cardiomyopathy/fibrosis in aged mice	54
αMHC-SENP5-Tg		↑	Dilated cardiomyopathy with mitochondrial abnormalities	55

ASD indicates atrial septal defect; E, embryonic stage; KO, knockout; α-MHC, α-myosin heavy chain; rAAV9, recombinant adeno-associated virus serotype 9; SENP, SUMO/sentrin-specific protease; SUMO, small ubiquitin-like modifier; Tg, transgenic; and VSD, ventricular septal defect.

expression levels. In fact, SUMO2 mRNA levels (70%–80%) are substantially higher than SUMO1 (15%–20%) or SUMO3 (2%–20%) in both embryonic and adult tissues. Accordingly, SUMO2 contributes the vast majority of total SUMO2/3 proteins at both stages. Despite its critical role in embryonic development, the SUMO2 KO mouse line does not show a specific cardiac phenotype, whereas the constitutive KO of SUMO1 led to specific cardiac septal defects (atrial septal defect and ventricular septal defect) with high penetrance.⁴⁴ The defects in SUMO1-mutant mice were rescued by transgenic cardiac-specific expression of SUMO1 arguing for an important and specific function of SUMO1 conjugation during normal cardiac development.⁴⁴ However, it is noteworthy that other groups failed to detect a phenotype in SUMO1 KO mice possibly because of differences in the genetic background and gene-targeting strategies.^{45,46}

The importance of a tightly regulated level of SUMOylation is also underlined by the observation of cardiac abnormalities in mice with cardiomyocyte-specific overexpression of the SUMO isopeptidase SENP2 (α -myosin heavy chain-SENP2-transgenic),⁵⁴ which decreases SUMO1 modification essentially mimicking the lack of SUMO1. In addition, cardiac-specific overexpression of SUMO1 correcting defective SUMO1 conjugation in α -myosin heavy chain-SENP2-transgenic mice rescues developmental defects and triggers cardiomyocyte proliferation⁵⁴ (Table). In summary, these data demonstrate that SUMO1 and SENP2 are key regulators of early cardiac morphogenesis. SENP2 acts as a deSUMOylating enzyme for a whole set of substrates, including GATA4 and other transcription factors that are critical for heart development.² The CHD observed in SENP2-transgenic mice might thus be the combined result of enforced deSUMOylation of a group of substrates. A prime candidate is Nkx2.5 because SENP2 was shown to inhibit Nkx2.5 transcriptional activity through promoting deconjugation of SUMO1.^{56–58} Accordingly, a number of Nkx2.5 targets are downregulated in SENP2 transgenic mice, and the compound mutant SENP2-transgenic/Nkx2.5^{+/-} mice exhibit a more severe phenotype.⁵⁴ On the contrary, mutations of Nkx2.5 linked to CHDs show an altered SUMOylation pattern.^{56–58} Taken together, the available data suggest that SUMO1—at least partially—exerts its function during embryogenesis by maximizing the activity of Nkx2.5. Additional candidates for critical targets of SUMO1 are serum responsive factor/myocardin or GATA4.^{44,59–62} It is worth noting that a subgroup of α -myosin heavy chain-SENP2-transgenic mice does not develop cardiac septal defects, but show cardiac hypertrophy and fibrosis (cardiomyopathy) during aging, indicating that balanced SUMOylation is also important for heart function in adult animals.⁵⁴ Because cardiac-specific overexpression of SUMO1-transgenic does only rescue the embryonic but not the adult heart phenotype of α -myosin heavy chain-SENP2-transgenic mice, it is tempting to speculate that enforced SENP2-mediated deconjugation of SUMO2/3 but not of SUMO1 primarily contributes to the adult phenotype. In support of this idea, cardiac-specific overexpression of SENP5, which deconjugates mainly SUMO2/3 from target proteins, does not result in embryonic heart defects, but induces cardiomyopathy in adult mice.⁵⁵

In contrast to the above-mentioned mutants resulting in decreased SUMO1- or SUMO2/3 conjugation in the heart, the consequences of enhanced SUMOylation for the heart have not yet been analyzed in great detail (Table). Ubc9 transgenic mice were investigated in the context of brain ischemia, where enhanced SUMOylation protects against ischemic damage.⁴³ At least in some pathological situations, enhanced modification by SUMO1 seems beneficial for cardiac function because cardiac-specific SUMO1-transgenic expression improved heart failure.^{44,47} In contrast, cardiac-specific SUMO2 overexpression induces premature death and severe cardiomyopathy, although no obvious embryonic phenotype has been described.⁵⁰ SENP1 KO mice, another animal model for unbalanced SUMO deconjugation, exhibit embryonic lethality possibly because of persistent SUMO1 conjugation of hypoxia-inducible factor 1 alpha (HIF1 α), which triggers its subsequent degradation followed by down-regulation of the HIF1 α target gene erythropoietin and defective erythropoiesis.⁵¹ Furthermore, SENP2 KO mice die at embryonic stage 10 because of decreased cardiomyocyte proliferation and defective heart formation⁵³ (Table). Knockout of SENP2 in embryonic hearts results in accumulation of SUMO1-conjugated hPC2/CBX4 (part of polycomb repressing complex 1), thereby increasing polycomb 2/chromobox homolog 4 occupancy on promoters of polycomb group target genes, which causes enhanced repression of GATA4 and GATA6, crucial regulators of cardiac development.^{53,63–65} It seems reasonable to assume that lack of SENP1 or SENP2 activities induces hyperSUMOylation of several substrates but apparently only few of them are critical for cardiac development. Moreover, orthologue, substrate, or tissue specificity of the SUMO proteases might play a distinctive role in proper SUMOylation in the embryonic heart because the phenotypes of SENP1 versus SENP2 mutants differ considerably.

