TGFBIp: more than meets the eye?

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After damage to the endothelium, platelets prevent excessive bleeding by adhering to the exposed ECM. In this issue of Blood, Kim and colleagues describe a novel function of TGFBIp that potentiates platelet adhesion and activation, a protein with a previously established role in corneal dystrophies.2

When platelets adhere, spread, and become activated, they form a procoagulant surface that stabilizes the wounded area. Platelets adhere to several extracellular matrix (ECM) proteins including collagen, von Willebrand factor (VWF), and fibrinogen.3 However, multiple interactions are necessary to secure platelet adhesion; mice deficient in fibrinogen and VWF still form occlusive thrombi.4 This suggests that other ECM or platelet-derived proteins contribute to adhesive events at the vascular wall. One of these proteins appears to be transforming growth factor beta–induced protein (TGFBIp). Interestingly, TGFBIp has been shown to affect sight. Abnormal deposition of TGFBIp mutants has been linked to blurred vision.2 In the current study, Kim et al go “outside the eye” to discover that platelets store TGFBIp and release it upon thrombin activation. Although future studies are necessary to resolve how TGFBIp accumulates inside platelets, the authors show that TGFBIp facilitates platelet adhesion and spreading and that soluble TGFBIp induces the expression of surface P-selectin and phosphatidylserine. Taken together, these data indicate that activated platelets may release TGFBIp, which, in turn, recruits additional platelets to the site of injury (see figure).

The mechanism behind TGFBIp-induced platelet activation requires further insight. The FAS1 domain of TGFBIp regulates platelet adhesion, in part through interactions with integrin α5β1. However, other partners likely participate since interruption of TGFBIp binding to α5β1 partially interferes with, but does not completely abrogate, platelet adhesion. Once adhesion has occurred, TGFBIp promotes platelet spreading through its RGD domain, most likely through interactions that involve integrin αIIbβ3. In this regard, the authors demonstrate that TGFBIp induces conformational activation of αIIbβ3. These intriguing observations indicate new roles for TGFBIp in platelet activation and provide an impetus for identifying the cognate TGFBIp receptor so that more in-depth mechanistic studies can be pursued.

Interestingly, too much TGFBIp may induce thrombosis. By simulating arterial shear rates, the authors demonstrate that platelets more readily adhere to collagen and form thrombi in the presence of TGFBIp. Furthermore, injections of epinephrine in mice in conjunction with increased levels of TGFBIp (through either coadministration of the recombinant protein or transgenic overexpression of TGFBIp) results in increased pulmonary emboli compared with mice receiving epinephrine alone.

So is TGFBIp an important player in arterial thrombosis? The pulmonary embolism model used by the authors provide evidence in favor of TGFBIp promoting increased platelet aggregation in the vasculature.7 Nevertheless, questions still remain about the in vivo relevance of TGFBIp in thrombus formation. Exploiting different thrombosis models, such as ferric chloride or laser-induced injury, may supply further support that elevated levels of TGFBIp foster thrombosis. The crux of its role, however, will need to be elucidated by other approaches that may include studies in...
murine platelets lacking TGFBIp or possibly in humans that possess mutations in the TGFBIp gene. These investigative avenues will help us visualize the exact function of TGFBIp in platelet biology and discern whether inhibitors that target TGFBIp may dampen a person’s risk for thrombosis.

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REFERENCES

Comment on Tremmel et al, page 5236

CD44: target for antiangiogenesis therapy

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In this issue of Blood, Tremmel and colleagues provide evidence that endothelial cells express a specific splice variant of the CD44 protein family, CD44v6, that constitutively associates with VEGF receptor-2 to regulate receptor activation and downstream receptor signaling. Significantly, the targeting of CD44v6 inhibited VEGF-dependent in vitro endothelial cell function as well as in vivo tumor growth and angiogenesis.1

