ATVB in Focus
Biomechanical Stress in Vascular Remodeling

Series Editors: Qingbo Xu and Peter Davies

Novel Mechanisms of Endothelial Mechanotransduction

Jun-ichi Abe, Bradford C. Berk

Abstract—Atherosclerosis is a focal disease that develops preferentially where nonlaminar, disturbed blood flow occurs, such as branches, bifurcations, and curvatures of large arteries. Endothelial cells sense and respond differently to disturbed flow compared with steady laminar flow. Disturbed flow that occurs in so-called atheroprone areas activates proinflammatory and apoptotic signaling, and this results in endothelial dysfunction and leads to subsequent development of atherosclerosis. In contrast, steady laminar flow as atheroprotective flow promotes expression of many anti-inflammatory genes, such as Kruppel-like factor 2 and endothelial nitric oxide synthase and inhibits endothelial inflammation and athrogenesis. Here we will discuss that disturbed flow and steady laminar flow induce pro- and antiatherogenic events via flow type–specific mechanotransduction pathways. We will focus on 5 mechanosensitive pathways: mitogen-activated protein kinases/extracellular signal–regulated kinase 5/Kruppel-like factor 2 signaling, extracellular signal–regulated kinase/peroxisome proliferator–activated receptor signaling, and mechanosignaling pathways involving SUMOylation, protein kinase C-ζ, and p90 ribosomal S6 kinase. We think that clarifying regulation mechanisms between these 2 flow types will provide new insights into therapeutic approaches for the prevention and treatment of atherosclerosis. **(*Arterioscler Thromb Vasc Biol*. 2014;34:2378-2386.)**

Key Words: ERK5 ■ flow ■ PKCζ ■ SUMOylation

The surface of the vasculature, which comprises 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 proinflammatory molecules, such as MCP-1 (monocyte chemotactic protein 1), PDGFs (platelet-derived growth factor), and endothelin-1 from EC, which promote leukocyte infiltration and smooth muscle proliferation, leading to the development of 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 nitric oxide, prostacyclin, and tissue-type plasminogen activator, which downregulate 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 (atheroprotective flow), whereas d-flow promotes atherosclerosis (atheroprone flow).[10]

Please see http://atvb.ahajournals.org/site/misc/ATVB_in_Focus.xhtml for all articles published in this series.

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 mechanosensitive genes in EC, and this process involves transcription factor regulation (eg, Kruppel-like factor [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, atheroprone/d-flow–induced signaling in which protein kinase C-ζ (PKCζ, p90 ribosomal S6 kinase (p90RSK), and increased levels of SUMOylation are involved is not activated by atheroprotective/s-flow,[14] suggesting that there must be specific mechanosensing and signaling systems for each type of flow. In this brief review, we will discuss some of the recent findings unique to the EC mechanotransduction system with respect to both atheroprone/d-flow and atheroprotective/s-flow.

S-Flow Activates ERK5 Kinase

MAPKs are highly conserved serine/threonine kinases. The MAPKs themselves require dual phosphorylation on a Thr-X-Tyr 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, has also been identified in EC.[15–17] MEK5 and ERK5 were first identified as 2 components of this new protein kinase–signaling cascade.[18,19] MEK5 is the

Received on: April 22, 2014; final version accepted on: August 19, 2014.
From the Aab Cardiovascular Research Institute, University of Rochester, NY.
Correspondence to Jun-ichi Abe, MD, PhD, Department of Cardiology, The University of Texas MD Anderson Cancer Center, 1515 Holcombe Blvd, Houston, TX 77030. E-mail jabe@mdanderson.org; or Bradford C. Berk, MD, PhD, Aab Cardiovascular Research Institute, Box CVRI, 601 Elmwood Ave, University of Rochester School of Medicine and Dentistry, Rochester, NY 14642. E-mail Bradford_Berk@urmc.rocheter.edu

Nonstandard Abbreviations and Acronyms

EC	endothelial cell
eNOS	endothelial nitric oxide synthase
ERK-5	extracellular signal–regulated kinase
KLF-2	Kruppel-like factor 2
MAPK	mitogen-activated protein kinases
NO	nitric oxide
p90RSK	p90 ribosomal S6 kinase
PKCζ	protein kinase C-ζ
PPAR	peroxisome proliferator–activated receptor
SUMO	Small ubiquitin-like modifier

only identified immediate upstream MAP kinase of ERK5. The critical role of JNK activation in endothelial inflammation and apoptosis has been reported.[20–24] We found that s-flow decreases inflammation in EC induced by tumor necrosis factor-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 on 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 coactivator with a unique C-terminus transactivation domain (Figure 1).[26,27] Although both ERK1/2 and ERK5 contain the same threonine/glutamic acid/tyrosine (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 antiapoptotic effect in cardiac, neuronal, and ECs through increasing Bad phosphorylation, but the detailed mechanism remains unclear.[25,28–30] Second, our studies have revealed that s-flow–induced ERK5 activation increases peroxisome proliferator–activated receptor (PPAR) γ transcriptional activity and KLF2/4 expression, with consequent anti-inflammatory and atheroprotective 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 2 activation function domains residing in the NH_2-terminus A/B domain (activation function-1) and the COOH-terminus E domain (activation function-2; Figure 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.[34–36] Phosphorylation of PPARγ Ser-82 by ERK1/2 significantly inhibits its transcriptional activation.[37] 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 upregulates PPARγ transcriptional activity by releasing the corepressor, SMRT (silencing mediator of retinoic acid and thyroid hormone receptor; Figure 2). Both PPARγ transcriptional activation and the release of its corepressor (transrepression) inhibit TNF-mediated NF-κB activation and subsequent inflammatory responses.[26,38,39] The detailed regulatory mechanism of transrepression was discussed extensively in other reviews.[40–43]

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[44] 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.

