Published in final edited form as:

Curr Opin Pharmacol. 2010 April ; 10(2): 146–155. doi:10.1016/j.coph.2009.12.001.

SUMO — A post-translational modification with therapeutic potential?

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Abstract

Sumoylation is a covalent modification, which is mediated by small ubiquitin-like modifier (SUMO) polypeptides. A growing body of evidence has shown that sumoylation affects the functional properties of many substrates in the regulation of cellular processes. Recent reports indicate the critical role of sumoylation in human diseases including familial dilated cardiomyopathy, suggesting that targeting of sumoylation would be of considerable interest for novel therapies. Even though hundreds of SUMO substrates have been identified, their pathophysiological roles remain to be determined. Among them, ERK5-sumoylation has recently been linked to diabetes and implicated in endothelial dysfunction and cardiomyocyte apoptosis *in vivo*. These findings support the idea that ERK5-sumoylation is a novel therapeutic target for the treatment of diabetes-related cardiovascular diseases.

Keywords

SUMO; sumoylation; ERK5; diabetes; cardiovascular disease

Introduction

SUMO (small ubiquitin-like modifier) covalently attaches to certain residues of specific target proteins and alters a number of different functions depending on the substrates. Sumoylation counteracts ubiquitination and subsequent proteosomal degradation via competition with the same lysine residue of substrates [1,2]. In addition to a decoy mechanism of sumoylation in the ubiquitin-proteasome system, sumoylation is involved in most cellular functions including subcellular localization, DNA-binding and/or transactivation of transcription factors [3,4]. Recent reports indicate that regulation of sumo-conjugation contributes to the pathogenesis and development of cardiovascular complications [5–8]. The purpose of this review is to summarize the physiological role of sumoylation-related molecules in the cardiovascular system, the pathological role of ERK5-sumoylation in diabetes-related cardiovascular diseases, and its therapeutic potential.

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Sumoylation process

Sumoylation is a dynamic and reversible process that is regulated by both conjugation and deconjugation pathways (Figure 1). The regulatory mechanism is analogous to ubiquitin-conjugation system, but sumoylation is regulated by a different set of enzymes. Most enzymes are evolutionary conserved from yeast to humans. To conjugate SUMO to the substrates, the proform of SUMO needs to be cleaved by sentrin/SUMO-specific proteases (SENPs) that hydrolyse C-terminal end to expose Gly-Gly motif [9]. SENP has not only a hydrolase activity but also an isopeptidase activity, which regulates deconjugation of sumoylated substrates [9,10]. The mature form of SUMO is activated in an ATP-dependent manner by an E1-activating enzyme, which consists of an SAE1-SAE2 heterodimer [11]. Activated SUMO is transferred to Ubc9 (E2 conjugase) to form a thioester bond and is subsequently attached to the ϵ -amino group of a lysine residue in the target substrates [12]. Even though Ubc9 itself associates with SUMO and transfers SUMO to targets, specific SUMO E3 ligases are required for efficient modification. Several classes of SUMO ligases have been identified: the family of protein inhibitor of activated STAT (PIAS; PIAS1, PIAS2(x), PIAS3, PIAS4(y)), Polycomb-2 protein (Pc2), and RanBP2/Nup358, a component of the nuclear pore complex. Since E1 and E2 enzyme are limited in number, it has been considered that E3 ligases and SENPs are responsible targets for fine-tuning the specific regulatory pathway of SUMO substrates.

Roles of sumoylation-related molecules in cardiovascular system SUMO

In contrast to yeast and non-vertebrates, the mammalian genome contains multiple SUMO genes (Table 1). The sequence identity between SUMO1 and SUMO2 is about 46%, but the mature form of SUMO2 and SUMO3 are nearly identical [13,14]. Multiple proteomic screenings of SUMO substrates showed that each gene is conjugated to different target proteins, suggesting the distinct features in substrate specificity [15,16]. It has been suggested that different localization of each gene might explain the substrate specificity (Table 1). SUMO1 is mainly conjugated to substrates *in vivo*, whereas a majority of SUMO2/3 exist as free forms and respond to stress stimuli such as oxidative stress and heat shock [17].

