Atheroprotective Signaling Mechanisms Activated by Steady Laminar Flow in Endothelial Cells

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Many studies of the human vascular tree have shown that atherosclerosis develops at an early age, with areas of the posterior aorta and branch sites showing the earliest fatty streaks. Closer analysis of the branch points showed that the flow dividers, regions of high shear stress with laminar flow, were relatively protected from fatty streak formation. In contrast, there was a predilection for atherosclerosis at the curvatures and lateral walls of branch points.[1] These are regions where disturbed flow occurs; importantly, when the time-averaged shear stress is low, the spatial shear stress gradient is very high.[1,2] Both monocyte adhesion and endothelial cell (EC) apoptosis also are highest in these areas.[3–6] Recently, Cheng et al[7] developed a vascular occluder that induced 3 different regions of altered flow: low, high, and low with oscillatory flow. They observed that lesions always developed in regions of low compared with high shear stress. However, the low shear stress was more likely to develop larger lesions with more lipids, expression of inflammatory mediators, and matrix metalloprotease activity than oscillatory flow. When viewed in concert with a study that showed that regions of low shear stress already had a greater number of inflammatory cells resident in the artery wall as a result of increased trafficking,[8] it is clear that low flow is proinflammatory and atherogenic. More sophisticated 3-dimensional analysis of flow patterns in human carotid bifurcations has identified prototypic arterial waveforms, "atheroprone" and "atheroprotective," representing the wall shear stresses in the carotid sinus, which is susceptible to atherosclerotic lesion development, and the distal internal carotid artery, which is resistant.[9] Characteristics of the atheroprone waveform include a high oscillatory shear index (≈0.45) and low time-averaged shear stress amplitude (≈0 dyne/cm^2). Thus, both the amplitude and flow pattern are key determinants of susceptibility to atherosclerosis.

Transcriptional Profiling of EC Reveals a Critical Role for Kruppel-Like Factor-2 in the Atheroprotective Response to Flow Pattern

Gene microarray technology represents a powerful tool to quantitatively analyze gene expression in ECs exposed to different flow patterns. Despite varying techniques for analysis, several transcriptional profiling studies share common conclusions. Overall, the most obvious and consistent result is that laminar high shear stress downregulates the expression of genes in ECs. These genes are inflammatory in nature and include cytokines, adhesion molecules, and genes that regulate apoptosis, proliferation, and migration. In contrast, ECs exposed to disturbed flow with low shear stress exhibit a marked increase in the expression of these inflammatory genes (although some protective genes such as antioxidant enzymes also are upregulated). These include several broad-acting inflammatory cytokines and receptors, in addition to elements of the nuclear factor-κB (NF-κB) system, consistent with a proinflammatory phenotype. In vitro, ECs subjected to disturbed laminar shear stress exhibit increased levels of nuclear localized NF-κB, Egr-1, c-Jun, and c-Fos compared with cells exposed to steady laminar flow or maintained under static conditions.[10] This differential regulation of transcription factor expression by disturbed versus steady laminar flow indicates that regional differences in blood flow patterns in vivo may represent important local modulators of endothelial gene expression at anatomic sites predisposed for atherosclerotic development.

More recently, transcriptional profiling studies identified the Kruppel-like factor-2 (KLF2) as being decreased by the inflammatory cytokine interleukin-1β and induced by laminar shear stress in cultured ECs.[11–13] Overexpression of KLF2 in ECs strongly induced endothelial nitric oxide (NO) synthase (eNOS) expression and inhibited induction of vascular cell adhesion molecule (VCAM)-1 and E-selectin. Consistent with these observations, in vitro flow assays demonstrate that binding of white blood cells was decreased in ECs transduced with KLF2. KLF2 also is important for the antithrombotic effects of flow.[14] Recently, Parmar et al[15] showed that ERK5 (also called BMK1) activation is required for the upregulation of KLF2. As discussed below, it appears that KLF2 expression is regulated by flow via a pathway involving MEK5-ERK5-MEF2C phosphorylation.[15]

