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Kinase-SUMO Networks in Diabetes-mediated Cardiovascular Disease

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Abstract

Type II diabetes mellitus (DM) is a common comorbidity in patients with cardiovascular disease (CVD). Epidemiological studies including the Framingham, UKPDS, and MRFIT studies have shown diabetes to be an independent risk factor for cardiovascular disease associated with increased incidence of morbidity and mortality. However, major randomized controlled clinical trials including ADVANCE, VAD, and ACCORD have failed to demonstrate a significant reduction in CVD complications from longstanding DM with strict glycemic control. This suggests that despite the strong clinical correlation between DM and CVD, the precise mechanisms of DM-mediated CVD pathogenesis remain unclear. Signal transduction investigations have shed some light on this question with numerous studies demonstrating the role of kinase pathways in facilitating DM and CVD pathology. Abnormalities in endothelial, vascular smooth muscle, and myocardial function from the pathological insults of hyperglycemia and oxidative stress in diabetes are thought to accelerate the development of cardiovascular disease. Extensive interplay between kinase pathways that regulate the complex pathology of DM-mediated CVD is heavily regulated by a number of post-translational modifications (PTMs). In this review, we focus on the role of a dynamic PTM known as SUMOylation and its role in regulating these kinase networks to provide a mechanistic link between DM and CVD.

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1. Introduction

Type II diabetes mellitus (DM) is a known cardiovascular disease (CVD) risk factor as shown in numerous epidemiological studies including the landmark Framingham Heart Study[1,2], United Kingdom Prospective Diabetes Study[3] (UKPDS), and Multiple Risk Factor Intervention Trial[4] (MRFIT). Compared to individuals without diabetes, type II diabetics are at greater risk of developing CVD including atherosclerosis, diabetic cardiomyopathy, stroke, renal disease, and myocardial infarction[5]. Type II diabetics often have multiple CVD risk factors including dyslipidemia, hypertension, and obesity.

Surprisingly, despite this epidemiological correlation as well as clinical evidence that strict glycemic control is beneficial for microvascular disease, such evidence is lacking for macrovascular disease. Three major randomized controlled clinical trials (ADVANCE[6], VADT[7], and ACCORD[8]) that examined near-normal glycemic control (A1C 6.4–6.9%) over a 3.5–6 year time period failed to demonstrate a significant reduction in CVD complications from longstanding DM. In contrast, post-trial observations from the UKPDS showed that there was a CVD risk reduction benefit with strict glycemic control in patients with newly diagnosed diabetes[9]. According to these results together with the epidemiological data from the Framingham, UKPDS, and MRFIT studies, we may suggest that although there is an association between DM and CVD, the precise mechanisms through which DM increases CVD risk remains unclear.

Signal transduction investigations have shed some light on this question with numerous studies demonstrating the role of kinase pathways in facilitating DM and CVD pathology[10]. Abnormalities in the endothelial, vascular smooth muscle, and myocardial functions caused by the pathological insults of hyperglycemia and oxidative stress in diabetes are thought to accelerate the development of cardiovascular diseases[11]. Numerous kinase signaling pathways are shared in DM and CVD pathology, several of which are heavily regulated by post-translational modifications (PTMs)[12]. In this review, we focus on the role of a dynamic PTM known as SUMOylation and its role in regulating certain kinase networks to provide a mechanistic link between DM and CVD.

2. SUMOyation – Dynamic Post-Translational Modification

Small Ubiquitin-related Modifier (SUMO) is a 10-kDa post-translational protein modifier[13,14]. SUMO covalently attaches to lysine (K) residues at a specific consensus motif ΨK-x-D/E in which Ψ is a hydrophobic residue, x is any amino acid, and D/E represent negatively charged aspartic acid and glutamic acid residues. Modification of target proteins can result in conformational changes or alteration of interaction surfaces allowing for regulation of protein functions including intracellular trafficking, cell cycle progression, DNA repair and replication, RNA metabolism, transcriptional regulation, apoptosis, protein stability, and kinase activity[13,15,16]. Four SUMO proteins are expressed in humans; SUMO1–3 are ubiquitously expressed whereas SUMO4 is limited to the kidney, lymph node, and spleen[14,15].

SUMO conjugation (SUMOylation) is a dynamic and reversible modification that allows for transient changes in signal transduction (Fig.1). It utilizes an enzymatic machinery similar to that of ubiquitination[15]. This cascade begins with the E1 activating enzyme and ends with the E3 ligating enzyme that leads to the conjugation of SUMO to lysine residues on target proteins[14]. Several SUMO E3 ligases have been identified including protein inhibitor of activated STAT (PIAS) family (PIAS1, PIAS2(x), PIAS3, PIAS4(y)), Polycomb-2 protein (Pc2), and RanBP2/Nup358[12].

Substrates are deconjugated by SUMO proteases sentrin/SUMO-specific proteases (SENP) and ubiquitin-like protein specific proteases (Ulp) [17,18]. This process is further discussed in the de-SUMOylation section.

3. Endothelial Dysfunction and Diabetes

Endothelial dysfunction is one of the hallmarks of DM and even found in diabetic patients without CVD risk factors[19,20]. Impaired endothelial function can be defined as a loss of vascular homeostasis leading to pathologic inflammation, apoptosis, permeability, and coagulation of the endothelium. Hyperglycemia induces pathologic changes in the endothelium through the production of AGEs (advanced glycation end-products) and ROS (reactive oxygen species)[10]. In vivo studies of the streptozotocin (STZ) induced diabetic rat model show an increase in leukocyte adhesion and rolling with increased expression of inflammatory cytokines and adhesion molecules under high glucose conditions[21]. Furthermore, hyperglycemia induces corresponding epigenetic changes of key inflammation regulators like NF-κB in endothelial cells leading to long-term upregulation of inflammatory gene expression[22,23]. Together these data suggest that hyperglycemia-induced endothelial dysfunction predisposes the vascular wall to atherogenesis. Below we review a few of the regulatory pathways of kinases and SUMO that link DM to CVD.

3.1 NF-κB and IKK

NF-κB is a master regulator of endothelial inflammation and plays an important role in DM and CVD pathogenesis. Hyperglycemia induces numerous epigenetic changes in the endothelium including the upregulation of NF-κB through its downstream mediators AGEs and ROS[22,23]. Similarly, atherogenesis occurs through NF-κB induced leukocyte recruitment, cytokine release, and adhesion molecule expression[24]. Overlap between the two mechanisms highlights the central role of inflammation in linking DM to CVD progression.

Under resting conditions, NF-κB exists in an inactivated state within the cytoplasm bound to IκB (Inhibitor of κB). Stimulants like TNF trigger the activation of IKK (IκB kinase) to phosphorylated IκB, leading to IκB's degradation via ubiquitination and unmasking of NF-κB's nuclear localization signal. This then frees NF-κB to translocate into the nucleus and transactivate pro-inflammatory genes including TNF, IL-1, IL-8, E-selectin, VCAM-1, and ICAM-1[25]. SUMOylation has been reported to be involved at different levels of NF-κB regulation. The NF-κB protein precursor p100 can be modified by SUMO to activate the non-canonical pathway[26]. IκBα can be modified by SUMO-1 to protect it from ubiquitination and degradation, limiting NF-κB activation[27]. In contrast, modification of IκBα by SUMO-2/3 confers the opposite effect and dissociates IκBα from NF-κB leading

to NF-κB activation[28]. Under high glucose conditions, IκBα SUMOylation is increased leading to further activation of NF-κB[29].

IκB kinase (IKK) is an important regulatory kinase in NF-κB activation that is activated during hyperglycemia[30,31]. Composed of two kinases (IKKα and IKKβ) and a regulator subunit NEMO/IKKγ (NF-κB Essential MOdulator), IKK has three total subunits. NEMO does not possess catalytic activity but is required for IKK activation and its subsequent phosphorylation of IκB leading to NF-κB activation[32]. SUMOylation of NEMO during genotoxic stress leads to increased IKK activation and NF-κB activation. Interestingly, the de-SUMOylation enzyme SENP2 is a downstream transcriptional target of NF-κB thereby creating a negative feedback mechanism for NF-κB activation through NEMO de-SUMOylation[33]. However, we did not detect any difference in SENP2 expression induced by disturbed blood flow, and this is further discussed in subsequent sections[34]. Another de-SUMOylation enzyme SENP6 attenuates TLR-triggered inflammation in the endotoxin-induced sepsis murine model via the de-SUMOylation of NEMO at the K277 residue[35].

