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Coordination of cellular localization-dependent effects of SUMOylation in regulating cardiovascular and neurological diseases

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Abstract

SUMOylation, a reversible post-transcriptional modification process, of proteins are involved in cellular differentiation, growth, and even motility by regulating various protein functions. SUMOylation is not limited to cytosolic proteins as recent evidence shows that nuclear proteins, those associated with membranes, and mitochondrial proteins are also SUMOylated. Moreover, it is now known that SUMOylation plays an important role in the process of major human ailments such as malignant, cardiovascular and neurological diseases. In this chapter, we will highlight and discuss how the localization of SUMO protease and SUMO E3 ligase in different compartments within a cell regulates biological processes that depend on SUMOylation. First, we will discuss the key role of SUMOylation in the nucleus, which leads to the development of endothelial dysfunction and atherosclerosis. We will then discuss how SUMOylation of plasma membrane potassium channel proteins are involved in epilepsy and arrhythmia. Mitochondrial proteins are known to be also SUMOylated, and the importance of dynamic-related protein 1 (DRP1) SUMOylation on mitochondrial function will be discussed. As we will emphasize throughout this review, SUMOylation plays crucial roles in different cellular compartments, which is coordinately regulated by the translocation of various SUMO proteases and SUMO E3 ligase. Comprehensive approach will be necessary to understand the molecular mechanism for efficiently moving around various enzymes that regulate SUMOylation within cells.

Keywords

Shear stress; atherosclerosis; SENP2; p90RSK; PKC ζ ; ERK5; p53; potassium channel; and DRP1

1. Introduction

SUMOylation is an important post-translational modification in which one or more of Small-Ubiquitin like modifier (SUMO) peptides are conjugated to a protein and contributes to the complexity of eukaryotic proteomes. There are four different SUMO family members

found in mammals, SUMO 1–4. A comparison of amino acid sequences of SUMO peptides has revealed that SUMO-1 shares 46–48% identity with SUMO-2 and -3, and SUMO-2 and -3 share 95% identity. Therefore, SUMO-2 and -3 together are considered to form a subfamily, which is distinct from SUMO-1. SUMO-4 is mainly expressed in the kidney(Bohren et al., 2004). The SUMO conjugation process is catalyzed by a specific set of enzymes comprising a SUMO activating enzyme called E1, a conjugating enzyme called E2, and a ligase named E3(Eifler and Vertegaal, 2015b, Eifler and Vertegaal, 2015a). SUMOylation is a dynamic and reversible process via conjugation and de-conjugation. First, the mature form of SUMO is activated by dimeric SUMO E1, SAE1/UBA2. Then, SUMO is transferred to Ubc9, an E2 conjugase that binds to SUMO by forming a thioester bond. The last step is regulated by E3 ligase whose function is to transfer SUMO to the free ϵ -amino group of a lysine residue of the target protein. E3 ligases include the family of protein inhibitors such as STAT and Pc2(Abe and Berk, 2014). Protein SUMOylation is reversible, and this is achieved by de-SUMOylation enzymes called sentrin/SUMO-specific proteases (SENPs; SENP1–7). The SENP family of proteins which consist of 7 enzymes catalyze de-conjugation of SUMOylated proteins. Certain SENPs are known to also edit SUMO precursors into matured forms by removing a short peptide from the C-terminus to expose a pair of glycine residues(Li and Hochstrasser, 1999, Yeh, 2009). In all isoforms of SENPs, the C-terminus is well conserved whereas the N-terminus is poorly conserved(Yeh, 2009), suggesting that the N-terminus is important for their enzymatic activity. However, it remains unclear how each specific SENP recognizes its substrate that leads to a variety of biological consequences. In addition, certain SENPs, especially SENP1 and 2 contain both nuclear localization and export signal domains, and shuttling of SENPs from one compartment of the cell to another has an effect on altering SUMOylation levels in different cellular regions. In this chapter, we will discuss the role of SENP2 in cardiovascular disease and epilepsy via regulating the SUMOylation levels of nuclear and membrane proteins and the regulatory mechanism of SENP2 nuclear import and export. In addition, we will discuss how mitochondrial translocation of SENP5 affects mitochondrial function.

2. SUMOylation in the nucleus regulates endothelial dysfunction and atherosclerosis

2.1. Steady laminar flow vs. disturbed flow

The luminal surface of blood vessels is made up of a thin monolayer of endothelial cells (ECs). ECs in general are atheroprotective as they prevent inappropriate activation of the coagulation system by producing antithrombotic factors (Uchiba et al., 2004, Selwyn, 2003, Dawes et al., 1982). This paradigm then suggests that the endothelium plays a central role in the initiation and development of inflammatory atherosclerosis when this thin tissue encounters risk factors for atherosclerosis. A certain form of hemodynamic shear stress is known to induce vascular pathologic conditions such as endothelial dysfunction(van Bussel et al., 2015) and progression of atherosclerosis (Davies et al., 2010, Heo et al., 2011a, Heo et al., 2013, Heo et al., 2015) via regulating local mechanotransduction mechanisms, ultimately activating the shear stress response promoter elements and transcription factors that modulate endothelial gene expression (Urbich et al., 2003, Huddleson et al., 2004, Nagel et al., 1999, Davis et al., 2003). Basically, there are two different types of flow; disturbed flow

(d-flow) and steady laminar flow (s-flow), which exert very different effects on endothelial function. For example, atherosclerotic plaque formation has been reported to be localized in the arterial vasculature where ECs experience d-flow (Libby et al., 2002). D-flow occurs at branch points, bifurcations, and curvatures along the arterial tree and not only down-regulates the atheroprotective mechanisms of ECs and vascular reactivity, but also increases EC inflammation (via upregulated expression of leukocyte adhesion molecules), apoptosis, and proliferation (Heo et al., 2016). In contrast, plaque formation is rare in areas exposed to s-flow ($10\text{--}20\text{ dyn/cm}^2$), which can stimulate ECs to release various factors including NO, PGI₂, and tPA to inhibit the inflammatory response of leukocytes, coagulation, and proliferation of smooth muscle cells while simultaneously promoting the survival of ECs (Garin et al., 2007, Reinhart-King et al., 2008, Frangos et al., 1985, Di Francesco et al., 2009, Korenaga et al., 1994, Diamond et al., 1989), all of which have anti-atherogenic effects. Thus, whereas s-flow is anti-atherogenic, d-flow is pro-atherogenic (Gimbrone et al., 2000), and understanding how various signaling pathways in ECs are affected by d-flow and s-flow is of crucial importance when elucidating the molecular mechanism for EC dysfunction and atherosclerosis.

