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Novel mechanisms of endothelial mechano-transduction

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Abstract

Atherosclerosis is a focal disease that develops preferentially where non-laminar, disturbed blood flow (d-flow) occurs such as branches, bifurcations, and curvatures of large arteries. Endothelial cells sense and respond differently to d-flow compared to steady laminar flow (s-flow). D-flow that occurs in so-called athero-prone areas activates pro-inflammatory and apoptotic signaling, and this results in endothelial dysfunction and leads to subsequent development of atherosclerosis. In contrast, s-flow as “athero-protective flow” promotes expression of many anti-inflammatory genes such as Kruppel-like factor 2 (KLF2) and endothelial nitric oxide synthase (eNOS) and inhibits endothelial inflammation and athrogenesis. Here we will discuss that d-flow and s-flow induce pro- and anti-atherogenic events via flow type-specific “mechanotransduction” pathways. We will focus on five mechano-sensitive pathways: MEK5 (MAPK/ERK kinase 5)-ERK5-KLF2 signaling, ERK5-PPAR (peroxisome proliferator-activated receptor) signaling, and mechano-signaling pathways involving SUMOylation, protein kinase C-ζ, (PKCζ), and p90 ribosomal S6 kinase (p90RSK). We believe that clarifying regulation mechanisms between these two flow types will provide new insights into therapeutic approaches for the prevention and treatment of atherosclerosis.

Keywords

flow; SUMOylation; ERK5; PKCζ; p90RSK

Introduction

The surface of the vasculature, which is comprised of a monolayer of endothelial cells (ECs), is constantly exposed to various forces as blood flows. It is well-established that atherosclerotic plaques localize to areas of disturbed flow (d-flow) found at regions where vessels curve and also at vessel bifurcations and branch points. Low endothelial nitric oxide synthase (eNOS) expression and increased adhesion molecule expression are observed in these particular areas[1, 2]. In addition, d-flow increases secretion of pro-inflammatory molecules such as MCP-1, PDGFs, and endothelin-1 from ECs, which promote leukocyte infiltration and smooth muscle proliferation, leading to the development of

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Disclosures
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atherosclerosis[3-5]. In contrast, atherosclerosis is rare in areas exposed to steady laminar flow (s-flow). EC stimulated by s-flow have been shown to increase the secretion of NO, PGI_2, and tPA, which down-regulate both thrombogenic and inflammatory cellular events[6-9]. The human coronary artery, especially at points of bifurcation, is exposed to d-flow and exhibits a susceptibility toward atherosclerosis. In essence, s-flow protects against atherosclerosis (athero-protective flow) while d-flow promotes atherosclerosis (athero-prone flow)[10].

D-flow promotes inflammation and apoptosis in EC, and this effect of d-flow is critical for the pathogenesis of many chronic inflammatory conditions and endothelial dysfunction in epicardial blood vessels (coronary arteries in the heart) and peripheral blood vessels (such as the carotid artery and femoral artery). Blood flow in these vessels leads to activation of mechano-sensitive genes in EC and this process involves transcription factor regulation (*e.g.,* KLF2/4, NF-κB, AP-1, early growth response-1, *c*-Jun, *c*-fos, and *c*-myc)[11-13]. Substantial evidence shows that these transcription factors are regulated by a family of mitogen activated protein kinases (MAPKs). Of note, athero-prone/d-flow-induced signaling in which PKCζ, p90RSK, and increased levels of SUMOylation are involved is not activated by athero-protective/s-flow[14], suggesting that there must be specific mechano-sensing and signaling systems for each type of flow. In this brief review, we will discuss some of the recent findings unique to the EC mechano-transduction system with respect to both athero-prone/d-flow and athero-protective/s-flow.

S-flow activates ERK5 kinase

Mitogen-activated protein kinases (MAPKs) are highly conserved serine/threonine kinases. The MAPKs themselves require dual phosphorylation on a Thr-X-Tyr (TXY) motif to become active. Three major MAPK cascades have been extensively studied in blood vessels: extracellular signal–regulated kinases (ERK1 and ERK2), c-Jun N-terminal kinases (JNK1 and JNK2), and p38 kinases. A fourth MAPK member, ERK5, also known as big MAPK-1 (BMK1), has also been identified in EC[15-17]. MEK5 and ERK5 were first identified as two components of this new protein kinase signaling cascade[18, 19]. MEK5 is the only identified immediate upstream MAP kinase kinase of ERK5. The critical role of JNK activation in endothelial inflammation and apoptosis has been reported [20-2223, 24]. We found that s-flow decreases inflammation in EC induced by TNF-α-mediated JNK activation and subsequent VCAM1 expression. Although the exact mechanism remains unclear, the s-flow-induced inhibition of the JNK pathway is dependent upon activation of the MEK5-ERK5, but not MEK1-ERK1/2, pathway [25].