Mechanosensing Mechanisms for Shear Stress

Although this review does not focus on mechanisms for mechanotransduction, recent studies have identified several candidate mediators.[16] Integrins, platelet EC adhesion molecule-1 (PECAM-1), tyrosine kinase receptors (especially

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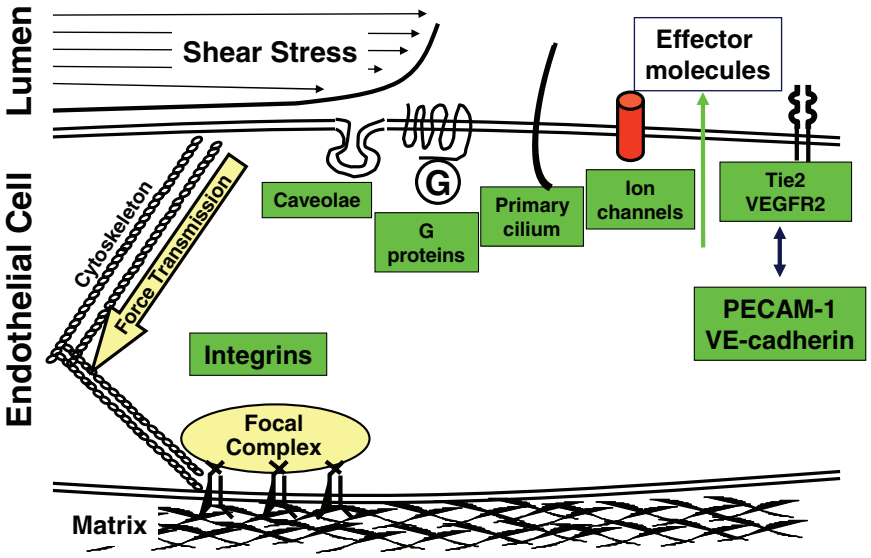

Figure 1. EC mechanosensing and mechanotransducing mechanisms.

vascular endothelial growth factor receptor 2 [VEGFR2]), vascular endothelial (VE)-cadherin, G proteins, primary cilium, and ion channels are known to be important for mechanosensing and mechanotransduction (Figure 1). These proteins are located at the surface of ECs, where they can sense directly and transduce mechanical forces across the membrane to the interior of the cells. Shear stress rapidly induces PECAM-1 tyrosine phosphorylation, accompanied by the binding of the PECAM-1 cytoplasmic tail to the phosphatase SHP-2. To further demonstrate a role for this protein in mechanosensing, direct application of mechanical force to PECAM-1 was shown to elicit its tyrosine phosphorylation.[17]

In addition, VEGFR2 was shown to mediate mechanotransduction. VEGFR2 is activated and phosphorylated very rapidly (1 to 2 minutes) after the onset of laminar flow.[18,19] One important question that has been generated is the coordination and differential roles of these molecules in sensing mechanical forces. Recently, studies by Tzima et al[20] showed that PECAM-1, VE-cadherin, and VEGFR2 make up a mechanosensory complex. In support of these observations in vivo, PECAM-1–knockout mice do not activate NF-κB or downstream inflammatory genes in regions of disturbed flow. Therefore, this mechanosensing pathway is required for the earliest known events in atherogenesis.[20]

Many studies have suggested that ion channels might play important roles in response to laminar shear stress. Ion channel activation is the most rapid response of ECs to shear stress, occurring almost immediately on the onset of flow. Shear stress–mediated activation of potassium channels,[21] calcium channels.[22,23] and an ATP-gated P2X4 ion channel[24] has been suggested to mediate flow sensing. Finally, a critical role for extracellular matrix in the EC response to flow has been demonstrated. ECs plated on fibronectin or fibrinogen activate NF-κB in response to flow, whereas cells on collagen or laminin do not.[25]