It has been reported that PECAM-1, VE-Cadherin, VEGFR, and integrin receptor are involved in mechanosensory systems of d-flow and s-flow. In particular, PECAM-1 has been established as a first-line mechanosensor (Tzima et al., 2005). PECAM-1 is a type 1 transmembrane glycoprotein with six extracellular Ig-like homology domains and a short cytoplasmic domain that contains two immune-receptor tyrosine-based inhibitory motifs (ITIMs). When the tyrosine in each ITIM is phosphorylated, ITIMs can recruit Src homology 2 (SH2) domain-containing proteins (Privratsky et al., 2010). Several lines of evidence support that PECAM-1 plays a role as a mechanosensor, but it is also clear that PECAM-1 is unable to distinguish between s-flow and d-flow. For example, PECAM-1 can accelerate the formation of atherosclerotic lesions in the lesser curvature of the aortic arch (d-flow area) (Stevens et al., 2008). In contrast, PECAM-1 can reduce atherosclerotic lesions in the descending thoracic and abdominal aorta (s-flow area) (Goel et al., 2008). These observations suggest that the different responses to s-flow and d-flow are not due to differential PECAM-1 responses to these flow patterns but are due to some downstream modification(s) exerted to PECAM-1 signaling (Osawa et al., 2002, Tzima et al., 2005). Piezo1 (Piezo-type mechanosensitive ion channel component 1) (Li et al., 2014), p130 Crk-associated substrate (Cas) (Sawada et al., 2006), and syndecan 4 (Baeyens et al., 2014) are also proposed as other candidates for mechanosensors. However, it remains unclear if d-flow and s-flow can differentially regulate signaling pathways activated by these mechanosensors.

Further investigation of d-flow mediated EC dysfunction has led to the discovery of post-translational modification (PTM) of proteins via phosphorylation and SUMOylation, which play a role in atherogenesis. Our group has reported that SUMOylation induced by d-flow affects key nuclear transcriptional molecules such as extracellular signal regulated kinases 5 (ERK5) and p53, resulting in EC inflammation and apoptosis (Heo et al., 2013). Recently, the crucial role of epigenetic factors in regulating flow signaling has become clear. Especially, d-flow-induced DNA methylation by chromatin-based mechanisms (Delgado-Olguin et al., 2014, Dunn et al., 2014, Rexhaj et al., 2013, Cheng et al., 2013, Kumar et al., 2013, Rao et al., 2011, Lee et al., 1998) plays a key role in the regulation of gene expression

in a DNA sequence-independent manner (Nazarenko et al., 2015, Hamm and Costa, 2015). In this chapter we will discuss the role of SUMOylation and DNA methylation on EC dysfunction under d-flow. We believe that the very different and unique physiological consequences are incited by d-flow and s-flow and that delineating signaling pathways activated by these two flow types is critical for understanding the hemodynamic contribution of vascular physiology and pathology.

2.2. Nuclear ERK5 SUMOylation and EC dysfunction

ERK5 is one of the mitogen-activated protein kinases (MAPKs), which along with other MAPKs, has been reported to regulate the downstream transcription factors of genes regulating the growth, proliferation, and differentiation of cells including ECs and cardiomyocytes (Abe et al., 2000). Kato et al. (1997) found that overexpressed ERK5 in (cell type) localizes in the cytoplasm in resting cells but when it is co-expressed with MEK5, which activates ERK5, ERK5 translocates to the nucleus (Kato et al., 1997). However, in ECs, ERK5 is exclusively localized in the nucleus. ERK5 is unique among the MAPK family of kinases because it is not only a kinase but also a transcriptional co-activator with a unique C-terminus transactivation domain (Fig. 1A) (Akaike et al., 2004, Kasler et al., 2000). When ERK5 is activated by s-flow in ECs, its transcriptional activity on peroxisome proliferator-activated receptor- γ (PPARs) and Kruppel-like factor 2 and 4 (KLF) is increased, resulting in decreased production of inflammatory chemokines and adhesion molecules while increasing the expression of athero-protective factors such as endothelial nitric oxide synthase (eNOS) (Akaike et al., 2004, Parmar et al., 2006). KLF2 and 4 induction in ECs has been noted to increase thrombomodulin (anti-thrombotic) production, control of vascular permeability, and EC barrier function (Lin et al., 2010). These observations clearly establish the athero-protective effect of ERK5 activation by s-low in ECs (Fig. 1B, left).

As explained above, SUMO is covalently attached to certain residues of specific target proteins and alters their functions including the site of protein activity (i.e. subcellular localization), interaction with other molecules including DNA, and transactivation functions of transcription factors (Hilgarth et al., 2004). Our group has reported that H₂O₂ (hydrogen peroxide) and AGE (advance glycation end products) inhibit ERK5 transcriptional activity and promote EC inflammation via up-regulating ERK5 SUMOylation (Woo et al., 2008). H₂O₂ triggers ERK5 SUMOylation at Lys6 and 22 (K6/22) residues, and this SUMOylation inhibits ERK5 transcriptional activity and down-regulates the ERK5/MEF2 (myocyte enhancer factor-2) pathway. Subsequently, the KLF2 promoter activity is reduced due to MEK/ERK5/MEF2/KLF2 inhibition, and this results in the inhibition of KLF2-mediated eNOS expression. Heo et al. reported an increase in ERK5 SUMOylation in ECs under d-flow and found that ERK5 SUMOylation played a critical role in the mechanism of decreased eNOS expression and increased VCAM-1 expression induced by d-flow (Heo et al., 2013). These studies suggest the importance of ERK5 SUMOylation by H₂O₂, AGE, and d-flow for down-regulating ERK5 transcriptional activity and subsequently up-regulating EC inflammation (Fig. 1B, right).

