analyzed RNA-sequencing data on cells expressing wild-type, hotspot, or rare mutant versions of SRSF2 and U2AF1. As the rare mutations were sometimes only observed in single MDS/AML patients, in addition to profiling primary patient material, they also generated human K562 cell lines that expressed wild-type, hotspot, or rare mutant versions of the factors.

Comparison of SRSF2 mutants unveiled remarkable overlap with most rare and hotspot mutations clustering together in their splicing pattern. Moreover, rare SRSF2 mutants alter exonic enhancer specificity, as was shown for hotspot mutations both in vivo and using biochemistry,[7,8] suggesting similar splicing mechanisms. In contrast, comparison of U2AF1 containing rare and hotspot mutations showed more divergence. Some rare U2AF1 mutants alter 3′ splice-site sequence preference, as was shown for hotspot mutations,[6] whereas others did not.

In many ways, the results that hotspot and rare mutations are similar are not surprising. The patients who were sequenced were selected by disease state. Thus, this in effect represents a genetic screen performed in humans by nature, with selective pressure for a highly specific phenotype. The results are reminiscent of the outcomes from many decades of yeast genetic screens for phenotypic splicing outcomes performed in the laboratory. It would be expected that mutations in splicing factors found in patients, no matter their location within those factors, would have similar outcomes on splicing, if splicing has anything to do with the disease phenotype. However, this has not been entirely clear. Indeed, the results from Pangallo et al provide a more compelling argument that it does for some splicing factors.

Although the outcomes for all of the SRSF2 mutants cluster together and support the model described herein, the more heterogeneous patterns observed with the U2AF1 mutants indicate a more complex situation. There also are "silent" mutations in both factors that do not significantly change splicing at all. It is unclear whether the clinical features of the patients with "silent" mutations are similar to those that phenocopy. If similar, these "silent" mutations could support the involvement of a nonsplicing function in disease etiology, which has been proposed by other groups.[9,10]

The results also raise the question of why mutations in hotspots are more frequent than those in rare positions. The answer, although not known, must be genomic context, likely the local DNA sequence and chromatin environment, both of which may influence mutation and DNA repair rates.

There are many spliceosome-associated factors whose function in splicing is still murky. As the current results demonstrate that messenger RNA changes from factor-specific mutations are quite canonical, could splicing pattern analysis in MDS/AML be used to identify factors with similar functionality as the major disease-associated splicing factors?

Overall, the work from Pangallo et al suggests that nonhotspot mutations should be considered similarly to common mutations, both in their mechanism and potential treatment.

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

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**RED CELLS, IRON, AND ERYTHROPOIESIS**

Heme A1M'ed at the kidney in sickle cell disease

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In this issue of *Blood*, Ofori-Acquah et al investigate hemolysis, hemopexin deficiency, and kidney function in sickle cell disease (SCD) and report that (1) acute elevations in heme lead to kidney damage in hemopexin-deficient states, and (2) a compensatory rise in α-1 microglobulin (A1M) relative to hemopexin concentration is associated with acute kidney injury.[1]

Acute kidney injury causes capillary loss, dysregulated apoptosis, and sustained proinflammatory and profibrotic signaling in animal models and leads to the subsequent development and progression of chronic kidney disease in the general population.[2] Acute kidney injury is observed in 5% to 17% of hospitalizations for vasoocclusive episodes in patients with SCD[3,4] and is associated with a 4.6-fold greater risk for chronic kidney disease progression.[4] The mechanisms for kidney injury are not well understood, and targeted therapies to prevent and ameliorate
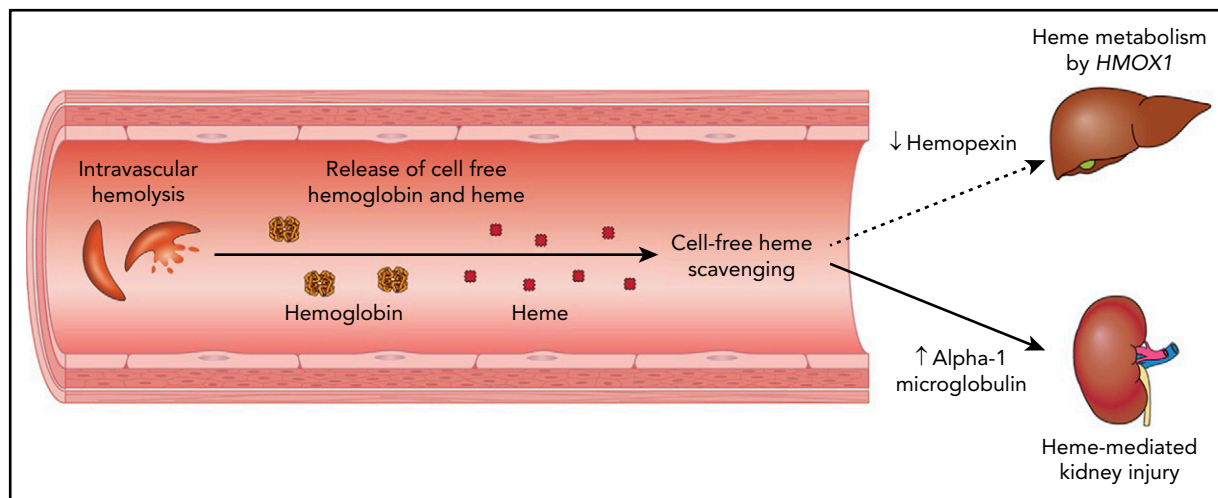
Acquired hemopexin deficiency, with a compensatory increase in A1M, leads to heme being transported to the kidney and causing tubular injury in SCD.