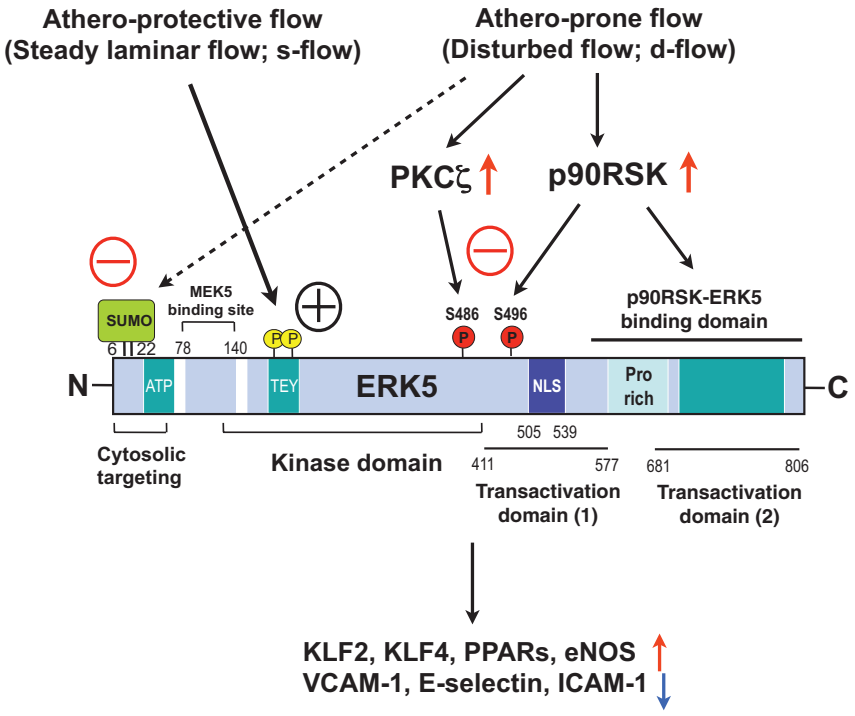

Figure 1. Primary structure of extracellular signal–regulated kinase (ERK5) and its regulation. The N-terminus region with small ubiquitin-like modifier (SUMO) modification inhibits its own transactivation. After ERK5 kinase activation induced by MEK5 binding and threonine/glutamic acid/tyrosine (TEY) motif phosphorylation with de-SUMOylation of K6/K22 sites, ERK5 transcriptional activity at the C-terminus region is fully activated. In contrast, atheroprone flow increases ERK5-SUMOylation and ERK5 S496 phosphorylation and inhibits ERK5 transcriptional activity. eNOS indicates endothelial nitric oxide synthase; KLF, Kruppel-like factor; p90RSK, p90 ribosomal S6 kinase; PKCζ, protein kinase C-ζ; and PPAR, peroxisome proliferator–activated receptor.

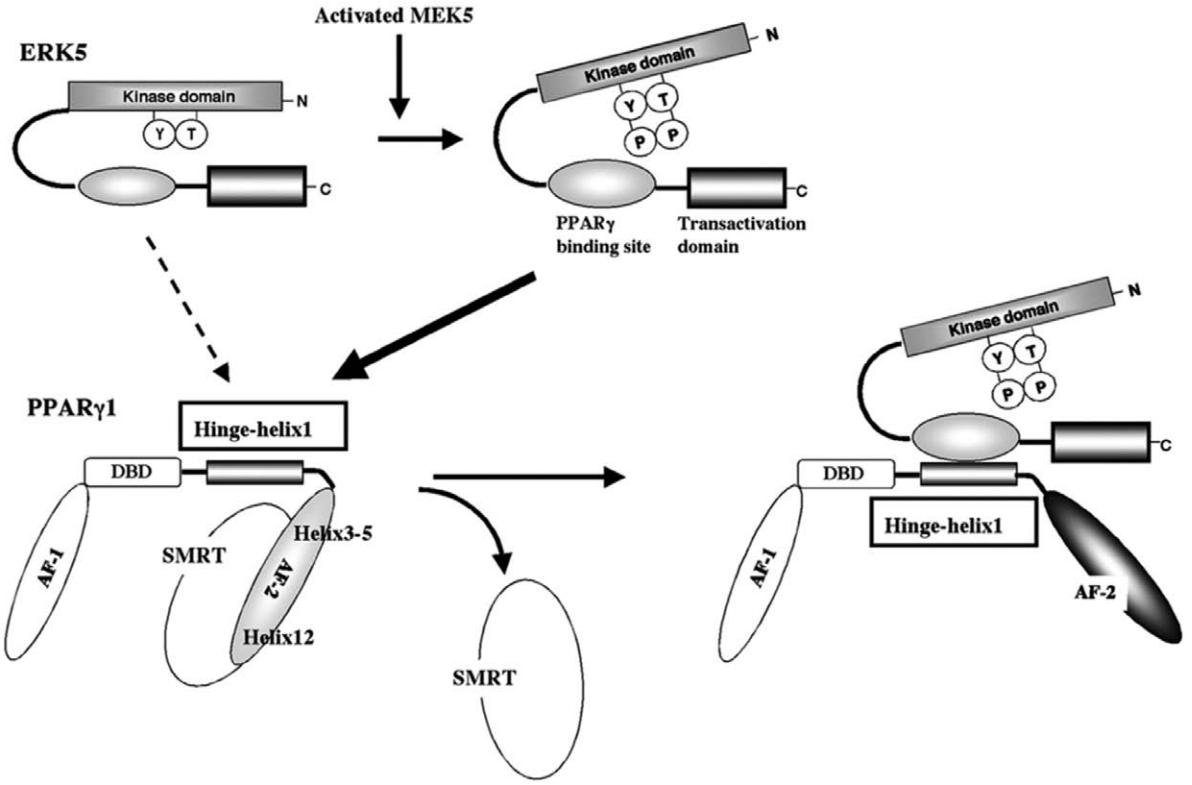

Figure 2. Model for the extracellular signal–regulated kinase (ERK5)-peroxisome proliferator–activated receptor (PPARγ) interaction-mediated PPARγ transactivation. The position of Helix 12 is regulated by ligand binding. When the PPARγ ligand binds to the receptor, Helix 12 folds back to form a part of the coactivator binding surface and inhibits corepressor (such as silencing mediator of retinoic acid and thyroid hormone receptor [SMRT]) binding to PPARγ[32] The corepressor interaction surface requires Helix 3–5.[33] 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 corepressor of SMRT and induces full activation of PPARγ[26] AF-1/2 indicates activating function (AF)-1/2 transactivation domain; and DBD, DNA binding domain. Reprinted and modified from Akaike et al[26] with permission of the publisher. Copyright © 2004, American Society for Microbiology.

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.[45] Dekker et al[46] first identified KLF2 as a gene regulated by s-flow in the endothelium, which is a key transcriptional regulator of EC inflammation. NF-κB is a key transcriptional factor that regulates expression of proinflammatory mediators, including cytokines, chemokines, and molecules that foster cell-to-cell adhesion.[47] KLF2 reduces NF-κB transcriptional activity and subsequent adhesion molecule expression via competing for the association of CBP/p300 cofactor with NF-κB.[48] Furthermore, Parmar et al[31] have reported that s-flow increases KLF2 expression via the MEK5-ERK5-MEF2 signaling pathway and impairs endothelial inflammation. 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.[49] A crucial role for KLF2 in inhibiting endothelial permeability by tight junction protein expression was also reported.[50] Consistent with such key roles of ERK5 in EC physiology in vitro, EC apoptosis and inflammation are accelerated in endothelial-specific ERK5 knockout mice,[30,51] and the deletion of ERK5 in ECs accelerates atherosclerosis formation in LDL receptor deficient mice.[52] These data strongly suggest that both ERK5 kinase activity and transcriptional activity play key roles in ECs achieving atheroprotective function. S-flow–induced ERK5 activation in ECs upregulates PPARs and KLF2 transcriptional activity, elicits anti-inflammatory responses, and maintains normal vascular reactivity and endothelial barrier function.