One of the interesting differences between SUMO1 and SUMO2/3 is the ability of SUMO2/3 but not SUMO1 to form polymeric chains. Tatham *et al.* have reported that only SUMO2/3, but not SUMO1, can form isopeptide bonds between the C-terminal glycine residue of one SUMO molecules and Lys 11 of another molecule of SUMO2/3, and lead to poly-sumoylation [18]. Interestingly, it has been reported that the functional consequence of poly- and monosumoylation might be different. Especially, the RING domain-containing ubiquitin E3 ligase, RNF4, only targets poly-SUMO-mediated proteins for degradation mediated by ubiquitin [19–21]. Therefore, since only SUMO2/3 can form polymeric chains, SUMO2/3-targeted substrates may have more chances to be ubiquitinated and subsequently degraded.

The functional roles of sumoylation in mammals are limited to SUMO1 because data of other gene knockouts is not available. Given the fact that SUMO1^{-/-} mice show no developmental defect, SUMO2/3 might be able to compensate for SUMO1 deficiency [22,23]. However, another group showed that haploinsufficiency of SUMO1 is related to the development of cleft lip and palate via Eya1-sumoylation in part [24,25]. Shao and co-workers reported that hypoxia could increase SUMO-1 expression and HIF1 α -sumoylation in mice hearts [26]. It is therefore interesting to investigate if SUMO1 deficiency affects cardiac dysfunction after ischemic injury via HIF1 α regulation in SUMO1^{-/-} mice. We will discuss the role of HIF1 α -sumoylation later in this review.

The human genome encodes a unique modifier of SUMO4, which is mainly expressed in the kidney, lymph node and spleen [27]. Genetic mutation of SUMO4 at Met 55 has been discovered and human study showed that SUMO4-M55V mutation is correlated to type 1 diabetes [27–29]. Guo *et al.* found that SUMO4-M55V mutation increased TNF α -induced nuclear factor κ B (NF- κ B) activation compared with wild type and proinflammatory *IL12B* expression is significantly higher in peripheral blood monocytes from individuals carrying SUMO4-M55V than in control individuals, suggesting the susceptibility for inflammation of SUMO4-M55V substitution [27]. The detailed mechanisms of SUMO4 were suggested by the transcriptional regulation of several transcription factors. For example, SUMO4 negatively regulates NF- κ B transcriptional activity via I κ B α -sumoylation, leading to inhibit its ubiquitin-dependent proteolysis and stabilize I κ B α [30,31]. It has been reported that the transcriptional activity of heat shock factor and activating protein-1 are also affected by SUMO4 [28,29], but the exact implications of SUMO4 sumoylation of two transcription factors in terms of susceptibility to type 1 diabetes remain to be determined.

Lamin A polymorphism associated with sumoylation in familial dilated cardiomyopathy

Aside from single nucleotide polymorphism (SNP) of SUMO4 gene, substitution of lamin A at Glu 203 (E203) has been identified [32,33]. Lamin A plays an important role in nuclear architecture and its mutations are involved in many human diseases, including familial dilated cardiomyopathy (FDC) and muscular dystrophies [34,35]. Recently Zhang and Sarge reported that Lys 201 is a specific target site of lamin A-sumoylation and sumoylation defective mutant (K201R) reveals abnormal nuclear localization, suggesting the critical role of sumoylation in nuclear function of lamin A [6]. Interestingly they found that two different SNPs of lamin A related with FDC, E203G and E203K, have a defect in lamin A-sumoylation. Since glutamic acid is also important for the SUMO-conjugation to the target lysine in the classical consensus sequence [31] (ψ KxE/D; ψ represent a bulky hydrophobic residue and E/D represent an acidic residue), it is possible that E203 is involved in SUMO-conjugation at K201 residue in 200-MKEE-203 consensus sequence of lamin A. Furthermore they provide clinical relevance with patient fibroblasts, which showed abnormal lamin A localization and nuclear morphology similar to cells expressing lamin A-K201R, -E203G, and -E203K mutants [6]. These findings provide a molecular insight of pathogenesis of lamin A-related FDC, indicating that sumoylation has a significant impact on cardiomyopathy *in vivo*.