The unique aspect of ERK5 is that it is not only a kinase, but also a transcriptional co-activator with a unique C-terminus transactivation domain (Fig. 1)[26, 27]. Although both ERK1/2 and ERK5 contain the same TEY dual phosphorylation sites and are crucial for regulating proliferation of several different cell types, many unique functions of ERK5, which are different from other MAP kinases, have been reported. First, activation of ERK5 is documented to have an anti-apoptotic effect in cardiac, neuronal, and ECs through increasing Bad phosphorylation, but the detailed mechanism remains unclear[25, 28,29, 30]. Second, our studies have revealed that s-flow-induced ERK5 activation increases

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peroxisome proliferator-activated receptor (PPAR) γ transcriptional activity and KLF2/4 expression, with consequent anti-inflammatory and athero-protective effects[26, 31].

S-flow activates PPARs transcriptional activity via ERK5

PPARs are ligand-activated transcription factors, which form a subfamily of the nuclear receptor gene family. PPARs contain two activation function (AF) domains residing in the NH_2-terminus A/B domain (AF-1) and the COOH-terminus E domain (AF-2) (Fig. 2). Three related PPAR isotypes have been identified to date: PPARα, PPARβ/δ, and PPARγ. It is well-established that PPARs possess anti-inflammatory effects via ligand-dependent and ligand-independent mechanisms[32-34]. Phosphorylation of PPARγ Ser-82 by ERK1/2 significantly inhibits its transcriptional activation[35]. In contrast to ERK1/2, ERK5 does not phosphorylate PPARγ, but instead, its binding with PPARγ regulates PPARγ transcriptional activity. We have found that s-flow increases the association of ERK5 with the hinge-helix 1 region of PPARγ and up-regulates PPARγ transcriptional activity by releasing the co-repressor, SMRT (Fig. 2). Both PPARγ transcriptional activation and the release of its co-repressor (trans-repression) inhibit TNF- mediated NF-κB activation and subsequent inflammatory responses[26, 36, 37]. The detailed regulatory mechanism of trans-repression was discussed extensively in other reviews[38-41].

In addition to PPARγ, ERK5 can also increase PPARδ transcriptional activation by its association with PPARδ, although the PPARδ binding site with ERK5 is not the hinge-helix 1 region unlike PPARγ[42]. ERK5-mediated PPARδ activation also contributes to anti-inflammatory responses induced by heme oxygenase 1. These data suggest that the ERK5-PPAR module play a crucial role in s-flow induced-anti-inflammatory processes.

ERK5, KLF2, and endothelial dysfunction

The KLF family is a group of zinc finger transcription factors with important biological roles in regulating blood vessel permeability, blood coagulation, and inflammation[43]. Dekker *et al*. first identified KLF2 as a gene regulated by s-flow in the endothelium, which is a key transcriptional regulator of EC inflammation[44]. NF-κB is a key transcriptional factor that regulates expression of pro-inflammatory mediators including cytokines, chemokines, and molecules which foster cell-to-cell adhesion[45]. KLF2 reduces NF-κB transcriptional activity and subsequent adhesion molecule expression via competing for the association of CBP/p300 co-factor with NF-κB[46]. Furthermore, Parmar *et al*. have reported that s-flow increases KLF2 expression via the MEK5-ERK5-MEF2 signaling pathway and impairs endothelial inflammation[31]. Another major endothelial function regulated transcriptionally by KLF2 is the control of vessel tone. KLF2 induces eNOS expression by direct association with the eNOS promoter with the recruitment of the coactivator CBP/p300[47]. A crucial role for KLF2 in inhibiting endothelial permeability by tight junction protein expression was also reported[48].

Consistent with such key roles of ERK5 in EC physiology *in vitro*, EC apoptosis and inflammation are accelerated in endothelial-specific ERK5 knockout mice[30, 49], and the deletion of ERK5 in ECs accelerates atherosclerosis formation in LDL receptor deficient ($LDLR^{-/-}$) mice[50]. These data strongly suggest that both ERK5 kinase activity and

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transcriptional activity play key roles in ECs achieving athero-protective function. S-flow-induced ERK5 activation in ECs up-regulates PPARs and KLF2 transcriptional activity, elicits anti-inflammatory responses, and maintains normal vascular reactivity and endothelial barrier function.