Altogether, one might speculate that SUMO1 plays a more specific role in cardiac development than SUMO2/3, whereas in adult hearts, disturbance of both SUMO1 and SUMO2/3 conjugation has detrimental effects. However, the specific and essential role of different SUMO orthologues (SUMO1 versus SUMO2) or various SENPs in embryogenesis has to be further clarified in more detail by systematic analysis of cardiac-specific KO and transgenic mouse lines.

Cardiac Metabolism

Proper cardiac function and contractility require a constant energy supply to fuel contraction/relaxation cycles. In the healthy adult, heart energy is mainly provided by oxidative phosphorylation, whereas cardiomyocytes in hypertrophic hearts or during heart failure undergo a metabolic switch and use mainly glucose instead of fatty acids.⁶⁶

Cardiac energy homeostasis is critically regulated by peroxisome proliferator-activator receptors (PPAR) both under physiological and pathological conditions (for comprehensive review, see Madrazo and Kelly⁶⁷ and Neels and Grimaldi⁶⁸). The PPAR family includes 3 members: PPAR α , β , and γ . Cardiomyocytes express high amounts of PPAR α and β , but less PPAR γ .⁶⁸ PPAR α was first described as a master regulator of genes controlling fatty acid oxidation.⁶⁹ Interestingly, its

expression decreases in several experimental models of cardiac hypertrophy.^{70–72} Conditional overexpression of PPAR β in adult hearts indicate that PPAR β plays a crucial role for the regulation of mitochondrial biogenesis and the enzymatic antioxidant defense system.^{73–75} Moreover, PPAR β promotes physiological cardiac remodeling.⁷⁶ Based on these findings, PPAR agonists were proposed to be beneficial for treatment of heart failure. However, the currently available PPAR agonists show significant side effects, thereby limiting a general use.^{77,78}

PPARs and their associated co-regulators are extensively regulated by PTMs, such as ubiquitylation and SUMOylation in different cells types. Although SUMO-dependent regulation of PPARs has not been studied in heart so far (for detailed review, see Wadosky and Willis⁷⁹), it seems reasonable to assume that this type of PPAR regulation also acts in cardiomyocytes.^{80,81} SUMO-modification represses the transcriptional activity of PPAR isoforms by modification of the ligand-binding domains in PPAR α and PPAR γ .^{79,82} Additional SUMOylation sites for SUMO1 conjugation are present in the D domain of PPAR α and in the activation domain of PPAR γ . In skeletal muscle cells, SENP2-regulated deSUMOylation of PPAR γ 1 controls fatty acid oxidation and ATP production,⁸³ whereas SENP2 inhibits glycolysis and induces glucose oxidation in mouse embryonic fibroblasts.⁸⁴ In light of these findings, it is tempting to speculate that SUMO-dependent repression of PPARs play a role in the control of cardiac metabolism.

Peroxisome proliferator-activated receptor coactivator-1 α (PGC-1 α), the co-activator of PPAR/RXR α (9-cis retinoic acid receptor α), plays a crucial role in heart physiology, as well as a potent regulator of mitochondrial biogenesis.^{85–87} PGC-1 α upregulation in long-term exercise represents a beneficial adaptive mechanism by which striated muscles increase the capacity of mitochondrial oxidation.^{87–90} PGC-1 α is SUMOylated by the E3 SUMO ligases PIAS1 and PIAS3, resulting in inhibition of its transcriptional activity by promoting interaction with the corepressor RIP140 (receptor-interacting protein 140).⁹¹ Enhanced deSUMOylation by transgenic overexpression of SENP1 in the heart causes activation of PGC-1 α and induces cardiomyopathy.⁵² However, at present, it is not clear whether the increased activity of PGC-1 α solely relies on deSUMOylation of PGC-1 α by SENP1⁵² or whether other regulatory events participate in this phenomenon. In fact, SENP1 also deSUMOylates MEF2C, a member of the MADS-box transcription factors, which controls expression of PGC-1 α in the heart.⁵² Because SUMOylation inhibits the activity of MEF2 factors,^{92–94} SENP1-mediated activation of MEF2C will increase PGC-1 α transcription. Future experiments might disclose the relative input of each pathway for SENP1-mediated activation of PGC-1 α in transgenic mouse hearts.⁵²