Ubc9 (E2)

Ubc9 is the single E2 conjugase for sumoylation process. The physiological roles of sumoylation in mammals have been addressed in mice deficient for Ubc9 E2 conjugase [36]. Homozygous Ubc9 deficiency reveals embryonic lethality due to the developmental defect at the early postimplantation stage. Ubc9-deficient blastocysts showed severe abnormality in nuclear organization, chromosome segregation, PML nuclear bodies and RanBP2-dependent nuclear pore complex, suggesting that most SUMO substrates are nuclear proteins and involved in cellular viability. Since Ubc9 itself conjugates SUMO to substrates, it is intriguing how Ubc9 regulates some of substrates without distinct E3 ligases.

E3 ligase

E3 ligases have a tuning function at the last step of sumoylation process [37]. In contrast to single E1 and E2 enzymes, many of E3 ligases have been identified. Mammalian PIAS (The protein inhibitor of activated STAT) family is a major SUMO E3 ligase, which consists of PIAS1, PIAS2 (PIASx), PIAS3, and PIAS4 (PIASy). In addition to PIAS family, RanBP2 and Pc2 have been identified as other types of SUMO E3 ligases. In this review we will focus on the role of PIAS1 and PIAS4 because both knock out mice were relatively well studied, as we will discuss below (Table 2).

PIAS1 was originally identified as a transcriptional repressor of STAT1 and NF- κ B by blocking the DNA-binding activity to specific promoters with SAP (scaffold attachment factor-A/B, acinus and PIAS) domain [38]. Later it turned out that PIAS family proteins have SUMO ligase activity characterized by an SP-RING domain, which is similar to that of ubiquitin E3 ligases [39]. Besides SAP and SP-RING domains three other conserved domains of PIASs are detected; 1) PINIT domain, which regulates nuclear retention of PIAS family, 2) AD (highly acidic domain) or SIM (SUMO-interacting motif) domain, which is the SUMO binding domain, and 3) the Ser/Thr rich region (Figure 2). PIAS1 null mice showed high level of STAT1 and NF- κ B activity, and were susceptible to viral and microbial infections [40]. However, the role of PIAS1 as the SUMO E3 ligase and its functional target *in vivo* remains unclear. Gene targeting mice in PIAS4 have no distinct phenotype and have a modest reduction in interferon and Wnt signaling compared to non-transgenic littermate control mice (NLC) [41]. However, another group reported that there are no significant changes in terms of interferon signaling [42]. It is possible that different methods for gene targeting may cause the functional difference. Interestingly, Tahk and colleagues found that the survival rate after lipopolysaccharide injection is significantly reduced in the same line of PIASy^{-/-} mice, indicating a specific response to cellular stress [43]. They also found that despite the mild phenotypes of single null mice, double transgenic mice (PIAS1^{-/-}/PIASy^{-/-}) are embryonic lethal. These results support the idea that PIAS family proteins have functional crosstalk with each other in the physiological condition.

The biochemical studies using mouse embryonic fibroblasts (MEFs) from PIAS4^{-/-} mice demonstrated that PIAS4 regulates cellular senescence and apoptosis in a p53-dependent manner [44]. They found that overexpression of PIAS4, but not other PIAS proteins, stimulates cellular senescence and apoptosis by p53-sumoylation and oncogenic RAS-induced senescence is reduced in PIAS4^{-/-} MEFs. In general p53 is a key regulator of cell death through its transcriptional activation. However, emerging evidence showed the transcription-independent role of p53 in apoptosis via p53-BCL2 binding, which leads to inhibition of BCL2 function in mitochondrial membrane integrity [45]. Recently we found that PKC ζ -PIAS4 signal nodule induces p53-sumoylation-dependent nuclear export, p53-BCL2 binding, and subsequent apoptosis in endothelial cells (ECs) (KS Heo *et al.*, unpublished). Further work will be necessary to prove the importance of PIAS4 and p53-sumoylation in atherosclerosis with PIAS4^{-/-} mice.