Atheroprotective Flow Patterns Limit Oxidative Stress, Inflammation, and Apoptosis

There is evidence that inflammation contributes at each stage to the development of clinically significant atherosclerosis.[26,27] For example, fatty streak formation is associated with the expression of the monocyte adhesion ligand, VCAM-1, on ECs.[28] During plaque progression, the monocytes present in the plaque proliferate, oxidize low-density lipoprotein, and generate multiple cytokines that act as chemoattractants for other inflammatory cells.[27] Thus, multiple recurrent inflammatory events contribute to atherosclerosis initiation and progression. Many studies suggest an important role for redox state (the balance between generation and removal of reactive oxygen species) in inflammation and vascular pathology. A major hypothesis discussed below is that steady laminar flow promotes a reducing environment in ECs that decreases inflammation and limits atherosclerosis (Figure 2). In addition, the role of redox state in EC apoptosis has been well established,[29] and the role of EC apoptosis in atherosclerosis has been demonstrated.[30–32] Importantly, in regions of disturbed flow with high shear gradients, there is increased EC apoptosis.[6] This has important functional consequences be-

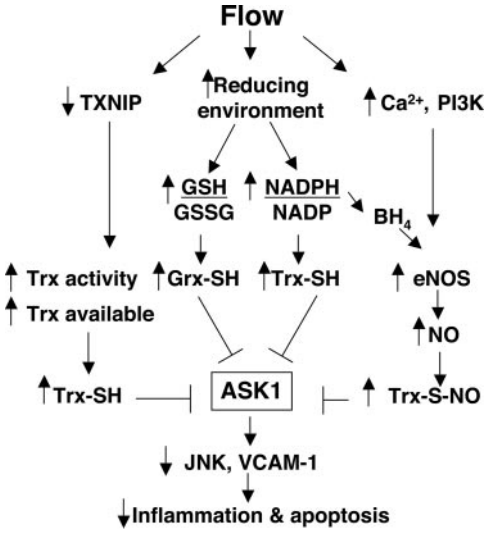

Figure 2. Flow inhibits ASK1 activation by multiple pathways that are redox regulated and enhance NO generation. GSH indicates glutathione; Trx, thioredoxin.

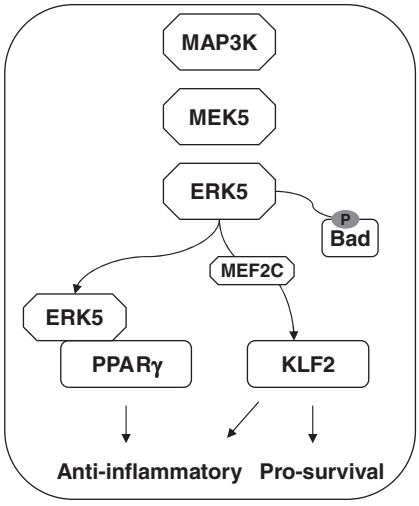

Figure 3. MEK5-ERK5 cascade mediates antiinflammatory and prosurvival effects of flow by activating several transcription factors.

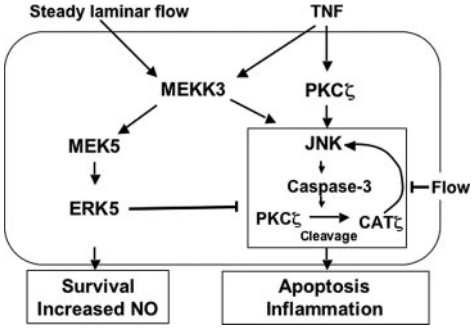

Figure 4. Crosstalk between MAPK family members. MEKK3 is activated by both flow and TNF, yet the downstream consequences are completely different. It is likely that flow recruits proteins (F) that specifically activate MEK5 and simultaneously may inhibit activation of TNF signals downstream of MEKK3. Here, we show that inhibition of PKCζ is 1 target specifically regulated by flow. Flow inhibits the activation of JNK, which is required for caspase-3 cleavage. This synergistically inhibits TNF signaling by preventing formation of CATζ, which normally is required to enhance JNK activation. Thus, the prosurvival effects of flow are mediated both by stimulating kinases such as ERK5 and by inhibiting other kinases such as PKCζ.