SUMOylation as a mechano-signaling mediator

Small ubiquitin-like modifier (SUMO) proteins covalently modify certain residues of specific target substrates to alter their functions. A substantial amount of evidence indicates that SUMOylation plays roles in flow-induced signaling and the pathogenesis and development of cardiovascular complications[51-53]. SUMOylation is a dynamic and reversible process mediated by both conjugation and de-conjugation enzymes. It is analogous to ubiquitination, but SUMO conjugation involves a different set of enzymes (Fig. 3). First, the mature form of SUMO is activated by E1-activating enzymes, a SAE1-SAE2 heterodimer[54]. After this activation, SUMO is transferred to Ubc9, an E2 conjugase, forming a thioester bond between Ubc9 and SUMO[55]. Lastly, Ubc9 transfers SUMO to the target substrate containing the free e-amino group of a lysine residue, which is regulated by several SUMO E3 ligases including the family of protein inhibitors such as activated STAT (PIAS1-4), Polycomb-2 protein (Pc2), and RanBP2/Nup358[56]. Sentrin/SUMO-specific proteases (SENPs; SENP1-7) catalyze deconjugation of SUMOylated substrates, or edit SUMO precursor into a matured form, which terminates with a pair of glycine (Gly) residues (Fig. 3)[57, 58]. As described above, the number of SUMO E1 and E2 enzymes is small compared with SUMO E3 ligases and SENPs. Therefore, the coordination of different SUMO E3 ligases and SENPs may be crucial for a specific EC function in which flow-induced protein SUMOylation plays a role.

ERK5-SUMOylation and d-flow

It is clear that SUMO influences many different biological processes, but particularly important in the present context is the regulation of transcription and protein kinase activity of modified proteins[53, 59]. As explained above, s-flow has a vasoprotective effect via ERK5-mediated KLF2 and eNOS expression[60, 61]. Our studies showed that treatment of ECs with H_2O_2, advanced glycation end products (AGE), or d-flow significantly increased ERK5 SUMOylation at Lys6 and Lys22 residues and that this SUMOylation inhibited ERK5/MEF2 transcriptional activity, and subsequent KLF2 promoter activity and KLF2-mediated eNOS expression[60]. Of note, both H_2O_2 and AGE increased ERK5 TEY motif phosphorylation as well as its protein kinase activity, suggesting that the inhibition of ERK5 transcriptional activity by H_2O_2 and AGE is an event independent of its protein kinase activity. We also found that the reduction of eNOS and KLF2 expression by H_2O_2 and AGE treatment was abolished in ECs expressing ERK5 K6/22R SUMOylation mutant, suggesting that ERK5 SUMOylation may down-regulate the vaso-protective effects of s-flow[60]. Furthermore, we found that ERK5 SUMOylation was increased by d-flow, but it was decreased by s-flow[62]. These data strongly suggest that ERK5 SUMOylation plays an important role in regulating endothelial inflammation and vascular tone and that d- and s-flow have, respectively, yin and yang effects on ERK5 SUMOylation.

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Role of p53 SUMOylation in d-flow-induced EC apoptosis

D-flow is able to increase both endothelial apoptosis and proliferation, which augments EC turnover and creates focal sites of increased endothelial permeability, inflammation, and dysfunction[63]. However, the mechanism by which d-flow regulates EC turnover, especially apoptosis, is unclear. To obtain some insights into this issue, we investigated the role of p53 in regulating d-flow-induced EC apoptosis (Fig. 4A). Acting as a sensor for DNA damage, the transcription factor p53 is a key molecule in determining cellular fate. p53 in the nucleus not only increases the expression of pro-apoptotic genes, but also is protective against cell death via up-regulating p21 expression[64]. In fact, Lin *et al*. reported that s-flow increased p53 expression and JNK-mediated p53 phosphorylation, which caused EC growth arrest via increasing GADD45 and $p21^{cip1}$ expression[65]. These data suggest that the athero-protective effect exerted by s-flow increases p21 via p53, inducing growth arrest, and may inhibit simultaneously apoptosis. It is important to note here that most of p53 <u>anti</u>-apoptotic effects has been explained by its function in the nucleus, especially under the resting condition[64]. We found increased levels of nuclear p53 and reduced numbers of apoptotic ECs in the area exposed to s-flow[51], which supports this general idea.

