Another disorder with convincing links to ribosomal dysfunction is Treacher Collins syndrome (TCS). Patients with Treacher Collins syndrome have craniofacial abnormalities that are similar to patients with Diamond Blackfan anemia, but do not develop bone marrow failure. TCOFI, the gene mutated in many patients with Treacher Collins syndrome, encodes a protein that is essential for the transcription of ribosomal DNA and may play a role in the methylation of rRNA. Moreover, mutations have recently been reported in Treacher Collins syndrome patients in genes encoding subunits of RNA polymerase I and III.

A central unanswered question is how defects in ribosome biogenesis lead to divergent clinical phenotypes. Both Diamond Blackfan anemia and Shwachman–Diamond syndrome cause bone marrow failure, but patients with the former have a more severe defect in erythropoiesis, while the latter tend to have worse neutropenia. Patients with Treacher Collins syndrome and some with Diamond Blackfan anemia develop craniofacial abnormalities but patients with Treacher Collins syndrome have normal hematopoiesis. Developmental and tissue–specific gene expression or transcriptional requirements may cause differential sensitivities to decreased expression of particular genes involved in ribosome function, but this remains to be elucidated.

The anemia in Diamond Blackfan anemia and the 5q– syndrome, as well as the craniofacial defects in Treacher Collins syndrome, appear to be caused by activation of p53 in distinct lineages. The degree to which p53 is pathologically activated in vivo by abnormal eIF6 release in Shwachman–Diamond syndrome remains to be determined. Despite the many unanswered questions, it is increasingly clear that genetic lesions causing specific defects in ribosome biogenesis are fundamental to the pathophysiology of multiple human disorders.

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Comment on Perseu et al, page 4454

HbA2: at the borderline of the KLF

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In this issue of Blood, Perseu et al provide new insights into our understanding of the genetic basis of elevated hemoglobin A2. This is a major step forward for physicians interpreting hemoglobin electrophoreses of patients with borderline hemoglobin A2, normal or slightly reduced mean corpuscular volume (MCV), and normal mean corpuscular hemoglobin (MCH).

These patients are a diagnostic dilemma. Does the borderline HbA2 represent an outlier in the normal population? Is there an inherited pathologic determinant increasing HbA2? Detailed studies have identified genetic variants in a minority of these patients, including α-globin chain triplication, β-globin gene promoter mutations, and β- or δ-globin gene variants. Should additional diagnostic evaluation, such as family studies and β-globin locus sequencing, be performed?

Perseu and colleagues studied 145 patients with borderline HbA2, normal or slightly reduced MCV, and normal MCH. They excluded associated variants including mutations of the β-globin or δ-globin gene, the β-globin promoter, and triplicated α-globin genes. Nucleotide sequence analysis of the KLF1 gene in these patients identified mutations in 52 (36%). Variants included nonsense mutations, in/del mutations, missense mutations in conserved amino acids in zinc finger 1 or 2, and mutation of a GATA-1 binding site in the KLF1 gene promoter. Expression profiling of cultured patient erythroblasts identified a large group of genes with altered mRNA expression including BCAM, which carries the Lutheran antigens, and CD44, which carries the Indian antigens. No other phenotypic abnormalities were described.

Reports of KLF1 mutations associated with nondeletional hereditary persistence of fetal hemoglobin (HPFH) and congenital dyserythropoietic anemia soon followed. The HPFH family carried a nonsense mutation, K288X, associated with KLF1 haplosufficienty and decreased BCL11A in erythroid cells. KLF1 is a direct activator of BCL11A, which represses γ-globin gene expression.

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KLF1 deficiency releases this repression, leading to elevated HbF. CDA patients had a mutation in a conserved residue, E325K, in the second zinc finger of KLF1. Erythroid cells from affected CDA patients exhibited failure of terminal erythroid differentiation, with elevated fetal and embryonic globins, and absent CD44 and AQP1 expression.