Notably, SUMO modification of ERK5 (extracellular signal-regulated kinase 5) may also contribute to SUMO-dependent repression of both PPARs and MEF2. ERK5 is an atypical mitogen-activated protein kinase functioning as a transcriptional co-activator for PPARs and MEF2 in the heart.^{95–97} SUMOylation of ERK5, which is strongly induced in diabetic animals, dampens its transcriptional activity. In

diabetic mice, this was linked to ventricular dysfunction after myocardial infarction.⁹⁶

Acetylation/deacetylation represents a different way to regulate the activity of PPARs and PGC-1 α . Sirtuin 1 (SIRT1), an NAD⁺-dependent histone deacetylase (HDAC), plays an important role in cardiac metabolism.⁹⁸ SIRT1 increases the activity of PPARs and PGC-1 α by deacetylation to induce fatty acid oxidation and mitochondrial biogenesis.⁹⁹ Hence, loss of SIRT1 activity leads to dilated cardiomyopathy in adult hearts accompanied with mitochondrial dysfunction and reduced mitochondrial gene expression.¹⁰⁰ Nevertheless, SIRT1 also controls the acetylation status of MEF2 transcription factors, which as outlined above regulate PGC-1 α expression. Intriguingly, Zhao et al showed that MEF2 is modified by either acetylation or SUMOylation. Deacetylation of MEF2 by SIRT1/HDAC4 at a specific lysine residue allows SUMOylation (acetylation/SUMOylation switch), thereby dampening MEF2 activity.¹⁰¹ Interestingly, SIRT1 itself undergoes SUMOylation at the C-terminal region (K734), which enhances its deacetylase activity at least 2-fold.¹⁰² However, it is not clear whether SUMO modification of SIRT1 causes a general increase in its activity or enhances affinity to a certain pool of substrates. On the other hand, SENP1 and SENP2 both interact with SIRT1, leading to its inactivation.¹⁰² Although this mechanism has not been analyzed in the heart to date, MEF2 might be activated by either enhanced acetylation (as a consequence of inactivated SIRT1 by SENP1) or by direct deSUMOylation via SENP1, thereby inducing PGC-1 α expression. Further experiments are necessary to study the relevance of SUMOylation/deSUMOylation in cardiac metabolism for the regulation of SIRT1/MEF2/PGC1- α /PPAR activity.

Adenosine monophosphate-activated protein kinase (AMPK) is a master regulator of cardiac metabolism (reviewed in Palomer et al¹⁰³ and Zaha and Young¹⁰⁴). Similar to SIRT1, AMPK positively regulates the PPAR/PGC-1 α axis, leading to the activation of mitochondrial functions.^{103,104} Very recently, Rubio et al demonstrated that SUMOylation is a novel PTM of AMPK.¹⁰⁵ The authors show that modification of AMPK depends on the E3 SUMO ligase PIAS4 and SUMO2 and propose that SUMOylation stimulates AMPK activity by preventing its ubiquitin-dependent degradation (SUMOylation/ubiquitylation switch). Because the AMPK β 1 isoform is not modified by SUMO2, the SUMOylation/ubiquitylation switch may represent a selective mechanism to activate certain isoforms of AMPK under specific conditions or in a cell type-specific manner. Activation of AMPK in the heart by SUMOylation is of outstanding interest because AMPK appears to act as a cardioprotective regulator in different pathological conditions. Modulation of the SUMOylation status of AMPK might thus be a future therapeutic approach to treat cardiac disease (ie, in hypertrophy, heart failure, ischemia/reperfusion [IR]).¹⁰⁴ In summary, SUMO modification of main regulators, such as PPARs, PGC-1 α , SIRT1, or AMPK, might play an important role in the control of cardiac metabolism, although much has to be learned to gain a comprehensive understanding of SUMOylation/deSUMOylation for metabolic decisions in the heart.