the damage are urgently needed. The findings by Ofori-Acquah et al highlight the central role of intravascular hemolysis and heme processing pathways in SCD-related kidney injury.

Cell-free hemoglobin is released during intravascular hemolysis and, if not efficiently scavenged by haptoglobin, undergoes autooxidation to ferric hemoglobin with release of free heme to the plasma. Hemopexin plays an essential role in sequestering plasma-free heme, thereby preventing direct heme-mediated oxidative damage or augmentation of inflammatory and immune response pathways, such as those mediated by toll-like receptor 4. Hemopexin delivers heme to hepatocytes through the lipoprotein receptor-related protein-1 receptor. Hepatocyte heme oxygenase-1 degrades heme, and the iron released in this process is recycled.[5]

Haptoglobin and hemopexin are both depleted in SCD, resulting in circulating concentrations of cell-free heme that range from 0 to 20 μM at steady state and increase up to 40 μM during a vaso-occlusive episode.[6,7] The kidneys may be continuously exposed to toxic cell-free hemoglobin and heme and damaged through direct oxidative injury, activation of inflammatory and immune response pathways, upregulation of endothelial cell adhesion molecules, and/or consumption of nitric oxide leading to vasculopathy.[5] In patients with SCD, increased markers of hemolysis are associated with iron deposition in the renal cortex as revealed by magnetic resonance imaging, glomerular dysfunction (albuminuria), and proximal tubular injury, as indicated by increased

urine levels of kidney injury molecule-1 (KIM-1).[5,8]

In this report, the investigators examined whether an acute elevation in plasma heme concentration leads to increased kidney heme processing and acute kidney injury in SCD. After an IV hemin challenge, excess heme was deposited primarily in the kidneys of SCD mice vs the liver of control mice. The SCD mice, but not the control mice, experienced significant rises in plasma creatinine, urine albumin, urine KIM-1, and histopathologic kidney tubular change, and a reduction in measured glomerular filtration after the hemin challenge. Heme-mediated kidney toxicity was further studied by transplanting hemoglobin SS bone marrow into hemopexin null and wild-type mice. The hemoglobin SS/hemopexin-null mice had the lowest measured glomerular filtration rate at baseline and the most severe reduction in the glomerular filtration rate after hemin challenge compared with hemoglobin SS/hemopexin wild-type mice or hemoglobin AA/hemopexin-null mice. Furthermore, infusion of hemopexin prior to a hemin challenge prevented significant changes in the glomerular filtration rate in the SCD mice.

This work builds upon the body of literature that heme promotes toxicity to the kidneys in hemopexin-deficient states. Increased lipid peroxidation and induction of heme oxygenase-1 have been observed in the kidneys of hemopexin knockout vs wild-type mice challenged with hemin.[9] Increased iron deposition, tubular cell damage, and lipid peroxidation

have been observed in the kidneys of hemopexin knockout vs wild-type mice after inducing intravascular hemolysis with phenylhydrazine.[10]

A new mechanism for how heme may be shuttled to the kidney and impair kidney function under hemolytic conditions is presented in this report (see figure). A1M is a 26-kDa protein that scavenges heme in the circulation and passes through the glomerular filtration barrier. In the current study, the authors demonstrate that A1M is increased 1.6-fold in SCD patients at steady state compared with healthy controls. In addition, a higher molar ratio of A1M:hemopexin correlates with elevated markers of hemolytic anemia and with increased levels of 2 tubular cell injury biomarkers, urine KIM-1 and urine neutrophil gelatinase-associated lipocalin, in SCD patients. Parallel with what was observed in SCD patients, a sevenfold increase in the A1M:hemopexin ratio was observed in SCD vs control mice. Consistent with transport of heme to the kidney by A1M in hemopexin-deficient states, the administration of A1M immediately prior to hemin infusion exacerbated the reduction in glomerular filtration rate by 39% in the SCD mice.