2.3. SUMOylation mediated p53 nuclear export leads to EC apoptosis

In addition to d-flow causing EC inflammation by modulating ERK5 SUMOylation as we described above, d-flow is known to induce EC apoptosis. Increased EC apoptosis results in increased EC turnover with accompanying endothelial inflammation and dysfunction (Chiu and Chien, 2011, Heo et al., 2011b). Transcription factor p53 has been demonstrated to have a key role in promoting cell death by increasing the production of pro-apoptotic factors and promoting cell arrest (via failed DNA repair) when DNA damage occurs (Garner and Raj, 2008). The role of p53 in ECs exposed to s-flow was investigated by Lin et al., who noted that ECs under prolonged s-flow had increased both a JNK-mediated phosphorylation of p53 and p53 expression itself. In addition, s-flow increases p21 and GADD45 (growth arrest and DNA damage inducible protein 45) expression, and consequently inhibits Rb phosphorylation, thus inhibiting cell cycle progression into S-phase. Taken together, p53 inhibits proliferation growth and possibly apoptosis in ECs exposed to s-flow (Lin et al., 2000). Interestingly, it should be noted that these effects of p53 on ECs occur when p53 is localized within the nucleus (Lin et al., 2000).

When cultured ECs are exposed to d-flow, we found that p53 is exported from the nucleus to the cytoplasm. Carter et al. reported that p53 has a NES (nuclear export sequence) on its C-terminus. Initially, p53 NES is masked by its own N-terminal lesion, thus preventing p53 from nuclear export. However, after mono-ubiquitination of p53 by E3 ligase MDM2 (douse double minute 2), SUMOylation of p53 by PIAS4 uncovers the masked p53 nuclear export signal (NES), which then allows p53 nuclear export (Carter et al., 2007). Once exported to the cytoplasm, p53 induces apoptosis by direct association and blocking of the Bax/Bcl anti-apoptotic function (Mihara et al., 2003, Heo et al., 2011b). Our group have reported the crucial role of protein kinase C ζ (PKC ζ) activation in p53 SUMOylation and consequent nuclear export (Fig. 2). D-flow activates PKC ζ in ECs which in turn, promotes the association between the PKC ζ C-terminus kinase domain (aa401–587) and the RING domain of PIAS4 (protein inhibitor of activated STAT4). Because the PIAS4 RING domain contains a catalytic site, the structure and enzymatic activity of PIAS4 might be altered by this association. The PIAS4-PKC ζ association causes an increase in p53 SUMOylation and once SUMOylated, p53 is exported to the cytoplasm, which then induces EC apoptosis (Heo et al., 2011b) (Fig. 2).

2.4. Nuclear export of de-SUMOylation enzyme SENP2 and its effects on nuclear ERK5 and p53

Among the six SENP isoforms that exist in humans (SENP 1–3 and SENP 5–7), we have characterized the functional role of SENP2 in controlling SUMOylation of ERK5 and p53 in ECs stimulated by flow (Heo et al., 2013). When SENP2 is deleted, d-flow-induced EC apoptosis and inflammation are up-regulated by increased SUMOylation of p53 and ERK5, respectively. Furthermore, aortas from SENP2^{-/-} mice exhibited accelerated atherosclerotic plaque formation in d-flow areas when compared to s-flow areas. As such, one might expect d-flow to decrease SENP2 expression. However, the expression of SENP2 is not regulated by d-flow. Because SENP2 has various nuclear localization/nuclear export signals (NLS/NES), one possibility is that SENP2 localization is altered and that the local concentration of SENP2 could be reflected in local de-SUMOylation activity (Itahana et al., 2006). Our group

found the possible role of p90 kDa ribosomal S6 kinases (p90RSK) in this possible process (Fig. 3).

The family of p90RSK is a unique serine/threonine kinase family that contains two functional kinase domains: the N-terminus and the C-terminus kinase domains (Frodin and Gammeltoft, 1999). The N-terminus kinase domain appears to belong to the AGC group of kinases (i.e. PKC and PKA) and phosphorylates p90RSK substrates. The C-terminus kinase domain is a member of the calcium/calmodulin dependent kinase group, which is involved in the p90RSK N-terminal kinase activation. The C-terminus tail contains a short docking motif which is activated by ERK1/2 (Blenis, 1993) (Fig. 4). It should be noted that p90RSK activation can be achieved by an ERK1/2-independent mechanism (Abe et al., 2000). Upon activation, p90RSK is able to phosphorylate transcription factors such as CREB, NF- κ B, and c-fos. In addition, more recent reports indicate phosphorylation of SENP2 and ERK5 by activated p90RSK (Heo et al., 2016, Heo et al., 2015, Le et al., 2013). Activated p90RSK binds the C-terminal transcriptional domain (aa 571–807) of ERK5 and phosphorylates ERK5 S496 with subsequent inhibition of ERK5 transcriptional activity (Le et al., 2013) (Fig. 1B). This inhibition of ERK5 transcriptional activity results in decreased KLF2/eNOS expression and at the same time increased adhesion molecules expression, all of which lead to EC dysfunction with accelerated atherosclerotic plaque formation. These effects are similar to what is observed in ECs during ERK5 SUMOylation and also in EC-specific ERK5 knockout mice, demonstrating the importance of ERK5 in preventing EC dysfunction.

