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THROMBOSIS AND HEMOSTASIS

Comment on Ishihara et al, page 2559

von Willebrand factor promotes wound healing

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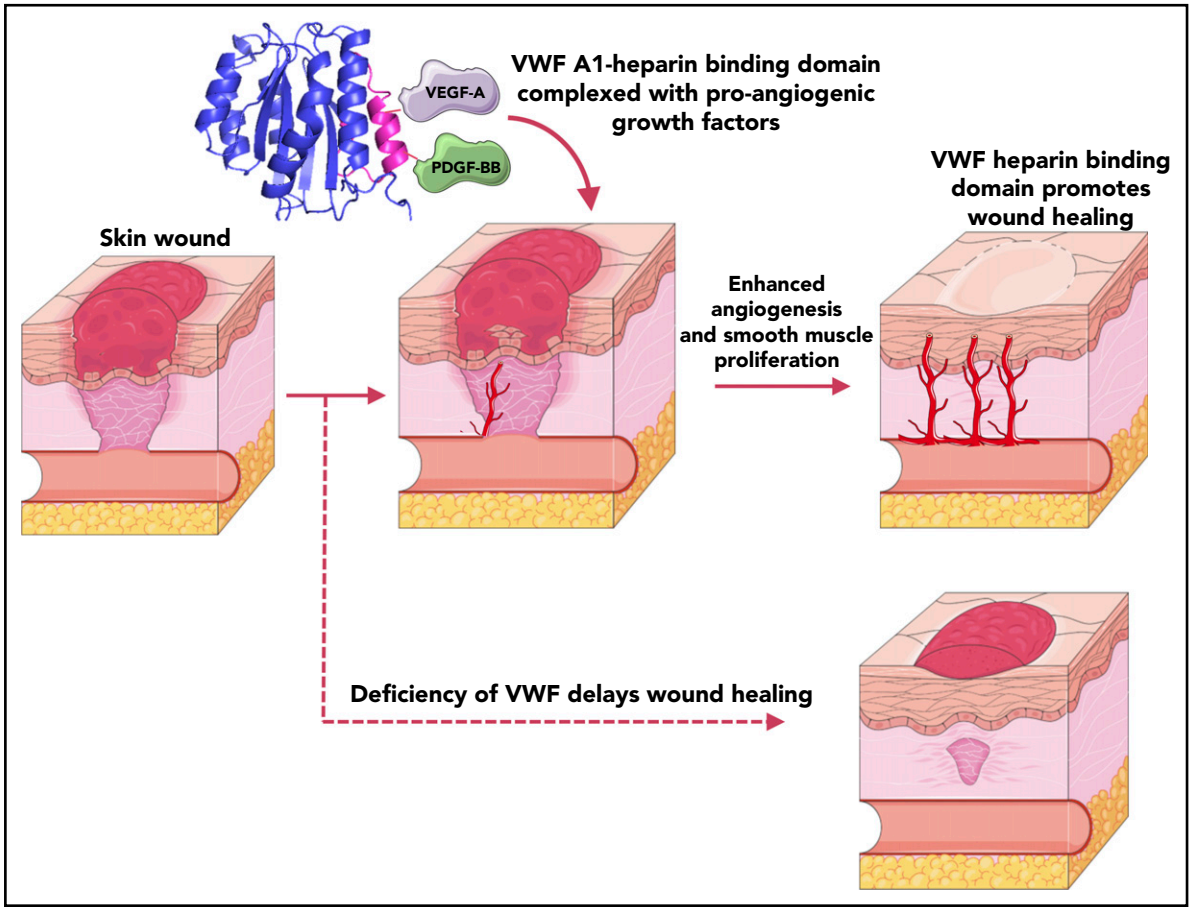
In this issue of *Blood*, Ishihara et al report an entirely novel role for von Willebrand factor (VWF) in promoting wound healing.[1] In particular, they demonstrate that the heparin-binding domain (HBD) within the A1 domain of VWF can bind to a variety of different growth factors, including vascular endothelial growth factor-A (VEGF-A) and platelet-derived growth factor-BB (PDGF-BB). Following a dermal skin injury, delayed wound healing, accompanied by reduced local growth factor concentrations and impaired local angiogenesis, was observed in VWF$^{-/-}$ mice compared with controls (see figure). In contrast, treatment of skin wounds with fibrin matrices functionalized with VWF HBD complexed with VEGF-A and PDGF-BB resulted in improved wound healing in both VWF$^{-/-}$ mice and type 2 diabetic mice. Collectively, these exciting findings suggest that VWF plays a critical role in recruiting growth factors to sites of injury and thereby in regulating tissue repair.