A report from Sardinia revealed the rich complexity of gene regulation by KLF1 and its alteration by different mutations. Missense mutations led to differing alterations in KLF1 protein structure and function. Missense mutations of the second zinc finger revealed their location and the change they impose on KLF1 activity. Missense mutations exert their influence by decreased zinc protoporphyrin.

The pleiotropic effects of KLF1 mutations can be attributed in part to quantitative and qualitative effects on KLF1 structure and function. KLF1 haploinsufficiency appears to be most commonly associated with the In(Lu) phenotype and with mild elevation in HbF. Missense mutations exert their influence by their location and the change they impose on KLF1 protein structure and function. Missense mutations may alter protein-protein or protein-DNA interactions. Study of KLF1 mutants in the second zinc finger revealed different mutations led to differing alterations in KLF1-DNA binding affinity. Clinical severity did not correlate with alterations in binding affinities in all cases. Some variants may alter composition of KLF1-associated protein complexes, affecting transcriptional regulators, chromatin-associated factors, and other important regulatory proteins. Missense mutations could also alter KLF1 by impairing trafficking, altering sites of posttranslational modification, or producing a mutant protein acting as a dominant negative.

Detailed expression and genomics studies indicate that there are many KLF1 targets in erythroid cells, including regulators of cell metabolism, structural membrane proteins, heme synthesis enzymes, and proteins responding to oxidative stress. Different mutations may perturb interactions based on variability in the quality of associated KLF1-DNA binding sites and their interactions. Other potential sources of clinical variability in KLF1 mutant patients include inheritance of other genetic modifiers. In some but not all kindreds with mutant KLF1, variability in HbF has been attributed, in part, to genetic variability at the BCL11A locus. Genetic variability may reside in factors regulated by KLF1, or in factors that are dysregulated when there is altered KLF1 structure or function.

The influence of KLF1 on HbA1c has not been examined in detail. Cultured erythroid cells from patients with elevated HbA1c showed an increase in the expression of the absolute amount of δ-globin mRNA relative to β-globin mRNA as differentiation progressed, suggesting a delay in the switch from δ-globin to β-globin. KLF1 or KLF1-regulated factors such as BCL11A could influence δ-globin gene interactions with the LCR and/or with local chromatin-associated proteins. The δ-globin gene could be a target of KLF1, although its promoter CACCC box is degenerate and no other KLF1 binding motifs are nearby.

The mysteries of KLF1 are now beginning to be revealed. When evaluating a patient with HPFH, atypical CDA, borderline HbA1c, In(Lu) blood group, or elevated zinc protoporphyrin, one should consider the presence of a KLF1 mutation. And now, the astute clinician will entertain defects of KLF1 when evaluating patients with otherwise unexplained erythrocyte disorders.

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**TRANSPANTATION**

**Comment on Merindol et al, page 4480**

**Resurrecting the recalcitrant T-cell**

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Delayed immune recovery after allogeneic hematopoietic stem cell transplantation (alloHSCT) is arguably the single greatest barrier to the successful use of this treatment modality. In this issue of Blood, Merindol et al dissect the phenotypic and functional patterns of T-cell recovery in recipients of partially HLA-matched unrelated donor umbilical cord blood (UCB).

While an effective treatment for selected malignant and nonmalignant disorders, alloHSCT is associated with a profound and prolonged immunodeficiency state. As a consequence, alloHSCT is associated with a high incidence of opportunistic infection. Furthermore, data suggest that a delay in immune recovery may also contribute the risk of relapse at least in some patient populations. It is already known that the recovery of antigen specific T cells after alloHSCT is dependent on donor and host factors, like HLA match, T-cell depletion of the allograft, degree of tissue damage from the conditioning regimen, and the development of acute graft-versus-host disease (GVHD). Based on studies in murine models, immune recovery after alloHSCT is characterized by an initial wave of thymic independent,
HbA$_2$: at the borderline of the KLF

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