Cardiac Contractility

Intracellular Ca^{2+} concentrations influence contraction and relaxation of the heart. Upon excitation, extracellular Ca^{2+} enters cardiomyocytes through L-type Ca^{2+} channels, promoting Ca^{2+} release from the endoplasmic reticulum (Ca^{2+} -induced Ca^{2+} release).¹⁰⁶ Relaxation occurs after removal of Ca^{2+} through the plasma membrane sodium-calcium exchangers and the sarcoplasmic/endoplasmic reticulum Ca^{2+} ATPase 2a (SERCA2a). Activity of SERCA2a is dynamically regulated by phospholamban phosphorylation, which increases the ATPase activity of SERCA2a, resulting in enhanced Ca^{2+} transport into the endoplasmic reticulum, thereby preparing the next contraction cycle.¹⁰⁷ Dysregulation of Ca^{2+} cycling and reduction of SERCA2a activity represent hallmarks of heart failure and are recognized as major pathogenic drivers in diseased hearts. As reviewed extensively elsewhere,^{6,107} Hajjar et al revealed that the levels of SUMO1 (but not SUMO2/3) are decreased in a murine heart failure model and that SERCA2a is critically and positively regulated by SUMO1 conjugation.⁴⁷ Identification and mutation of the 2 major SUMOylation sites in SERCA2a (K480R and K585R) revealed that loss of SUMOylation significantly decreases ATP-binding affinity and ATPase activity of SERCA2a, respectively. Moreover, it was proposed that SUMOylation of SERCA2a (similar to AMPK) prevents ubiquitylation and subsequent proteasomal degradation, thereby increasing the stability of SERCA2a. Consistent with these data, elevated SUMO1 or SERCA2a levels induced by viral transfection increase SERCA2a activity and improve heart failure in mice. Importantly, SUMO1 does not improve cardiac parameters in a heart failure model induced by SERCA2a downregulation, demonstrating that SERCA2a is indeed the key target in this pathway.

In further support of this concept, Hajjar's group used a gene therapy approach to express SUMO1 and SERCA2a in an IR heart failure model in pigs,¹⁰⁸ which resulted in functional improvements, although the increase in ejection fraction did not reach statistically significant levels. Nevertheless, gene delivery of SUMO1 and SERCA2a might play a beneficial role in human cardiac disease. Moreover, a potential new strategy has been recently described using a small molecule activator (N106) that targets SERCA2a SUMOylation to improve ventricular function in mice with heart failure.¹⁰⁹

A more complex view of the regulation of contractility by SUMOylation has emerged by analyzing the cross talk of acetylation and SUMOylation similar to the regulation of the SIRT1-MEF2-PGC-1 α axis as discussed earlier.¹¹⁰ It is known that HDAC inhibitors exert cardioprotective effects by promoting Lys acetylation.^{111,112} More recent studies also uncovered increased global SUMOylation (mainly by SUMO1) in cardiomyocytes after HDAC inhibitor treatment, which seems to be mediated by HDAC2.¹¹⁰ Because the steady state level of SENPs did not change after inhibition of HDAC2, increased SUMOylation enhanced conjugation rather than reduced deconjugation appears to be the primary mode of action. However, changes in the level of acetylation might also have a more direct impact on the activity of SERCA2a. Hajjar et al postulated that acetylation of SERCA2a¹¹³ can be reversed by the Class III HDAC enzyme, SIRT1.⁴⁷ Future experiments

will clarify the significance of reversible acetylation versus SUMOylation of SERCA2a or other critical targets (eg, MEF2) known to regulate heart contractility.

Cardiac Protein Quality Control

Protein quality control plays an essential role in regulating cell homeostasis by preventing accumulation of misfolded or damaged proteins. The protein quality control system works in a sequential manner with molecular chaperones representing the first defence line by refolding abnormal proteins and eventually disassembling protein aggregates. Failure of chaperone function leads to activation of 2 major pathways, the ubiquitin–proteasome system (UPS) and selective macroautophagy, which are responsible for the removal of misfolded proteins.¹¹⁴ Recent work points to an interconnection of the SUMO system with the UPS for removal of misfolded or aggregated proteins via the SUMO-targeted E3 ubiquitin ligase pathway. In this pathway, the aberrant proteins are first polySUMOylated by specific E3 SUMO ligases of the tripartite motif family. PolySUMOylation serves as a signal for recognition by distinct E3 ubiquitin ligases, such as RNF4 or RNF111 (ring finger protein-4/111) that bind to the SUMO2/3 chains through tandem SUMO-interacting motifs, ubiquitylate misfolded proteins, and direct them to UPS degradation.^{115–119}

There is increasing evidence that misfolded proteins (forming the so-called preamyloid oligomers) contribute to the pathogenesis of cardiac disease and heart failure under various conditions.^{120–123} Work from Gupta et al indicates that the SUMO system triggers cardiac quality control by activating protein degradation through the proteasome system.^{124,125} Knock down of the E2 SUMO conjugating enzyme Ubc9 in cardiomyocytes causes accumulation of protein aggregates, and lack of Ubc9 impairs cardiac function in a heart model of proteotoxicity, whereas overexpression of Ubc9 reduces formation of protein aggregates by stimulation of the UPS system. Although the underlying mechanisms are not completely clear yet, it is plausible that Ubc9 stimulates SUMO-targeted E3 ubiquitin ligase-mediated clearing of protein aggregates in cardiomyocytes.^{124,125} Ubc9-mediated SUMOylation of other components of the UPS or the autophagy machinery may also trigger protein quality control.¹²⁶ The Beclin1-VPS34 (vacuolar protein sorting 34) complex, which serves a critical function in autophagosome formation, is stabilized by SUMOylation,¹²⁷ another possible mechanism by which SUMOylation might regulate removal of toxic protein aggregates. Understanding the mechanisms that determine specificity of the SUMO system for protein aggregates might lead to novel therapeutic interventions in cardiac disease that are linked to protein aggregates.^{128–130}

Cardiac Stress Adaptation

The heart uses various adaptation mechanisms to cope with cellular stress. However, metabolic, hypertrophic, ischemic, or oxidative stress responses might also lead to pathological adaptation, resulting in cardiac dysfunction and heart failure. Identification of cardioprotective factors, which prevent or reverse pathological adaptations, is an important goal in translational medicine.