cause EC turnover exposes the underlying intima, which may contain collagen, tissue factor, and other procoagulant and proinflammatory mediators. Conversely, previous reports from our group and others showed that steady laminar flow, in part through inhibition of cytokine-mediated signaling,[33–35] prevents EC apoptosis.[36,37] Thus, a second concept is that flow-mediated promotion of EC survival is a key atheroprotective mechanism (Figures 3 and 4).

Redox and NO Pathways Are Critical for Atheroprotection

Recent reviews[38] have discussed the multiple mechanisms by which reactive oxygen species modulate EC function to promote atherosclerosis. In brief, flow promotes a reducing state characterized by increased levels of reduced glutathione. Glutathione levels are maintained by stimulation of pathways that promote its synthesis and maintain it in a reduced state (Figure 2). Glutathione is the major low-molecular-weight thiol antioxidant in EC. It serves as a substrate for glutathione peroxidase to eliminate lipid hydroperoxides and H_2O_2. After being oxidized, glutathione is converted to glutathione disulfide (GSSG). Normally, ECs exist in a reduced condition because glutathione is present in very high concentrations ($\approx$1 mmol/L), whereas GSSG is maintained at levels $<$1% of total glutathione. Steady laminar flow inhibits H_2O_2-induced jun N-terminal kinase (JNK) activation, partly by increasing the ratio between reduced glutathione and oxidized glutathione (glutathione/GSSG) in a glutathione reductase–dependent manner.[29] This is supported by the fact that a glutathione reductase inhibitor, but not a thioredoxin reductase inhibitor, blocked the inhibitory effect of flow on H_2O_2-mediated JNK activation.[29] Although it is not clear how flow regulates the activity of glutathione reductase, the impact is significant in that it is at least partly responsible for the inhibition of both H_2O_2- and tumor necrosis factor (TNF)–induced cell death. Recently, Mueller et al[39] showed that the multidrug resistance protein-1 is the major exporter of GSSG. Inhibiting multidrug resistance protein-1 expression or function prevented the decline in intracellular glutathione associated with oscillatory flow and reduced apoptosis. These findings suggest that increased glutathione/GSSG is critical for maintaining EC in a reduced condition, which is essential for atheroprotection.

Glutathione peroxidase is a selenoprotein that plays an important role in defense mechanisms against oxidative damage by catalyzing the reduction of hydroperoxides using glutathione as the reducing substrate. Glutathione peroxidase is upregulated in both disturbed and laminar flow patterns.[40] Interestingly, this response is shear stress specific because stretch did not change either the expression or activity of glutathione peroxidase.[41]

Superoxide dismutase is a key enzyme family that catalyzes the dismutation of superoxide into hydrogen peroxide and oxygen. Of the 3 superoxide dismutase isoforms found in the vascular wall, extracellular superoxide dismutase and Cu/Zn superoxide dismutase expression is upregulated when ECs are exposed to laminar shear stress.[42,43] Decreased superoxide levels will have important effects on limiting both protein oxidation and formation of peroxynitrite.