In addition to the direct role of p90RSK in ERK5 S496 phosphorylation, p90RSK also mediates SENP2 T368 phosphorylation, which also leads to increased p53 and ERK5 SUMOylation; events exhibited by ECs undergoing d-flow-induced inflammation and apoptosis (Heo et al., 2015) (Fig. 3). As already discussed in this chapter, decreased SENP2 expression increases p53 and ERK5 SUMOylation with subsequent EC apoptosis and inflammation, respectively (Heo et al., 2013). Although d-flow increases p53 and ERK5 SUMOylation, surprisingly, reduced SENP2 expression was not observed. Therefore, it was hypothesized that it is SENP2 post-translational modification (PTM), which plays a critical role in this process. In vitro, SENP2-mediated reduction of p53 SUMOylation is inhibited in ECs over-expressing p90RSK. However, the observed inhibitory effect of p90RSK over-expression is lost in ECs expressing the SENP2 T368A phosphorylation mutant, demonstrating that SENP2 phosphorylation at T368 is important for its de-SUMOylating function. Furthermore, this study noted that when p90RSK is not able to bind and phosphorylate SENP2 T368 due to over-expression of a decoy fragment (SENP2 aa 131–300 fragment), SUMOylation of p53 and ERK5 and subsequent EC apoptosis and inflammation are inhibited. Inhibition of p90RSK activation by FMK-MEA (p90RSK specific inhibitor) or by over-expression of dominant negative p90RSK adenovirus (Ad-DN-p90RSK) both abolish d-flow induced p53 and ERK5 SUMOylation via inhibiting SENP2 T368 phosphorylation. Also as previously hypothesized, SENP2 export from the nucleus to the cytoplasm indeed does occur when p90RSK mediated SENP2 T368 phosphorylation occurs. The same phenotype was observed in ECs exposed to d-flow. In contrast, SENP2 is localized in the nucleus in ECs exposed to s-flow. ECs isolated from wild type p90RSK transgenic mice (WT-p90RSK) have increased SENP2 T368 phosphorylation, increased adhesion molecule as well as caspase-3 expression (at both protein and mRNA levels), and decreased

eNOS expression. In addition, the p90RSK transgenic mice exhibit increased atherosclerotic lesion size in the aortic arch compared to controls. Overall, these data suggest that p90RSK-mediated SENP2 T386 phosphorylation induces SENP2 nuclear export and plays an important role in atherosclerotic plaque formation in d-flow areas via up-regulating SUMOylation of nuclear p53 and ERK5, which leads to EC apoptosis and endothelial inflammation, respectively(Heo et al., 2015) (Fig. 3).

2.5. D-flow and DNA methylation in the nucleus

DNA methylation at the 5 position of cytosine is a dynamic postsynthetic covalent modification, and more than 98% of DNA methylation occurs in cytosine–phosphate–guanine (CpG) dinucleotides (Guza et al., 2011) in mammals. Methylation of cytosine can cause gene transcriptional silencing via interfering with binding of transcriptional factors or inducing (or forming) a repressive chromatin structure within or near the promoter region (Weber et al., 2005, Jaenisch and Bird, 2003). Gene promoters with a cluster of unmethylated CpG dinucleotides are about 50 % of genomic DNA, which allow transcription. The dynamic process of DNA methylation is regulated by both methylation and demethylation enzymes (Fig. 5).

DNA methyltransferases (DNMTs) which methylate DNA are encoded by different genes on distinct chromosomes: DNMT1, DNMT3A, and DNMT3B (Fig. 6). DNMT3A and DNMT3B catalyze de novo methylation during early embryonic development while DNMT1 is crucial to maintaining DNA methylation throughout replication (Okano et al., 1999). DNMT3L has no catalytic activity and belongs to the family of DNMT3A and 3B. It has an important role for stabilizing DNMT3A (Xi et al., 2009). The ten eleven translocation (TET) methylcytosine dioxygenase gene plays a major role in regulating DNA methylation by oxidizing 5-methylcytosine to 5-hydroxymethylcytosine (Okano et al., 1999). The involvement of DNA methylation in various cancers (Chik and Szyf, 2011, Roll et al., 2008, Robert et al., 2003, Mizuno et al., 2001), immune disorders (Januchowski et al., 2004), neurodegeneration (Martin and Wong, 2013, Chestnut et al., 2011), and d-flow-induced EC dysfunction (Jiang et al., 2014) has been reported. Jiang et al showed different levels of DNA methylation in ECs isolated from swine aortas and human aortas exposed to d-flow and s-flow (Jiang et al., 2014). They found the key role of DNA methylation of CpG islands within the KLF4 promoter in d-flow-mediated inhibition of KLF4 transcription. Using two different DNMT inhibitors, RG-108 and 5-Aza, Jiang et al found that the reduction of premature KLF4 transcripts was totally recovered but that mature KLF4 was only partially recovered by DNMT inhibition. These data do not only suggest the key role of DNMT activity in regulating d-flow-induced reduction of KLF4 expression, but also the existence of another posttranscriptional inhibition of KLF4 mRNA induced by d-flow(Dunn et al., 2014). In addition, d-flow induces DNMT activation and consequent DNA hypermethylation of the KLF4 promoter, which inhibits the expression of eNOS, thrombomodulin, and monocyte chemoattractant protein 1(Dunn et al., 2014).

The crucial role of DNMTs in d-flow signaling has been reported by Dunn et al and Zhou et al, but there are some discrepancies in terms of expression of DNMT isoforms(Dunn et al., 2014, Zhou et al., 2014). Dunn et al reported that d-flow increased both DNMT1 mRNA and

protein (Dunn et al., 2014), while Zhou et al did not observe an increase in DNMT1 protein although they did find up-regulation of DNMT1 mRNA expression and nuclear translocation (Zhou et al., 2014). These differences may come from different experimental systems employed. Using ECs isolated from the area exposed to either d-flow or s-flow in swine aortas (Jiang et al., 2014), Jiang et al found no significant differences in DNMT (DNMT1, 3A, and 3B) expression or cytosine demethylation enzyme mRNA (TET1–3, TDG1, GADD45B, MBD4, and SMUG1) expression in ECs from the d-flow region, but they found a significant increase in DNMT3A protein levels without any change in mRNA levels (Jiang et al., 2014). In contrast, Dunn et al. used the partial carotid ligation model to generate d-flow and compared gene expression and DNA methylation in the carotid artery with (d-flow) or without (s-flow) partial ligation. In in vitro studies, Zhou et al used a human umbilical vein endothelial cell culture system and Jiang et al used ECs isolated from swine aorta, which may explain the difference between these studies. Although d-flow-induced induction of nuclear translocation of DNMT1 was reported by Zhou et al, the regulatory mechanism of this nuclear translocation is not clear. It has been reported that IL-6 causes DNMT1 nuclear translocation by AKT-mediated phosphorylation at the DNMT1 nuclear localization signal site (Hodge et al., 2007), suggesting possible involvement of other forms of PTMs including SUMOylation as we explain later in d-flow-induced DNMT1 nuclear translocation.