Enhanced cellular SUMOylation seems to play a beneficial role to protect the brain during ischemia, stroke, or hibernation torpor (for detailed review, see Guo and Henley¹³¹ and Lee and Hallenbeck¹³²), which might represent a paradigm for the heart as well. Data obtained in animal models and human patients suggest that balanced SUMO conjugation/deconjugation is critical for cardiac stress adaptation.^{47,50,124,125} A beneficial effect of the cardiotropic recombinant adeno-associated virus-SUMO1 transgene has been described in the experimental model of transverse aortic constriction, in which cardiac hypertrophy and heart failure is induced by pressure overload. Enhanced SUMOylation suppresses the hypertrophic phenotype in this model, and SUMO1 expression in cultivated cardiomyocytes inhibits hypertrophic responses.⁴⁸ Although the full range of SUMO1 targets in the heart is not known, it is likely that SERCA2a is one of the relevant substrates by which enforced SUMO1 modification exerts its cardioprotective effects in certain pathological conditions.⁴⁷

However, another mouse model with constitutive heart-specific expression of SUMO2 revealed a more complex scenario characterized by dose-dependent induction of hypertrophy and cardiomyopathy.⁵⁰ Increased global SUMO2/3 conjugation seems to induce apoptosis via modification of calpain-2 and calpastatin, crucial components of the proteolytic system that is activated upon cell death. Increased expression of SUMO2 represses calpastatin by inducing its breakdown, which in turn enhances the enzymatic activity of calpain-2 normally inhibited by calpastatin.⁵⁰ It is possible that the different outcomes of forced SUMO1 or SUMO2 expression result from modification of distinct sets of substrates targeted by either SUMO1 or SUMO2. In addition, the expression level and the time course of expression is certainly a critical factor. Because many cellular processes require coordinated cycles of SUMO conjugation and deconjugation of key regulators, a supraphysiological expression level of any component of the SUMOylation system will most likely cause unwanted adverse effects.

The deSUMOylating enzyme SENP1 seems to play an important role for balancing SUMO conjugation/deconjugation in cardiomyocytes in response to hypertrophic stimuli as indicated by the increased expression of SENP1 in hypertrophic and failing hearts of human patients.⁵² The calcineurin-NFAT3 (nuclear factor of activated T-cells-3) pathway, which is activated during cardiac hypertrophy, induces expression of SENP1. This might be a compensatory effect in the initial phase of hypertrophy, limiting the glycolytic metabolic switch in dysfunctional heart, because SENP1 enhances cardiac PGC-1 α expression and subsequent mitochondrial gene activation. However, uncontrolled mitochondrial biogenesis ultimately causes loss of sarcomeric structure, cardiomyopathy, and heart failure. Therefore, the prolonged SENP1 expression observed in human failing hearts might contribute to cardiac dysfunction by altering mitochondrial function.⁵² This conclusion is also supported by the finding that forced expression of SENP1 through a virus-based approach induces dilated cardiomyopathy and mitochondrial abnormalities in adult mice⁵² (Table).

Similar deleterious effects of enhanced SUMO deconjugation in adult heart were also observed after forced expression of SENP2 or SENP5 in the heart, leading to cardiomyopathies and cardiac dysfunction^{54,55} (Table). Increased expression of