These data suggest SUMOylation plays an integral role in regulating NF-κB itself at the fundamental level. Conditions of high glucose are able to activate different components of the NF-κB mechanism leading to their regulation by SUMOylation. However these pathways remain not well characterized in vascular tissue and so further investigation is needed to clarify this. Other upstream kinase pathways involved in DM and CVD that lead to downstream NF-κB activation are discussed below.

3.2 Protein Kinase C in DM and CVD

Protein kinase C (PKC) isoforms consists of family of kinases that are chronically activated by hyperglycemia in diabetes. They play an integral role in vascular biology and are responsible for regulating cell growth, apoptosis, inflammation, permeability, and contractility in various cell types including endothelial cells and cariomyocytes[36]. PKC is a serine/threonine kinase that is part of the AGC (protein kinase, A, G, and C). Several isoforms of PKC exist that are subdivided into subgroups that have varying activation requirements. Most PKC isoforms require some combination of diacylglycerol (DAG), calcium, and phospholipid for their activation. The conventional group (α, β1, β2, and γ) require all three components while the novel subtypes (δ, δ1, δ2, δ3, ε, ε, and σ) only require DAG. Atypical isoforms (η, δ, N1, N2, and N3) are the exception and do not need DAG or calcium but require phosphotidylserine for activation[37].

Hyperglycemia triggers chronic activation of PKC due to elevated levels of DAG, a downstream product of the glycolysis intermediate dihydroxyacetone phosphate (DHAP). Diabetic patients have elevated levels of DAG in several organ systems including the heart, large vessels, kidneys, liver, and skeletal muscle[36]. Oxidative stress signaling from H_2O_2 can also trigger PKC activation[38]. In atherosclerosis, PKC plays an important role in regulating hepatic lipid metabolism, gluconeogenesis, insulin resistance, and key components of vascular homeostasis as described above[39–43]. Dysregulation of PKC signaling with diabetes therefore has many pathological consequences that may accelerate DM-mediated CVD.

PKCβ has been studied extensively for its role in diabetes and atherosclerotic plaque formation. PKCβ expression is strongly induced by high glucose in retinal endothelial cells[44]. Diabetic patients have elevated baseline vascular endothelial PKCβ expression compared to non-diabetics leading to impaired eNOS activation. This was rescued by the PKCβ specific synthetic inhibitor LY379196[39]. Activation of PKCβ leads to phosphorylation of IRS2 (insulin receptor substrate 2) at the S303 and S375 sites and inhibits insulin-induced eNOS expression in vascular endothelial cells[45]. Knockout of PKCβ in the T2D obesity leptin-deficient mouse model (ob/ob) is protective against insulin resistance and fat accumulation[43]. Deficiency of PKCβ in ApoE$^{-/-}$ mice resulted in decelerated plaque formation with reduced expression of Egr-1 (early growth response protein-1) which is needed in adhesion molecule expression and matrix metalloproteinase-2 (MMP2)[46]. Subsequent study by the same group found that hyperglycemic STZ-induced ApoE$^{-/-}$ mice had elevated PKCβ expression in their aortas compared to non-treated euglycemic ApoE$^{-/-}$ mice. Pharmacological inhibition of PKCβ with ruboxistaurin reduced plaque lesion size in these diabetic mice. Global PKCβ$^{-/-}$ knockout reduced expression of inflammatory molecules, decreased macrophage content in plaques, and diminished lesion size in these diabetic PKCβ$^{-/-}$/ApoE$^{-/-}$ mice[47]. In addition, stimulation of endothelial cells with oxLDL induces PKCβ phosphorylation and activation[42]. These observations suggest a common pathway shared between diabetic and atherogenic mediators and that perhaps PKCβ plays a critical role in accelerating atherosclerosis in DM through inflammatory signaling (Fig.2).

Despite all of the experimental evidence for the role of PKCβ in DM-mediated atherogenesis, clinical trials with the PKCβ inhibitor ruboxistaurin have not shown improved endothelial function. One randomized clinical trial of 13 T2D patients without CVD treated with ruboxistaurin versus 15 healthy controls failed to show an improvement in endothelial function parameters including vasodilation, fibrinolysis, inflammation, or oxidative stress[48]. Although there are severe limitations of this study, it also suggests that regulation of PKCβ in diabetes most likely involves other regulatory mechanism like PTMs.

PKC contains multiple putative SUMOylation sites and modification by SUMO can affect its activity. PKCα can undergo SUMOylation at the K465 site and also de-SUMOylation by SENP1. Activation of PKC depended on its de-SUMOylation by SENP1 which allowed it to be activated by calcium in a rodent spinal cord neuron cell model[49]. This shows that SUMOylation can play an inhibitory role in modifying PKC kinase function. More studies on the role of SUMOylation and PKCβ in DM and CVD may improve pharmacological therapies in the future.

3.3 ERK5 SUMOylation and DM-mediated Endothelial Inflammation

ERK5 is a mitogen-activated protein kinase (MAPK) with an important role in endothelial homeostasis. Also known as MAPK7, ERK5 is a serine/threonine kinase with only one identified upstream kinase, MEK5[50]. It belongs to a mechano-transduction pathway through which laminar shear stress (steady flow) from blood flow confers anti-inflammatory and atheroprotective signaling on the endothelium. Significant clinical evidence has shown that atherosclerotic plaques localize to areas of the vasculature that experience turbulent and

disturbed flow, primarily areas where the vessels curve or branch[51]. These areas experience significant endothelial dysfunction with increased expression of pro-inflammatory molecules including NF-κB, VCAM1 (vascular cell adhesion molecule-1), MCP1 (monocyte chemotactic protein-1). In contrast, areas of steady laminar flow rarely develop atherosclerosis and are associated with secretion of atheroprotective factors including nitric oxide, prostacyclin, and tissue-type plasminogen activator[50,52].

What is unique about ERK5 in protecting the endothelium from atherosclerosis is that it not only functions as a kinase but also as a transcriptional coactivator[53,54]. As with all MAPKs, ERK5 requires dual phosphorylation on its Thr-X-Tyr motif for activation and has the identical Thr-Glu-Tyr (TEY) sequence as ERK1/2. In the C-terminus is a transactivation domain that allows ERK5 to regulate anti-inflammatory genes[53]. ERK5 provides a mechanistic link between DM and atherosclerosis through its regulation of peroxisome proliferator-activated receptors (PPAR) and Krüppel-like factors (KLF). Its transcriptional function is activated by protective signaling from steady flow but inhibited by disturbed flow, oxidative stress, and hyperglycemia.

PPARs are a family of nuclear receptors (α, β/δ, γ) that function as ligand-activated transcriptional factors involved in regulating inflammatory pathways in endothelial cells[55]. It is well-established that PPARγ plays an important role in DM in terms of regulating glucose metabolism and fatty acid storage, making it a therapeutic target for thiazolidinediones. ERK5 regulates PPARγ's transcriptional activity through a binding mechanism that is independent of phosphorylation. Binding of ERK5 to the hinge-helix 1 region of PPARγ releases its corepressor SMRT (silencing mediator of retinoic acid and thyroid hormone receptor) leading to PPARγ inhibitory signaling of NF-κB activation[54,56–58]. In addition, ERK5 regulates PPARδ transactivation of anti-inflammatory genes including heme oxygenase-1 through a similar binding mechanism but not involving the hinge-helix 1 region[59].