Recently, our laboratory has found important roles for glutaredoxin in limiting EC inflammation and apoptosis.[44,45] Glutaredoxin is a ubiquitous redox molecule that is unique in its ability to regulate S-glutathiolation of proteins. Exposure of ECs to steady laminar flow for 5 minutes significantly increased glutaredoxin activity and increased Akt and eNOS phosphorylation. Inhibiting glutaredoxin decreased stimulation of Akt and eNOS phosphorylation induced by flow, whereas overexpression of glutaredoxin increased Akt and eNOS phosphorylation. These data suggest that glutaredoxin is an important mediator for flow-induced Akt and eNOS activation and that glutaredoxin activity depends on glutathione reductase–mediated changes in the EC redox state. We next studied the effect of glutaredoxin on caspase-3 glutathiolation.[45] Glutaredoxin activity was significantly upregulated by TNF in ECs, and decreased caspase-3 glutathiolation was associated with increased caspase-3 cleavage. Cysteine-to-serine mutations (C163S, C184S, and C220S) of caspase-3

that were predicted to prevent glutathiolation showed increased cleavage compared with wild-type caspase-3. These findings demonstrate a novel mechanism of caspase-3 regulation by glutaredoxin in TNF-induced apoptosis and suggest that glutaredoxin regulates EC apoptosis.

Finally, S-nitrosylation is an important posttranslational modification, highly regulated by flow, that likely plays a critical role in EC survival. In the presence of endogenous NO, free-sulfhydryl groups in proteins can be modified to S-NO.[46,47] When exposed to laminar shear stress, the level of S-nitrosylated proteins increases significantly, in part because shear stress stimulates eNOS activity.[48,49] Target proteins regulated by S-nitrosylation include the catalytic p17 subunit of caspase-3 and thioredoxin.[48] Increased protein S-nitrosylation inhibits caspase-3 activity and enhances thioredoxin activity. Because Haendeler et al[50] showed that increased S-nitrosylation of thioredoxin enhanced its ability to inhibit ASK1 and prevent EC apoptosis, these data suggest that S-nitrosylation is a key atheroprotective effect of laminar shear stress.

Crosstalk Among Mitogen-Activated Protein Kinase Pathways Regulates EC Response to Flow and TNF

Crosstalk among the ubiquitous mitogen-activated protein kinases (MAPKs) is a key mechanism for the regulation of multiple signal inputs. Of interest, flow stimulates ERK1/2, p38, and ERK5 but inhibits JNK.[34] On the basis of this finding, we have studied the mechanisms by which flow regulates JNK activation to gain insight into the atheroprotective mechanisms induced by flow.

Mechanisms of JNK Inhibition

There are multiple mechanisms by which JNK activation may be controlled. They are divided into 5 categories: (1) alterations in upstream MAP3K that activate JNK such as ASK1, MLK3, and MEKK1; (2) crosstalk among MAPKs such as ERK1/2 and ERK5; (3) alterations in upstream phosphatases that activate or inhibit JNK such as SHP2; (4) alterations in downstream phosphatases that directly inactivate JNK, including PP2C and the MAP kinase phosphatases; and (5) alterations in binding proteins such as JIP1 that may modulate the ability of JNK to interact with activators.

ASK1 as a Mediator of TNF Signaling in ECs

ECs are primary cellular targets for the actions of proinflammatory cytokines such as TNF. Binding of TNF to the p55 TNF receptor that is expressed by ECs leads to EC activation and apoptosis. Because both responses are initiated by ligand binding to a single receptor, it is clear that TNF activates multiple signal transduction pathways. JNK is activated by dual phosphorylation mediated by one of the MAP2Ks (MKK4 and MKK6). MKK4/6 in turn is activated through phosphorylation by MAP3Ks (including MEKK1, TAK1, and ASK1).[51–53] Some MAP3Ks such as MEKK1 and TAK1 can activate both NF-κB and JNK cascades, and these MAP3Ks are direct targets of TNF receptor–associated factor molecules.[54–56] However, some MAP3Ks such as ASK1 appear to be involved in JNK activation only in response to proinflammatory cytokines and stress stimuli.[57–59] Among the MAP3K, our data[60,61] and published results from other laboratories suggest that ASK1 may be particularly important in vessels.[58,62,63] ASK1 is a 170-kDa protein that functionally is composed of an inhibitory N-terminal domain, an internal kinase domain, and a C-terminal regulatory domain.[58] The C-terminal domain of ASK1 binds to the TNF receptor–associated factor domain, and this association is required for ASK1 activation by TNF receptor–associated factor-2 and TNF receptor–associated factor-6.[59] Overexpression of ASK1 induces apoptotic cell death, and a catalytically inactive form of ASK1 inhibits TNF-induced apoptosis.

