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 kinase/extracellular signal–regulated kinase 5 (PKCζ)/NF-κB signaling, extracellular signal–regulated kinase 5/peroxisome proliferator–activated receptor signaling, and mechanosignaling pathways involving SUMOylation, protein kinase Cζ, 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. 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. 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. 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). Inflamed arterial plaques are driven by increased levels of 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-xB, AP-1, early growth response-1, c-Jun, c-fos, and c-myc). 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 Cζ (PKCζ), p90 ribosomal S6 kinase (p90RSK), and increased levels of SUMOylation are involved is not activated by atheroprotective/s-flow, 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. MEK5 and ERK5 were first identified as 2 components of this new protein kinase–signaling cascade. MEK5 is the 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. 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. 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. 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).

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-xB, AP-1, early growth response-1, c-Jun, c-fos, and c-myc). 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 Cζ (PKCζ), p90 ribosomal S6 kinase (p90RSK), and increased levels of SUMOylation are involved is not activated by atheroprotective/s-flow, 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. MEK5 and ERK5 were first identified as 2 components of this new protein kinase–signaling cascade. MEK5 is the 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. 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. 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. 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).

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-xB, AP-1, early growth response-1, c-Jun, c-fos, and c-myc). 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 Cζ (PKCζ), p90 ribosomal S6 kinase (p90RSK), and increased levels of SUMOylation are involved is not activated by atheroprotective/s-flow, 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. MEK5 and ERK5 were first identified as 2 components of this new protein kinase–signaling cascade. MEK5 is the
have revealed that s-flow–induced ERK5 activation increases 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. 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.

**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 NH2-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. Phosphorylation of PPARγ Ser-82 by ERK1/2 significantly inhibits its transcriptional activation. 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.

The detailed regulatory mechanism of transrepression was discussed extensively in other reviews. 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. 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. 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. NF-κB is a key transcriptional factor that regulates expression of proinflammatory mediators, including cytokines, chemokines, and molecules that foster cell-to-cell adhesion. KLF2 reduces NF-κB transcriptional activity and subsequent adhesion molecule expression via competing for the association of CBP/p300 cofactor with NF-κB. Parmar et al. 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 cofactor with NF-κB. Furthermore, Parmar et al. 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 cofactor with NF-κB. A crucial role for KLF2 in inhibiting endothelial permeability by tight junction protein expression was also reported. Consistent with such key roles of ERK5 in EC physiology in vitro, EC apoptosis and inflammation are accelerated in endothelial-specific ERK5 knockout mice, and the deletion of ERK5 in ECs accelerates atherosclerosis formation in LDL receptor deficient mice. 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. 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. After this 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. The corepressor interaction surface requires Helix 3–5. 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. The corepressor interaction surface requires Helix 3–5. 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. The corepressor interaction surface requires Helix 3–5.

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. The corepressor interaction surface requires Helix 3–5. 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. The corepressor interaction surface requires Helix 3–5. 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. The corepressor interaction surface requires Helix 3–5. 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. The corepressor interaction surface requires Helix 3–5.
family of protein inhibitors, such as activated STAT (PIAS1-4), Polycomb-2 protein (Pc2), and RanBP2/Nup358. Sentrin/SUMO-specific proteases (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). 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. Our studies showed that treatment of ECs with H2O2, 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. Of note, both H2O2 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 H2O2 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 H2O2 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. Furthermore, we found that ERK5 SUMOylation was increased by d-flow, but it was decreased by s-flow. 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. 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. In fact, Lin et al reported that s-flow increased p53 expression and JNK-mediated p53 phosphorylation, which led to p53 nuclear export. These results show that the atheroprotective effect exerted by s-flow increases p21 via p53, inducing the vaso-protective effects of s-flow.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 apoptotic. 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.68 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 localization 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 (SENP1-7) catalyze deconjugation of newly synthesized SUMO protein. The primary subcellular localization of each SENP is also listed. Reprinted and modified from Woo et al with permission of the publisher. Copyright © 2010, Elsevier.

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 (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). 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.
PKCζ was recently reported to have an important function in EC. Magid and Davies reported that this PKC isoform was highly expressed in EC in the atheroprone areas of porcine aorta. Frey et al 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. 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. 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. 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.
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 TNF-α-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 activation and subsequent PKCζ–PIAS4 binding are crucial for d-flow–induced p53 SUMOylation and ECs apoptosis.53

SENPE and Atheroprone D-Flow

SENPE2 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 (SENPE1-3 and 5–7). 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 SENPE isoform recognizes its specific substrates and causes different functional consequences. Among the 6 isoforms, the functions of SENPE1 and SENPE2 have been relatively well studied. Li et al83 showed that TNFα transiently induced SENPE1 translocation from the cytosol to the nucleus and subsequently increased JNK activation and apoptosis via Homeodomain Interacting Protein Kinase 2 de-SUMOylation in EC. SENPE1−/− embryos are severely anemic because of diminished erythropoietin production, and this leads to SUMOylation-induced HIF1α degradation.64 The deletion of SENPE2 in mouse causes defects in cardiac development by inhibiting Gata4 and Gata6 expression and accumulation of SUMOylated Pargc/CBX4 (a polycomb repressive complex 1 subunit). HIF1α stabilization is not affected in SENPE2−/− mouse embryonic fibroblasts, demonstrating the substrate specificity between SENPE1 and SENPE2.

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 SENPE2 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 SENPE2 expression to be downregulated by d-flow, but we did not observe this effect in EC exposed to d-flow.86 We think that d-flow likely regulates the de-SUMOylation activity...
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). 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. p90RSK is located downstream of the Raf-MEK-ERK1/2 signaling pathway, 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.

The activation and nuclear translocation of p90RSK are concomitant with immediate early gene expression. p90RSK is also involved in the activation of NF-kB by phosphorylation of Ik-B or phosphorylation of transcription factors, including c-Fos, Nur77, and CREB. Although ERK5 can regulate p90RSK kinase activation as an upstream kinase like ERK1/2 under certain conditions, we have reported that p90RSK also directly phosphorylates ERK5 S496 and inhibits its transcriptional activity. 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 H₂O₂-induced reduction of ERK5 transcriptional activity were inhibited. 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. 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-traduction 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 atheroprobe 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**


Molecular mechanism of endothelial growth arrest by laminar shear stress.


Ranganathan A, Pearson GW, Christensen CA, Sturgill TW, Cobb MH. The MAP kinase ERK5 binds to and phosphorylates p90 RSK. Arch Biochem Biophys. 2006;449:8–16.


Yeh ET. SUMO-specific protease 2 is essential for stabilization of HIF1alpha during hypoxia. Cell. 2007;131:584–595.


Ranganathan A, Pearson GW, Christensen CA, Sturgill TW, Cobb MH. The MAP kinase ERK5 binds to and phosphorylates p90 RSK. Arch Biochem Biophys. 2006;449:8–16.


Yeh ET. SUMO-specific protease 2 is essential for stabilization of HIF1alpha during hypoxia. Cell. 2007;131:584–595.
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
Copyright © 2014 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/34/11/2378

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in *Arteriosclerosis, Thrombosis, and Vascular Biology* can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to *Arteriosclerosis, Thrombosis, and Vascular Biology* is online at:
http://atvb.ahajournals.org//subscriptions/