Krüppel-like factor (KLF) family of zinc finger transcription factors is another ERK5 target that plays a crucial role in regulating endothelial inflammation, vascular tone, and permeability[54,60]. KLF2/4 inhibits inflammation via competition with the CBP/p300 cofactor for binding to NF-κB. KLF2/4-mediated down-regulation of NF-κB transcriptional activity attenuates the expression of pro-inflammatory mediators including cytokines, chemokines, and adhesion molecules[61]. In addition, the induction of KLF2/4 expression up-regulates eNOS expression which has vasodilatory, anti-inflammatory, and anti-coagulatory properties[62–64]. Both KLF2 and KLF 4 are highly expressed in the endothelium and induced by steady flow. In contrast, their expression is inhibited by disturbed flow and diabetic mediators (H_2O_2 and AGEs). Activation of ERK5 leads to MEF2 (myocyte enhancer factor-2) transactivation. MEF2 then upregulates KLF2/4, which inhibits endothelial inflammation[60,65]. Endothelial-specific ERK5 knockout mice demonstrate accelerated endothelial inflammation and apoptosis[66,67]. Indeed, we have recently shown that deletion of endothelial ERK5 in LDL receptor knockout mice accelerates atherosclerotic plaque formation[68].

SUMOylation regulates ERK5 by inhibiting its transcriptional ability. Endothelial cells stimulated with either DM mediators (H_2O_2 and AGEs) or atherogenic mediator (disturbed flow) both induced ERK5 SUMOylation and inhibited ERK5 transcriptional activity with reduction of KLF2-mediated eNOS expression. This was reversed with the expression of ERK5-SUMOylation site mutant (K6/22R) that protected the cells from these pathogens and rescued KLF2 expression. Furthermore, ERK5 SUMOylation was increased in the aortas of diabetic mice[34,69].

Together these data highlight the protective role of ERK5 in the endothelium through PPARγ and KLF2/4 regulation and that the pathological loss of this function with SUMOylation by diabetic mediators (Fig.3). The important role of ERK5 in endothelial function suggests that this is a common pathway shared in DM and CVD pathology and that perhaps DM-mediated endothelial dysfunction may predispose the endothelium to the pro-atherosclerotic effects of disturbed flow. Further studies will be needed to clarify the interplay between these mechanisms.

3.4 De-SUMOylation: SENPs in regulating inflammation

The mechanisms described so far have focused on the role of SUMO conjugation in modifying substrates leading to changes in pathological pathways. It is important to note that de-SUMOylation can also play a key role in regulating these pathways. Sentrin/SUMO-specific proteases (SENP) are a family of enzymes that catalyze the de-conjugation of SUMO from target proteins. Six isoforms of SENP (1–3, 5–7) exist in humans and their specificity can be regulated through localization, substrate shielding, and environmental stimuli[18]. For example, both SENP1 and SENP2 are self-regulated through transcriptional feedback loops. Under conditions of genotoxic stress, there is an early increase in SENP2 mRNA followed by de-SUMOlyation of NEMO/IKKγ by SENP2. NEMO then decreases SENP2 transcription thereby creating a negative feedback loop to prevent the survival of damaged cells[33].

While it is true that SENPs may have certain overlapping substrates, they can also have a high degree of substrate specificity. In response to cell hypoxia, SENP1 levels rise in a manner dependent on hypoxia inducible factor 1α (HIF1α). SUMOylation of HIF1α decreases its stability leading to its degradation in response to hypoxia. SENP1 enhances HIF1α stability through de-SUMOylation. $SENP1^{-/-}$ deficient mouse embryos therefore show increased SUMOylated HIF1α and decreased stabilization. What highlights the specificity of these pathways is that HIF1α stability is not affected in $SENP2^{-/-}$ embryos[70,71]. Furthermore, SENP2 de-SUMOylates PPARγ at the K107 residue in vascular smooth muscle cells to inhibit proliferation, migration, and apoptosis as well as reduce neointimal formation after balloon injury[72,73].

Hyperglycemia, oxidative stress, and disturbed flow induce SUMOylation of ERK5 leading to endothelial dysfunction from inflammation, and SUMOylated ERK5 is not present under resting conditions[34,69]. SENP2 protects the endothelium by removing this pathological modification by SUMO on ERK5. Knockdown of SENP2 expression increased ERK5 SUMOylation leading to endothelial inflammation and apoptosis. The effects of disturbed flow-mediated endothelial dysfunction were inhibited *in vitro* in endothelial cells

overexpressing ERK5-SUMOylation sites mutant SENP2 deficient mice showed a significant increase of endothelial inflammation and apoptosis with accelerated formation of atherosclerotic lesions in the aortic arch. Lesions in the aortic arch were also larger than those of the descending aorta suggesting the critical role of disturbed flow in driving SUMOylation of ERK5 in plaque formation. Of note, disturbed flow did not show any effect on SENP2 expression despite the increase in ERK5 SUMOylation[34]. Together these results suggest that SENP2's SUMO protease activity on ERK5, but not SENP2 expression, is actively inhibited by disturbed flow.

While diabetic conditions were not used in this study using SENP2 deficient mice, based on a previous study that hyperglycemic conditions were a strong inducer of ERK5 SUMOylation, it would be a reasonable hypothesis that SENP2 would be regulated in a similar fashion in DM. Further studies examining the role of DM in regulating this mechanism are warranted.

3.5 p90RSK regulates ERK5 and SENP2 in diabetes-mediated atherosclerosis

p90RSK (p90 ribosomal s6 kinase) is a serine/threonine kinase that is unique for its two different functional kinase domains at the terminal ends. The N-terminus kinase belongs to the AGC (protein kinase, A, G, and C) family of kinases and is responsible for the majority of p90RSK's kinase function. In contrast, the C-terminus kinase is a part of the calcium/calmodulin-dependent kinase group and has a minor role in phosphorylation but is required for full activation of p90RSK[74]. Activation of p90RSK occurs from the Raf-MEK-ERK1/2 signaling cascade when ERK1/2 binds to a docking motif on p90RSK's C-terminus leading to full activation of the N-terminus kinase function[50,74].

In DM-mediated CVD, p90RSK plays a key role in mediating endothelial dysfunction. High glucose and H_2O_2 inhibit the transactivation of anti-inflammatory genes by ERK5 through p90RSK[68]. Specifically, p90RSK binds to ERK5's C-terminus and phosphorylates ERK5 at the S496 residue leading to enhanced VCAM-1 expression and reduced eNOS expression. Aortas from diabetic mice showed elevated activity and expression of p90RSK. These phenotypes were rescued by the p90RSK-specific kinase inhibitor FMK-MEA (fluoromethyl ketone-methoxyethylamine) with decreased leukocyte recruitment and vascular reactivity in these diabetic mice. Plaque formation was reduced in FMK-MEA treated ApoE$^{-/-}$ mice and an endothelial-specific ERK5 deletion demonstrated resistance to these protective effects of FMK-MEA. Together these results suggest that p90RSK plays an important role in regulating ERK5 function in DM-mediated endothelial dysfunction.

Furthermore, p90RSK regulates ERK5 SUMOylation through the SENP2 dependent mechanism described earlier[34,75]. Activation of p90RSK leads to SENP2 phosphorylation at the T368 residue, causing a loss of SENP2's de-SUMOylation protease function and an increase in ERK5 SUMOylation. Inhibition of ERK5's transactivation function leads to a loss of anti-inflammatory gene expression and increased endothelial inflammation, apoptosis, and dysfunction (Fig.3). Endothelial-specific overexpression of p90RSK increased endothelial cell dysfunction and plaque formation in mouse aortas that was not present in dominant-negative p90RSK mice. Depletion of SENP2 in DN-p90RSK mice eliminated this atheroprotective phenotype[75].

4. Cardiomyopathy

Chronic exposure of the myocardium to hyperglycemia and oxidative stress results in pathological changes that cause cardiomyopathy in diabetics. Development of systolic and diastolic ventricular dysfunction occurs from a combination of endothelial dysfunction, fibrosis, altered glucose and fatty acid metabolism, mitochondrial defects, and increased ROS. As shown in previous epidemiological studies, there is no evidence that rigid glycemic control alters the progression of diabetic heart failure[6–9].