SENP5 is linked to alterations in mitochondrial dynamics and mitochondrial fission, which is assumed to rely on enhanced recruitment of mitochondria and oligomerization of the dynamin-related protein (DRP1).¹³³ Several lines of evidence indicate that transient SUMOylation of DRP1 controls the dynamic association of DRP1 with mitochondria. McBride and co-workers proposed that SUMO1 conjugation enhances retention of DRP1 on the membrane after recruitment of DRP1 to mitochondria, followed by disassembly of the DRP1 oligomer via de-SUMOylation once fission is complete.^{134–136} Recently, SENP5, SENP3, and SENP2 were identified as SUMO deconjugases of DRP1 (Figure 2). It was proposed that recruitment of SENP5 to mitochondria, which normally occurs during mitosis, drives mitochondrial fragmentation by enhancing the SUMO cycle on DRP1 in concert with the SUMO E3 ligase MAPL (mitochondrial-anchored protein ligase).^{135–137} Furthermore, mitochondrial-anchored protein ligase SUMOylation of DRP1 stabilizes an endoplasmic reticulum/mitochondrial platform required for cell death.¹³⁸ In contrast, lack of SENP5 traps SUMO1-conjugated DRP1 at the mitochondrial membrane, ultimately leading to apoptotic cell death. In a cardiac-specific transgenic mouse model, Kim et al confirmed that forced expression of SENP5 decreases the level of SUMO2/3-conjugated DRP1 in the heart, which according to their model results in enlarged mitochondria with several functional abnormalities and enhanced apoptotic cell death⁵⁵ (Figure 2). Along the same line, reduced SUMO2/3 conjugation of DRP1 mediated by SENP3 in an *in vitro* cellular model of neuronal IR resulted in mitochondrial association, cytochrome c release, and cellular apoptosis^{139,140} (Figure 2). Again, paralogue-specific effects of different SUMO family members might explain these discrepancies. McBride et al mainly focused on DRP1-SUMO1 conjugates, whereas in the SENP5 mouse model and in the neuronal cell culture model, DRP1-SUMO2/3 conjugates were studied. Conjugation of SUMO2/3 to DRP1 might prevent its association with mitochondria and cell death, whereas SUMO1 modification might promote residency of DRP1 at mitochondria to induce apoptosis. In line with this idea, neuron-specific SENP2 KO mice suffer from neurodegeneration via dysregulation of mitochondrial functions, which was attributed to removal of SUMO1 from DRP1 by SENP2 counteracting neuronal apoptosis.¹⁴¹ To date, the available data suggest that an imbalance of SUMO conjugation/deconjugation affects cell functions by disturbing mitochondrial dynamics. Such a mechanism might be particularly relevant for the response of the heart to hypertrophic or ischemic stimuli, but a full understanding of the role of SUMO-dependent signaling in this process will require further system-wide proteomic approaches. The data on DRP1 also exemplify that the outcome of an unbalanced SUMO conjugation or deconjugation critically depends on the cell type, as well as the physiological or pathological context. Hence, lack of SUMOylation and enhanced SUMOylation may not necessarily cause opposing phenotypes. In fact, disruption of the modification-demodification cycle in either way seems to affect target protein function in a very similar manner.

Mitochondrial abnormalities induce not only metabolic alterations or cellular apoptosis but profoundly influence the production of reactive oxygen species (ROS). Increased ROS

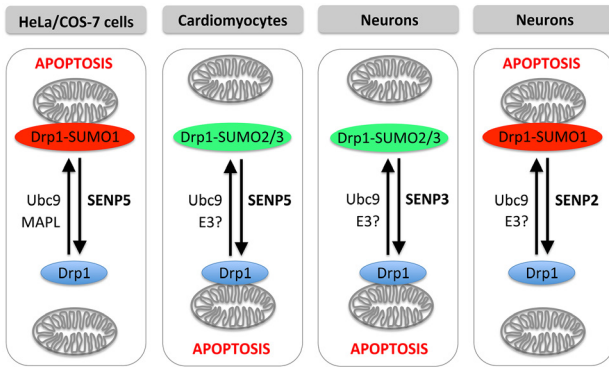


Figure 2. Effect of differential SUMOylation of dynamin-related protein (Drp1) on apoptotic cell death in various cell types. Mitochondrial fission is achieved through mitochondrial recruitment and oligomerization of the DRP1. Transient SUMOylation controls the dynamic association of DRP1 with mitochondria affecting both mitosis and apoptosis. SENP5, SENP2, and SENP3 were identified as SUMO deconjugases of DRP1 in different cell types. Silencing of SENP5 in COS-7 or HeLa cells increases modification of DRP1 by SUMO1 in a process mediated by the E3 SUMO ligase mitochondrial-anchored protein ligase (MAPL), which enhances binding to mitochondria, eventually resulting in mitochondrial fragmentation and cellular apoptosis. Cardiac-specific overexpression of SENP5 in mouse hearts decreases DRP1-SUMO2/3 levels, which in turn promotes association of de-SUMOylated DRP1 to mitochondria, thereby inducing apoptosis. Similarly, cortical neurons subjected to in vitro ischemia re-express SENP3 after re-oxygenation. Induced deconjugation of DRP1-SUMO2/3 increases the residence time of DRP1 at mitochondria and promotes neuronal apoptosis. In contrast, the cytoplasmic isoform of SENP2 (SEN2S) exerts neuroprotective effects by deconjugating DRP1-SUMO1. SUMO1 conjugation of DRP1 might thus enhance mitochondrial association and promote fragmentation and apoptosis, whereas SUMO2/3 conjugation seems to decrease mitochondrial localization of DRP1 and reduce cell death. SENP indicates SUMO/sentrin-specific protease; and SUMO, small ubiquitin-like modifier.

generation from different sources plays a major role in various cardiac pathologies, including cardiac hypertrophy and IR injury.^{142,143} In fact, ROS are a major cause of IR injury in heart, as well as in brain.¹⁴⁴ Importantly, SUMO2/3 conjugation increases during cerebral IR injury in animal models, although it is still debated whether SUMOylation occurs mainly during reperfusion or already during the initial ischemia period.^{131,132,145}