CD44 represents a family of class I transmembrane glycoproteins that are expressed by a wide variety of cells.2-4 Multiple CD44 isoforms arise from extensive alternative exon splicing that most often involves the tandem insertion of sequences coded by variant exons v1 to v10 into the membrane proximal region of the extracellular domain. Posttranslational modifications, including N- and O-linked glycosylation, glycosaminoglycan additions, and sulfation, dependent on the cell type and growth conditions, lead to further molecular diversity. CD44 has long been recognized as one of the primary cell-surface receptors for hyaluronan (HA), while its cytoplasmic domain engages the cytoskeleton through interactions with linker molecules such as ankyrin and ERM (ezrin, radixin, and moesin) proteins.5

The CD44 proteins participate in the regulation of a number of diverse cellular processes, including the regulation of cell proliferation, differentiation, survival, and motility.2-4,6 They have therefore been implicated in developmental events (eg, neuronal axon guidance, fetal lymphogenesis, and limb-bud development), physiologic processes (eg, hematopoiesis, leukocyte recruitment, and lymphocyte homing), and pathologic conditions (eg, tumor growth and spread).

With respect to the growth and spread of tumors, CD44 proteins may be involved in at least 3 ways.2-4 First, CD44 may stimulate tumor cell proliferation, motility, and/or invasiveness. This may include mechanisms in which the molecule functions to recruit and then enable the activity of surface-associated matrix metalloproteinases to promote tumor invasiveness or growth factor receptor activation.1,8 Second, variants of CD44 may function as coreceptors for the activation of growth–promoting tumor receptor tyrosine kinases.7 Third, specific CD44 variants may function as tumor cell–surface ligands that interact with endothelial selectins to mediate the initial capture and arrest of circulating tumor cells at a secondary organ.10 Last, by regulating the function of endothelial cells (ECs)11 and/or the recruitment or activity of inflammatory cells,6 CD44 might promote tumor angiogenesis.

A role for CD44 in angiogenesis was first suspected given the involvement of low-molecular-weight HA species in blood vessel formation,2 a suspicion that was further reinforced by the finding that anti-CD44 antibodies block in vitro endothelial cell functions such as cell proliferation and tube formation.13 However, direct confirmation of CD44’s participation during in vivo angiogenesis has only more recently come from investigations of CD44-null mice.14 Specifically, these studies demonstrated that the absence of CD44 inhibits angiogenesis, including the vascularization of tumors, an effect that appears to be due principally to the loss of endothelial CD44. In the context of these previous studies, Tremmel et al not only provide further confirmation of the involvement of CD44 in tumor angiogenesis, but also present some additional mechanistic insights into the activity of endothelial CD44 in the formation of new vessels.1

In their paper, Tremmel et al demonstrate that human ECs express CD44 isoforms bearing sequences coded by variant exon 6 (designated as CD44v6). Coimmunoprecipitation studies revealed a constitutive association between vascular endothelial growth factor receptor-2 (VEGFR-2) and CD44v6 (unlike the inducible interaction between CD44v6 and c-Met, the receptor for hepatocyte growth factor [HGF]), which did not require heparan sulfate modification of CD44. Importantly, VEGFR-2 and c-Met activation (but not PDGF–dependent activation) were suppressed by a CD44v6 peptide and a soluble CD44v6 ectodomain. In addition, downstream signaling after VEGFR-2 activation was abrogated by the coexpression of a CD44 cytoplasmic domain construct, an effect that was lost by mutation of the ERM binding sequence. Subsequent studies demonstrated that the targeting of CD44v6 inhibited VEGF-dependent in vitro endothelial cell migration, sprouting, and tube formation. Further, in SCID mice, CD44v6 peptide also suppressed the assembly of human vessels in Matrigel/fibrin implants containing human ECs, as well as the growth and vascularization of human pancreatic tumors. Together, these data implicate CD44v6 variant isoforms as coreceptors in the VEGF–dependent signaling involved in endothelial cell functions required for (tumor) angiogenesis. Issues beyond the scope of this
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