Apoptosis of cardiomyocytes is one of the key pathological events in the progression of diabetic cardiomyopathy. Loss of myocytes through apoptosis represents a pathological transition from cardiac hypertrophy to heart failure. Failing human hearts have an increased activation of neurohormonal systems including the sympathetic nervous system (SNS) and the renin-angiotensin-aldosterone system (RAAS) to maintain circulatory homeostasis in the face of declining cardiac output. Circulating catecholamines and hormones activate a number of kinase pathways that precipitate the vicious cycle of worsening heart failure. Below we discuss some of the kinase pathways in which SUMOylation is involved in regulating cardiac apoptosis in diabetic cardiomyopathy.

4.1 PDE3A-ICER and ERK5-SUMOylation in Cardiomyocyte Apoptosis

Cyclic AMP signaling plays an important role in transmitting signaling from neurohormonal molecules in heart failure. Failing human hearts demonstrate significantly reduced expression of cAMP hydrolyzing enzymes including phosphodiesterase 3A (PDE3A) but increased expression of inducible cAMP early repressor (ICER). ICER is an important regulator of myocyte apoptosis because it is a proapoptotic transcriptional repressor that inhibits the transactivation of cAMP response element binding protein (CREB) leading to downregulation of Bcl2. In addition, ICER represses PDE3A gene transcription leading to increased cAMP availability and upregulation of PKA signaling, forming an autoregulatory positive feedback loop (Fig.4). Angiotensin II and isoproterenol (β-AR agonist) activate this mechanism by downregulating PDE3A and upregulating ICER, providing a mechanism of how the activation of neurohormonal systems in heart failure affects myocyte apoptosis[76,77].

ERK5 plays a critical role in regulating this cardiac apoptosis pathway. As discussed before, ERK5 is a unique kinase that possesses transcriptional activity that allows it to upregulate anti-inflammatory and anti-apoptotic genes to maintain endothelial homeostasis. SUMOylation regulates ERK5 by inhibiting its transcriptional ability. Just as in endothelial cells, diabetic mediators (H_2O_2 and AGEs) increased ERK5 SUMOylation and inhibited its transactivation of anti-inflammatory genes in cardiomyocytes[34,69,78]. Cardiac-specific ERK5 knockouts experience accelerated cardiac apoptosis and dysfunction after thoracic aorta constriction[79]. Overexpression of cardiac-specific constitutively active MEK5 (CA-MEK5), ERK5's direct upstream kinase, in mice inhibited ICER induction and decreased myocyte apoptosis in the pressure-overload and myocardial infarction model[78,80]. Activation of ERK5 with insulin growth factor-1 also reduced myocyte apoptosis, and this protective effect was abolished by ERK5 siRNA or DN-ERK5 overexpression[80].

ERK5 SUMOylation was increased in the cardiomyocytes of STZ-induced diabetic mice versus control. Induction of MI in these diabetic mice (DM + MI) with left coronary artery ligation demonstrated increased ERK5 SUMOylation along with increased LV dysfunction and cardiomyocyte apoptosis, but this was not present in the non-diabetic MI group. This DM + MI phenotype was rescued in diabetic CA-MEK5 transgenic mice, which showed significantly decreased ERK5 SUMOylation, improved LV function, and decreased apoptosis[78]. Combined with the data that ERK5 is a negative regulator of the PDE3A-ICER mechanism and thus cardioprotective by inhibiting apoptosis, the inhibitory effect of SUMOylation on ERK5 suggests that ERK5 SUMOylation is an important event in regulating cardiac apoptosis in diabetes.

4.2 ERK5-CHIP Promote ICER Degradation

Degradation of proteins by post-translational modification ubiquitination is an important mechanism for protein quality control but is also important in signal transduction. Transient changes in signaling can be implemented through degradation of unnecessary molecules such as activating signaling by degrading repressor molecules, inhibiting signaling by degrading active molecules, etc. Regulation of the PDE3A-ICER mechanism by ERK5 occurs in this manner through an E3 ubiquitin (Ub) ligase named CHIP (carboxyl terminus of HSP70-interacting protein). CHIP has an important cardioprotective role in limiting myocardial damage from ischemia/reperfusion injury after MI by inhibiting apoptosis. Transgenic CHIP knockouts had increased infarct sizes and decreased survival compared to wild-type[81].

Activation of ERK5 decreased ICER protein stability through ubiquitin-mediated degradation. It also turns out that ICER is a CHIP substrate and that ERK5 is required to bind and activate CHIP as disruption of ERK5-CHIP binding with a peptide fragment inhibited CHIP's Ub ligase activity. In diabetic mice induced by MI (DM + MI), CHIP Ub activity was decreased but this effect was not present in diabetic CA-MEK5 transgenic mice[82]. MEK5 is the direct upstream kinase of ERK5 and diabetic CA-MEK5 transgenic mice have a cardioprotective phenotype with decreased cardiac apoptosis after MI[78]. Furthermore, ERK5 activation increased CHIP Ub ligase activity and decreased ICER expression after MI[82]. Together these studies provide a mechanism how ERK5 and CHIP interact to protect the myocardium by promoting ICER degradation through ubiquitination leading to the inhibition of myocyte apoptosis.

4.3 p90RSK Inhibits ERK5-CHIP to Promote Cardiac Apoptosis

p90RSK plays an important role in the diabetic heart. It is strongly activated in cardiomyocytes by mechanical and oxidative stress under conditions for ischemia/reperfusion, cardiac hypertrophy, and heart failure[83–86]. Failing human hearts have increased activation of p90RSK[87]. In diabetic mice, cardiac p90RSK is significantly activated versus non-DM mice. Transgenic mice overexpressing cardiac-specific p90RSK have significant cardiac dysfunction with increased interstitial fibrosis and apoptosis, decreased contractility, and increased hypertrophy compared to control[88].

We have previously mentioned that angiotensin II was a strong upregulator of ICER leading to cardiomyocytes apoptosis[76,77]. It turns out that this mechanism is dependent on angiotensin II induction of p90RSK activation, leading to an increase in ICER protein levels and increased apoptosis. Activation of p90RSK reduces CHIP's Ub ligase activity in diabetic mice and this occurs due to a direct competition with CHIP for ERK5 binding and p90RSK's phosphorylation of the ERK5 S496 residue. Cardiac-specific knockout of either ERK5 or overexpression of p90RSK reduced CHIP Ub ligase activity and increased ICER levels after MI. This means that cardiac-specific knockouts accelerate cardiac apoptosis after MI but that remarkably, this phenotype is reversed by activation of ERK5[89]. When combined with the previous mechanisms described, these studies suggest that in the diabetic heart elevated, neurohormonal signaling from the failing heart through the SNS or RAAS activates a kinase cascade through p90RSK that disrupts ERK5-CHIP leading to an upregulation of ICER and subsequent cardiac apoptosis (Fig.4).

5. Conclusion

Diabetes and cardiovascular disease have a strong clinical correlation based on numerous epidemiological studies[1–4,6–9]. Patients with cardiovascular disease complicated by diabetes have poorer clinical outcomes than those without and are associated with increased morbidity and mortality[2,5]. However there is conflicting evidence on the role of glycemic control in improving DM-complicated CVD outcomes suggesting a multifactorial etiology in the association[6–9]. In this review, we have highlighted the role of SUMOylation in modifying the kinase pathways that link diabetes with cardiovascular disease. Examining these pathways, it is clear that pathological mediators of diabetes like hyperglycemia and oxidative stress contribute to the development cardiovascular disease. Kinases like PKC, ERK5, and p90RSK are activated under diabetic conditions and play central roles in regulating a complex network of signal transduction that is continuously modified by SUMOylation as it is evident from the mouse transgenic models (Table 1).

Each kinase-SUMOylation-related signaling described here can reveal different consequences in different cell systems by regulating different down-stream events. For example, there are differences between the roles of p90RSK in regulating ERK5 in endothelial cells versus cardiomyocytes. In endothelial cells, p90RSK regulation of ERK5 SUMOylation either directly or through SENP2 leads to a downstream cascade of events that up regulate endothelial inflammation leading to atherosclerosis. In cardiomyocytes, the same p90RSK-ERK5-SUMOylation results in cardiomyocyte apoptosis through the PDE3A-ICER mechanism. Although it would be reasonable to speculate the similar functional consequence of these pathways among different cell types, there have not been studies to investigate the role of this pathway in different cell systems. PDE3A-ICER has primarily been studied in cardiomyocytes due to its role in the neurohormonal signaling in heart failure, but its role in endothelial cells is not well-defined. In addition, there are only limited studies on site-specific SUMOylation mutant knockin *in vivo* and the identification of site-specific small-target SUMO inhibitors. Studies so far have focused primarily on using non-specific global SUMO inhibitors or *in vitro* mechanistic studies of SUMOylation site mutants. Therefore, further investigation using site-specific SUMOylation mutant knockin *in vivo* will allow for the identification of new therapeutic targets to improve clinical outcomes.