Several cellular factors, including AIP-1, 14-3-3, and thioredoxin, have been reported to inhibit ASK1 activity.[60,62–64] Thioredoxin in a reduced form binds to the N-terminal part of ASK1 and blocks activation of ASK1 by TNF.[65–67] A phosphoserine-binding molecule, 14-3-3 binds to ASK1 specifically via Ser-967 and has been reported to inhibit ASK1-induced apoptosis.[68] We found that ASK1 is required for TNF-mediated JNK activation in ECs.[60] TNF activates ASK1 in part by dissociating ASK1 from its inhibitor 14-3-3, which is facilitated by the ASK1-inteacting protein.[63] Steady laminar flow inhibited TNF activation of both ASK1 and JNK. Inhibition of ASK1 by flow correlated with increased association of ASK1 with 14-3-3. A constitutively active form of ASK1 lacking Ser-967, which is the 14-3-3 binding site (ASK1-ΔNS967A), failed to interact with its inhibitor, 14-3-3. Moreover, the kinase activity of ASK1-ΔNS967A was not inhibited by flow. These data establish ASK1 as a target for flow-mediated inhibition of cytokine signaling. Interestingly, ASK1 in cytoplasm and mitochondria mediates distinct apoptotic pathways induced by TNF, and thioredoxin-1 and thioredoxin-2 cooperatively inhibit ASK1 activities.[64]

More recently, we and others have shown a critical role for the thioredoxin-interacting protein (TXNIP) as a key integrator of flow and redox signaling in ECs.[61,69] TXNIP is a stress-responsive protein that inhibits thioredoxin activity. When rabbit aortas or ECs were exposed to normal flow (12 dynes/cm^2 for 24 hours), TXNIP expression decreased, whereas thioredoxin-1 activity increased compared with low flow (0.4 dyne/cm^2). Normal flow inhibited TNF activation of JNK and VCAM-1 expression. In cultured ECs, reducing TXNIP expression by small interference RNA increased thioredoxin-1 binding to ASK1 and inhibited TNF activation of JNK and VCAM-1 expression. Conversely, overexpressing TXNIP stimulated JNK. In aortas from TXNIP-deficient mice, TNF-induced VCAM-1 expression was inhibited. In cardiomyocytes, TXNIP was found to promote apoptosis,[70,71] demonstrating that its downregulation by flow is antiapoptotic. These data suggest that TXNIP and thioredoxin-1 are key components of biomechanical signal transduction and establish TXNIP and thioredoxin-1 as novel regulators of TNF signaling (Figure 2).

MAPK Crosstalk Inhibits TNF Signaling by Multiple Mechanisms

Several investigators, including those in our laboratory, have found that 1 MAPK (eg, p38 or JNK) may inhibit or oppose the activation of another MAPK (eg, ERK1/2).[72–74] In fact, a

general antagonism in signal transduction has been proposed for ERK1/2 (progrowth and antiapoptotic) and p38/JNK (proapoptotic) so that the dynamic balance between growth factor–activated ERK and stress-activated JNK-p38 pathways may be important in determining whether a cell survives or undergoes apoptosis.[74] Recent data suggest that ERK5 also is primarily progrowth and antiapoptotic.[75–77] MAPK crosstalk is likely mediated by events that involve upstream MAP2K and MAP3K. Regulation of MLK3 and MEKK1 is suggested by findings that ERK1/2 inhibits activity of exchange factors such as Sos and C3G. Okada et al[78] found that insulin dynamically regulated the relative activation of Ras and Rap1 by altering the association of C3G and Crk.