E3 ligases are key regulators for sumoylation and functional consequences *in vivo*. However, what is lacking is the molecular mechanism how ligase activity is regulated in physiological condition. IKK α -dependent phosphorylation site of PIAS1 has been identified at Ser 90 and this modification plays a role in the negative regulation of NF- κ B through repression [46]. TNF α -induced NF- κ B activation was enhanced in PIAS1 phosphorylation mutant and this mutant lost the binding affinity to NF- κ B promoter. Another group reported that PIAS1-mediated PPAR γ -sumoylation has an anti-inflammatory effect in monocytes/macrophages via the transrepression of NF- κ B promoter activity [47]. These data support the potential implication of PIAS1 modulator as therapeutic interventions in inflammation-related cardiovascular diseases including atherosclerosis. Recently, Stehmeier *et al.* suggested a novel mechanism that casein kinase 2 (CK2)-mediated phosphorylation of PIAS1/3 increased its binding affinity to SUMO and SUMO substrates, suggesting that kinase-mediated signal cascade may govern PIAS ligase activity to specific substrates [48]. Since CK2 has been recognized as a therapeutic target of cardiac hypertrophy [49], it would be interesting to identify the specific substrates of CK2-PIAS module-mediated sumoylation in cardiac hypertrophy.

SENPs

SUMO conjugation occurs through an enzymatic cascade, but this reaction is reversible in the process regulated by SENP. The hydrolytic activity of SENP is regulated by well-conserved C-terminus catalytic domain, but N-terminus is poorly conserved, suggesting the regulatory role of this region [9,50]. Li and colleagues showed that TNF α transiently induced SENP1 translocation from cytosol to nucleus and subsequently resulted in JNK activation and apoptosis through HIPK2-desumoylation in endothelial cells, indicating the dynamic regulation of desumoylation process in response to extracellular stimuli [51]. It has been intensively studied how SENP recognizes the specific substrates, but the molecular mechanism and physiological role of SENP regulation remain to be established. Among 7 isoforms, the functions of SENP1 and SENP2 were studied most using genetic mutations in mouse. The physiological role of SENP1 was addressed with a loss of function study by a retroviral insertional mutation of mouse SENP1 [52]. The mutant mice revealed placental defects with widespread cell death and led to death between *e12.5* and *e14.5*. Recently another group observed a similar phenotype regarding embryonic lethality in SENP1^{-/-} mice and suggested the molecular insight that SENP1 is critical for stabilization of HIF-1 α during hypoxia.

At normoxic condition, HIF1 α is hydroxylated by prolyl hydroxylase domain (PHD) proteins, which leads to the interaction with ubiquitin ligase von Hippel Lindau protein (VHL). VHL mediates poly-ubiquitination and degradation of HIF1 α and inhibits its activity. Under the hypoxic condition, PHD activity is inhibited and HIF1 α is stabilized by the inhibition of the interaction of VHL and HIF1 α . Interestingly, Cheng *et al.* have reported that hypoxia induced HIF1 α -sumoylation, and VHL recognizes not only hydroxylation but also this sumoylation form of HIF1 α and ubiquitinates/degradates it. Therefore, to make HIF1 α stabilized under hypoxia, de-sumoylation enzyme SENP1 becomes critical. SENP1 de-sumoylates HIF1 α and inhibits VHL-HIF1 α interaction and stabilizes it [53]. HIF1 α is a master transcriptional factor to regulate erythropoiesis, angiogenesis, and glucose metabolism, especially under hypoxia. Therefore, it is very reasonable that SENP1^{-/-} embryos showed severe fetal anemia stemming from deficient erythropoietin production. The interplay between hydroxylation and sumoylation of HIF1 α remains unclear. It is also intriguing to point out that hypoxia-mediated HIF-1 α stabilization is not affected in SENP2^{-/-} MEFs, indicating the substrate specificity between SENP1 and SENP2 although it is hard to distinguish the deconjugation activity *in vitro*.