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Abbreviations

AGE	advanced glycation end-products
CVD	cardiovascular disease
DM	Type II diabetes mellitus
EC	endothelial cell
ERK5	extracellular-signal regulated kinase 5
KLF	Krüppel-like factor
KO	knock-out
ICER	inducible cAMP early repressor
NEMO	NF-κB Essential MOdulator
NF-κB	nuclear factor kappa B
p90RSK	p90 ribosomal S6 kinase
PDE3A	phosphodiesterase 3A
PKC	protein kinase C
PIAS	protein inhibitor of activated STAT
PPAR	peroxisome proliferator-activated receptor
PTM	post-translational modification
ROS	reactive oxygen species
SENP	sentrin-specific protease
STZ	streptozotocin
SUMO	Small Ubiquitin-related Modifier
TAC	transverse aortic constriction
VE-cadherin	vascular endothelial cadherin

References

1. Preis SR, et al. Trends in cardiovascular disease risk factors in individuals with and without diabetes mellitus in the Framingham Heart Study. Circulation. 2009; 120:212–220. [PubMed: 19581493]

2. Kannel WB, McGee DL. Diabetes and cardiovascular risk factors: the Framingham study. Circulation. 1979; 59:8–13. [PubMed: 758126]
3. UK Prospective Diabetes Study (UKPDS) Group. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). Lancet. 1998; 352:837–853. [PubMed: 9742976]
4. Stamler J, Vaccaro O, Neaton JD, Wentworth D. Diabetes, other risk factors, and 12-yr cardiovascular mortality for men screened in the Multiple Risk Factor Intervention Trial. Diabetes care. 1993; 16:434–444. [PubMed: 8432214]
5. Smith SC Jr, et al. AHA/ACC Guidelines for Preventing Heart Attack and Death in Patients With Atherosclerotic Cardiovascular Disease: 2001 update. A statement for healthcare professionals from the American Heart Association and the American College of Cardiology. Journal of the American College of Cardiology. 2001; 38:1581–1583. [PubMed: 11691544]
6. Group AC, et al. Intensive blood glucose control and vascular outcomes in patients with type 2 diabetes. The New England journal of medicine. 2008; 358:2560–2572. [PubMed: 18539916]
7. Duckworth W, et al. Glucose control and vascular complications in veterans with type 2 diabetes. The New England journal of medicine. 2009; 360:129–139. [PubMed: 19092145]
8. Ismail-Beigi F, et al. Effect of intensive treatment of hyperglycaemia on microvascular outcomes in type 2 diabetes: an analysis of the ACCORD randomised trial. Lancet. 2010; 376:419–430. [PubMed: 20594588]
9. Holman RR, Paul SK, Bethel MA, Matthews DR, Neil HA. 10-year follow-up of intensive glucose control in type 2 diabetes. The New England journal of medicine. 2008; 359:1577–1589. [PubMed: 18784090]
10. Shaw A, Doherty MK, Mutch NJ, MacRury SM, Megson IL. Endothelial cell oxidative stress in diabetes: a key driver of cardiovascular complications? Biochemical Society transactions. 2014; 42:928–933. [PubMed: 25109981]
11. Paneni F, Beckman JA, Creager MA, Cosentino F. Diabetes and vascular disease: pathophysiology, clinical consequences, and medical therapy: part I. Eur Heart J. 2013; 34:2436–2443. [PubMed: 23641007]
12. Gao C, Huang W, Kanasaki K, Xu Y. The role of ubiquitination and sumoylation in diabetic nephropathy. BioMed research international. 2014; 2014:160692. [PubMed: 24991536]
13. Hay RT. SUMO: a history of modification. Molecular cell. 2005; 18:1–12. [PubMed: 15808504]
14. Guo B, Yang SH, Witty J, Sharrocks AD. Signalling pathways and the regulation of SUMO modification. Biochemical Society transactions. 2007; 35:1414–1418. [PubMed: 18031234]
15. Geiss-Friedlander R, Melchior F. Concepts in sumoylation: a decade on. Nature reviews. Molecular cell biology. 2007; 8:947–956. [PubMed: 18000527]
16. Chang E, et al. MK2 SUMOylation regulates actin filament remodeling and subsequent migration in endothelial cells by inhibiting MK2 kinase and HSP27 phosphorylation. Blood. 2011; 117:2527–2537. [PubMed: 21131586]
17. Yeh ET. SUMOylation and De-SUMOylation: wrestling with life's processes. The Journal of biological chemistry. 2009; 284:8223–8227. [PubMed: 19008217]
18. Hickey CM, Wilson NR, Hochstrasser M. Function and regulation of SUMO proteases. Nature reviews. Molecular cell biology. 2012; 13:755–766. [PubMed: 23175280]
19. Makimattila S, et al. Chronic hyperglycemia impairs endothelial function and insulin sensitivity via different mechanisms in insulin-dependent diabetes mellitus. Circulation. 1996; 94:1276–1282. [PubMed: 8822980]
20. Clarkson P, et al. Impaired vascular reactivity in insulin-dependent diabetes mellitus is related to disease duration and low density lipoprotein cholesterol levels. Journal of the American College of Cardiology. 1996; 28:573–579. [PubMed: 8772741]
21. Smolock AR, Mishra G, Eguchi K, Eguchi S, Scalia R. Protein kinase C upregulates intercellular adhesion molecule-1 and leukocyte-endothelium interactions in hyperglycemia via activation of endothelial expressed calpain. Arteriosclerosis, thrombosis, and vascular biology. 2011; 31:289–296.