In contrast to this, EC exposed to d-flow have decreased levels of nuclear p53 localization and become apoptosis. We have reported that d-flow induces EC apoptosis via p53 SUMOylation in a PKCζ-dependent manner[51]. Previously, Carter *et al*. reported a role of p53 SUMOylation in regulating p53 localization[66]. They showed that in its unmodified form, the p53 C-terminus nuclear export signal (NES) was masked by its own C-terminus region and that this caused persistent nuclear localization. A low level of ubiquitination by MDM2 (mouse double minute 2) exposed the NES, promoting p53 to interact with PIAS4 and causing further modification by SUMOylation which led to p53 nuclear export. These results show that p53 nuclear export is regulated by SUMOylation (Fig. 4B)[66]. Cytosolic p53 has non-transcriptional pro-apoptotic activities. It has been reported that cytoplasmic p53 directly interacts with Bcl-2 (B cell lymphoma/leukemia-2) family member proteins, Bcl-xL and Bcl-2, and blocks their well-known anti-apoptotic function[67, 68]. We have reported that d-flow induces p53 nuclear export, p53-Bcl-2 binding, and apoptosis in a p53 SUMOylation-dependent manner[51].

The next question is how d-flow increases p53 SUMOylation. We found that athero-prone flow increased PKCζ binding to the E3 SUMO ligase PIAS4 and induced p53-SUMOylation[69]. Among the PKC family members, atypical PKCζ was recently reported to have an important function in EC[70, 71]. Magid and Davies reported that this PKC isoform was highly expressed in EC in the athero-prone areas of porcine aorta[70]. Frey *et al*. demonstrated involvement of PKCζ in oxidant generation in ECs via NADPH oxidase activation[71]. Consistent with these results, endothelial PKCζ activation was elevated in atherosclerotic lesions[69, 72]. Therefore we investigated the interactions of PKCζ with SUMO ligases and discovered that d-flow increased PKCζ binding to the E3 SUMO ligase PIAS4 and stimulated p53-SUMOylation[69]. <u>It is likely that</u> PIAS4 activation by PKCζ is likely to be phosphorylation-independent because we did not observe PIAS4 phosphorylation by PKCζ. It is noteworthy that active protein kinases may regulate signaling pathways and cell

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functions not only by phosphorylating substrates, but also by direct protein-protein interactions.

It has been reported that PKCζ contains a pseudosubstrate autoinhibitiry sequence (amino acids 116-122), and the release of the kinase domain (amino acids 268-587) from this auto-inhibitory domain leads to PKCζ activation[73, 74] (Fig. 4C). We found that the C-terminus kinase domain of PKCζ (amino acids 401-587) was a PIAS4 binding site, and the deletion of the N-terminus autoinhibitory domain (amino acids 1-200) increased PKCζ-PIAS4 association[51]. Therefore, in addition to its protein kinase activation, the subsequent release of the PKCζ N-terminus auto-inhibitory domain is necessary for the PKCζ-PIAS4 association. PKCζ associates with the catalytic site, RING domain, of PIAS4, which recruits the cognate E2 conjugating enzyme into the PIAS4/substrate complex to facilitate SUMO conjugation. Therefore, the association of PKCζ with PIAS4 may alter the structure and enzymatic activity of PIAS4. Taken together, PKCζ activation and subsequent PKCζ-PIAS4 binding are crucial for d-flow-induced p53 SUMOylation and ECs apoptosis [51].

Other PKCζ that mediat e endothelial dysfunction

We have discussed the mechanisms by which PKCζ mediates d-flow-induced endothelial apoptosis in the previous section. Here, we discuss other PKCζ functions in ECs. PKCζ regulates not only endothelial apoptosis but also TNFα-induced endothelial dysfunction, particularly under s-flow conditions[61]. TNF-α promotes association between PKCζ and ERK5 and also increases ERK5 S486 phosphorylation. ERK5 S486 site, when phosphorylated, evokes eNOS protein degradation, leading to endothelial dysfunction. Although several mechanisms including calcium-dependent calpain-mediated degradation have been proposed for eNOS protein degradation[75, 76], it remains unclear exactly how PKCζ-ERK5-pS486 mediates eNOS degradation.

In addition to ERK5, we also reported the importance of p62 on TNF-α-induced PKCζ activation[77]. p62 is a scaffold protein containing a Phox/Bem1p (PB1) domain in its NH_2-terminus region, which can interact with other PB1 domain containing proteins via PB1-PB1 interaction[78]. PKCζ also contains a PB1 domain, and the p62-PKCζ association is critical for the activation of PKCζ downstream events such as JNK and caspase 3 activation[77]. The precise role of this p62-PKCζ module in s-flow and d-flow needs further investigation.