5-Aza is an inhibitor of DNMTs and has been shown to inhibit formation of atherosclerosis in the mouse partial carotid ligation model (Dunn et al., 2014). In this study, DNA methylation in 11 gene promoters was shown to increase by d-flow, and this increase was reversed by 5-Aza. Since a system biology analysis by MetaCore predicted these 11 genes to be regulated by cAMP response element binding protein (CREB1), these authors investigated the CRE site within the promotor region of these genes. Five of the 11 genes contained a CRE site in its differentially methylated regions, and promoters of HoxA5, Klf3, Cmkrl1, and Acvr11 at the CRE CG site were hypermethylated by d-flow, which was also inhibited by 5-Aza. Although a possible role for HoxA5 in vascular remodeling and angiogenesis via EC inflammation has been reported (Dunn et al., 2014), the actual pathological role of d-flow-mediated hypermethylation for each gene promoter remains unclear.

It has been reported that the function of DNMTs can be regulated by SUMOylation (Fig. 6). For example, as we discussed above, Jiang et al have reported that a significant increase in DNMT3A protein levels without changing its mRNA levels, and they have suggested that DNMT3A SUMOylation may contribute to this process, because SUMOylation can increase the half-life of DNMT3A (Jiang et al., 2014, Ling et al., 2004). Interestingly, DNMT3A SUMOylation does not only delay its degradation, but also disrupt the ability of DNMT3A to interact with histone deacetylases (HDACs) and repress transcription of a reporter gene (Ling et al., 2004). These data suggest the possible role of DNMT3A SUMOylation in the nucleus, which can up-regulate d-flow-induced transcriptome by hypermethylation of promoters. Not only DNMT1 phosphorylation but also the significant role of DNMT1 SUMOylation in regulating DNA methylation activity has been reported (Lee and Muller, 2009). The relationship between DNMT1 phosphorylation and SUMOylation and the way in which this activity stimulates the methylation activity of DNMT1 remain unclear. As we

discussed above, DNMTs can shuttle between the cytoplasm and the nucleus, and d-flow may induce DNMTs nuclear translocation(Zhou et al., 2014). Because we have found that d-flow elicits SENP2 nuclear export and increases nuclear ERK5 and p53 SUMOylation, it is reasonable to speculate that d-flow also induces DNMT SUMOylation, which may increase DNA hypermethylation in the nucleus. Further investigation is needed.

2.6. Nuclear inducible cAMP early repressor (ICER) is regulated by ERK5-SUMOylation in heart

Reduced expression of cAMP hydrolyzing enzymes including phosphodiesterase 3A (PDE3A) and increased expression of inducible cAMP early repressor (ICER) have been observed in failing hearts. ICER down-regulates Bcl2 via inhibiting the transactivation of cAMP response element binding protein (CREB) and leads to down-regulation of Bcl2 and PDE3A expression. The reduction of PDE3 expression increases cAMP availability and up-regulates PKA signaling, forming an autoregulatory positive feedback loop. Angiotensin II and isoproterenol (β -AR agonist) activate this positive feedback loop, providing a mechanism of how the activation of neurohormonal systems in heart failure affects myocyte apoptosis(Ding et al., 2000, Ding et al., 2005).

Our group has reported that ERK5 plays a critical role in regulating this cardiomyocyte apoptosis pathway (Fig. 7). Mice with cardiac-specific ERK5 knockout show accelerated cardiac apoptosis and dysfunction after thoracic aorta constriction(Kimura et al., 2010), while transgenic mice overexpressing cardiac-specific constitutively active MEK5 (CA-MEK5, ERK5's direct upstream kinase) show reduced levels of ICER induction and myocyte apoptosis upon induction of pressure-overload and myocardial infarction (MI)(Yan et al., 2007, Shishido et al., 2008a). Regulation of the PDE3A-ICER mechanism by ERK5 is achieved by an E3 ubiquitin (Ub) ligase called CHIP (carboxyl terminus of HSP70-interacting protein). CHIP has an important cardioprotective role in limiting myocardial damage due to ischemia/reperfusion injury after MI by inhibiting apoptosis. Transgenic CHIP knockout mice exhibit increased infarct sizes and decreased survival compared to wild-type(Zhang et al., 2005). Activation of ERK5 decreased ICER protein stability through CHIP-mediated degradation. ERK5 activation increased ERK5-CHIP binding and subsequently up-regulated CHIP Ub ligase activity and decreased ICER expression after MI(Woo et al., 2010). In diabetic mice with induced MI (DM + MI), CHIP Ub activity and PDE3 expression was decreased, while ERK5-SUMOylation and ICER expression was increased, suggesting that ERK5-SUMOylation may directly inhibit CHIP Ub activity and increase ICER expression(Shishido et al., 2008b). Further investigation is necessary to clarify the role of ERK5-SUMOylation on the CHIP-ICER signaling pathway.

3. SUMOylation of potassium channels at the plasma membrane

In the previous section, we have discussed the role of SENP2 in the nucleus, and the nuclear export of SENP2 may enhance SUMOylation of nuclear ERK5 and p53. Although SENP1 and 2 can translocate from the nucleus to the cytosol/membrane, the consequence of this translocation on extra-nuclear proteins remains unclear. In this section, we will review the

role of SENP1 and 2 in regulating several potassium channel proteins, which are localized in the plasma membrane.