SUMOylation as a Mechanosignaling 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.[53–55] SUMOylation is a dynamic and reversible process mediated by both conjugation and deconjugation enzymes. It is analogous to ubiquitination, but SUMO conjugation involves a different set of enzymes (Figure 3). First, the mature form of SUMO is activated by E1-activating enzymes, a SAE1-SAE2 heterodimer.[57] After this activation, SUMO is transferred to Ubc9, an E2 conjugase, forming a thioester bond between Ubc9 and SUMO.[58] Finally, 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

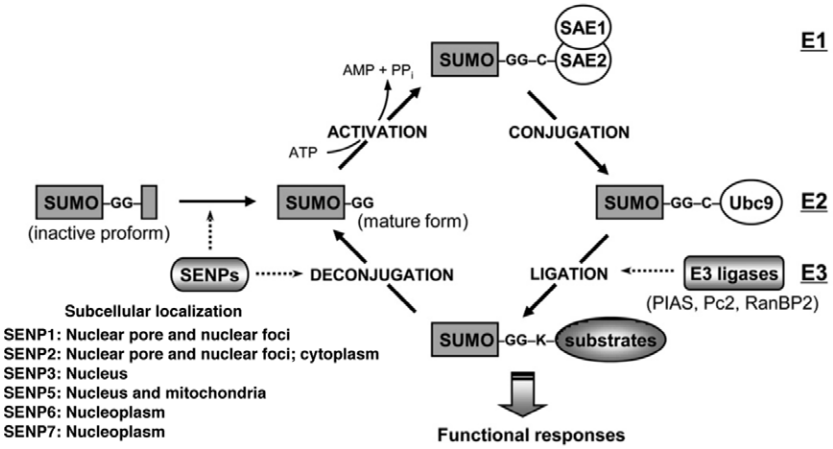

Figure 3. The regulation of SUMOylation pathway. Protein SUMOylation is achieved by a recycle system consisting of conjugation and deconjugation pathway. Small ubiquitin-like modifier (SUMO) conjugation to a target substrate requires an enzymatic cascade, which involves 3 classes of enzymes (E1→E2→E3). 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.[56] Reprinted and modified from Woo et al[55] with permission of the publisher. Copyright © 2010, Elsevier.

family of protein inhibitors, such as activated STAT (PIAS1-4), Polycomb-2 protein (Pc2), and RanBP2/Nup358.[59] 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 (Figure 3).[60,61] 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.[55,62] As explained above, s-flow has a vasoprotective effect via ERK5-mediated KLF2 and eNOS expression.[63,64] Our studies showed that treatment of ECs with H_2O_2, advanced glycation end products, 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.[63] Of note, both H_2O_2 and advanced glycation end products 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 advanced glycation end products 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 advanced glycation end products treatment was abolished in ECs expressing ERK5 K6/22R SUMOylation mutant, suggesting that ERK5 SUMOylation may downregulate the vaso-protective effects of s-flow.[63] Furthermore, we found that ERK5 SUMOylation was increased by d-flow, but it was decreased by s-flow.[65] 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.

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.[66] 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 (Figure 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 proapoptotic genes, but also is protective against cell death via upregulating p21 expression.[67] 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.[68] These data suggest that the atheroprotective 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 antiapoptotic effects has been explained by its function in the nucleus, especially under the resting condition.[67] We found increased levels of nuclear p53 and reduced numbers of apoptotic ECs in the area exposed to s-flow,[53] 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.[53] Previously, Carter et al reported a role of p53 SUMOylation in regulating p53 localization.[69] They showed that, in its unmodified form, the p53 C-terminus nuclear export signal was masked by its own C-terminus region and that this caused persistent nuclear localization. A low level of ubiquitination by mouse double minute 2 exposed the nuclear export signal, 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 (Figure 4B).[69] Cytosolic p53 has nontranscriptional proapoptotic 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 antiapoptotic function.[70,71] We have reported that d-flow induces p53 nuclear export, p53-Bcl-2 binding, and apoptosis in a p53 SUMOylation-dependent manner.[53]

The next question is how d-flow increases p53 SUMOylation. We found that atheroprone flow increased PKCζ binding to the E3 SUMO ligase PIAS4 and induced p53-SUMOylation.[72] Among the PKC family members, atypical