SENP2 and athero-prone d-flow

SENP2 is a de-SUMOylation enzyme, which is important for both processing new SUMO proteins for conjugation as well as deconjugation of SUMO from SUMOylated proteins. Six isoforms exist in human (SENP1-3 and 5-7)[79]. In contrast to the C-terminus that contains the well-conserved catalytic domain, the N-terminus is poorly conserved among different isoforms, suggesting that the enzyme is regulated by the N-terminus[58], but it remains unclear how each SENP isoform recognizes its specific substrates and causes different functional consequences. Among the six isoforms, the functions of SENP1 and SENP2 have been relatively well studied. Li *et al*. showed that TNFα transiently induced SENP1 translocation from the cytosol to the nucleus and subsequently increased JNK activation and apoptosis via Homeodomain Interacting Protein Kinase 2 (HIPK2) de-SUMOylation in

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EC[80]. SENP1$^{-/-}$ embryos are severely anemic due to diminished erythropoietin production, and this leads to SUMOylation-induced HIF1α degradation[81]. The deletion of SENP2 in mouse causes defects in cardiac development by inhibiting Gata4 and Gata6 expression and accumulation of SUMOylated Pc2/CBX4 (a polycomb repressive complex 1 subunit). HIF1α stabilization is not affected in SENP2$^{-/-}$ mouse embryonic fibroblasts, demonstating the substrate specificity between SENP1 and SENP2.

As we explained above, we found that d-flow induced p53 and ERK5 SUMOylation, leading to EC apoptosis and inflammation, respectively[51, 60]. Interestingly, reduced expression of SENP2 increased both endothelial p53 and ERK5 SUMOylation, hence increased EC dysfunction and inflammation, and accelerated atherosclerotic plaque formation[62]. In addition, we found that d-flow-induced adhesion molecule expression and EC apoptosis were inhibited in cultured ECs overexpressing p53 or ERK5 SUMOylation mutant[62]. In contrast, s-flow inhibited ERK5 SUMOylation[62]. Taken together, we may conclude that SUMOylation of p53 and ERK5 is both necessary and sufficient to promote endothelial apoptosis and inflammation under the conditions of d-flow. One might expect SENP2 expression to be down-regulated by d-flow, but we did not observe this effect in EC exposed to d-flow[62]. We believe that d-flow likely regulates the de-SUMOylation activity of SENP2 or the cellular localization of SENP2, but further studies will be needed to clarify these points.

ERK5 and its inhibitory kinase, p90RSK, under d-flow

p90RSK is a serine/threonine kinase containing two functional kinase domains (Fig. 5)[82]. The N-terminus kinase belongs to the AGC group of kinases (i.e., protein kinase A [PKA] and protein kinase C [PKC]). Within this AGC group, p70S6K has the greatest sequence identity (~ 60%) within the p90RSK N-terminus kinase region. The C-terminus kinase belongs to the calcium/calmodulin-dependent kinase group. These two p90RSK kinase domains possess different functional properties. The N-terminus kinase has the most activity since it directly phosphorylates p90RSK substrates. The C-terminus kinase domain, conversely, plays only a minor direct role in phosphorylation, but its presence, together with the linker region, is required for full activation of the N-terminus kinase. The C-terminus tail contains a short docking motif for the specific association between p90RSK and ERK1/2[82-84]. p90RSK is located downstream of the Raf-MEK-ERK1/2 signaling pathway[85], 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 has also been suggested[86].

The activation and nuclear translocation of p90RSK are concomitant with immediate early gene expression[87, 88]. p90RSK is also involved in the activation of NF-κB by phosphorylation of Iκ-B[89] or phosphorylation of transcription factors including c-Fos[90], Nur77[91], and CREB[92]. Although ERK5 can regulate p90RSK kinase activation as an upstream kinase like ERK1/2 under certain conditions[93], we have reported that p90RSK also directly phosphorylates ERK5 S496 and inhibits its transcriptional activity[50]. In this study we found that p90RSK associated with the ERK5 C-terminus transcriptional activation domain (amino acids 571-807). When we overexpressed this C-terminus fragment as a

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decoy, both p90RSK-ERK5 association and H_2O_2-induced reduction of ERK5 transcriptional activity were inhibited[50]. These data suggest that inhibition of ERK5 transcriptional activity depends on p90RSK-ERK5 binding. In addition, phosphorylation of ERK5 S496 by p90RSK inhibits ERK5 transcriptional activity as well as eNOS expression. Lastly, we also found increased p90RSK activation in regions of d-flow in the aortic arch, indicating that p90RSK activation and atherosclerosis are closely linked. The inhibition of 90RSK activation by FMK-MEA (a p90RSK specific inhibitor) significantly reduced atherosclerosis plaque formation[50]. Further studies are necessary to elucidate the precise mechanism by which d-flow regulates the function of p90RSK that leads to endothelial dysfunction.