Recently, Chiu *et al.* have reported that SENP2 is critical in development of trophoblast layers [54]. SENP2 knock out mice showed a defect in the cell cycle G1-S transition during placentation. The authors found a significant increase of Mdm2-sumoylation in SENP2 knock out mice. The sumoylation of Mdm2, which is a ubiquitin E3 ligase of p53, inhibited Mdm2 ubiquitin ligase activity, stabilized p53, inhibited DNA synthesis and trophoblast development [55]. Since both SENP1^{-/-} and SENP2^{-/-} die midgestation, the functional role of SENP remains largely unknown in adult organs including the heart tissue.

ERK5-sumoylation in cardiovascular diseases

Atherosclerosis, the primary cause of heart failure and stroke, is one of the most global burdens for human health care today. Epidemiological studies over the past decade have revealed that diabetes significantly affects this process via EC inflammation. Inflammation is regarded as an important initiating factor in the pathogenesis of atherosclerosis and its cardiovascular complications, and anti-inflammatory therapy has gained increasing attention in the drug development of cardiovascular diseases [56]. There is a lot of evidence showing that diabetic stimuli activate proinflammatory signaling pathways, while little is known about diabetes-mediated inhibition of anti-inflammatory responses. Our lab reported that

steady laminar flow stimulation of PPAR γ 1 activity, via activation of ERK5, contributes to the anti-inflammatory and atheroprotective effects of flow [57]. However, the molecular mechanism of the role of ERK5 in diabetes-mediated inflammatory responses remains largely unknown. Recently, we found that diabetic stimuli can inhibit ERK5 transcriptional activation through ERK5-sumoylation [7]. Here we will discuss the novel mechanism of diabetes-induced ERK5-sumoylation in the cardiovascular system, which may explain the causal relationship between diabetes and EC inflammation as well as exacerbation of cardiac dysfunction in diabetic heart.

ERK5 in Diabetes: ERK5-sumoylation

ERK5 has dual phosphorylation sites, like ERK1/2, containing TEY motif that are phosphorylated by the upstream kinase, MEK5 (Figure 3). However, ERK5 is not only a kinase but also a transcriptional coactivator characterized with a unique C-terminal transactivation domain, suggesting that its regulation and function may be different from ERK1/2 (Figure 3) [58]. ERK5 null mice resulted in embryonic lethality, which was caused by blood leakage from the vessels due to endothelial apoptosis, indicating the essential role of ERK5 in endothelial survival [59]. Previously, our group found that steady laminar flow potentially activates ERK5 [60] and flow inhibits leukocyte binding, as well as ICAM-1 and VCAM-1 expression via ERK5 activation [61]. We have recently shown that hydrogen peroxide (H₂O₂) and advanced glycation end products (AGE) inhibit flow-induced ERK5 transcriptional activation as well as the subsequent KLF2 and eNOS expression via ERK5-sumoylation in ECs [7] (Figure 4). Interestingly, both stimuli increased ERK5 phosphorylation and kinase activity, suggesting the novel mechanism of kinase activity-independent regulation of ERK5 transcriptional activity. Lysine residues 6 and 22 of ERK5 have been identified as SUMO targeting sites. The inhibitory effect of H₂O₂ and AGE in ERK5 transcriptional activity is significantly reduced in the sumoylation-defect mutant form of ERK5 (ERK5-K6/22R) compared to wild type. In addition, depletion of PIAS1 and ERK5-K6/22R mutant reversed the inhibition of KLF2 and eNOS expression in response to diabetic stimuli. Furthermore we found that ERK5-sumoylation is increased in the aortic endothelial cells from diabetic mice. These data demonstrate that inhibition of ERK5-sumoylation might be a novel therapeutic target for the treatment of diabetes-related vascular diseases including atherosclerosis.