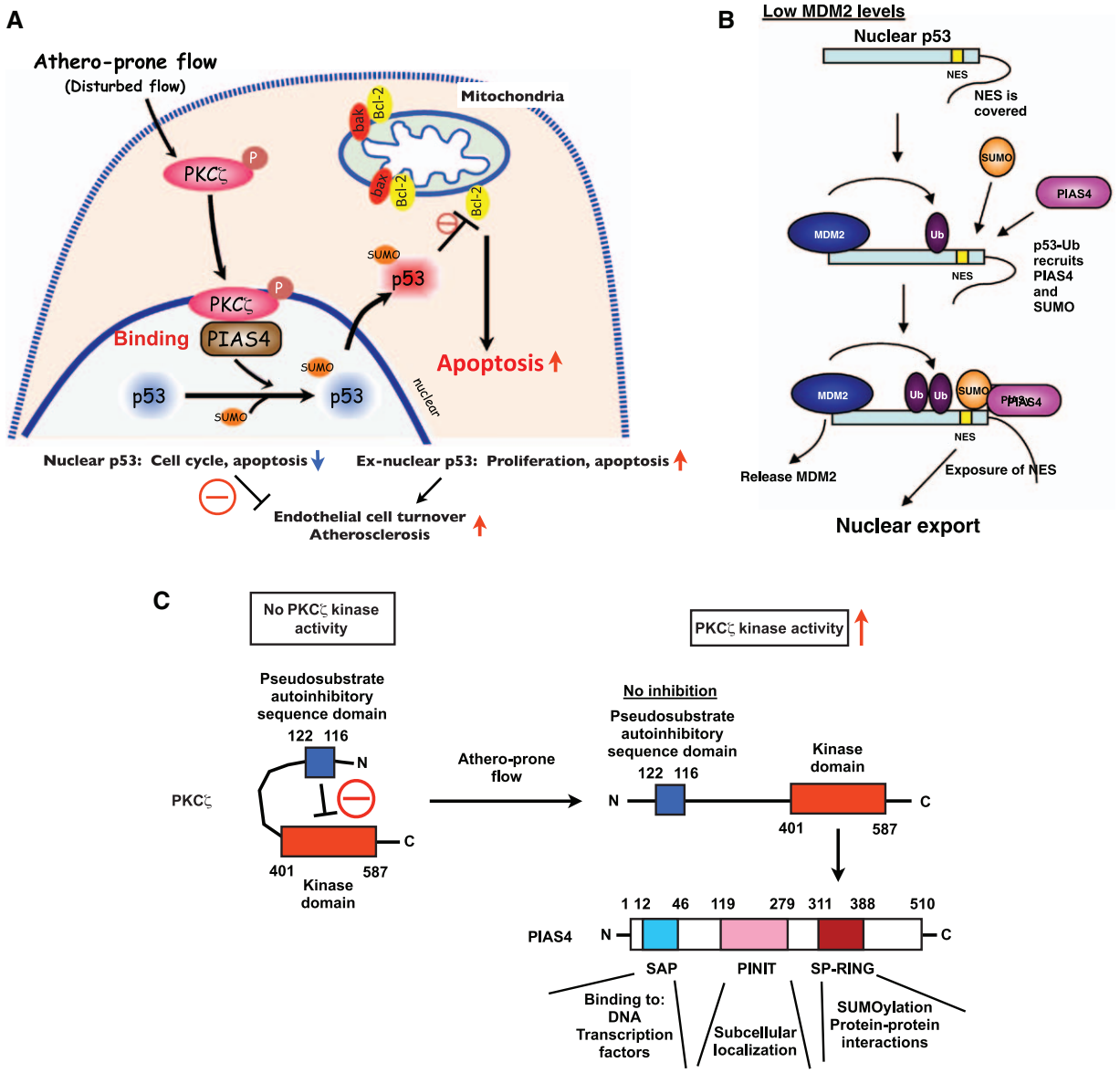

Figure 4. Atheroprone flow increases p53 SUMOylation via protein kinase C-ζ (PKCζ)–PIAS4 binding. **A**, Atheroprone flow uniquely activates PKCζ, which increases PKCζ–PIAS4 binding and PIAS4 small ubiquitin-like modifier (SUMO) E3 ligase activity and subsequently increases p53 SUMOylation. SUMOylation causes p53 nuclear export and binds to B cell lymphoma/leukemia-2 (Bcl-2), which inhibits antiapoptotic function of Bcl-2 and increases apoptosis. **B**, p53 nuclear export and stabilization. Masking of the C-terminus nuclear export signal (NES) results in nuclear localization of unmodified p53, but a low level of ubiquitination by mouse double minute 2 (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.[69] These data suggest important roles of SUMOylation in p53 stabilization, localization, and subsequent apoptosis. Reprinted and modified from Carter et al[69] with permission of the publisher. Copyright © 2007, Macmillan Publishers Ltd. **C**, PKCζ-mediated p53 SUMOylation requires PKCζ–PIAS4 binding at SP-RING domain, not PKCζ-mediated phosphorylation of PIAS4. SAP indicates scaffold attachment factor-A/B, acinus, and PIAS domain; and SP-RING, Siz/PIAS-RING domain.[55]

PKCζ was recently reported to have an important function in EC.[73,74] Magid and Davies reported that this PKC isoform was highly expressed in EC in the atheroprone areas of porcine aorta.[73] Frey et al[74] demonstrated involvement of PKCζ in oxidant generation in ECs via NADPH (nicotinamide adenine dinucleotide phosphate) oxidase activation. Consistent with these results, endothelial PKCζ activation was elevated in atherosclerotic lesions.[72,75] 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.[72] It is likely that 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 functions not only by phosphorylating substrates, but also by direct protein–protein interactions.

It has been reported that PKCζ contains a pseudosubstrate autoinhibitory 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[76,77]

(Figure 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.[53] Therefore, in addition to its protein kinase activation, the subsequent release of the PKCζ N-terminus autoinhibitory 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.[53]

Other PKCζ That Mediate 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.[64] 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,[78,79] 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.[80] 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.[81] PKCζ also contains a PB1 domain, and the p62-PKCζ association is critical for the activation of PKC downstream events, such as JNK and caspase 3 activation.[80] The precise role of this p62-PKCζ module in s-flow and d-flow needs further investigation.

SENP2 and Atheroprone 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).[82] 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,[61] but it remains unclear how each SENP isoform recognizes its specific substrates and causes different functional consequences. Among the 6 isoforms, the functions of SENP1 and SENP2 have been relatively well studied. Li et al[83] 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 de-SUMOylation in EC. $SENP1^{-/-}$ embryos are severely anemic because of diminished erythropoietin production, and this leads to SUMOylation-induced HIF1α degradation.[84] 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, demonstrating 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.[53,63] Interestingly, reduced expression of SENP2 increased both endothelial p53 and ERK5 SUMOylation, hence increased EC dysfunction and inflammation, and accelerated atherosclerotic plaque formation.[65] 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.[65] In contrast, s-flow inhibited ERK5 SUMOylation.[65] 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 downregulated by d-flow, but we did not observe this effect in EC exposed to d-flow.[65] We think that d-flow likely regulates the de-SUMOylation activity

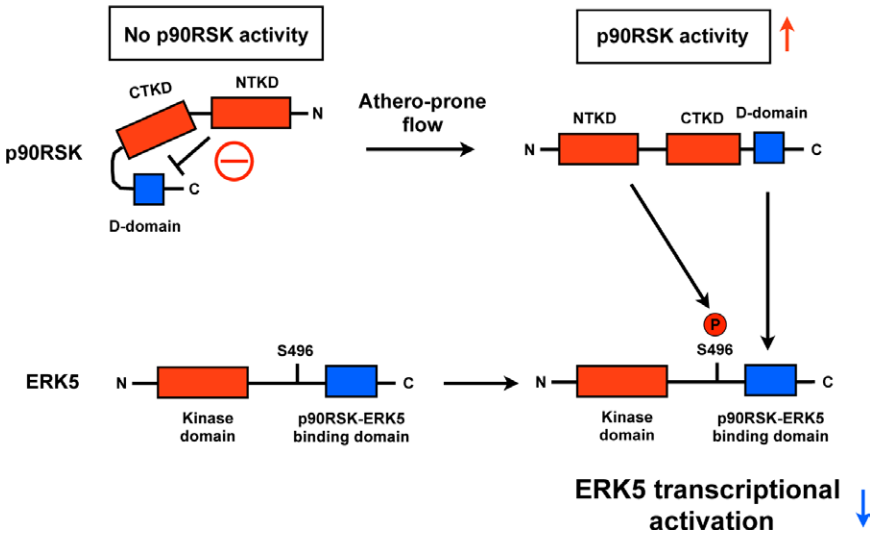

Figure 5. Atheroprone flow increases p90 ribosomal S6 kinase (p90RSK) activation, leading to p90RSK-extracellular signal–regulated kinase (ERK5) association and ERK5 S496 phosphorylation, and subsequently decreases in ERK5 transcriptional activity. At the basal level, inactive p90RSK inhibits the D-domain 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[86] and increases ERK5 S496 phosphorylation, which inhibits ERK5 transcriptional activity.[87] CTKD indicates COOH-terminal kinase domain; D-domain, NH_2-terminal docking domain; and NTKD, NH_2-terminal kinase domain.

of SENP2 or the cellular localization of SENP2, but additional studies will be needed to clarify these points.