Flow Inhibits TNF-Mediated JNK Activation

We investigated the effect of flow on activity of 4 MAPKs.[34] Flow alone stimulated ERK1/2, p38, and ERK5 activity but decreased JNK activity compared with static controls (both human umbilical vein ECs and bovine aortic ECs). TNF alone activated ERK1/2, p38, and JNK maximally at 15 minutes. Pre-exposing human umbilical vein ECs to flow inhibited TNF activation of JNK by >50% but had no significant effect on p38 or ERK1/2 activation by TNF. Incubation of ECs with PD98059 or U0126, specific MAP2K inhibitors (MEK1 and MEK5), blocked flow-mediated inhibition of TNF-stimulated JNK. Using transfection experiments with a constitutive-active form of MEK5, we found that TNF-mediated activation of JNK was inhibited by MEK5.[79] These findings indicate that flow inhibits TNF-mediated signaling events in ECs by a mechanism that is dependent on the activation of ERK1/2 and ERK5 signaling pathways and independent of receptor activation, NO production, or cyclic nucleotide generation.

ERK5 Prevents EC Inflammation and Apoptosis by Activating Transcription Factors (KLF2 and Peroxisome Proliferated–Activated Receptor-γ)

Flow regulates the activity of numerous transcription factors, including NF-κB, KLF2, and peroxisome proliferated–activated receptor (PPAR)-γ. Here, we focus on KLF2 and PPARγ because several recent reports link the MEK5-ERK5 pathway to these factors. ERK5 has become the MAPK most associated with EC survival because the ERK5-knockout mice display defects in vessel formation and cardiac development with abnormal EC morphology, whereas other MAPK knockouts have no obvious vascular phenotype.[80,81] We showed that ERK5 protects ECs from apoptosis induced by growth factor deprivation and incubation with proinflammatory stimuli and that ERK5 is an important mediator for the antiapoptotic effect of steady flow.[37] The antiapoptotic mechanism of flow and ERK5 activation likely involves phosphorylation of Bad on Ser-136 and Ser-112 because increasing ERK5 activity stimulated Bad phosphorylation and inhibited apoptosis. Further strengthening the role for ERK5 is the report that the flow-mediated increase in KLF2 expression occurs via a MEK5-ERK5-MEF2 signaling pathway (Figure 4).[15] Of interest, mice lacking KLF2 die during embryogenesis, exhibiting defects in assembly of the vessel wall that lead to massive hemorrhage, similar to the ERK5 knockout. Parmar et al[15] suggest that flow activates MEK5, which in turn phosphorylates ERK5, resulting in activation of the MEF2 family at the KLF2 promoter. Binding of MEF2 members to the KLF2 promoter is constitutive and not significantly altered by flow, indicating that this family likely binds constitutively to the KLF2 promoter and acts as a switch on phosphorylation by ERK5. In support of this concept, a recent study showed that MEF2 factors bind to the KLF2 promoter and stimulate transcriptional activity.[82] Furthermore, inhibition of MEF2 function by p65 and histone deacetylases (HDAC4 and HDAC5) accounts for the reduction in KLF2 expression observed in ECs treated with TNF.[82]

Recent data suggest that PPARγ is another key transcriptional mediator of the atheroprotective effects of flow. Ligand-dependent activation of PPARγ attenuates lesion formation in mice and monocyte/macrophage recruitment during atherogenesis.[83] In ECs, laminar flow activates PPARγ activity and interestingly also stimulates PPARγ ligand production through the phospholipase A2–cytochrome P450 pathway.[84] Endothelial activation of PPARγ by flow is believed to exert antiinflammatory effects because PPARγ ligands reduce TNF-induced VCAM-1 expression.[85] Akaike et al[86] showed that flow-induced PPARγ activation is dependent on ERK5 (Figure 3) and is functionally important because expression of the dominant-negative form of PPARγ significantly decreased the inhibitory effect of ERK5 on VCAM-1 expression.