22. El-Osta A, et al. Transient high glucose causes persistent epigenetic changes and altered gene expression during subsequent normoglycemia. The Journal of experimental medicine. 2008; 205:2409–2417. [PubMed: 18809715]
23. Bornfeldt KE, Tabas I. Insulin resistance, hyperglycemia, and atherosclerosis. Cell metabolism. 2011; 14:575–585. [PubMed: 22055501]
24. Collins T, Cybulsky MI. NF-kappaB: pivotal mediator or innocent bystander in atherogenesis? The Journal of clinical investigation. 2001; 107:255–264. [PubMed: 11160146]
25. Xiao L, Liu Y, Wang N. New paradigms in inflammatory signaling in vascular endothelial cells. American journal of physiology. Heart and circulatory physiology. 2014; 306:H317–325. [PubMed: 24285111]
26. Vatsyayan J, Qing G, Xiao G, Hu J. SUMO1 modification of NF-kappaB2/p100 is essential for stimuli-induced p100 phosphorylation and processing. EMBO reports. 2008; 9:885–890. [PubMed: 18617892]
27. Desterro JM, Rodriguez MS, Hay RT. SUMO-1 modification of IkappaBalpha inhibits NF-kappaB activation. Molecular cell. 1998; 2:233–239. [PubMed: 9734360]
28. Culver C, et al. Mechanism of hypoxia-induced NF-kappaB. Molecular and cellular biology. 2010; 30:4901–4921. [PubMed: 20696840]
29. Huang W, et al. High glucose induces activation of NF-kappaB inflammatory signaling through IkappaBalpha sumoylation in rat mesangial cells. Biochemical and biophysical research communications. 2013; 438:568–574. [PubMed: 23911785]
30. Ramana KV, Friedrich B, Srivastava S, Bhatnagar A, Srivastava SK. Activation of nuclear factor-kappaB by hyperglycemia in vascular smooth muscle cells is regulated by aldose reductase. Diabetes. 2004; 53:2910–2920. [PubMed: 15504972]
31. Mohan S, Konopinski R, Yan B, Centonze VE, Natarajan M. High glucose-induced IKK-Hsp-90 interaction contributes to endothelial dysfunction. American journal of physiology. Cell physiology. 2009; 296:C182–192. [PubMed: 18945937]
32. Israel A. The IKK complex, a central regulator of NF-kappaB activation. Cold Spring Harbor perspectives in biology. 2010; 2:a000158. [PubMed: 20300203]
33. Miyamoto S. Nuclear initiated NF-kappaB signaling: NEMO and ATM take center stage. Cell research. 2011; 21:116–130. [PubMed: 21187855]
34. Heo KS, et al. De-SUMOylation enzyme of sentrin/SUMO-specific protease 2 regulates disturbed flow-induced SUMOylation of ERK5 and p53 that leads to endothelial dysfunction and atherosclerosis. Circulation research. 2013; 112:911–923. [PubMed: 23381569]
35. Liu X, Chen W, Wang Q, Li L, Wang C. Negative regulation of TLR inflammatory signaling by the SUMO-deconjugating enzyme SENP6. PLoS pathogens. 2013; 9:e1003480. [PubMed: 23825957]
36. Geraldes P, King GL. Activation of protein kinase C isoforms and its impact on diabetic complications. Circulation research. 2010; 106:1319–1331. [PubMed: 20431074]
37. Newton AC. Regulation of the ABC kinases by phosphorylation: protein kinase C as a paradigm. The Biochemical journal. 2003; 370:361–371. [PubMed: 12495431]
38. Konishi H, et al. Activation of protein kinase C by tyrosine phosphorylation in response to H2O2. Proceedings of the National Academy of Sciences of the United States of America. 1997; 94:11233–11237. [PubMed: 9326592]
39. Tabit CE, et al. Protein kinase C-beta contributes to impaired endothelial insulin signaling in humans with diabetes mellitus. Circulation. 2013; 127:86–95. [PubMed: 23204109]
40. Schmitz-Peiffer C. The tail wagging the dog--regulation of lipid metabolism by protein kinase C. FEBS J. 2013; 280:5371–5383. [PubMed: 23587021]
41. Hennige AM, et al. Enforced expression of protein kinase C in skeletal muscle causes physical inactivity, fatty liver and insulin resistance in the brain. J Cell Mol Med. 2010; 14:903–913. [PubMed: 20569275]
42. Shi Y, et al. Oxidized low-density lipoprotein activates p66Shc via lectin-like oxidized low-density lipoprotein receptor-1, protein kinase C-beta, and c-Jun N-terminal kinase kinase in human endothelial cells. Arteriosclerosis, thrombosis, and vascular biology. 2011; 31:2090–2097.

43. Huang W, Bansode RR, Bal NC, Mehta M, Mehta KD. Protein kinase Cbeta deficiency attenuates obesity syndrome of ob/ob mice by promoting white adipose tissue remodeling. J Lipid Res. 2012; 53:368–378. [PubMed: 22210924]
44. Park JY, et al. Induction of endothelin-1 expression by glucose: an effect of protein kinase C activation. Diabetes. 2000; 49:1239–1248. [PubMed: 10909984]
45. Park K, et al. Serine phosphorylation sites on IRS2 activated by angiotensin II and protein kinase C to induce selective insulin resistance in endothelial cells. Mol Cell Biol. 2013; 33:3227–3241. [PubMed: 23775122]
46. Harja E, et al. Mice deficient in PKCbeta and apolipoprotein E display decreased atherosclerosis. FASEB journal : official publication of the Federation of American Societies for Experimental Biology. 2009; 23:1081–1091. [PubMed: 19036858]
47. Kong L, et al. PKCbeta promotes vascular inflammation and acceleration of atherosclerosis in diabetic ApoE null mice. Arteriosclerosis, thrombosis, and vascular biology. 2013; 33:1779–1787.
48. Beckman JA, et al. Inhibition of protein kinase Cbeta does not improve endothelial function in type 2 diabetes. J Clin Endocrinol Metab. 2010; 95:3783–3787. [PubMed: 20444914]
49. Sun H, et al. Kainate receptor activation induces glycine receptor endocytosis through PKC deSUMOylation. Nat Commun. 2014; 5:4980. [PubMed: 25236484]
50. Abe J, Berk BC. Novel mechanisms of endothelial mechanotransduction. Arteriosclerosis, thrombosis, and vascular biology. 2014; 34:2378–2386.
51. Jongstra-Bilen J, et al. Low-grade chronic inflammation in regions of the normal mouse arterial intima predisposed to atherosclerosis. The Journal of experimental medicine. 2006; 203:2073–2083. [PubMed: 16894012]
52. Heo KS, Fujiwara K, Abe J. Disturbed-flow-mediated vascular reactive oxygen species induce endothelial dysfunction. Circulation journal : official journal of the Japanese Circulation Society. 2011; 75:2722–2730. [PubMed: 22076424]
53. Kasler HG, Victoria J, Duramad O, Winoto A. ERK5 is a novel type of mitogen-activated protein kinase containing a transcriptional activation domain. Molecular and cellular biology. 2000; 20:8382–8389. [PubMed: 11046135]
54. Akaike M, et al. The hinge-helix 1 region of peroxisome proliferator-activated receptor gamma1 (PPARgamma1) mediates interaction with extracellular signal-regulated kinase 5 and PPARgamma1 transcriptional activation: involvement in flow-induced PPARgamma activation in endothelial cells. Molecular and cellular biology. 2004; 24:8691–8704. [PubMed: 15367687]
55. Hamblin M, Chang L, Fan Y, Zhang J, Chen YE. PPARs and the cardiovascular system. Antioxidants & redox signaling. 2009; 11:1415–1452. [PubMed: 19061437]
56. Wang N, et al. Constitutive activation of peroxisome proliferator-activated receptor-gamma suppresses pro-inflammatory adhesion molecules in human vascular endothelial cells. The Journal of biological chemistry. 2002; 277:34176–34181. [PubMed: 12107164]
57. Lee CH, et al. Transcriptional repression of atherogenic inflammation: modulation by PPARdelta. Science. 2003; 302:453–457. [PubMed: 12970571]
58. Straus DS, Glass CK. Anti-inflammatory actions of PPAR ligands: new insights on cellular and molecular mechanisms. Trends in immunology. 2007; 28:551–558. [PubMed: 17981503]
59. Woo CH, et al. ERK5 activation inhibits inflammatory responses via peroxisome proliferator-activated receptor delta (PPARdelta) stimulation. The Journal of biological chemistry. 2006; 281:32164–32174. [PubMed: 16943204]
60. Parmar KM, et al. Integration of flow-dependent endothelial phenotypes by Kruppel-like factor 2. The Journal of clinical investigation. 2006; 116:49–58. [PubMed: 16341264]
61. SenBanerjee S, et al. KLF2 Is a novel transcriptional regulator of endothelial proinflammatory activation. The Journal of experimental medicine. 2004; 199:1305–1315. [PubMed: 15136591]
62. Lin Z, et al. Kruppel-like factor 2 regulates endothelial barrier function. Arteriosclerosis, thrombosis, and vascular biology. 2010; 30:1952–1959.
63. Sen-Banerjee S, et al. Kruppel-like factor 2 as a novel mediator of statin effects in endothelial cells. Circulation. 2005; 112:720–726. [PubMed: 16043642]

64. Villarreal G Jr, et al. Defining the regulation of KLF4 expression and its downstream transcriptional targets in vascular endothelial cells. Biochemical and biophysical research communications. 2010; 391:984–989. [PubMed: 19968965]

65. Ohnesorge N, et al. Erk5 activation elicits a vasoprotective endothelial phenotype via induction of Kruppel-like factor 4 (KLF4). The Journal of biological chemistry. 2010; 285:26199–26210. [PubMed: 20551324]

66. Hayashi M, et al. Targeted deletion of BMK1/ERK5 in adult mice perturbs vascular integrity and leads to endothelial failure. The Journal of clinical investigation. 2004; 113:1138–1148. [PubMed: 15085193]

67. Sohn SJ, Sarvis BK, Cado D, Winoto A. ERK5 MAPK regulates embryonic angiogenesis and acts as a hypoxia-sensitive repressor of vascular endothelial growth factor expression. The Journal of biological chemistry. 2002; 277:43344–43351. [PubMed: 12221099]