ERK5 and Its Inhibitory Kinase, p90RSK, Under D-Flow

p90RSK is a serine/threonine kinase containing 2 functional kinase domains (Figure 5).[85] The N-terminus kinase belongs to the AGC group (protein kinase A, G, and C families group) of kinases (ie, 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 2 p90RSK kinase domains possess different functional properties. The N-terminus kinase has the most activity because 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 [85–89]. p90RSK is located downstream of the Raf-MEK-ERK1/2 signaling pathway,[90] 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.[91]

The activation and nuclear translocation of p90RSK are concomitant with immediate early gene expression.[92,93] p90RSK is also involved in the activation of NF-κB by phosphorylation of Iκ-B[94] or phosphorylation of transcription factors, including c-Fos,[95] Nur77,[96] and CREB.[97] Although ERK5 can regulate p90RSK kinase activation as an upstream kinase like ERK1/2 under certain conditions,[98] we have reported that p90RSK also directly phosphorylates ERK5 S496 and inhibits its transcriptional activity.[52] In this study, we found that p90RSK is associated with the ERK5 C-terminus transcriptional activation domain (amino acids 571–807). When we overexpressed this C-terminus fragment as a decoy, both p90RSK-ERK5 association and H_2O_2-induced reduction of ERK5 transcriptional activity were inhibited.[52] 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. Finally, 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.[52] Further studies are necessary to elucidate the precise mechanism by which d-flow regulates the function of p90RSK that leads to endothelial dysfunction.

Conclusions

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 mechano-trasduction initiated by different patterns of flow, but the exact nature of signaling that d-flow and s-flow initiate in ECs has to date evaded investigators. In this review, we have discussed several molecules and signaling events, which seem to be differentially regulated by atheroprone and atheroprotective 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 2 different types of flow may be the final key needed to unlock the door which stands between EC dysfunction and atherosclerosis formation.

Acknowledgments

We thank the current and past members of our group who have contributed to the work in improving the understanding of shear stress–induced signal transduction pathways. We also thank Drs Scott Cameron and Keigi Fujiwara, and Walter Knight for critical reading of this article.

Sources of Funding

This study was supported by a grant from National Institutes of Health to Drs Bradford C. Berk (HL-064839, HL 106158), Jun-ichi Abe (HL-064839, HL-108551, HL-102746).

Disclosures

None.

References

1. Won D, Zhu SN, Chen M, Teichert AM, Fish JE, Matouk CC, Bonert M, Ojha M, Marsden PA, Cybulsky MI. Relative reduction of endothelial nitric-oxide synthase expression and transcription in atherosclerosis-prone regions of the mouse aorta and in an *in vitro* model of disturbed flow. *Am J Pathol*. 2007;171:1691–1704.
2. Jongstra-Bilen J, Haidari M, Zhu SN, Chen M, Guha D, Cybulsky MI. Low-grade chronic inflammation in regions of the normal mouse arterial intima predisposed to atherosclerosis. *J Exp Med*. 2006;203:2073–2083.
3. Bao X, Lu C, Frangos JA. Temporal gradient in shear but not steady shear stress induces PDGF-A and MCP-1 expression in endothelial cells: role of NO, NF kappa B, and egr-1. *Arterioscler Thromb Vasc Biol*. 1999;19:996–1003.
4. Wang GX, Cai SX, Wang PQ, Ouyang KQ, Wang YL, Xu SR. Shear-induced changes in endothelin-1 secretion of microvascular endothelial cells. *Microvasc Res*. 2002;63:209–217.
5. Hsiai TK, Cho SK, Reddy S, Hama S, Navab M, Demer LL, Honda HM, Ho CM. Pulsatile flow regulates monocyte adhesion to oxidized lipid-induced endothelial cells. *Arterioscler Thromb Vasc Biol*. 2001;21:1770–1776.
6. Frangos JA, Eskin SG, McIntire LV, Ives CL. Flow effects on prostacyclin production by cultured human endothelial cells. *Science*. 1985;227:1477–1479.
7. Di Francesco L, Totani L, Dovizio M, Piccoli A, Di Francesco A, Salvatore T, Pandolfi A, Evangelista V, Dercho RA, Seta F, Patrignani P. Induction of prostacyclin by steady laminar shear stress suppresses tumor necrosis factor-alpha biosynthesis via heme oxygenase-1 in human endothelial cells. *Circ Res*. 2009;104:506–513.
8. Korenaga R, Ando J, Tsuboi H, Yang W, Sakuma I, Toyo-oka T, Kamiya A. Laminar flow stimulates ATP- and shear stress-dependent nitric oxide production in cultured bovine endothelial cells. *Biochem Biophys Res Commun*. 1994;198:213–219.
9. Diamond SL, Eskin SG, McIntire LV. Fluid flow stimulates tissue plasminogen activator secretion by cultured human endothelial cells. *Science*. 1989;243:1483–1485.
10. Gimbrone MA Jr, Topper JN, Nagel T, Anderson KR, Garcia-Cardeña G. Endothelial dysfunction, hemodynamic forces, and atherogenesis. *Ann N Y Acad Sci*. 2000;902:230–9; discussion 239.
11. Hsieh HJ, Li NQ, Frangos JA. Pulsatile and steady flow induces c-fos expression in human endothelial cells. *J Cell Physiol*. 1993;154:143–151.
12. Khachigian LM, Resnick N, Gimbrone MA Jr, Collins T. Nuclear factor-kappa B interacts functionally with the platelet-derived growth