VWF is a complex multimeric plasma glycoprotein that plays critical roles in maintaining normal hemostasis. For many years, it has been recognized that quantitative or qualitative VWF deficiency results in the bleeding disorder von Willebrand disease (VWD).[2] More recently, evidence from several independent groups has defined a number of other novel biological functions for VWF. For example, accumulating data suggest that VWF plays important roles in regulating inflammatory responses and tumor cell biology.[3,4] Moreover, Starke et al previously reported that VWF also functions as a negative regulator of angiogenesis.[5] Thus, inhibition of VWF expression in endothelial cells (ECs) resulted in significantly enhanced proliferation and migration velocity in response to VEGF-induced signaling. Furthermore, increased angiogenesis in vivo was observed in VWF$^{-/-}$ mice.[5] These findings have direct clinical relevance in that recurrent gastrointestinal bleeding due to angiodysplasia constitutes a well-recognized complication in VWD. Although the molecular mechanisms underpinning the precise roles of VWF in regulating angiogenesis remain poorly understood, preliminary studies using blood outgrowth ECs derived from patients with different types of VWD have confirmed a proangiogenic phenotype.[6,7]

Following on from the evidence that VWF is involved in angiogenesis, Ishihara et al now propose an additional novel role for VWF in regulating wound healing. In particular, they report significantly delayed healing of dermal skin wounds in VWF$^{-/-}$ mice compared with controls. Importantly, in the absence of VWF, wounds demonstrated attenuated EC and smooth muscle proliferation. In addition, there was a significant reduction in VEGF-A and fibroblast growth factor-2 (FGF-2) in the wounds of VWF$^{-/-}$ mice. Subsequent in vitro studies confirmed that VWF binds a variety of specific growth factors, including members of the PDGF/VEGF, FGF, and transforming growth factor-β families. Binding of these growth factors was mediated in large part via the HBD in the VWF A1 domain (Tyr1328-Ala1350). Interestingly, in contrast to the VWF-GpIBα interaction, which requires shear-induced unfolding, growth factors bind to the VWF A1 domain in its native state. In keeping with this observation, Ishihara et al further demonstrate that VWF circulates in complex with VEGF-A in normal human plasma. Collectively, these findings suggest that VWF may regulate recruitment of growth factors to sites of vascular injury, thereby promoting local angiogenesis and effective tissue regeneration. The putative role of the VWF HBD in this context is consistent with previous studies that have implicated HBDs in other extracellular matrix proteins (eg, laminin and fibrinogen) in modulating angiogenesis by regulating local growth factors concentrations.

Based on their data, Ishihara et al propose that the ability of VWF to promote wound healing is likely modulated in a significant part through enhanced local angiogenesis. This hypothesis contrasts with the previous studies in which VWF was shown to be an inhibitor of angiogenesis.[5,6] Interestingly, in vivo data from other animal models of ischemia have also suggested paradoxical pro- and antiangiogenic roles for VWF under specific settings. For example, VWF was shown to inhibit angiogenesis in a mouse model of cerebral ischemia,[8] but conversely promoted angiogenesis in a hind limb ischemia model.[9] Further studies will be required to elucidate the mechanisms through which these variable effects of VWF on EC biology and angiogenesis are regulated, and how these functions may vary between different tissues.

A biological role for VWF in promoting wound healing raises a number of important clinical questions. For example, it is well recognized that plasma VWF levels vary over a wide range in the general population. Moreover, plasma VWF levels are significantly influenced by age,



VWF promotes growth factor recruitment and wound healing. The HBD of VWF-A1 binds to proangiogenic growth factors, including VEGF-A and PDGF-BB. This interaction promotes sequestration and slow release of growth factors at sites of wound healing to enhance angiogenesis and smooth muscle proliferation, and ultimately, to accelerate tissue repair. Conversely, VWF deficiency results in reduced levels of growth factors at the wound site and delayed wound healing.

ethnicity, and ABO blood group.[10] It remains unclear whether these fluctuations in VWF levels may influence wound healing. In addition, further studies will be needed to determine whether wound healing may be abnormal in patients with VWD, and whether any such pathology may vary across different VWD subtypes. Previous studies have demonstrated that normal vascular development requires regulated local expression of VEGF-A. Consequently, Ishihara et al hypothesize that decreased VWF levels, or indeed reduced ability of VWF to bind and regulate angiogenic growth factor release at sites of blood vessel formation, may contribute to the molecular pathogenesis underpinning angiodysplasia in patients with VWD. Interestingly, type 2B VWD mutations affecting R1341 within the HBD of the VWF A1 domain significantly attenuated VEGF-A interaction. This finding raises the intriguing possibility that other VWD mutations may also result in altered affinity for specific growth factors. Finally, Ishihara et al have used the high-affinity VWF–growth factor interaction in order to develop a novel proangiogenic therapeutic in which a fibrin matrix was functionalized with VWF HBD complexed with VEGF-A and PDGF-BB, respectively. Inclusion of the VWF HBD was shown to lead to slower VEGF-A and PDGF-BB release from the fibrin matrix and thus improved wound healing in both $VWF^{-/-}$ mice and type 2 diabetes mice.

