aggressive subset of lymphoma, with long-term cure rates of ~35%.[5] In patients with relapsed/refractory PTCL, few treatment options are available. Response rates with US Food and Drug Administration–approved therapies in PTCL are low. The HDACi's romidepsin and belinostat have ORRs of 25% and 29%, respectively, whereas the folate antagonist pralatrexate has an ORR of 29%.[6-9] The only exception is anaplastic large cell lymphoma, which universally expresses CD30, which is an exquisite target for the CD30 drug–antibody conjugate brentuximab vedotin. In the relapsed refractory setting, ORR with brentuximab vedotin approaches 80%.[10] In CTCL, while the course of the disease is often more indolent, once the pace of the disease accelerates, remissions are short, there is high morbidity, and the disease can transform to a more aggressive and eventually fatal subtype. Novel therapeutics are critically needed in relapsed/refractory TCLs. To date, PI3K inhibitors such as the PI3K-δ inhibitor idelalisib have not demonstrated clinical success in TCLs and have limited efficacy in CLL and iNHL. Although the number of TCL patients treated with duvelisib in these studies is relatively low, these results suggest a new therapeutic approach for patients with these rare diseases. This is particularly important given recent investigations that suggest that PD-1 blockade may paradoxically lead to progression in TCLs.[11,12] Prospective studies with central review of clinical responses in larger populations of patients are needed to conclusively evaluate the role of duvelisib in TCLs and other lymphoma subtypes.

These 2 studies demonstrate that dual inhibition of both PI3K-δ and PI3K-γ induces clinically meaningful antitumor responses, even in relapsed/refractory hematologic malignancies. Furthermore, these effects are seen in multiple diseases, including TCLs. Phase 2 clinical trials are underway with duvelisib in CLL and iNHL, with one currently planned in TCLs. These results will ultimately demonstrate whether dual inhibition of this critical pathway can impact clinical outcomes.

Conflict-of-interest disclosure: The author declares no competing financial interests. ■

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RED CELLS, IRON, AND ERYTHROPOIESIS

Comment on Aschemeyer et al, page 899

How does hepcidin hinder ferroportin activity?

De-Liang Zhang and Tracey A. Rouault | Eunice Kennedy Shriver National Institute of Child Health and Human Development

In this issue of *Blood*, Aschemeyer et al found that hepcidin bound to the central cavity of ferroportin (Fpn) and occluded its iron export activity, thereby revealing a previously unrecognized mechanism for Fpn regulation.[1]

Fpn, the sole iron exporter known in vertebrates to date, is highly expressed in intestinal epithelial cells, reticuloendothelial macrophages, and hepatocytes to regulate iron absorption, recycling, and storage. Hepcidin, a small peptide hormone secreted by hepatocytes, regulates the abundance of Fpn in response to iron overload, inflammation, and erythropoiesis to optimize systemic iron homeostasis.[2] In the circulation, hepcidin binds to Fpn on the plasma membrane to induce its ubiquitination, internalization, and degradation, which reduces iron influx to the blood. Gene mutations that either disrupt hepcidin expression or change Fpn activity cause hereditary iron disorders, emphasizing the essential role of the hepcidin-Fpn axis in systemic iron hemostasis. Fpn mutations are a common cause of hemochromatosis.[3] Gain-of-function mutations that impair Fpn degradation cause high intestinal iron uptake, iron overload in hepatocytes and other tissues, high transferrin saturations, and iron deficiency in macrophages.[3] Conversely, loss-of-function mutations that reduce Fpn activity result in iron overload in reticuloendothelial macrophages, hyperferritinemia, and low to normal transferrin saturations.

Aschemeyer et al analyzed gain-of-function Fpn mutations in stably transfected cell lines. They found that these mutations either impaired hepcidin binding to Fpn or interfered with Fpn ubiquitination and degradation following hepcidin treatment.

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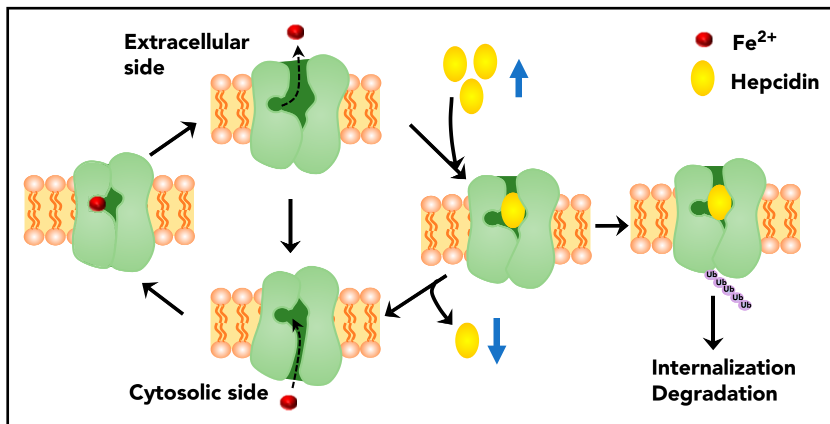

Hypothetical model showing how hepcidin regulates the activity of the iron exporter ferroportin.[4] Intracellular Fe^{2+} ions approach and bind to a site buried in the N-lobe of Fpn in its inward-facing state, which converts the Fpn conformation to an outward-facing state that permits export of iron. Apo-Fpn then reverts to the inward-facing state to transport another intracellular iron ion. When hepcidin levels are high, hepcidin binds and occludes the central cavity, which prevents the conformational transition and iron export. When hepcidin levels decrease under iron deficiency, hepcidin does not occupy the central cavity, which enables Fpn to resume iron export. Hepcidin binding also triggers a conformational change that exposes several ubiquitination sites, and the ubiquitination initiates the internalization and degradation of Fpn.