68. Le NT, et al. A crucial role for p90RSK-mediated reduction of ERK5 transcriptional activity in endothelial dysfunction and atherosclerosis. Circulation. 2013; 127:486–499. [PubMed: 23243209]

69. Woo CH, et al. Extracellular signal-regulated kinase 5 SUMOylation antagonizes shear stress-induced antiinflammatory response and endothelial nitric oxide synthase expression in endothelial cells. Circulation research. 2008; 102:538–545. [PubMed: 18218985]

70. Li X, et al. SENP1 mediates TNF-induced desumoylation and cytoplasmic translocation of HIPK1 to enhance ASK1-dependent apoptosis. Cell death and differentiation. 2008; 15:739–750. [PubMed: 18219322]

71. Cheng J, Kang X, Zhang S, Yeh ET. SUMO-specific protease 1 is essential for stabilization of HIF1alpha during hypoxia. Cell. 2007; 131:584–595. [PubMed: 17981124]

72. Lim S, et al. Effect of a peroxisome proliferator-activated receptor gamma sumoylation mutant on neointimal formation after balloon injury in rats. Atherosclerosis. 2009; 206:411–417. [PubMed: 19339015]

73. Nigro P, et al. PKCzeta decreases eNOS protein stability via inhibitory phosphorylation of ERK5. Blood. 2010; 116:1971–1979. [PubMed: 20538799]

74. Frodin M, Gammeltoft S. Role and regulation of 90 kDa ribosomal S6 kinase (RSK) in signal transduction. Molecular and cellular endocrinology. 1999; 151:65–77. [PubMed: 10411321]

75. Heo KS, et al. Disturbed flow-activated p90RSK kinase accelerates atherosclerosis by inhibiting SENP2 function. The Journal of clinical investigation. 2015

76. Ding B, et al. Left ventricular hypertrophy in ascending aortic stenosis mice: anoikis and the progression to early failure. Circulation. 2000; 101:2854–2862. [PubMed: 10859293]

77. Ding B, et al. A positive feedback loop of phosphodiesterase 3 (PDE3) and inducible cAMP early repressor (ICER) leads to cardiomyocyte apoptosis. Proc Natl Acad Sci U S A. 2005; 102:14771–14776. [PubMed: 16186489]

78. Shishido T, et al. Effects of MEK5/ERK5 association on small ubiquitin-related modification of ERK5: implications for diabetic ventricular dysfunction after myocardial infarction. Circulation research. 2008; 102:1416–1425. [PubMed: 18467627]

79. Kimura TE, et al. Targeted deletion of the extracellular signal-regulated protein kinase 5 attenuates hypertrophic response and promotes pressure overload-induced apoptosis in the heart. Circulation research. 2010; 106:961–970. [PubMed: 20075332]

80. Yan C, et al. Activation of extracellular signal-regulated kinase 5 reduces cardiac apoptosis and dysfunction via inhibition of a phosphodiesterase 3A/inducible cAMP early repressor feedback loop. Circulation research. 2007; 100:510–519. [PubMed: 17272811]

81. Zhang C, Xu Z, He XR, Michael LH, Patterson C. CHIP, a cochaperone/ubiquitin ligase that regulates protein quality control, is required for maximal cardioprotection after myocardial infarction in mice. American journal of physiology. Heart and circulatory physiology. 2005; 288:H2836–2842. [PubMed: 15665051]

82. Woo CH, et al. Novel role of C terminus of Hsc70-interacting protein (CHIP) ubiquitin ligase on inhibiting cardiac apoptosis and dysfunction via regulating ERK5-mediated degradation of inducible cAMP early repressor. FASEB J. 2010; 24:4917–4928. [PubMed: 20724525]

83. Yamazaki T, et al. Mechanical stress activates protein kinase cascade of phosphorylation in neonatal rat cardiac myocytes. The Journal of clinical investigation. 1995; 96:438–446. [PubMed: 7615816]

84. Seko Y, Tobe K, Ueki K, Kadowaki T, Yazaki Y. Hypoxia and hypoxia/reoxygenation activate Raf-1, mitogen-activated protein kinase kinase, mitogen-activated protein kinases, and S6 kinase in cultured rat cardiac myocytes. Circulation research. 1996; 78:82–90. [PubMed: 8603510]

85. Takeishi Y, et al. Differential regulation of p90 ribosomal S6 kinase and big mitogen-activated protein kinase 1 by ischemia/reperfusion and oxidative stress in perfused guinea pig hearts. Circulation research. 1999; 85:1164–1172. [PubMed: 10590243]

86. Takeishi Y, et al. Src and multiple MAP kinase activation in cardiac hypertrophy and congestive heart failure under chronic pressure-overload: comparison with acute mechanical stretch. J Mol Cell Cardiol. 2001; 33:1637–1648. [PubMed: 11549343]

87. Takeishi Y, et al. Activation of mitogen-activated protein kinases and p90 ribosomal S6 kinase in failing human hearts with dilated cardiomyopathy. Cardiovasc Res. 2002; 53:131–137. [PubMed: 11744021]

88. Itoh S, et al. Role of p90 ribosomal S6 kinase-mediated prorenin-converting enzyme in ischemic and diabetic myocardium. Circulation. 2006; 113:1787–1798. [PubMed: 16585392]

89. Le NT, et al. p90RSK targets the ERK5-CHIP ubiquitin E3 ligase activity in diabetic hearts and promotes cardiac apoptosis and dysfunction. Circulation research. 2012; 110:536–550. [PubMed: 22267842]

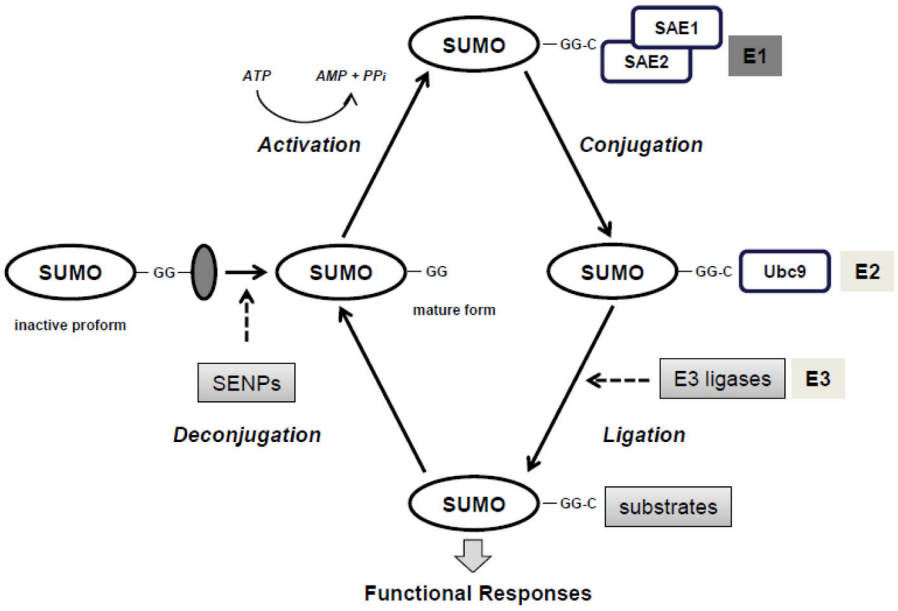

Fig.1. Mechanism of SUMOylation

SUMO conjugation (SUMOylation) is a dynamic and reversible modification utilizing an E1, E2, and E3 mechanism to regulate target proteins leading to changes in signal transduction. This cascade begins with an E1 activating enzyme that is a heterodimer containing SAE1/SAE2 subunits, which activate SUMO in an ATP-dependent manner. Next, activated SUMO is transferred to the E2 conjugating enzyme (Ubc9) to facilitate SUMO protein attachment to lysine (K) residues on target proteins. The final role is played by the E3 ligating enzyme that catalyzes efficient modification by binding to the E2 enzyme. Dotted lines denote catalyst role of respective enzymes.