factor B-chain shear-stress response element in vascular endothelial cells exposed to fluid shear stress. *J Clin Invest*. 1995;96:1169–1175.
13. Lan Q, Mercurius KO, Davies PF. Stimulation of transcription factors NF kappa B and AP1 in endothelial cells subjected to shear stress. *Biochem Biophys Res Commun*. 1994;201:950–956.
14. Heo KS, Fujiwara K, Abe J. Disturbed-flow-mediated vascular reactive oxygen species induce endothelial dysfunction. *Circ J*. 2011;75:2722–2730.
15. Abe J, Baines CP, Berk BC. Role of mitogen-activated protein kinases in ischemia and reperfusion injury: the good and the bad [editorial; comment]. *Circ Res*. 2000;86:607–609.
16. Abe J, Berk BC. Reactive oxygen species as mediators of signal transduction in cardiovascular disease. *Trends Cardiovasc Med*. 1998;8:59–64.
17. Yan C, Takahashi M, Okuda M, Lee JD, Berk BC. Fluid shear stress stimulates big mitogen-activated protein kinase 1 (BMK1) activity in endothelial cells. Dependence on tyrosine kinases and intracellular calcium. *J Biol Chem*. 1999;274:143–150.
18. Zhou G, Bao ZQ, Dixon JE. Components of a new human protein kinase signal transduction pathway. *J Biol Chem*. 1995;270:12665–12669.
19. English JM, Vanderbilt CA, Xu S, Marcus S, Cobb MH. Isolation of MEK5 and differential expression of alternatively spliced forms. *J Biol Chem*. 1995;270:28897–28902.
20. De Cesaris P, Starace D, Starace G, Filippini A, Stefanini M, Ziparo E. Activation of Jun N-terminal kinase/stress-activated protein kinase pathway by tumor necrosis factor alpha leads to intercellular adhesion molecule-1 expression. *J Biol Chem*. 1999;274:28978–28982.
21. Ahmad M, Theofanidis P, Medford RM. Role of activating protein-1 in the regulation of the vascular cell adhesion molecule-1 gene expression by tumor necrosis factor-alpha. *J Biol Chem*. 1998;273:4616–4621.
22. Min W, Pober JS. TNF initiates E-selectin transcription in human endothelial cells through parallel TRAF-NF-kappa B and TRAF-RAC/CDC42-JNK-c-Jun/ATF2 pathways. *J Immunol*. 1997;159:3508–3518.
23. Wong HK, Fricker M, Wyttenbach A, Villunger A, Michalak EM, Strasser A, Tolkovsky AM. Mutually exclusive subsets of BH3-only proteins are activated by the p53 and c-Jun N-terminal kinase/c-Jun signaling pathways during cortical neuron apoptosis induced by arsenite. *Mol Cell Biol*. 2005;25:8732–8747.
24. Fan G, Merritt SE, Kortenjann M, Shaw PE, Holzman LB. Dual leucine zipper-bearing kinase (DLK) activates p46SAPK and p38mapk but not ERK2. *J Biol Chem*. 1996;271:24788–24793.
25. Li L, Tatake RJ, Natarajan K, Taba Y, Garin G, Tai C, Leung E, Surapisitchat J, Yoshizumi M, Yan C, Abe J, Berk BC. Fluid shear stress inhibits TNF-mediated JNK activation via MEK5-BMK1 in endothelial cells. *Biochem Biophys Res Commun*. 2008;370:159–163.
26. Akaike M, Che W, Marmarosh NL, Ohta S, Osawa M, Ding B, Berk BC, Yan C, Abe J. 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. *Mol Cell Biol*. 2004;24:8691–8704.
27. Kasler HG, Victoria J, Duramad O, Winoto A. ERK5 is a novel type of mitogen-activated protein kinase containing a transcriptional activation domain. *Mol Cell Biol*. 2000;20:8382–8389.
28. Pi X, Garin G, Xie L, Zheng Q, Wei H, Abe J, Yan C, Berk BC. BMK1/ERK5 is a novel regulator of angiogenesis by destabilizing hypoxia inducible factor 1alpha. *Circ Res*. 2005;96:1145–1151.
29. Lee JD, Ulevitch RJ, Han J. Primary structure of BMK1: a new mammalian map kinase. *Biochem Biophys Res Commun*. 1995;213:715–724.
30. Hayashi M, Kim SW, Imanaka-Yoshida K, Yoshida T, Abel ED, Eliceiri B, Yang Y, Ulevitch RJ, Lee JD. Targeted deletion of BMK1/ERK5 in adult mice perturbs vascular integrity and leads to endothelial failure. *J Clin Invest*. 2004;113:1138–1148.
31. Parmar KM, Larman HB, Dai G, Zhang Y, Wang ET, Moorthy SN, Kratz JR, Lin Z, Jain MK, Gimbrone MA Jr, García-Cardeña G. Integration of flow-dependent endothelial phenotypes by Kruppel-like factor 2. *J Clin Invest*. 2006;116:49–58.
32. Schulman IG, Juguilon H, Evans RM. Activation and repression by nuclear hormone receptors: hormone modulates an equilibrium between active and repressive states. *Mol Cell Biol*. 1996;16:3807–3813.
33. Hu X, Lazar MA. The CoRNR motif controls the recruitment of corepressors by nuclear hormone receptors. *Nature*. 1999;402:93–96.
34. Piqueras L, Sanz MJ, Perretti M, Morcillo E, Norling L, Mitchell JA, Li Y, Bishop-Bailey D. Activation of PPARbeta/delta inhibits leukocyte recruitment, cell adhesion molecule expression, and chemokine release. *J Leukoc Biol*. 2009;86:115–122.