It remains unclear how H₂O₂ and AGE induce ERK5-sumoylation. Several previous studies suggested that sumoylation could be regulated by crosstalk with other types of post-translational modifications including phosphorylation or association with binding partners that recruit sumoylation-related molecules. For example, phosphorylation-dependent sumoylation motif (PDSM: ψ KxE/DxxS/TP) has been defined as a regulatory mechanism by which proline-directed kinase-mediated phosphorylation of serine residue in PDSM increases SUMO conjugation to target substrates [62,63]. However, we failed to find any PDSM in ERK5 sequence. There is another mechanism regulated by phosphorylation that phosphorylations of PIAS proteins alter target protein functions via either sumoylation-dependent or -independent manner [46,48]. Thus, it is interesting to identify the specific kinases that are activated in diabetes and phosphorylate sumoylation-linked enzymes.

ERK5-sumoylation increases myocyte apoptosis

Epidemiological studies strongly indicate that diabetes mellitus (DM) is an independent risk factor for both mortality and morbidity following myocardial infarction (MI) [64,65], especially since post-MI left ventricular (LV) function is significantly worse in diabetic patients. However, what is lacking is a plausible relationship between diabetes and any of the known regulators of myocyte apoptosis known to play a significant role in the post-MI cardiac dysfunction. Our group has demonstrated that down-regulation of phosphodiesterase

3A (PDE3A) is associated with apoptosis and induction of inducible cAMP early repressor (ICER), a proapoptotic transcriptional repressor, providing a mechanistic framework for how angiotensin II regulates myocyte apoptosis [66,67]. Sustained elevation of ICER favors apoptosis through inhibition of cAMP response element binding protein (CREB)-mediated transcription and down-regulation of Bcl-2 (Figure 5). Interactions between PDE3A and ICER constitute an auto regulatory positive feedback loop (PDE3A-ICER feedback loop) likely to determine the fate of injured myocytes (Figure 5). However, the mechanism between DM-mediated exacerbation of LV dysfunction and ERK5-mediated ICER reduction remains unknown. This question has been addressed with DM + MI model which was established by MI surgery one week after streptozotocin injection [8]. We found that streptozotocin-induced hyperglycemia exacerbates LV dysfunction and mortality after MI [8]. In support of the idea that PDE3A-ICER cycle plays an important role in myocyte apoptosis and heart failure, we found a reduction of PDE3A and ICER induction in diabetic heart after MI. Since diabetic stimuli induced ERK5-sumoylation in ECs, it can be assumed that the level of ERK5-sumoylation is elevated in diabetic hearts after MI. Not surprisingly, ERK5-sumoylation was significantly increased in diabetic heart after MI and this induction is reversed with insulin treatment, suggesting the specific role of ERK5-sumoylation, but not the toxic effect of streptozotocin, on the exaggerated LV dysfunction and development of heart failure after MI in DM mice. Biochemical studies showed that oxidative stress and high glucose increase ERK5-sumoylation and decrease ERK5 transcriptional activity. We also found that H₂O₂-induced myocyte apoptosis is significantly inhibited in the presence of si-PIAS1 and adeno-viral vector expressing ERK5-K6/22R mutant compared to wild type. These results indicate that ERK5-sumoylation is a key modulation in myocyte apoptosis through ICER induction.