They mapped these mutations onto computational models of human Fpn structure and discovered that 1 class of mutations hindering the hepcidin binding was located in the central cavity, whereas the class of mutations impairing the ubiquitination was positioned at the helix-helix interfaces of the multitransmembrane transporter, which likely interfered with the conformational changes proposed to occur following binding to hepcidin.[4] Hepcidin binding to the central cavity would likely interfere with the conformational transition of Fpn from an outward- to an inward-facing conformation that permits cytosolic Fe^{2+} ion access to an internal binding site buried in the N-lobe near the central transport cavity, which is essential for the iron export process to initiate.[4] Accordingly, researchers speculated that hepcidin binding to the central cavity would occlude the iron export activity of Fpn.[4] To distinguish between the contributions of the well-known Fpn degradation pathway and the effects of occlusion alone to the regulation of Fpn activity, Aschemeyer et al used K8R Fpn, an Fpn mutant known to be resistant to hepcidin-induced degradation; they also used *Xenopus oocytes*, which likely do not internalize Fpn. Their experiments demonstrated that hepcidin inhibited the iron export activity of Fpn, thereby demonstrating that hepcidin-mediated occlusion plays an important regulatory role.

Recently, our laboratory found that Fpn was highly abundant in mature red blood cells (RBCs).[5] RBCs lack the ubiquitination and proteasomal degradation machinery needed for Fpn degradation and thus represent an ideal model in which the occlusive effect of hepcidin could be assessed on endogenous Fpn. Using mature human RBCs, Aschemeyer et al showed that hepcidin incubation for 24 hours reduced the non–transferrin-bound iron in the medium in a dose-dependent manner, confirming that hepcidin inhibited the iron export activity of endogenous Fpn. This result was consistent with our observation that hepcidin incubation for 1 hour inhibited the export of iron-55 from preloaded RBCs by 30%.[5] Aschemeyer et al also showed that minihepcidins, which are more stable and potent than hepcidin, dramatically reduced serum iron levels of mice without changing Fpn protein levels in the spleen and duodenum up to 36 hours after the minihepcidin infusion. Because hepcidin is known to reduce Fpn expression in vivo,[6-8] minihepcidins apparently do not promote Fpn degradation efficiently, an observation that will likely inspire further studies of the hepcidin degradation pathway. Collectively, these observations confirmed the hypothesis that binding of hepcidin to the central cavity of Fpn inhibits its iron export activity (see figure).

These findings have revealed a novel mode of hepcidin in the regulation of Fpn activity that will likely have a significant influence on the understanding of Fpn regulation in normal physiology and pathophysiology. The findings indicate that hepcidin or its mimetics represent promising approaches to treat nonclassical Fpn diseases because they can directly inhibit the iron export activity of Fpn. Fpn is ubiquitously expressed, although expression levels are relatively low in multiple cells, including cardiomyocytes, renal proximal tubules, neurons, pancreatic β cells, endothelial cells, and bronchial epithelial cells. Although the response of Fpn levels to hepcidin in these cells may not be as sensitive as in splenic macrophages, hepcidin binding occludes the iron export activity and may have important and undefined roles in physiology.

These findings also raise some interesting questions. What are the relative contributions of the hepcidin-occlusion effect vs the hepcidin-induced degradation effect on blood iron status in vivo? Aschemeyer et al attempted to assess the significance of the occlusion effect by using minihepcidin infusions, which did not cause the same magnitude of effect as hepcidin in the spleen. The estimated affinity of the hepcidin-Fpn interaction is relatively low (500 nM),[9] perhaps because the binding between hepcidin and Fpn regulates a highly dynamic process that must be versatile and responsive. Also, once hepcidin has bound, how does it initiate the proposed conformational change, and what causes hepcidin to dissociate to unblock the Fpn export pathway?

In summary, these findings have revealed a new steric mechanism for how hepcidin regulates Fpn activity, which may have significant implications for understanding the regulation of systemic iron homeostasis.