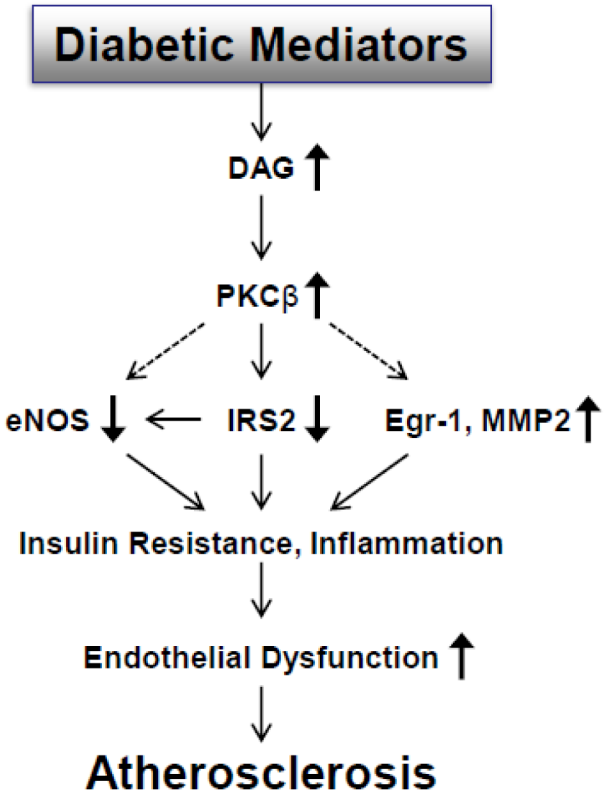

Fig.2. Endothelial Dysfunction: PKCβ induces insulin resistance and endothelial inflammation

Hyperglycemia triggers the chronic activation of PKCβ due to elevated levels of DAG leading to insulin resistance via IRS2 phosphorylation, inflammation from Erg-1 and MMP2 activation, and reduced eNOS expression; events that promote endothelial dysfunction and atherosclerosis.

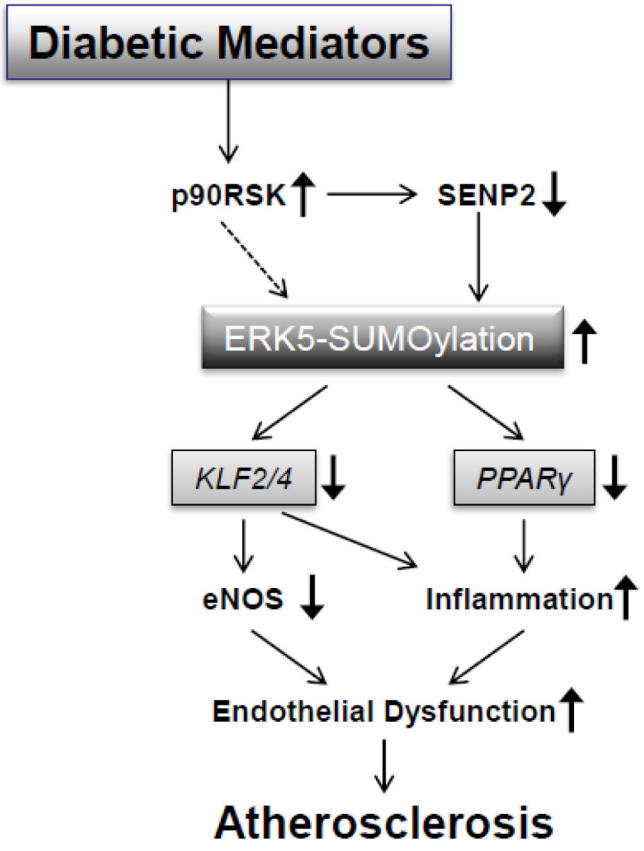

Fig.3. Endothelial Dysfunction: p90RSK regulates ERK5-SUMOylation and SENP2 in DM-mediated atherosclerosis

Diabetes mediators activate p90RSK to regulate SENP2 and ERK5 to induce ERK5-SUMOylation. This causes a downregulation of ERK5's transcriptional activity leading to transrepression of the atheroprotective genes KLF2/4 and PPARγ and subsequent development of endothelial dysfunction and atherosclerosis.

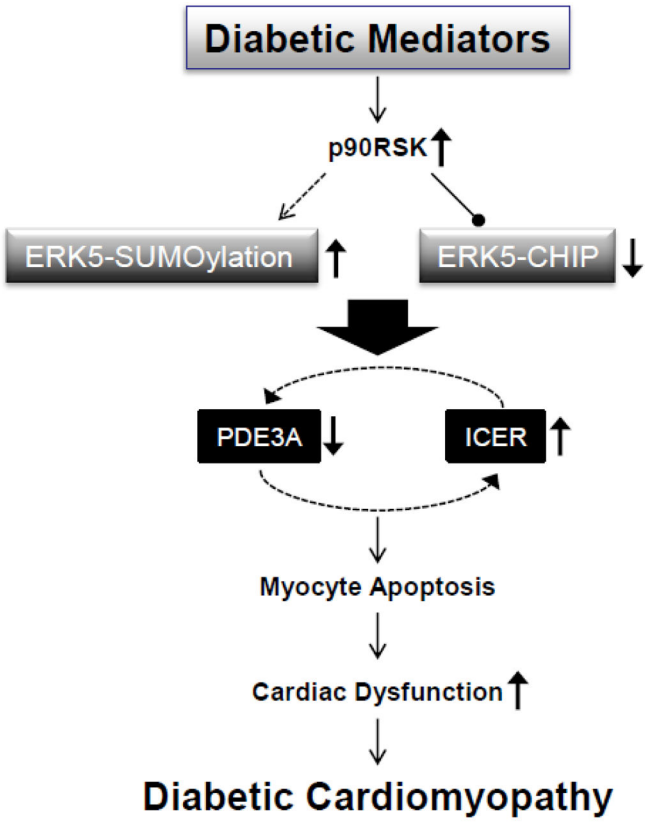

Fig.4. Diabetic Cardiomyopathy: p90RSK disrupts ERK5-SUMOylation and ERK5-CHIP to upregulate ICER and subsequent cardiac apoptosis

Activation of p90RSK by diabetic mediators leads to the disruption of ERK5-CHIP binding and upregulation of ERK5-SUMOylation. These two events disrupt the PDE3A-ICER feedback loop leading to an upregulation of proapoptotic ICER that causes myocyte apoptosis and cardiac dysfunction that contribute to diabetic cardiomyopathy.

Table 1

Summary of Transgenic Models and Phenotypes

Kinase	Transgenic Model	Model Characteristics	Cardiovascular Phenotype
PKCβ	PKCβ$^{-/-}$ in ob/ob$^{-/-}$	• Non-lethal • Increased thermogenesis and enhanced insulin sensitivity[43] • Decreased fat accumulation[43]	• Not de
	PKCβ$^{-/-}$ in ApoE$^{-/-}$	• Non-lethal	• Decele plaque format with re macro conten • Increa MMP2 expres
ERK5	ERK5$^{-/-}$	• Embryonically lethal	• Loss o vascul integri EC misali and le due to in angiog
	EC-specific ERK5$^{-/-}$ in Ldlr$^{-/-}$	• EC-specific ERK5 KO flox mice under VE-cadherin promoter	• Increa endoth inflam and ap with accele plaque format
	Cardiac-specific ERK5$^{-/-}$	• Cardiac-specific ERK5 KO flox mice under α-MHC promoter • No cardiac abnormalities in morphology or function up to 6months of age	• Attenu cardia hypert remod with accele cardia apopto dysfun after T • Reduc CHIP ligase and in ICER MI[89]
p90RSK	EC-specific p90RSK overexpression	• EC-specific p90RSK KO flox mice under VE-cadherin promoter	• Increa dysfun and accele

Kinase	Transgenic Model	Model Characteristics	Cardiovascular Phenotype
			plaque formati
	Cardiac-specific p90RSK overexpression	• Cardiac-specific p90RSK overexpression under α-MHC promoter	• Signifi cardiac dysfun with in fibrosi apopto hypert • Accele cardiac apopto MI wi reduce Ub lig activit increas ICER