Several lines of evidence suggest that the dynamic regulation of SUMOylation serves as a cytoprotective pathway.^{131,132} For example, transgenic mice overexpressing Ubc9 show a diminished infarct size in the brain after focal cerebral ischemia when compared with control animals.⁴³ Moreover, in hibernating animals, which provide a model of natural tolerance to ischemia, massive SUMOylation is observed in brain.¹⁴⁶ The mechanisms that trigger the dynamics of SUMOylation in ischemia and reoxygenation and elicit cytoprotection are not yet fully understood. However, there is accumulating evidence that the stability and activity of the enzymatic components of the SUMO system are tightly regulated by the cellular redox status. One key example is the SUMO isopeptidase SENP3 which is one of the most prominent genes upregulated on the very first day after traumatic brain injury.¹⁴⁷ Under basal conditions,

SENP3 is rapidly degraded via the UPS.¹⁴⁸ Mild oxidative stress by oxidation of cysteine 243 and 274 in SENP3 protects SENP3 from degradation, thus enhancing SENP3-mediated deSUMOylation events^{149,150} (Figure 3). In contrast, SENP3 activity is inhibited by oxidation of the catalytic cysteine residue during severe oxidative stress (Cys532)^{149,150} (Figure 3). Hence, SENP3 deSUMOylates SUMO2/3 targets, such as the coactivator p300, during mild oxidative stress, thereby increasing p300 binding to HIF1 α and enhancing its transcriptional activity. In contrast, strong oxidative stress prevents p300 from deSUMOylation and HIF1 α activation (Figure 3). The biphasic redox sensing of SENP3 may also contribute to cardiac IR injury, but clear experimental evidence is still missing. In contrast, the cardiac function of SENP1 is better understood. IR injury in human and mouse hearts and in isolated cardiomyocytes leads to increased SENP1 levels, and heterozygous SENP1^{+/-} mice develop larger myocardial infarct lesion than control animals, indicating that SENP1 limits IR injury.^{151,152} Because SENP1-mediated de-SUMOylation stabilizes HIF1 α under hypoxic conditions⁵¹ and elevated HIF1 α protects against cardiac IR injury, deSUMOylation of HIF1 α by SENP1 or SENP3 might be critical for cardioprotection.^{152,153} Furthermore, low levels of ROS activate SENP1 similar to SENP3, whereas high ROS levels induce SENP1 inactivation (Figure 3). SENP1 acts also as a factor to develop tolerance to hypoxia, contributing to hypoxia-driven vascular endothelial growth factor expression and angiogenesis in endothelial cells,^{154,155} and protects against hydrogen peroxide-induced cell death, whereas its depletion enhances apoptosis in vitro.^{156,157} It is, therefore, obvious that the simple model of a beneficial role of enhanced SUMOylation

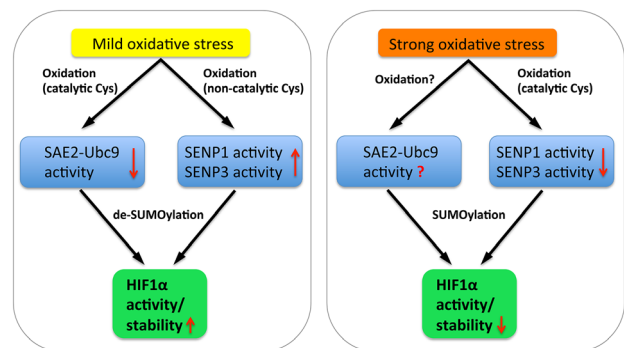


Figure 3. Regulation of hypoxia-inducible factor 1 alpha (HIF1 α) by small ubiquitin-like modifier (SUMO) conjugation/deconjugation in mild versus strong oxidative stress. The SUMOylation status of HIF1 α is highly redox-sensitive and regulated by the relative activities of SUMO conjugating (SAE2, Ubc9) versus deconjugating enzymes (SENP1, SENP3). Mild oxidative stress inhibits both SAE2 and Ubc9 enzymes by reversible covalent crosslinking of catalytic cysteine (Cys) residues. In contrast, oxidation of a noncatalytic Cys residue protects both SENP1 and SENP3 from degradation under the same conditions. SENP1 directly deSUMOylates HIF1 α and increases its stability, whereas SENP3 deSUMOylates p300, enhancing its co-activator function towards HIF1 α . In contrast, SENP1 and SENP3 are inactivated or degraded during strong oxidative stress, resulting in enhanced SUMOylation of both p300 and HIF1 α , which in turn inhibit HIF1 α activity and stability, respectively. SAE2 indicates SUMO activating enzyme 2; SENP, SUMO/sentrin-specific protease; and Ubc9, ubiquitin conjugating enzyme 9.

after post-ischemic injury needs to be refined because, as discussed earlier, deSUMOylation by SENP1 can be protective as well. Instead, based on both cardiac and noncardiac studies, a more complex picture emerges, indicating that timely controlled and finely balanced SUMOylation of specific substrates but not bulk conjugation/deconjugation is critical. Moreover, a general note of caution should be raised when it comes to the interpretation of SENP overexpression or depletion phenotypes. SENPs function not only in demodification, but also in processing of the SUMO precursors.¹⁵⁸ Depletion of a given SENP, therefore, does not necessarily lead to enhanced conjugation in all cases, but may also inhibit conjugation. The consequence of SENP depletion on modification of a given substrate must, therefore, always be experimentally validated.