First, it is known that Kv1.5 (potassium voltage-gated channel subfamily A member 5, KCNA5) is responsible for the I_{Kur} repolarizing current in atrial myocytes and also regulates vascular tone in peripheral vascular beds. Benson et al have reported that SENP2 can de-SUMOylate Kv1.5 and lead to a substantial hyperpolarizing shift in the voltage dependence of steady-state inactivation (Benson et al., 2007). Of note, they did not see any significant V_{50} shift of (wild type) Kv1.5 in the depolarizing direction by overexpressing SUMO3 and Ubc9, suggesting that neither Ubc9 nor SUMO is a limiting factor for regulating Kv1.5 function. In this study, SENP2 deletion mutant of the first N-terminus residues (SENP2 aa71–590), which shows enhanced de-SUMOylation activity against multiple SUMOylated substrates and also localizes to the cytoplasm, was used. Coexpression with the this mutant with wild type Kv1.5 decreased Kv1.5 SUMOylation, then caused a significant hyperpolarization shift in the voltage dependence of inactivation without altering the total current density or voltage dependence of Kv1.5 activation. Therefore, it is likely that SENP2 nuclear export can regulate Kv1.5 function via changing its cellular localization. Kv1.5 is widely expressed in the cardiovascular system (Overturf et al., 1994). A loss-of-function mutant of Kv1.5 expressed in the atrium causes a familial form of atrial fibrillation (Olson et al., 2006), and a critical role of Kv1.5 in the pulmonary vasculature for the oxygen-sensitive regulation of arterial tone has also been reported (Hong et al., 2005). Further investigation is necessary to determine the role of Kv1.5 SUMOylation and SENP2 in these processes.

Another plasma membrane potassium channel family which can be regulated by SUMOylation is potassium voltage-gated channel subfamily Q member (KCNQ). Five KCNQ genes (KCNQ1 to KCNQ5) codify a family of 5 different voltage-gated potassium ion channels (KV7.1 to KV7.5), which are mainly expressed in the nervous and cardiac systems (Brown and Passmore, 2009). Using mice homozygous for the floxed SENP2 allele with a neomycin insert (SENP2^{fxN/fxN}), Yeh's group has elucidated SENP2's role in sudden death. These SENP2^{fxN/fxN} mice appear healthy at birth, but develop convulsive seizures followed by sudden death at 6–8 weeks of age. The neomycin cassette insertion caused a reduction in SENP2 transcription and protein levels. Reduced expression of SENP2 protein induced an accumulation of SUMO-1 or SUMO-2/3 proteins in the brain and heart, thus leading to the formation of a hyper-SUMOylation environment (Qi et al., 2014). SENP2 is abundant in the hippocampal region, an area of the brain of great relevance to seizure, but the exact location with increased SUMO proteins in the hippocampus has not yet been determined. In SENP2^{fxN/fxN} mice, SENP2 levels are markedly reduced and as the result, SUMOylation of Kv7.2 is enhanced in hippocampal neurons. Hyper-SUMOylation of Kv7.2 potassium channels diminished the M-current (conducted by Kv7 channels), leading to a more positive resting membrane potential and increased excitability of hippocampal neurons. These data suggest the pathophysiological role of SENP2 in epilepsy via regulating plasma membrane Kv7.2 function.

The last potassium channel which can be SUMOylated is potassium channel subfamily K member 1 (KCNK1). Both KCNK1 K274E SUMOylation site mutation and overexpression of SENP1 increase KCNK1 current (Rajan et al., 2005), suggesting an inhibitory effect of

SUMOylation(Plant et al., 2010). KCNK1 is widely expressed in the heart and the central nervous system and regulates background leak currents stabilizing neuronal excitability(Silveirinha et al., 2013). Although the possible involvement of SENP1 in regulating KCNK1 has been suggested, it is not clear how the SUMOylation status of the plasma membrane KCNK1 current is regulated.

In summary since all the reported SUMOylated potassium channels can be regulated by de-SUMOylation enzymes of SENP1 and 2, how SENP1 and 2 change their de-SUMOylation activity or localization and regulate the plasma membrane potassium channel function need further investigation.

4. SUMOylation of Mitochondrial Proteins

4.1. Overview of mitochondrial fission and fusion

Cardiomyocyte mitochondria form a diverse network that are integral to maintaining appropriate cardiomyocyte activity. Mitochondria occupy nearly 33% of cardiac cell volume and produce the energy required to sustain cardiac function(Ong et al., 2015a, Ong et al., 2015b, Ong et al., 2015c). This structure is a dynamic organelle that persistently changes its membrane morphology in response to cellular activity (Jayashankar et al., 2016). These conformational changes are necessary for mitochondrial replication and membrane integration. The mitochondrion undergoes both fission and fusion in order to maintain normal cell function. Mitochondrial fusion is achieved by the process of integrating separate membranes, and fission by the process of separating intact membranes(Twig et al., 2008b, Twig et al., 2008a). The fusion of mitochondrial membranes is mediated by GTPase proteins from the dyamin family including mitofusin 1 (Mfn1), mitofusin 2 (Mfn2), and optic atrophy-1 (Opa1)(Mishra, 2016, Mishra and Chan, 2016). The overall process of fusion is mainly regulated by ubiquitination via ubiquitin ligases such as membrane-associated RING finger 5 (MARCH5)(Nagashima et al., 2014). Fission of mitochondria occurs in a sequential manner. First, constriction of mitochondrial tubules takes place. Next, the GTPase called dynamic-related protein 1 (Drp1) is mobilized from the cytosol to the outer membrane of mitochondria via several receptor proteins(Ong et al., 2015b). Upon reaching the outer membrane, Drp1 assembles into a scission complex by forming a spiral that surrounds the constricted tubules. Then, functioning in a GTP dependent manner, the Drp1 complex further constricts the tubule to cause scission(Ong et al., 2015b)(Friedman et al., 2011). Lastly, the complex is disassembled. (Fig. 8)

4.2. The roles of DRP1 SUMOylation in mitochondrial fission; SUMO1 vs SUMO2/3

DRP1 SUMOylation regulates the process of fission by modifying DRP1 function. In Cos7 and Hela cells, DRP1 SUMO1 modification stimulates mitochondrial fission by enhancing retention of DRP1 on the membrane after its recruitment to mitochondria, followed by disassembly of the Drp1 oligomer via de-SUMOylation (SUMO1) once fission is completed(Wasiak et al., 2007, Zunino et al., 2007, Zunino et al., 2009). Mitochondrial-anchored protein ligase (MAPL) is a 40 kDa protein located on the outer mitochondrial membrane(Zungu et al., 2011) (Fig. 9). Although MAPL can participate into the process of both ubiquitination and SUMOylation, under physiologic conditions MAPL preferentially

functions as a SUMO E3 ligase for DRP1 SUMOylation (SUMO1)(Braschi et al., 2009). MAPL-mediated DRP1 SUMOylation (SUMO1) increases mitochondrial fission and hyper-fragmentation(Zungu et al., 2011, Braschi et al., 2009, Neuspiel et al., 2008). MAPL-mediated DRP1 SUMOylation (SUMO1) has also been identified to play a role in apoptosis. Cytochrome c functions as the terminal trigger for apoptotic cell death and is located in the intermembrane space(Chipuk et al., 2006). Release of cytochrome c has been shown to depend on MAPL-mediated SUMOylation (SUMO1) of DRP 1(Prudent et al., 2015).