All together, these findings reveal further intriguing insights into the complicated relationship that exists between VWF and angiogenesis and define an entirely novel role for VWF in regulating wound healing. Further studies in this setting will undoubtedly provide better understanding of the importance of altered wound healing in the pathophysiology underlying VWD. Given that the clinical management of angiodysplasia-related bleeding in patients with VWD continues to present major clinical challenges, elucidating the pathogenic importance of VWF-regulated growth factor release in this context may also offer novel therapeutic opportunities.

Conflict-of-interest disclosure: The authors declare no competing financial interests. ■

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DOI 10.1182/blood.2019001175

CLINICAL TRIALS AND OBSERVATIONS

Comment on Winkler et al, page 2575

Eltrombopag: wielding a double-edged sword?

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In this issue of *Blood*, Winkler et al[1] report that extending eltrombopag therapy to at least 24 weeks can improve hematologic responses in patients with refractory severe aplastic anemia (rSAA), a lymphocyte-mediated bone marrow failure syndrome. Genetic analysis of patients from this and the initial rSAA cohorts[2,3] warn of cytogenetic evolution in 18% of eltrombopag-treated rSAA patients.

Until the recent approval of eltrombopag, "grave" was an apt description of the prognosis of SAA patients refractory to standard immunosuppressive therapy (IST) with antithymocyte globulin and cyclosporine A. Salvage therapies for patients ineligible for bone marrow transplant were limited to modest efficacy options, such as androgens and alternative immunosuppressants.[4] In 2012, a breakthrough phase 1/2 study demonstrated that a 12- to 16-week treatment with a small molecule thrombopoietin mimetic eltrombopag could produce hematologic responses in ~40% of rSAA patients, including several bi- and trilineage hematologic responses.[2,3] The mechanism of multilineage responses to eltrombopag is believed to be mediated by thrombopoietin receptor c-MPL signaling in the remaining hematopoietic stem and progenitor cells (HSPCs) (see figure). The surprising efficacy of eltrombopag despite the already elevated endogenous thrombopoietin levels in SAA patients was explained by the recent discovery of steric inhibition of endogenous thrombopoietin signaling by interferon-γ, which is bypassed by eltrombopag.[5]

Extended follow-up of patients in the initial National Institutes of Health cohort,[2] as well as the emerging real-world experience with eltrombopag in Europe,[6] suggested that longer treatment with eltrombopag may improve response rates in rSAA and could rescue patients who would have been deemed refractory after 3 or 4 months of therapy. To evaluate the effectiveness of extended therapy, Winkler et al treated 40 rSAA patients for 24 weeks with a 150-mg daily dose of eltrombopag. The authors found that extending therapy from 12 to 24 weeks improved hematologic responses from 40% to 50% and nearly doubled the rate of multilineage responses, including higher neutrophil counts (see figure). Many responding patients continued eltrombopag beyond 24 weeks, and, remarkably, 9 of the 20 responders (>20% of the study cohort) eventually met the complete response criteria.

Despite these successes, concerns remain about the long-term health of HSPCs recovered with eltrombopag in patients with rSAA. The initial National Institutes of Health cohort had an alarmingly high rate of early cytogenetic evolution,[2,3] also confirmed by this study. Nearly 1 in 5 patients (18%) developed chromosomal abnormalities or overt myelodysplastic syndrome/acute myeloid leukemia (MDS/AML) transformation within the 24-week treatment period. Most of the cytogenetic changes appeared within the first 12 weeks of eltrombopag therapy, and nearly one-half involved a complete or a partial loss of chromosome 7. The 18% rate of cytogenetic evolution over a 6-month study period exceeds the expected rate of chromosomal abnormalities in SAA. Historical studies found cytogenetic abnormalities in approximately 10% of aplastic anemia patients, ranging from 3% to 26%.[7] The high rate of cytogenetic abnormalities, particularly those involving chromosome 7, in close temporal association with eltrombopag treatment, suggests a causative link between thrombopoietin receptor signaling and cytogenetic evolution. Possible underlying mechanisms include an eltrombopag-mediated selection of preexisting cytogenetically aberrant cells or an increase in genetic instability by stimulating HSPCs beyond the limits of replicative senescence. Alternatively, some rSAA patients may have an occult inherited bone marrow failure syndrome, rendering them both refractory to immunosuppression and potentially more likely to develop genetic instability.

In contrast to cytogenetic abnormalities, the prevalence of somatic mutations was unchanged during the 6-month eltrombopag therapy, and no significant clonal expansion of pathogenic variants was seen within the cohort overall. Interestingly, nearly one-half of the patients did have the emergence of new or the disappearance of previously detected variants, indicative of a dynamic hematopoietic environment. Importantly, aside from abnormalities of chromosome 7, the clinical significance of other changes is unclear. Most patients had no immediate clinical sequela and no morphologic findings of myelodysplasia at the 24-week primary end point.

The crucial unanswered question, beyond the time frame of the present study, concerns the impact of eltrombopag on