35. Takata Y, Liu J, Yin F, Collins AR, Lyon CJ, Lee CH, Atkins AR, Downes M, Barish GD, Evans RM, Hsueh WA, Tangirala RK. PPARdelta-mediated antiinflammatory mechanisms inhibit angiotensin II-accelerated atherosclerosis. *Proc Natl Acad Sci U S A*. 2008;105:4277–4282.
36. Wang N, Verna L, Chen NG, Chen J, Li H, Forman BM, Stemerman MB. Constitutive activation of peroxisome proliferator-activated receptor-gamma suppresses pro-inflammatory adhesion molecules in human vascular endothelial cells. *J Biol Chem*. 2002;277:34176–34181.
37. Camp HS, Tafuri SR. Regulation of peroxisome proliferator-activated receptor gamma activity by mitogen-activated protein kinase. *J Biol Chem*. 1997;272:10811–10816.
38. Barish GD, Yu RT, Karunasiri MS, et al. The bcl6-smrt/ncor cistrome represses inflammation to attenuate atherosclerosis. *Cell Metab*. 2012;15:554–562
39. Lee CH, Chawla A, Urbiztondo N, Liao D, Boisvert WA, Evans RM, Curtiss LK. Transcriptional repression of atherogenic inflammation: modulation by PPARdelta. *Science*. 2003;302:453–457.
40. Glass CK, Saijo K. Nuclear receptor transrepression pathways that regulate inflammation in macrophages and T cells. *Nat Rev Immunol*. 2010;10:365–376.
41. Straus DS, Glass CK. Anti-inflammatory actions of PPAR ligands: new insights on cellular and molecular mechanisms. *Trends Immunol*. 2007;28:551–558.
42. Ricote M, Glass CK. PPARs and molecular mechanisms of transrepression. *Biochim Biophys Acta*. 2007;1771:926–935.
43. Pascual G, Glass CK. Nuclear receptors versus inflammation: mechanisms of transrepression. *Trends Endocrinol Metab*. 2006;17:321–327.
44. Woo CH, Massett MP, Shishido T, Itoh S, Ding B, McClain C, Che W, Vulapalli SR, Yan C, Abe J. ERK5 activation inhibits inflammatory responses via peroxisome proliferator-activated receptor delta (PPARdelta) stimulation. *J Biol Chem*. 2006;281:32164–32174.
45. Suzuki T, Aizawa K, Matsumura T, Nagai R. Vascular implications of the Krüppel-like family of transcription factors. *Arterioscler Thromb Vasc Biol*. 2005;25:1135–1141.
46. Dekker RJ, van Soest S, Fontijn RD, Salamanca S, de Groot PG, VanBavel E, Pannekoek H, Horrevoets AJ. Prolonged fluid shear stress induces a distinct set of endothelial cell genes, most specifically lung Krüppel-like factor (KLF2). *Blood*. 2002;100:1689–1698.
47. Rothwarf DM, Karin M. The NF-kappa B activation pathway: a paradigm in information transfer from membrane to nucleus. *Sci STKE*. 1999;1999:RE1.
48. SenBanerjee S, Lin Z, Atkins GB, Greif DM, Rao RM, Kumar A, Feinberg MW, Chen Z, Simon DI, Luscinskas FW, Michel TM, Gimbrone MA Jr, García-Cardeña G, Jain MK. KLF2 Is a novel transcriptional regulator of endothelial proinflammatory activation. *J Exp Med*. 2004;199:1305–1315.
49. Sen-Banerjee S, Mir S, Lin Z, Hamik A, Atkins GB, Das H, Banerjee P, Kumar A, Jain MK. Kruppel-like factor 2 as a novel mediator of statin effects in endothelial cells. *Circulation*. 2005;112:720–726.
50. Lin Z, Natesan V, Shi H, Dong F, Kawanami D, Mahabeleshwar GH, Atkins GB, Nayak L, Cui Y, Finigan JH, Jain MK. Kruppel-like factor 2 regulates endothelial barrier function. *Arterioscler Thromb Vasc Biol*. 2010;30:1952–1959.
51. 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. *J Biol Chem*. 2002;277:43344–43351.
52. Le NT, Heo KS, Takei Y, et al. A crucial role for p90rsk-mediated reduction of erk5 transcriptional activity in endothelial dysfunction and atherosclerosis. *Circulation*. 2013;127:486–499
53. Heo KS, Lee H, Nigro P, Thomas T, Le NT, Chang E, McClain C, Reinhart-King CA, King MR, Berk BC, Fujiwara K, Woo CH, Abe J. PKCζ mediates disturbed flow-induced endothelial apoptosis via p53 SUMOylation. *J Cell Biol*. 2011;193:867–884.
54. Le NT, Corsetti JP, Dehoff-Sparks JL, Sparks CE, Fujiwara K, Abe J. Reactive Oxygen Species, SUMOylation, and Endothelial Inflammation. *Int J Inflam*. 2012;2012:678190.
55. Woo CH, Abe J. SUMO–a post-translational modification with therapeutic potential? *Curr Opin Pharmacol*. 2010;10:146–155.
56. Hickey CM, Wilson NR, Hochstrasser M. Function and regulation of SUMO proteases. *Nat Rev Mol Cell Biol*. 2012;13:755–766.
57. Johnson ES, Schwienhorst I, Dohmen RJ, Blobel G. The ubiquitin-like protein Smt3p is activated for conjugation to other proteins by an Aos1p/Uba2p heterodimer. *EMBO J*. 1997;16:5509–5519.
58. Johnson ES, Blobel G. Ubc9p is the conjugating enzyme for the ubiquitin-like protein Smt3p. *J Biol Chem*. 1997;272:26799–26802.