SENP5, SENP3, and SENP2 can be a de-SUMOylation enzyme for DRP1(Mendler et al., 2016, Harder et al., 2004). Several experiments have suggested that SENP5 plays a role in regulation of fission via its interaction with DRP1(Di Bacco et al., 2006, Zunino et al., 2009). SENP5 participates in de-SUMOylation of DRP1 with the ability to remove SUMO1, SUMO2, or SUMO3(Zunino et al., 2009, Gong and Yeh, 2006), but the functional consequence of conjugation of SUMO1 and SUMO2/3 to DRP1 on regulating mitochondrial function is not the same among these isoforms(Mendler et al., 2016). The depletion of SENP5 in COS7 or Hela cells increases DRP1 SUMO1 modification induced by MAPL, leading to mitochondrial binding of DRP1 and increasing mitochondrial fragmentation and cellular apoptosis(Wasiak et al., 2007, Zunino et al., 2007, Zunino et al., 2009). In contrast, cardiac-specific overexpression of SENP5 inhibits DRP1 SUMO2/3 modification, which induces apoptosis via promoting the association of DRP1 with mitochondria(Kim et al., 2015) (Fig. 9). It has been suggested that DRP1 SUMO2/3 modification prevents DRP1 association with mitochondria, whereas DRP1 SUMO1 modification induces DRP1 binding to mitochondria and induce apoptosis(Mendler et al., 2016). SUMOylation of mitochondrial proteins is an area of ongoing research. Especially, elucidation of detailed mechanisms for different roles of DRP1-SUMO1 and DRP1-SUMO2/3 remains to be critical.

SENP5 is localized primarily to the nucleus, but there is also a substantial amount of this enzyme in the cytosol(Zunino et al., 2007). Zunino et al have reported that SENP5 translocation from the nucleus to the mitochondria specifically occurs at the G2/M transition (Zunino et al., 2009). Although the regulatory mechanism for SENP5 translocation from the nucleus to the mitochondria remains unclear, this provides another example that translocation of SUMO proteases between different intracellular compartments can regulate various cell responses by modifying SUMOylation.

Conclusions

In this chapter, we have highlighted the role of SUMOylation in different cellular locations particularly in cardiovascular disease and epilepsy. Kinases like PKC ζ and p90RSK are activated under d-flow or diabetic conditions and play central roles in regulating a complex network of signal transduction that is continuously modified by SUMOylation. As discussed in this chapter, SUMOylation is an important and dynamic posttranslational protein modification occurring at different compartments of cells and this is tightly regulated by the localization of SUMOylation and de-SUMOylation enzymes. Different roles of SUMO1 and SUMO2/3 in regulating DRP1 function was reviewed, but the contribution of SENP5 mitochondrial translocation and how SENP5 differentially regulate DRP1 modification with SUMO1 and SUMO2/3 remains unclear. Further investigations focused on different roles of

SUMOylation in different cellular location and how de-SUMOylation enzymes including SENP2 and 5 coordinately regulate these processes by changing their localization will be necessary.

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Figure 1. Primary structure of ERK5 and its regulation by shear stress

A. ERK5 is twice the size of other MAPKs and hence the largest kinase within its group. It possesses a catalytic N-terminal domain including the MAPK-conserved threonine/glutamic acid/tyrosine (TEY) motif in the activation loop with 50% homology with ERK1/2, and a unique C-terminal tail including transactivation domains. The activation of ERK5 occurs via interaction with and dual phosphorylation in its TEY motif by MEK. On the other hand, inflammatory stimuli or athero-prone flow (d-flow) leads to ERK5 deactivation via phosphorylation of Ser486 or Ser496, respectively. The N-terminus K6 and K22 sites with small ubiquitin-like modifier (SUMO) modification inhibit its own transactivation. B. After ERK5 kinase activation induced by MEK5 binding or athero-protective flow (s-flow) stimulation and TEY motif phosphorylation with de-SUMOylation, ERK5 transcriptional activity at the C-terminus region is fully activated. In contrast, d-flow increases ERK5 SUMOylation and ERK5 Ser496 phosphorylation and inhibits ERK5 transcriptional activity. eNOS, endothelial nitric oxide synthetase; KLF, Kruppel-like factor; p90RSK, p90 ribosomal S6 kinase; PKC ζ , protein kinase C- ζ ; and PPAR, peroxisome proliferator-activated receptor. Reprinted and modified from Heo et al (Heo et al., 2016) with permission of the publisher..

Figure 2. The scheme of PKC ζ -mediated p53 SUMOylation and consequent EC apoptosis by atheroprone flow

Atheroprone flow (d-flow) uniquely activates PKC ζ via up-regulating ONOO⁻, which increases PKC ζ -PIAS4 binding at the SP-RING domain and PIAS4 small ubiquitin-like modifier (SUMO) E3 ligase activity, subsequently increasing p53 SUMOylation. Upon binding SUMOylated p53, p53 translocates to the cytosol, and the anti-apoptotic effect of Bcl-2 is inhibited, leading to caspase activation and apoptosis. PIAS, protein inhibitor of activated STAT; SAP, scaffold attachment factor-A/B, acinus, and PIAS domain; PINIT, Pro-Ile-Asn-Ile-Thr motif; SP-RING, Siz/PIAS-RING domain(Abe and Berk, 2014).