MEK5-ERK5 interaction protects cardiac dysfunction

It has been shown that ERK5 activation prevents MEF2-sumoylation via phosphorylation leading to an increase in its transcriptional activity [68]. Thus, we checked the effect of MEK5 activation in ERK5-sumoylation and found that CA-MEK5 α inhibits sumoylation of both wild type as well as kinase dead mutant form of ERK5, suggesting the novel mechanism of MEK5 activation of ERK5 via reduction of ERK5-sumoylation which is not related to phosphorylation of TEY motif. The molecular basis has been proposed by two different ERK5 deletion mutants which lack the MEK5-ERK5 association sites. Even though both mutants were sumoylated, CA-MEK5 α no longer inhibited sumoylation compared to wild type, indicating the critical role of MEK5-ERK5 association, but not ERK5 kinase activation, on MEK5 activation-mediated inhibition of ERK5-sumoylation. In agreement with *in vitro* data, we observed that ERK5-sumoylation, PDE3A-ICER cycle, and myocyte apoptosis was inhibited in CA-MEK5 α -Tg mice compared with NLC. We also found that cardiac dysfunction and mortality in DM+MI mice was significantly improved in CA-MEK5 α -Tg mice, suggesting the critical role of MEK5 activation in ameliorating the exacerbation of LV dysfunction in diabetic heart after MI. Taken together, our physiological data indicate that ERK5-sumoylation is significantly increased after ischemic injury and this induction may have an impact on the exacerbation of cardiac dysfunction and heart failure after MI in diabetic mice. Thus, a novel mechanism with the regulation of myocyte apoptosis via ERK5-sumoylation in diabetes may provide an important therapeutic target in diabetic cardiomyopathy.

Conclusions and perspectives

Although the molecular mechanism of sumoylation has been extremely well defined, the pathophysiological roles of sumoylation *in vivo* just begin to explore. Global knock down of sumoylation in Ubc9^{-/-} mice demonstrated that sumoylation is a fatal post-translational

modification for development and nuclear integrity. Specific genetic mutation analysis showed that PIAS proteins share substrates and can compensate for the deficiency of other genes *in vivo*. On the other hand some phenotypes shown in single gene knock out mice only appeared under the various challenges, indicating that each PIAS isoform has specific substrates and the pathological roles of PIAS proteins in cardiovascular diseases remain an open question. Also it is hard to define the pure roles of PIAS-mediated sumoylation with gene targeting technique due to the sumoylation-independent function of PIAS proteins such as DNA binding to promoters with SAP domain. In contrast to knock out model of PIAS proteins, both embryos from *SEN1*^{-/-} and *SEN2*^{-/-} mice died before/at midgestation. It can be assumed that SENP-mediated maturation of SUMO proforms plays a critical role in the developmental process or different isoforms have specific substrates that are fatal in cellular functions. However, relatively little is known about the detailed mechanism how SUMO E3 ligase and SENP activity is regulated in pathophysiological condition. Our recent data demonstrated that diabetic stimuli increase ERK5-sumoylation via PIAS1 leading to EC dysfunction and cardiomyocyte apoptosis (Figure 6). Several kinases have been known to be SUMO substrates including ERK5, HIPK2 and FAK although most SUMO substrates are restricted to nuclear proteins that function as transcriptional regulators. It is therefore intriguing whether sumoylation can interplay with kinase and sumoylation affects kinase activity. Particularly, studies using knock in system with sumoylation defective mutant forms of specific target molecules will provide the precise role of sumoylation *in vivo*.

Acknowledgments

This work was supported by grants from the American Heart Association to Dr. Woo (Fellowship 0625957T, SDG 0930360N), and from the National Institute of Health to Dr. Abe (HL-088637, HL-064839, and HL-077789). Dr. Abe is a recipient of Established Investigator Awards of the AHA (0740013N).

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Figure 1. The regulation of sumoylation pathway

Protein sumoylation is achieved by the recycle system consisting conjugation and deconjugation pathway. SUMO conjugation to target substrates requires an enzymatic cascade that involves three classes of enzymes (E1→E2→E3). The sentrin/SUMO-specific proteases (SENPs) are responsible for the deconjugation pathway as well as the maturation process of newly synthesized SUMO proteins.