In the present study, Ofoto-Acquah et al provide compelling evidence that acute rises in cell-free heme are primarily handled by the kidneys and contribute to kidney injury in SCD. The findings from this study strengthen the observation that kidney damage is mediated by intravascular hemolysis and provide novel insight that A1M may transport toxic cell-free heme to the kidneys in

hemopexin-deficient states. Other non-SCD models of acute intravascular hemolysis, such as red blood cell transfusion or phenylhydrazine-induced hemolysis, have demonstrated that haptoglobin, the first line of defense against intravascular hemolysis, may be more effective than hemopexin in preventing acute kidney injury.[5] Future studies evaluating the protective benefits of cell-free hemoglobin vs heme scavenging, or the combination of both, may provide additional insight into the mechanisms of kidney damage in SCD and guide strategies to mitigate acute kidney injury.

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TRANSPLANTATION

Comment on Jodele et al, page 1049

Compliments to complement blockade for TA-TMA

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In this issue of *Blood*, Jodele et al report the largest ever cohort series using eculizumab, a humanized monoclonal immunoglobulin G antibody inhibitor of complement protein C5, as first-line therapy for pediatric patients with high-risk transplant-associated thrombotic microangiopathy (TA-TMA).[1] These researchers were innovators in TA-TMA research by originally defining biologically linked, biomarker-driven diagnostic criteria and using these criteria to routinely monitor for TA-TMA and to inform practice-changing treatment strategies.[2]

TA-TMA is a representative example of a toxicity syndrome linked to overactivation of complement pathways and endothelial injury resulting in thrombosis, hemolysis, and often multiorgan dysfunction syndrome that has historically been associated with considerable morbidity, high rates of health care use, and mortality after allogeneic hematopoietic cell transplantation (allo-HCT).[2,3] TA-TMA has been difficult to reliably diagnose and treat in clinical practice, given an initially limited understanding of its pathobiology; variable, nonspecific, and inconsistently adopted diagnostic criteria; and for quite some time, ineffective therapies.[4]

By using their previously defined criteria, the authors identified 177 (31%) of 566 patients who developed TA-TMA after allo-HCT between 2012 and 2018 at their center. Of these patients, 64 (36%) were deemed to have high-risk TA-TMA which, on the basis of their previous work, would be expected to result in a dismal 1-year overall survival of <20%.[2] With an innovative pharmacokinetically (PK) and pharmacodynamically (PD)–targeted therapeutic strategy, 41 patients (64%) with high-risk TA-TMA responded to eculizumab, with the majority (56%) achieving complete remission. Patients with the highest levels of complement activation, as measured by elevated serum sC5b-9, at TA-TMA diagnosis and at the start of therapy were less likely to respond to and required longer courses of eculizumab. Even in eculizumab responders, the authors detail the substantially increased toxicities faced by these patients, including fluid overload with serositis, gastrointestinal bleeding, central nervous system symptoms, acute kidney injury requiring renal replacement therapy, and the need for frequent anti-hypertensives and blood product transfusions. About one-third of treated patients developed a bacterial bloodstream infection, a rate comparable to that of patients with untreated TA-TMA. Although fungal infections were seen in 6 patients, most had concurrent steroid-refractory graft-versus-host disease (GVHD), which required additional immunosuppression, suggesting that eculizumab therapy may not greatly increase the risk of serious infections. In the most remarkable result of the study, overall survival at 1 and 3 years was 66% and 53%, respectively, a stark improvement when compared with that in patients with untreated TA-TMA in the era before complement inhibition.[2]

What makes this study so compelling is the strong evidence that a rigorous prospective TA-TMA diagnostic, monitoring, and treatment program targeting high-risk patients can turn a previously nearly universally fatal condition into a more manageable one for several reasons (see figure). First, it allows for more frequent and earlier identification of TA-TMA post-HCT and more readily stratifies patients into low- and high-risk groups for further study. Second, the PK/PD-targeted approach allows for more precise, personalized dosing that is driven by both clinical and serum biomarker responses. This manner of dosing may have the