Changes in the cellular redox status not only affect the deconjugating enzymes, but also influence the SUMO conjugation system in a dose-dependent way. Moderate doses of ROS inhibit global SUMOylation by reversible covalent cross-linking of the catalytic cysteine residues of SUMO-activating enzyme 2 and Ubc9 enzymes,¹⁵⁹ which might potentially contribute to decreased SUMOylation events induced by mild oxidative stress in the heart (Figure 3). In contrast to the global effect on SUMOylation after oxidation of catalytically relevant Cys residues, PTMs of Ubc9 distinctively modulate its affinity toward specific substrates.^{129,130} Hypoxia has recently been shown to redirect Ubc9 to a subset of substrates, including CBP or Elk-1, thus limiting their transcriptional activity by enhanced SUMOylation. Targeting of Ubc9 to these substrates is directed through SIRT1-mediated deacetylation of lysine 65, which is triggered in hypoxic cells.¹³⁰ These data highlight the dynamics of PTMs of the main actors of the SUMO conjugation system, modulating SUMO-dependent regulatory mechanisms under various stress conditions. It is certainly of crucial importance to determine the relevance of these pathways for homeostatic and pathological regulatory processes in the heart.

The SUMOylation target SIRT1 does not only play an important role in the cardiac metabolism but also acts as a cardioprotective and adaptive factor under different cardiac stress conditions (for detailed review, see Kwon and Ott¹⁶⁰, Chong et al¹⁶¹, and Yang et al¹⁶²). Nucleocytoplasmic shuttling is essential to target SIRT1 into the nucleus where it exerts antioxidant and antiapoptotic functions. In the aging heart, nuclear translocation of SIRT1 is impaired during ischemic stress, hindering adaptive responses.¹⁶³ SUMOylation of SIRT1 increases its deacetylase activity, which is essential for adapting to genotoxic stress.¹⁰² At present, it is not known whether SUMOylation regulates the nuclear versus cytoplasmic localization of SIRT1 or increases the activity of SIRT1 by other means. Similarly, the dominant form of SIRT1-SUMOylation in the heart (SUMO1- or SUMO2/3-conjugation) has not been identified. On the other hand, SIRT1 activity is regulated by SENP1 and SENP2,¹⁶² and oxidative and genotoxic stress lead to increased interaction with SENP1 inducing deSUMOylation and subsequent inactivation of SIRT1.¹⁰² Further research focusing on the interplay of acetylation/deacetylation and SUMOylation/deSUMOylation events will open new insights into the complex machinery controlling stress responses in the heart.

Concluding Remarks and Future Perspectives

Several key findings in the last few years have unravelled an important role of the SUMO system for organ function under physiological and pathological conditions. Current evidence suggest that SUMO1 maintains normal cell physiology in the brain, whereas SUMO2/3 is involved in cellular stress responses.^{8,164} Enhanced global SUMOylation (mainly by SUMO2/3) exerts neuroprotective effects in IR injury, highlighting the potential therapeutic value of the SUMO system. In contrast, the role of SUMOylation in cardiovascular system is just emerging. Unfortunately, very little is known about the exact function of SUMO1 versus SUMO2/3 in the heart and their specific cellular targets. In the past, it has been challenging to identify and analyze endogenous SUMO targets because of their low abundance, but novel mass spectrometry techniques allow highly sensitive, proteome-wide identification of SUMO targets in tissue culture and animal models,¹²⁸ which will help to define the SUMO proteome in cardiac tissues under physiological and pathological settings. The spray or group modification theory of SUMOylation predicts that a set of functionally related and spatially linked proteins are simultaneously regulated by SUMO.¹⁶⁵ Therefore, it will be a challenge to distinguish functionally relevant SUMO targets from less relevant byproducts in a given cellular setting. The developmental master regulators GATA4, Nkx2.5, and MEF2, as well as key regulators in the adult heart (SERCA2a, PPARs, PGC-1 α , SIRT1, AMPK, DRP1, and HIF1 α), might already represent the most significant SUMO targets, but it seems very likely that additional important target proteins will be disclosed. Moreover, several contradictory results have been published, indicating the needs to conduct careful system-wide biochemical approaches in established animal models. We are confident that a comprehensive understanding of SUMO functions in the heart under physiological and pathological conditions will eventually result in novel SUMOylation-based therapeutic strategies to combat heart diseases.

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Disclosures

None.

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