Conclusion

It is apparent now from multiple studies that ECs sense and respond differently to s-flow and d-flow. Many studies have also sought to define molecular mechanisms responsible for mechanotrasduction initiated by different patterns of flow, but the exact nature of signaling that d-flow and s-flow initiate in ECs has so far evaded investigators. In this review we have discussed several molecules and signaling events, which appear to be differentially regulated by athero-prone and athero-protective blood flow patterns. Molecules that may be involved in flow pattern specific signaling include PKCζ and p90RSK for d-flow-initiated signaling and ERK5, KLF2/4, and PPARs for s-flow. Understanding the interplay among these molecules under the two different types of flow may be the final key needed to unlock the door which stands between endothelial cell dysfunction and atherosclerosis formation.

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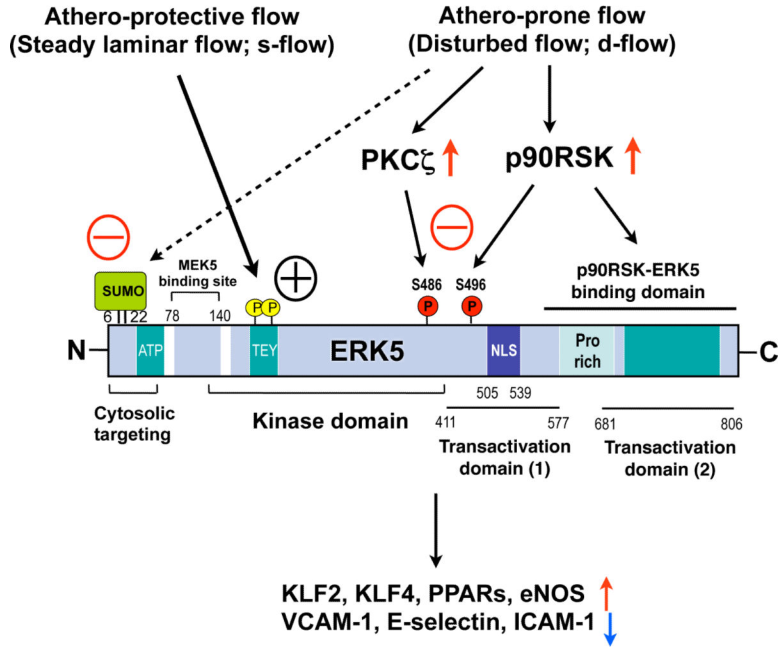

Figure 1. Primary structure of ERK5 and its regulation

The N-terminus region with SUMO modification inhibits its own transactivation. After ERK5 kinase activation induced by MEK5 binding and TEY motif phosphorylation with de-SUMOylation of K6/K22 sites, ERK5 transcriptional activity at the C-terminus region is fully activated. In contrast athero-prone flow increases ERK5-SUMOylation and ERK5 S496 phosphorylation and inhibits ERK5 transcriptional activity.

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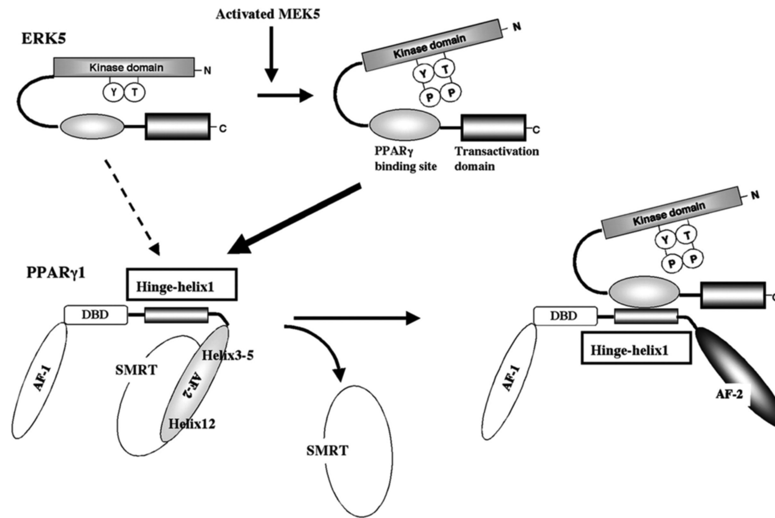

