Flow Perturbation Is Linked to Endothelial PAR Signaling

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Although several KLFs are expressed in the vasculature, so far only KLF2 deletion results in embryonic lethality attributable to vascular failure.5 The prominent abnormality in KLF2-deficient vessels is diminished recruitment or retention of vascular smooth muscle cells starting after embryonic day 12.5 with subsequent rupture of the thinning endothelial cell lining of larger vessels.6 Similar phenotypes of vascular failure result from deletion of PDGF-B7 or the sphingosine 1 phosphate receptor 1 (S1PR1, EDG1)8 both of which play critical roles in the communication of endothelial cells with mural cells. Indeed KLF2 upregulates PDGF-B expression.9 KLF2 is thus a crucial endothelial cell expressed transcription factor that regulates adaptation of the vessel wall to the increasing hemodynamic forces during cardiovascular development. A series of recent publications9–13 have now elucidated the central role of KLF2 as a master regulator that links mechanosensing to the fine tuning of inflammatory and procoagulant responses of the endothelium.

Perturbed flow at atherosclerosis prone sites indeed leads to downregulation of KLF2 with concordant loss of vascular protective mechanisms.9,11 Laminar flow induces KLF2 through activation of the MEK5-ERK5-MEF2C pathway,9 and overexpression of KLF2 to a large extent mimics the atheroprotective antiinflammatory, antithrombotic, and antiproliferative phenotype of differentiated endothelial cells under flow.9,11 KLF2 induces endothelial cell nitric oxide synthase (eNOS) and reduces caveolin levels that suppresses eNOS activity. KLF2 thus regulates vascular tone.13 Moreover, KLF2 specifically prevents the upregulation of vascular cell adhesion molecule (VCAM) and E-selectin in response to interleukin (IL)-1β, TNFα, and endotoxin by sequestering the NF-κB coactivator CBP/p300, thereby reducing leukocyte and T-cell recruitment and local inflammation.13 KLF2 is barrier protective and counteracts VEGF-induced hyperpermeability.14 Furthermore, KLF2 increases thrombomodulin and suppresses antifibrinolytic plasminogen activator inhibitor (PAI)-1 expression while preventing cytokine-induced tissue factor (TF) expression (Figure). Together, these changes maintain the anticoagulant balance of the endothelium.12 In this issue, Lin et al add to this emerging theme by describing an unexpected regulatory role KLF2 in suppressing thrombin signaling through protease activated receptor (PAR) 1.15

Lin et al show that overexpression of KLF2 abolishes thrombin-dependent induction of inflammatory cytokines IL-8, MCP-1, and, to a lesser degree, IL-6. Importantly, thrombin-mediated TF upregulation is also suppressed by KLF2. Because thrombin synergizes with mediators released from activated platelets such as sphingosine 1 phosphate to induce TF,16 KLF2 may suppress a vicious cycle of sustained endothelium procoagulant alteration at sites susceptible to thrombosis. Unlike KLF2’s suppression of cytokine-induced...
VCAM and E-selectin expression through sequestration of NF-κB coactivators, KLF2 attenuated thrombin signaling by downregulating mRNA levels and cell surface expression of the thrombin receptor PAR1. Conversely, knock-down of KLF2 upregulated PAR1 and increased thrombin-mediated TF induction. These experiments thus demonstrate control of PAR expression by the central mechanosensing pathway of endothelial cells.

However, suppression of thrombin signaling by KLF2 is not uniform, and thrombin still induces CD40L release, essentially reversing suppressed baseline secretion in KLF2 overexpressing cells. Consistent with a more complex regulation of PAR1 by shear forces, Dekker et al described that PAR1 mRNA levels are upregulated when endothelial cells are switched from static to flow conditions.3 Similarly, Weibel-Palade body release by thrombin is not inhibited by KLF2 overexpression, although a shift in the dose response for thrombin was observed.11 Taken together, these results indicate that KLF2 modulates, rather than shuts off, PAR1 signaling completely. It is of interest that KLF2 suppresses PAR2 mRNA expression even more pronounced than PAR1 levels.9 PAR2 is more susceptible to inflammatory cytokine upregulation, and PAR2 activation potently induces TF.17,18 In addition, PAR2 is involved in recruiting leukocytes to activated endothelium, thus closely linking PAR2 to inflammatory pathways.19 Although PAR2 is not cleaved by thrombin, PAR2 can be crossactivated by thrombin-cleaved PAR1.20 In addition, certain thrombin responses are dependent on synergistic effects of PAR1 and PAR2 activation.21,22 The significant downregulation of thrombin-induced proinflammatory and procoagulant responses by KLF2 in endothelial cells may thus result from the concordant modulation of PAR1 and PAR2 expression. TF-dependent PAR2 signaling regulates vascular regeneration,23 and downregulation of PAR2 as well as TF may be part of the coordinated program by which KLF2 suppresses angiogenesis.14

KLF2 upregulates thrombomodulin (TM) which shifts the balance from thrombin to activated protein C (APC) generation and thus favors vascular protective signaling of APC through PAR1. Thrombin signaling is attenuated by KLF2-mediated downregulation of PAR1. Efficient disruption of the proinflammatory and procoagulant circuits may depend on the simultaneous suppression of PAR2 by KLF2.

It will be of interest for further studies to evaluate whether the significant upregulation of thrombomodulin by KLF2 maintains the vascular protective signaling properties of the downstream activated protein C (APC) pathway. Endothelial protein C receptor (EPCR)-bound APC activates PAR1.24 APC signaling is barrier protective through sphingosine 1 phosphate receptor cross-activation,25 whereas thrombin signaling through PAR1 is barrier disruptive.26 The effect of KLF2 on thrombin-induced permeability is of particular interest when considering the strong barrier protective effects of KLF2 in VEGF-induced hyperpermeability.14 APC-PAR1 signaling induces an antiapoptotic and endothelial protective gene profile that is distinct from thrombin-mediated PAR1 signaling in cytokine stimulated endothelial cells.27 Whether suppression of the proinflammatory signaling of thrombin in endothelial cells by KLF2 shifts the balance favorably to the APC signaling pathway should be of interest. These future directions will add to our understanding on the role of mechanosensing pathways to regulate the proinflammatory signaling balance of the endothelium.

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