Protein Kinase Cζ is a Novel Mediator of EC Apoptosis, Counterregulated by Flow

Our laboratory became interested in the role of protein kinase C ζ (PKCζ) in flow-mediated atheroprotection for 3 reasons. First, among the PKC family members, the atypical PKCζ has recently emerged as an important isoform in ECs. PKCζ promotes the adhesive phenotype of ECs when activated by TNF[87] via stimulation of NF-κB–dependent intracellular adhesion molecule-1 expression.[87,88] Second, a recent study demonstrated a correlation between PKCζ activity and the flow pattern in pig arteries,[89] with lower PKCζ activity in ECs exposed to laminar flow compared with ECs exposed to disturbed flow.[89] Third, PKCζ contains a Phox and Bem1p (PB1) domain that also is present in MEKK2 and MEK5. PB1 domains are an evolutionary conserved protein-protein interaction module found in the atypical PKC isoforms ζ and λ, members of MAPK modules (eg, MEK5, MEKK2/3), p40(phox), and several scaffolds, including p62 and Par6. Proteins containing PB1 domains interact with each other. Thus, the scaffold proteins p62 and Par6 use their PB1 domains to interact specifically with the PB1 domain of atypical PKCs.[90] The Par6 protein physically links the atypical PKCs to the Rho family GTPases Cdc42 and Rac1, thus forming signaling complexes involved in cell polarity decisions.[90] PB1 domain interactions also play important roles in MAPK modules because MEKK2, MEKK3, and MEK5 contain PB1 domains in their N-terminal regions. PKCζ is cleaved by caspases after TNF stimulation to generate a 50-kDa truncated form (catalytic domain of PKCζ [CATζ]) with a higher kinase activity than the full-length protein, likely as a result of the loss of autoinhibition (Figure 4). We

hypothesized that flow would inhibit TNF-mediated PKCζ cleavage and thereby CATζ formation. We found that PKCζ activity was required for TNF-mediated JNK and caspase-3 activation in ECs.[91] PKCζ was rapidly cleaved after stimulation with TNF to generate CATζ in cultured ECs and in intact rabbit vessels. This truncated form of PKCζ enhanced JNK and caspase-3 activation. Interestingly, PKCζ cleavage was prevented by inhibitors of PKCζ, JNK, and caspase activities, suggesting that these enzymes, by regulating CATζ formation, modulate caspase-3 activity in ECs. Finally, we found that flow reduced caspase-dependent processing of PKCζ and caspase-3 activation, markedly inhibiting EC apoptosis induced by CATζ.[91] These results define a novel role for PKCζ as a shared signaling mediator for flow and TNF that is likely important for flow-mediated inhibition of proinflammatory and apoptotic events in EC.

Conclusions

In summary, recent data suggest that there are multiple potentially synergistic mechanisms by which steady laminar flow inhibits signal transduction by proinflammatory stimuli such as TNF and interleukin-1. These mechanisms are likely to represent important intracellular pathways for atheroprotection mediated by flow. The ability to sense and transduce local hemodynamic forces is unique to ECs, suggesting that the underlying mechanisms represent important therapeutic targets. Future studies that comprehensively define the processes involved in flow-mediated signaling and highlight specific flow-regulated signaling events that occur in vivo represent exciting opportunities for basic and clinical research.

Note Added in Proof

During the preparation of this article, Woo et al published the evidence that H_2O_2 and advanced glycation end products inhibited ERK5 function by increasing ERK5-SUMOylation.[92] SUMOylation of ERK5 decreased MEF2 transcriptional activity as well as flow-mediated KLF2 promoter activity and eNOS expression. ERK5-SUMOylation is a novel mechanism by which flow-mediated atheroprotection is prevented, which may be especially important in diabetes.

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Disclosures

None.

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