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

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THROMBOSIS AND HEMOSTASIS

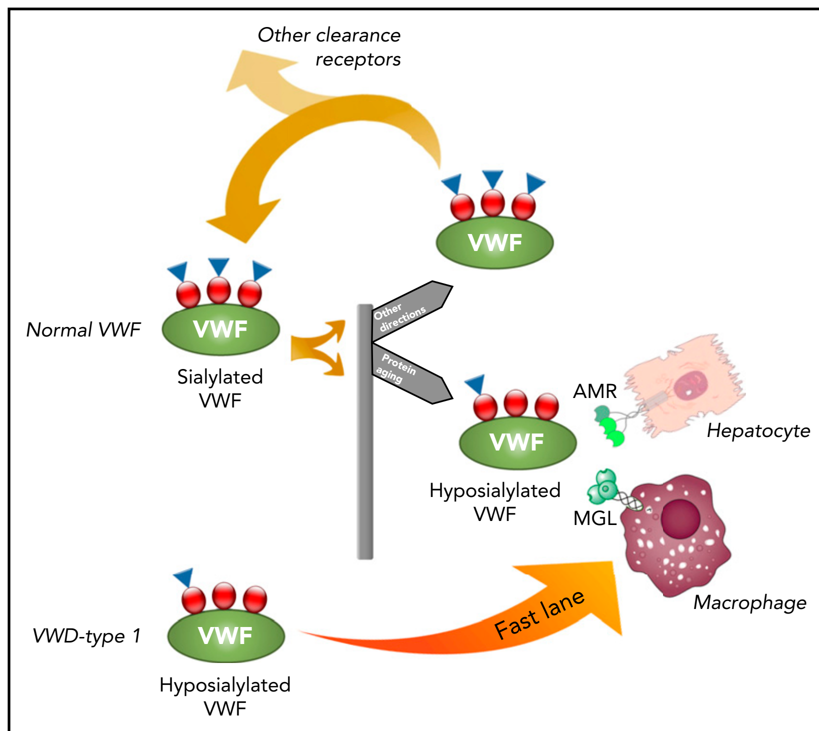
Comment on Ward et al, page 911

VWF clearance: it's glycomplicated

Cécile V. Denis and Peter J. Lenting | INSERM U1176

In this issue of *Blood*, Ward et al demonstrate that a loss of sialic acids turns von Willebrand factor (VWF) into a ligand for the scavenger-receptor macrophage galactose-type lectin (MGL).[1]

VWF is a protein that is mostly known for its involvement in the hemostatic pathway, and its deficiency is associated with severe bleeding complications in a disorder known as von Willebrand disease (VWD). In the early 2000s, it was found that abnormal clearance of VWF contributes to the pathogenesis of VWD. Together with the notion that VWF regulates clearance of coagulation factor VIII (FVIII; which is highly relevant for treatment of hemophilia A), studies on VWF clearance have therefore drawn increasing attention.[2] In particular, the role of VWF glycosylation has been of particular interest for 2 reasons. First, we know that ABO-blood group glycan structures are a major determinant of VWF plasma levels. Second, in the 1970s, it had already been found that the presence of sialic acids was pertinent to the in vivo survival of VWF, a finding that has since been confirmed in several other studies.[3-5]



The role of MGL in the clearance of VWF. VWF contains glycan structures (red dots) that are capped by sialic acids (blue triangles). Circulating VWF may lose some of the terminal sialic acids over time and becomes hyposialylated. Hyposialylated VWF is a target for the AMR on hepatocytes. However, in this issue, Ward and colleagues now show that MGL is also an active receptor for hyposialylated VWF, mediating rapid macrophage-mediated clearance. Interestingly, van Schooten et al[5] reported previously that type 1 VWD is often associated with reduced sialylation of *O*-linked glycans and concomitant increased clearance (fast lane). It is therefore tempting to speculate that MGL also contributes to the increased clearance of such hyposialylated VWF in type 1 VWD.

Loss of sialic acids generates hyposialylated VWF, in which exposed galactose residues can be recognized by several lectin-like receptors, with the Ashwell-Morell receptor (AMR) being their quintessential representative. The active participation of the AMR in eliminating hyposialylated VWF has been shown in an elegant study by Grewal et al.[6] However, in the present study, Ward et al noticed that deficiency of the AMR was insufficient to correct increased clearance of hyposialylated VWF, irrespective of whether all sialic acids were removed or only those on *O*-linked glycans. This led them to conclude that besides the AMR, other lectin receptors should also contribute to the removal of hyposialylated VWF. In their search for alternative receptors, Ward et al found that chemical depletion of macrophages markedly enhanced survival of hyposialylated VWF, pointing to a macrophage-specific receptor. They further focused on a lectin-like receptor named MGL, which is expressed on macrophages and dendritic cells. Several lines of evidence confirmed that MGL plays an active role in removing hyposialylated VWF from the circulation.[1] For instance, the presence of anti-MGL antibodies impairs clearance of sialidase-treated VWF in AMR-deficient mice. Furthermore, basal VWF levels are significantly increased in mice lacking the murine ortholog of human MGL (*Mgl1*$^{-/-}$ mice). Finally, clearance of VWF was delayed in *Mgl1*$^{-/-}$ mice.[1]

Having established MGL as a novel clearance receptor for VWF, the next step would be to define its relative contribution in relation to the spectrum of other VWF receptors previously described. Ward et al first refer to the recent description of an intrinsic pathway of protein aging and

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