59. Johnson ES. Protein modification by SUMO. *Annu Rev Biochem*. 2004;73:355–382.
60. Li SJ, Hochstrasser M. A new protease required for cell-cycle progression in yeast. *Nature*. 1999;398:246–251.
61. Yeh ET. SUMOylation and De-SUMOylation: wrestling with life's processes. *J Biol Chem*. 2009;284:8223–8227.
62. Chang E, Heo KS, Woo CH, Lee H, Le NT, Thomas TN, Fujiwara K, Abe J. MK2 SUMOylation regulates actin filament remodeling and subsequent migration in endothelial cells by inhibiting MK2 kinase and HSP27 phosphorylation. *Blood*. 2011;117:2527–2537.
63. Woo CH, Shishido T, McClain C, Lim JH, Li JD, Yang J, Yan C, Abe J. Extracellular signal-regulated kinase 5 SUMOylation antagonizes shear stress-induced antiinflammatory response and endothelial nitric oxide synthase expression in endothelial cells. *Circ Res*. 2008;102:538–545.
64. Nigro P, Abe J, Woo CH, Satoh K, McClain C, O'Dell MR, Lee H, Lim JH, Li JD, Heo KS, Fujiwara K, Berk BC. PKCzeta decreases eNOS protein stability via inhibitory phosphorylation of ERK5. *Blood*. 2010;116:1971–1979.
65. Heo KS, Chang E, Le NT, Cushman H, Yeh ET, Fujiwara K, Abe J. 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. *Circ Res*. 2013;112:911–923.
66. Chiu YJ, Kusano K, Thomas TN, Fujiwara K. Endothelial cell-cell adhesion and mechanosignal transduction. *Endothelium*. 2004;11:59–73.
67. Garner E, Raj K. Protective mechanisms of p53-p21-pRb proteins against DNA damage-induced cell death. *Cell Cycle*. 2008;7:277–282.
68. Lin K, Hsu PP, Chen BP, Yuan S, Usami S, Shyy JY, Li YS, Chien S. Molecular mechanism of endothelial growth arrest by laminar shear stress. *Proc Natl Acad Sci U S A*. 2000;97:9385–9389.
69. Carter S, Bischof O, Dejean A, Vousden KH. C-terminal modifications regulate MDM2 dissociation and nuclear export of p53. *Nat Cell Biol*. 2007;9:428–435.
70. Mihara M, Erster S, Zaika A, Petrenko O, Chittenden T, Pancoska P, Moll UM. p53 has a direct apoptogenic role at the mitochondria. *Mol Cell*. 2003;11:577–590.
71. Miyashita T, Krajewski S, Krajewska M, Wang HG, Lin HK, Liebermann DA, Hoffman B, Reed JC. Tumor suppressor p53 is a regulator of bcl-2 and bax gene expression *in vitro* and in vivo. *Oncogene*. 1994;9:1799–1805.
72. Heo KS, Lee H, Nigro P, Thomas T, Le NT, Chang E, McClain C, Reinhart-King CA, King MR, Berk BC, Fujiwara K, Woo CH, Abe J. PKCζ mediates disturbed flow-induced endothelial apoptosis via p53 SUMOylation. *J Cell Biol*. 2011;193:867–884.
73. Magid R, Davies PF. Endothelial protein kinase C isoform identity and differential activity of PKCzeta in an athero-susceptible region of porcine aorta. *Circ Res*. 2005;97:443–449.
74. Frey RS, Rahman A, Kefer JC, Minshall RD, Malik AB. PKCzeta regulates TNF-alpha-induced activation of NADPH oxidase in endothelial cells. *Circ Res*. 2002;90:1012–1019.
75. Nigro P, Abe JI, Woo CH, Satoh K, McClain C, O'Dell MR, Lee H, Lim JH, Li JD, Heo KS, Fujiwara K, Berk BC. Pkc{zeta} decreases enos protein stability via inhibitory phosphorylation of erk5. *Blood*. 2010
76. Newton AC. Protein kinase C: structural and spatial regulation by phosphorylation, cofactors, and macromolecular interactions. *Chem Rev*. 2001;101:2353–2364.
77. Smith L, Wang Z, Smith JB. Caspase processing activates atypical protein kinase C zeta by relieving autoinhibition and destabilizes the protein. *Biochem J*. 2003;375(Pt 3):663–671.
78. Averna M, Stifanese R, De Tullio R, Passalacqua M, Salamino F, Pontremoli S, Melloni E. Functional role of HSP90 complexes with endothelial nitric-oxide synthase (eNOS) and calpain on nitric oxide generation in endothelial cells. *J Biol Chem*. 2008;283:29069–29076.
79. Dong Y, Wu Y, Wu M, Wang S, Zhang J, Xie Z, Xu J, Song P, Wilson K, Zhao Z, Lyons T, Zou MH. Activation of protease calpain by oxidized and glycated ldl increases the degradation of endothelial nitric oxide synthase. *Journal of cellular and molecular medicine*. 2009;13:2899–2910
80. Kim GY, Nigro P, Fujiwara K, Abe J, Berk BC. p62 binding to protein kinase C ζ regulates tumor necrosis factor α-induced apoptotic pathway in endothelial cells. *Arterioscler Thromb Vasc Biol*. 2012;32:2974–2980.
81. Moscat J, Diaz-Meco MT, Wooten MW. Of the atypical PKCs, Par-4 and p62: recent understandings of the biology and pathology of a PB1-dominated complex. *Cell Death Differ*. 2009;16:1426–1437.
82. Kang X, Qi Y, Zuo Y, Wang Q, Zou Y, Schwartz RJ, Cheng J, Yeh ET. SUMO-specific protease 2 is essential for suppression of polycomb group protein-mediated gene silencing during embryonic development. *Mol Cell*. 2010;38:191–201.
83. Li X, Luo Y, Yu L, Lin Y, Luo D, Zhang H, He Y, Kim YO, Kim Y, Tang S, Min W. SENP1 mediates TNF-induced desumoylation and cytoplasmic translocation of HIPK1 to enhance ASK1-dependent apoptosis. *Cell Death Differ*. 2008;15:739–750.
84. 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.
85. Frödin M, Gammeltoft S. Role and regulation of 90 kDa ribosomal S6 kinase (RSK) in signal transduction. *Mol Cell Endocrinol*. 1999;151:65–77.
86. Ranganathan A, Pearson GW, Chrestensen CA, Sturgill TW, Cobb MH. The MAP kinase ERK5 binds to and phosphorylates p90 RSK. *Arch Biochem Biophys*. 2006;449:8–16.
87. Le NT, Takei Y, Shishido T, et al. P90rsk targets the erk5-chip ubiquitin e3 ligase activity in diabetic hearts and promotes cardiac apoptosis and dysfunction. *Circ Res*. 2012;110:536–550
88. Zhao Y, Bjorbaek C, Moller DE. Regulation and interaction of pp90(rsk) isoforms with mitogen-activated protein kinases. *J Biol Chem*. 1996;271:29773–29779.
89. Smith JA, Poteet-Smith CE, Malarkey K, Sturgill TW. Identification of an extracellular signal-regulated kinase (ERK) docking site in ribosomal S6 kinase, a sequence critical for activation by ERK in vivo. *J Biol Chem*. 1999;274:2893–2898.
90. Blenis J. Signal transduction via the MAP kinases: proceed at your own RSK. *Proc Natl Acad Sci U S A*. 1993;90:5889–5892.
91. Abe J, Okuda M, Huang Q, Yoshizumi M, Berk BC. Reactive oxygen species activate p90 ribosomal S6 kinase via Fyn and Ras. *J Biol Chem*. 2000;275:1739–1748.
92. Chen RH, Sarnecki C, Blenis J. Nuclear localization and regulation of erk- and rsk-encoded protein kinases. *Mol Cell Biol*. 1992;12:915–927.
93. Chen RH, Tung R, Abate C, Blenis J. Cytoplasmic to nuclear signal transduction by mitogen-activated protein kinase and 90 kDa ribosomal S6 kinase. *Biochem Soc Trans*. 1993;21:895–900.
94. Ghoda L, Lin X, Greene WC. The 90-kDa ribosomal S6 kinase (pp90rsk) phosphorylates the N-terminal regulatory domain of IkappaBalpha and stimulates its degradation *in vitro*. *J Biol Chem*. 1997;272:21281–21288.
95. Chen RH, Chung J, Blenis J. Regulation of pp90rsk phosphorylation and S6 phosphotransferase activity in Swiss 3T3 cells by growth factor-, phorbol ester-, and cyclic AMP-mediated signal transduction. *Mol Cell Biol*. 1991;11:1861–1867.
96. Fisher TL, Blenis J. Evidence for two catalytically active kinase domains in pp90rsk. *Mol Cell Biol*. 1996;16:1212–1219.
97. Xing J, Ginty DD, Greenberg ME. Coupling of the RAS-MAPK pathway to gene activation by RSK2, a growth factor-regulated CREB kinase. *Science*. 1996;273:959–963.
98. Pearson G, English JM, White MA, Cobb MH. Erk5 and erk2 cooperate to regulate nf-{kappa}b and cell transformation. *J Biol Chem*. 2000;15:15

Arteriosclerosis, Thrombosis, and Vascular Biology

JOURNAL OF THE AMERICAN HEART ASSOCIATION

Novel Mechanisms of Endothelial Mechanotransduction

Jun-ichi Abe and Bradford C. Berk

Arterioscler Thromb Vasc Biol. 2014;34:2378-2386; originally published online October 9, 2014;
doi: 10.1161/ATVBAHA.114.303428
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

Print ISSN: 1079-5642. Online ISSN: 1524-4636