Figure 2. Primary domain structure of PIAS1 and PIAS4

PIAS family proteins have unique SP-RING domain which execute sumoylation by enzymatic process. N-terminus of PIAS1 can be phosphorylated by IKK α and this modification regulates NF- κ B transrepression while PIAS4 does not have a conserved phosphorylation site although PIAS4 also has function of transrepression for NF- κ B transcriptional activity. C-terminus of PIAS family has CK2-mediated phosphorylation site which resulted in the increased SUMO-binding affinity. Other regions are not fully characterized yet.

SAP: scaffold attachment factor-A/B, acinus and PIAS; SP-RING: Siz/PIAS-RING domain; SIM: SUMO interacting module; S/T: serine/threonine rich region; ND: not determined.

Figure 3. Primary domain structure of ERK5

Instead of kinase domain which are similar to ERK1/2, ERK5 has long C-terminal region containing arginine rich domain, NLS, proline rich region, and transcriptional activation domain. These domains reveal unique features of ERK5 pathway including PPAR and MEF2. In particular the end of N-terminal region has sumoylation sites characterized by LKEE and VKAE in human.

N: nuclear localization signal; P: proline rich region; ATP: ATP binding domain; TEY: TEY phosphorylation motif.

Figure 4. A signaling scheme describing the relationship between laminar flow-mediated ERK5/MEF2/KLF2/eNOS pathway and H₂O₂ or AGE-mediated ERK5-SUMOylation

Figure 5. Schematic diagram of “PDE3A-ICER cycle” in myocyte apoptosis

Angiotensin II, Isoproterenol (ISO), DM+MI mouse model and PDE3 inhibitors induce sustained down-regulation of PDE3A expression and up-regulation of ICER, which is regulated by PDE3A-ICER positive feedback loop. Persistent ICER elevation resulted in myocyte apoptosis via the inhibition of Bcl-2 which is an important anti-apoptotic regulator. IGF-1/MEK5-mediated ERK5 activation inhibits ICER induction through regulating ICER protein stability.

Figure 6. Model of the role of ERK5-sumoylation in diabetes-related cardiovascular diseases
Diabetic stimuli increase ERK5-sumoylation via PIAS1 regulation. In case of endothelial cells, ERK5-sumoylation down-regulates the expression of KLF2 as well as eNOS which are important in endothelial function. In addition ERK5-sumoylation induces cardiac apoptosis and dysfunction through PDE3A-ICER feed back loop.a

Table 1

Comparison of human SUMO genes [3•,13••].

Modifier	% of identity (vs SUMO2)	Cellular localization	Tissue distribution	Phenotypic effects of genetic mutation	Ref
SUMO1	100	NM >> NB ≈ C	ubiquitous	SUMO1 ^{-/-} no developmental defect	[22•,23]
SUMO2	46.1 (100)	NB >> C > NM	ubiquitous	Haploinsufficiency and human SNP : cleft lip and palate	[24,25]
SUMO3	45.0 (87.5)	C > NB > NM	ubiquitous	ND	
SUMO4	41.0 (85.3)	ND	kidney, spleen, lymph node	Human SNP (SUMO4-M55V) : susceptibility to Type 1 diabetes	[27••,29]

NM: nuclear membrane; NB: nuclear bodies; C: cytosol, ND: not determined.

Table 2

Functional phenotype of genetic deletion mutant mice against PIAS and SENP [9,37•].

Modulator	Classification	Phenotypic effects of genetic mutation	Ref
PIAS1	E3 ligase	Systemic PIAS1 ^{-/-} susceptible against viral and microviral infection, high level of NF-κB and STAT1 transcriptional activity	[40]
PIAS4	E3 ligase	Systemic PIAS4 ^{-/-} no developmental defect, susceptible against septic-shock	[41,43•]
SENP1	deconjugase	Systemic SENP1 ^{-/-} embryonic lethal and defect in erythropoiesis	[52,53•]
SENP2	deconjugase	Systemic SENP2 ^{-/-} embryonic lethal and defect in cell cycle G1-S transition	[54•]