Figure 2. Model for the ERK5-PPARγ interaction-mediated PPARγ transactivation

The position of Helix 12 is regulated by ligand binding. When the PPARγ ligand binds to the receptor, Helix 12 folds back to form a part of the co-activator binding surface, and inhibits corepressor (such as SMRT) binding to PPARγ[94]. The co-repressor interaction surface requires Helix 3-5[95]. We found a critical role of the PPARγ hinge-helix 1 domain in ERK5-mediated PPARγ transactivation. The inactive N-terminus kinase domain of ERK5 inhibits its own transactivation and PPARγ binding. After ERK5 activation the inhibitory effect of the N-terminus domain decreases, and subsequently the middle region can fully interact with the hinge-helix 1 region of PPARγ. The association of ERK5 with the hinge-helix 1 region of PPARγ releases co-repressor of SMRT and induces full activation of PPARγ[26]. AF-1/2: Activating function (AF)-1/2 transactivation domain, DBD: DNA binding domain. Reprinted and modified from Akaike et al[26].

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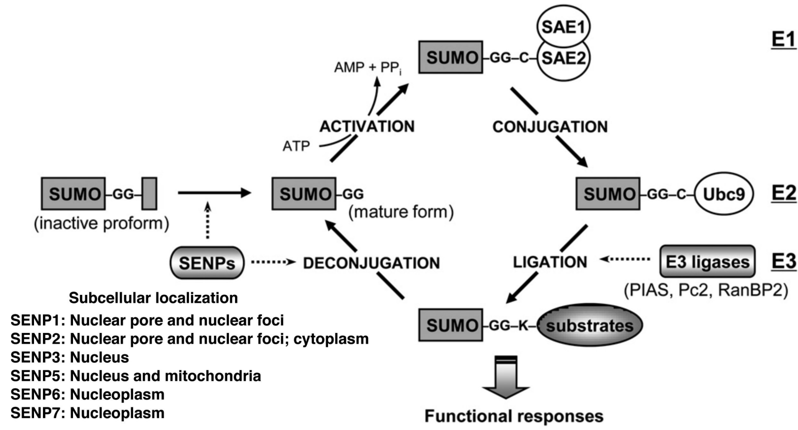

Figure 3. The regulation of SUMOylation pathway

Protein SUMOylation is achieved by a recycle system consisting of conjugation and deconjugation pathway. SUMO conjugation to a target substrate requires an enzymatic cascade, which involves three classes of enzymes (E1→E2→E3). The sentrin/SUMO-specific proteases (SENPs) are responsible for the deconjugation pathway as well as the maturation process of newly synthesized SUMO protein. The primary subcellular localization of each SENP is also listed[96]. Reprinted and modified from Woo et al. with permission of the publisher[53].

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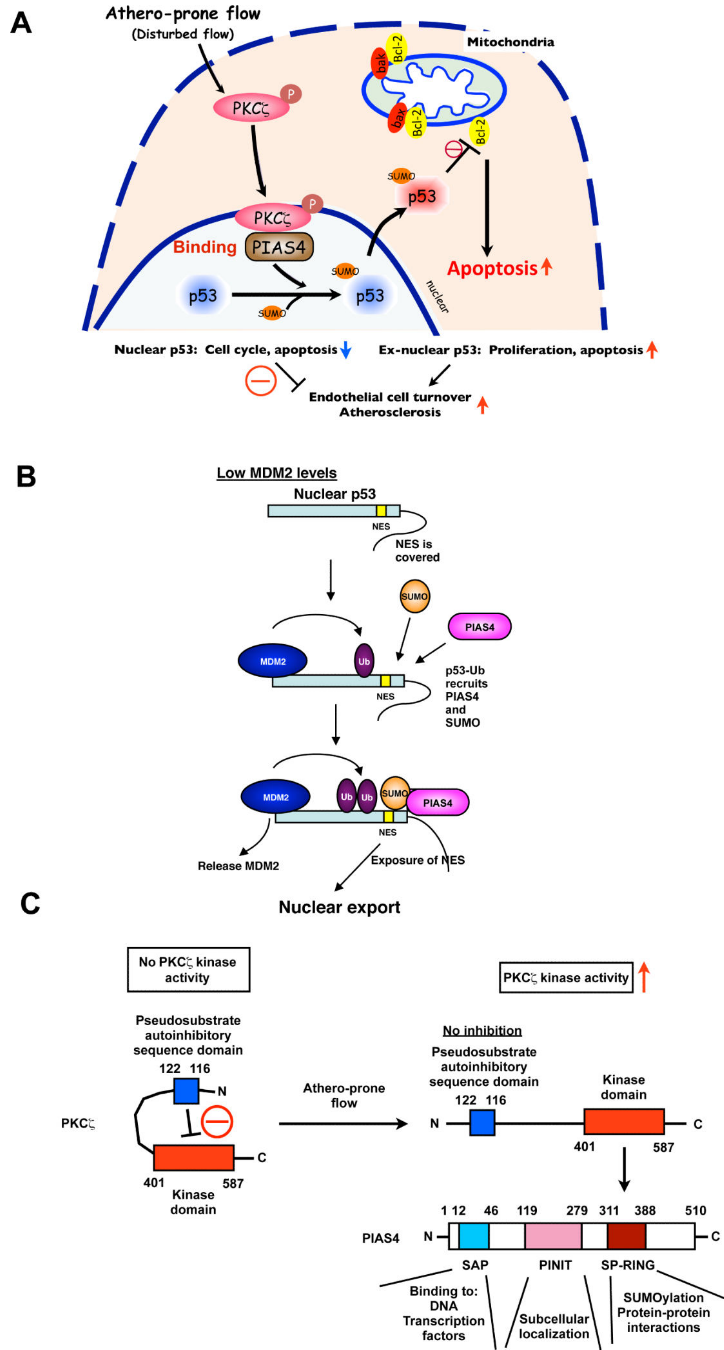

