A Nuclear Attack on Thrombosis and Inflammation

Edward M. Conway

Thrombomodulin is a transmembrane glycoprotein expressed on the luminal surface of endothelial cells, where it maintains vascular homeostasis via its anti-inflammatory, anticoagulant, and anti-fibrinolytic properties. These effects of thrombomodulin are achieved through dynamic interactions primarily with thrombin, protein C, thrombin activatable fibrinolysis inhibitor, complement components, and the proinflammatory danger signal high mobility group box 1 (HMGB1).1 When bound to thrombomodulin, thrombin loses its procoagulant/proinflammatory properties, while efficiently generating activated protein C and activated thrombin activatable fibrinolysis inhibitor. Activated protein C is a potent anticoagulant, anti-inflammatory and cytotoxic protein. Activated thrombin activatable fibrinolysis inhibitor inhibits fibrinolysis, and inactivates proinflammatory mediators and anaphylatoxins. The lectin-like domain of thrombomodulin also damps inflammation by blocking HMGB1 and suppressing complement activation. Diminished expression of thrombomodulin is a feature of endothelial cell dysfunction, and it is a driver in the pathogenesis of several disorders, including venous thromboembolic disease, sepsis, disseminated intravascular coagulation (DIC), atherosclerosis, stroke, inflammatory arthritis and colitis, thrombotic microangiopathies, and diabetic nephropathy. To offset the imbalance associated with reduced thrombomodulin, and with the aim of preventing organ damage, systemic administration of recombinant forms of thrombomodulin has shown efficacy in several preclinical models of thrombosis and inflammation, and in humans with DIC and sepsis.2

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Yang et al3 have taken a different approach to augment endothelial thrombomodulin and limit disease, particularly focusing on thrombosis. Going nuclear, they examined the role of 2 transcription factors, Nur77 and Nor1, members of the family of nuclear orphan NR4A receptors. These are constitutively active, early response genes that encode transcription factors that have multiple effects, but are implicated in modulating vascular function.4 Yang et al showed that gene delivery of either Nur77 or Nor1 on the surface of HUVECs (human umbilical vein endothelial cells), but interestingly, via distinctly different mechanisms. Nor1 caused an increase in thrombomodulin gene transcription by enhancing expression of Kruppel-like factors (KLF)-2 and KLF-4. In contrast, Nur77 had no effect on thrombomodulin gene transcription, but stabilized thrombomodulin mRNA, likely via interactions with the adenyrate-uridylate-rich elements in the 3′-untranslated region of thrombomodulin. Overexpression of Nur77 or Nor1 prevented tumor necrosis factor α-induced downregulation of thrombomodulin and upregulation of the procoagulant tissue factor, endowing the endothelial cells with antithrombotic properties. The authors then extended their studies in vivo. Mice deficient in Nur77 had reduced expression of thrombomodulin, and they were, as one would expect, more susceptible to thrombosis in a carotid artery injury model. Gene delivery of either of these nuclear receptors, Nor1 or Nur77, in wild-type mice, upregulated thrombomodulin and prolonged the time to formation of an occlusive arterial thrombus. These exciting observations uncover a potential novel strategy of targeting the orphan nuclear receptors, Nor1 or Nur77, to treat or prevent vascular thrombosis.

How do these findings mesh with our understanding of thrombomodulin regulation? Are there other nuclear receptors that are similarly amenable to therapeutic targeting? Are there downsides to their findings? Because of its central role in modulating coagulation and inflammation, the complex mechanisms by which thrombomodulin are regulated are a topic of intense study.5 At a transcriptional level, thrombomodulin expression requires the presence of the coactivator complex, p300/CAMP response element-binding protein.6 Cytokine-mediated suppression of thrombomodulin is achieved by activating nuclear factor-κB (NF-κB), which in turn sequesters p300/CAMP response element-binding protein, preventing it from binding to the thrombomodulin promoter. Like Nur77 and Nor1, tumor necrosis factor α-induced suppression of thrombomodulin can be offset by activation of several nuclear receptors, including the retinoic acid receptor-α,7 the vitamin D receptor,8 the peroxisome proliferator–activated receptors (PPAR)-α and PPAR-γ,9,10 and the farnesoid X receptor,11 as well as by the histone deacetylase, Sirt1 (Figure).12 These can all induce expression of KLF-2 or KLF-4 and repress NF-κB pathway activity, and in turn enhance thrombomodulin gene transcription.13,14 KLF-2/4 can directly promote thrombomodulin transcription by binding to its promoter, or by recruiting p300/CAMP response element-binding protein from NF-κB.15 KLF-2 is also vasculoprotective by inducing expression of endothelial nitric oxide synthase, and suppressing PAI-1, von Willebrand factor, and tissue factor.16,17 The latter also observed with Nor1. Clearly, Nor1 and Nur77 are not the only nuclear receptors that upregulate thrombomodulin, able to switch the vasculature to an anti-inflammatory/antithrombotic phenotype. However, Yang et al3 are the first to convincingly...
demonstrate the therapeutic potential of these factors in a mouse model of arterial thrombosis.

NR4A receptors are widely expressed in many cells beyond the endothelium, and they have diverse properties that may complicate interpretation of the underlying mechanisms and responsible cell(s). For Nur77, even though it is upregulated in injured endothelial cells, smooth muscle cells and monocytes/macrophages, there is overwhelming in vitro and in vivo evidence that supports Yang et al’s findings that Nur77 is vasculoprotective. By directly upregulating KLF-2, activating target genes by binding to their promoter(s), and enhancing expression of the NF-κB inhibitor IκBα, Nur77 induces an anti-inflammatory endothelial cell phenotype, damps vascular smooth muscle cell proliferation and migration, and suppresses leukocyte activation and infiltration. The caveat to this is that transgenic mice in which Nur77 is overexpressed in endothelial cells, exhibit enhanced vascular permeability because of increased endothelial nitric oxide synthase and reduced expression of endothelial junctional proteins.

The story is more complicated for Nor1. This nuclear receptor is, such as Nur77, highly expressed in atherosclerotic or balloon injury lesion endothelial cells, smooth muscle cells and macrophages. Nor1-deficient mice are, however, resistant to atherosclerosis in apoE−/− mice and neointimal hyperplasia in a carotid artery injury model. Deficiency of Nor1 in vascular smooth muscle cells dampens Rb phosphorylation and E2F activity, thereby restricting DNA replication. Nor1 overexpression induces endothelial cell activation, with increased expression of proadhesive molecules VCAM-1 and ICAM-1 (intercellular adhesion molecule 1), findings that would be considered at odds with the findings of Yang et al. However, contrasting these reports that define Nor1 as proinflammatory in the vasculature, transgenic overexpression of Nor1 in mice attenuates the inflammatory response to lipopolysaccharide, reducing IκBα phosphorylation/degradation and inhibiting phosphorylation and nuclear translocation of NF-κB.

Taken together, the roles of the nuclear receptors, Nor1 and Nur77, in the vasculature are complex, often opposing, and undoubtedly dictated by context. Further complicating the picture is the fact that these NR4A members also variably exhibit tumor suppressor or oncogenic properties. In spite of these challenges, the observations of Yang et al are provocative and exciting, as they reveal pathways that have hitherto not been explored in the setting of thrombosis. Teasing out the cell- and stress-specific responses mediated by Nor1 and Nur77 in different thrombosis models, and further clarifying the relevant pathways, will hopefully uncover novel preventative and therapeutic strategies for thrombotic and inflammatory diseases.

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Disclosures

None.

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