Figure 3. Regulation of p90RSK-SENTP2 to increase EC dysfunction by athero-prone flow
 p90RSK is uniquely activated by athero-prone (d-flow) flow. SENTP2 contains several NLS and NES domains, and we found that p90RSK activation induces SENTP2 nuclear export by phosphorylation of SENTP2 Thr386 and direct binding to SENTP2 aa131–300. This SENTP2 nuclear export subsequently up-regulates SUMO modulation of nuclear p53 and ERK5, and increase apoptosis and EC inflammation, respectively. In addition, the increase of ERK5 SUMOylation decreases eNOS expression.

Figure 4. Scheme of p90RSK functional domains

The N-terminus kinase belongs to the AGC group of kinases (i.e., protein kinase A [PKA] and protein kinase C [PKC]), while the C-terminus kinase belongs to the calcium/calmodulin-dependent kinase group. p90RSK is located downstream of the Raf-MEK-ERK1/2 signaling pathway (65), and ERK1/2 activates the C-terminus kinase of p90RSK, leading to full activation of the N-terminus kinase and subsequent substrate phosphorylation. However, the involvement of an ERK1/2-independent pathway and the role of fyn kinase in regulating ROS-induced p90RSK activation have also been suggested (66). Recently, we have reported that p90RSK is activated by d-flow, but not by s-flow (67). Reprinted and modified from Heo et al (Heo et al., 2016) with permission of the publisher.

Figure 5. DNA methylation/demethylation enzymes

Methylation of the promoter regions of genes significantly suppresses transcription by direct inhibition of transcription factor binding and recruitment of methyl-CpG-binding proteins within their recognition sites of transcription factors. DNA methylation occurs at carbon 5 of cytosine (5-methylcytosine [5mC]) in cytosine-phosphate-guanine dinucleotides (CpG) dinucleotides. DNA (cytosine-5-)-methyltransferase 1 (DNMT1) maintains DNA methylation patterns during cell proliferation via methylation of a hemi-methylated nascent DNA strand. DNMT3A and DNMT3B are required for genome-wide de novo methylation and play crucial roles in the establishment of DNA methylation patterns. Methylation by DNMTs is counterbalanced by DNA demethylation. TET oxidizes 5mC to 5-hydroxymethylcytosine (5hmC) and subsequently to 5-formyl cytosine (5fC) and 5-carboxy cytosine (5caC). The carboxyl group of 5caC is excised by thymine DNA glycolase (TDG) to restore cytosine. An active demethylation pathway through consecutive oxidation of 5-methylcytosine (5mC) mediated by TET (ten eleven translocation) proteins and subsequent base excision repair (BER) in mammalian systems DNA methylation dynamics. Reprinted and modified from Heo et al(Heo et al., 2016) with permission of the publisher.

A. DNMT1 **B. DNMT3A** **DNMT3B** **Figure 6. Post-translational regulation of mammalian DNA methyltransferases**

DNMTs protein domain structure and SUMylation. A. DNMT1; DMAP1 domain, PCNA domain, nuclear localization signal domain (NLS), DNA replication foci-targeting domain, CXXC- zinc finger region, bromo-adjacent homology domains (BAH1 and BAH2), and catalytic domain. More than 10 SUMOylation sites throughout DNMT1 sequence were suggested. B. DNMT 3A and 3B; a proline-tryptophan-proline domain (PWWP), an ATRX-DNMT3-DNMT3L-type zinc finger domain (ADD), and catalytic domain. SUMOylation of the N-terminal regulatory region including the PWWP domain was reported. Reprinted and modified from Heo et al (Heo et al., 2016) with permission of the publisher.

Figure 7. p90RSK regulates ERK5-CHIP module

(A) A model of myocardial infarction under diabetes (DM + MI) or angiotensin II (Ang II)-mediated p90RSK-ERK5-CHIP signal transduction pathway that regulates cardiac apoptosis and subsequent cardiac dysfunction. (B) A scheme depicting p90RSK-mediated regulation of the ERK5-CHIP module. At the basal level, inactive p90RSK inhibits the D-domain to bind with ERK5 (Gao et al., 2010). p90RSK-free ERK5 associates with CHIP at its linker and U-box domain and maintains its CHIP Ub ligase activity to prevent ICER induction and subsequent apoptosis¹³. However, once p90RSK gets activated, the inhibition of the kinase domain is released (Gao et al., 2010, Woo et al., 2010), and the D-domain of p90RSK associates with the ERK5 COOH-terminal domain, leading to compete with ERK5-CHIP association and ERK5-S496 phosphorylation, which disrupts ERK5-CHIP interaction. The disruption of ERK5-CHIP interaction inhibits CHIP Ub ligase activity (Woo et al., 2010), increases ICER induction, and induces apoptosis (Woo et al., 2010, Esser et al., 2005). Reprinted from Le et al (Le et al., 2012) with permission of the publisher.

Figure 8. SUMO1 modification of Dynamin Related Protein (DRP1) and mitochondrial fission
 The process of mitochondrial fission is regulated by the recruitment and oligomerization of the DRP1. SUMO1 modification of DRP1 increases DRP1 activity, which is regulated by a SUMO E3 ligase MAPL, and deconjugating enzymes include SENP5 (a SUMO protease). The crucial role of GTPase Opa1 and mitofusin has been reported, but the role of SUMOylation in this process remains unclear (Braschi and McBride, 2010).

Figure 9. SUMOylation of dynaminrelated protein (Drp1) in regulating mitochondrial fission
 SENP5 was identified as SUMO deconjugases of DRP1 in HeLa, Cos7, and cardiomyocytes. Silencing of SENP5 In COS-7 or HeLa cells SUMO1 modification of DRP1 is increased by the depletion of SENP5 and the E3 SUMO ligase mitochondrialanchored protein ligase (MAPL). SUMO1 modification of DRP1 enhances DRP1 binding to mitochondria, leading to mitochondrial fragmentation and cellular apoptosis. In contrast, in cardiomyocytes SENP5 inhibits SUMO2/3 modification of DRP1. De-SUMOylated DRP1 can bind to mitochondria, and subsequently induced mitochondrial fragmentation and apoptosis(Mendler et al., 2016).