Figure 4. Athero-prone flow increases p53 SUMOylation via PKCζ-PIAS4 binding

(A) Athero-prone flow uniquely activates PKCζ, which increases PKCζ-PIAS4 binding and PIAS4 SUMO E3 ligase activity, and subsequently increases p53 SUMOylation. SUMOylation causes p53 nuclear export and binds to Bcl-2, which inhibits anti-apoptotic function of Bcl-2 and increases apoptosis. (B) p53 nuclear export and stabilization. Masking of the C-terminus NES results in nuclear localization of unmodified p53, but a low level of ubiquitination by MDM2 exposes the NES, causing the p53-Ub fusion protein to come out of the nucleus. When MDM2 levels are low, ubiquitination promotes the interaction of p53

with PIAS4 and further modification of p53 by SUMOylation that causes the release of MDM2 and nuclear export, which may increase the cytoplasmic apoptotic function of p53. Under conditions of high MDM2, persistent binding and activity of MDM2 leads to polyubiquitination and degradation of p53[66]. These data suggest important roles of SUMOylation in p53 stabilization, localization, and subsequent apoptosis. Reprinted and modified from Carter et al with permission of the publisher[66]. (C) PKCζ-mediated p53 SUMOylation requires PKCζ-PIAS4 binding at SP-RING domain, not PKCζ-mediated phosphorylation of PIAS4. SAP: scaffold attachment factor-A/B, acinus and PIAS domain; SP-RING: Siz/PIAS-RING domain[53].

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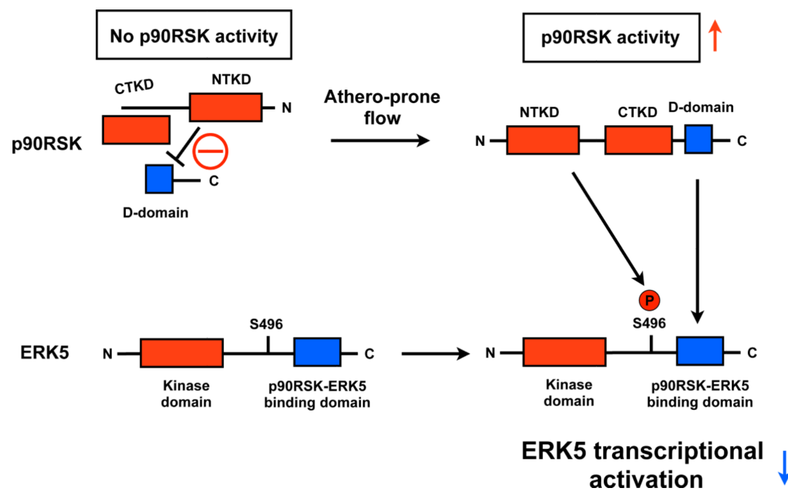

Figure 5. Athero-prone flow increases p90RSK activation, leading to p90RSK-ERK5 association and ERK5 S496 phosphorylation, and subsequently decreases in ERK5 transcriptional activity

At the basal level, inactive p90RSK inhibits the D-domain to bind ERK5. Once p90RSK is activated, the inhibition of the kinase domain is released and the D-domain of p90RSK associates with the ERK5 C-terminus domain[97] and increases ERK5 S496 phosphorylation, which inhibits ERK5 transcriptional activity[98]. NTKD: NH_2-terminal kinase domain, CTKD: COOH-terminal kinase domain, D-domain: NH_2-terminal docking domain.