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Erythroleukemia: all roads lead to GATA1?

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In this issue of *Blood*, Fagnan et al investigate the genetic and transcriptional landscape of 33 acute erythroleukemia (AEL) patients and propose a transcriptome-based space that helps distinguish AEL from other myeloid leukemias.[1] They also provide intriguing evidence for leukemic transformation in AEL being a result of aberrant transcription factor expression impinging on GATA1 functions in erythropoiesis.

AEL is an aggressive, rare hematologic malignancy associated with poor prognosis and is characterized by the proliferation of erythroid and myeloid blasts in the bone marrow.[2] Classification of AEL is based on morphological criteria that can vary; this led to its reclassification by the World Health Organization (WHO) in 2016 as a myelodysplastic syndrome (MDS), or acute myeloid leukemia (AML) not otherwise specified.[3] A handful of recent studies exploring the genetic basis of AEL provided evidence for a molecular complexity of AEL that challenges present classification by WHO,[4] at the same time allowing glimpses into AEL leukemogenesis.[5] The work by Fagnan et al in this issue of *Blood* adds to these studies by describing a spectrum of genetic lesions in a cohort of 33 patients that can be classified into 3 distinct molecular subgroups characterized by alterations in (i) the TP53 gene; (ii) genes coding for the epigenetic modifiers DNMT3A or TET2, often accompanied by mutations in splicing factor genes; and (iii) other genes not associated with those identified in (i) and (ii). In line with previous work,[4,5] these findings provide further evidence as to the genetic heterogeneity of AEL and the extent of distinct, as well as overlapping, genetic lesions in relation to MDS and AML.

Follow-up transcriptomic analysis revealed a prominent erythroid transcriptional signature in the majority (20 out of 33) of the AEL samples. Interestingly, when comparing the AEL transcriptomes to erythroid or myeloid cellular expression trajectories drawn from ENCODE data, Fagnan et al found that, although all AEL samples mapped in the space between the 2 trajectories, the majority (25 out of 33) mapped closer to the erythroid trajectory. Importantly, similar patterns were obtained when transcriptomic data from a bigger AEL cohort[5] were reanalyzed in this study, thus providing further validation. Taken together, this part of the study defines a transcriptomic space that can distinguish AEL from MDS and other AML subtypes and improve their classification according to WHO 2016 criteria.

These observations create a paradox as the predominantly erythroid transcriptomes of AEL leukemic cells cannot be easily explained by the underlying genetic lesions, given also their overlap with MDS and AML. To address this, Fagnan et al interrogated further the AEL transcriptomic data and identified a number of transcription factors and cofactors known to suppress erythropoiesis (ERG, GFI1, RUNX1T1/ETO, ETO2, SPI1) and/or to act antagonistically to GATA1 (SKI), that were expressed at abnormally high levels, in small subsets of (1 or 2) AEL patients, which, together, account for nearly one-third of the patients. These findings suggest that AEL often presents with genetic or transcriptional alterations in factors that affect erythropoiesis, potentially by affecting GATA1 activity. In an elegant set of experiments, Fagnan et al provide additional evidence to support these observations. First, they showed that ectopic expression of these transcription factors in murine erythroid progenitors led to their immortalization at an immature erythroid stage. Injection of such immortalized erythroblasts in lethally irradiated mice led to an AEL-like lethal disease. Importantly, proerythroblast immortalization by these factors was accompanied by changes in chromatin consistent with reduced or absent GATA1 binding to its normal erythroid target sites. Furthermore, ectopic expression of these factors in GATA1-null murine proerythroblasts blocked erythroid maturation driven by restoring GATA1 expression, providing further evidence for AEL leukemogenesis interfering with GATA1 functions in erythropoiesis.

These observations are important, as they suggest a mechanism for acute erythroid leukemogenesis that directly impinges on GATA1 erythroid functions. There is precedent for reduced GATA1 levels leading to erythroleukemia in mice[6]; however, the findings of Fagnan et al in murine erythroblasts need to be confirmed directly in patients' samples, at both the epigenetic (ie, GATA1 occupancies in chromatin) and biochemical (ie, GATA1 interactions with SKI, ETO2 etc) levels. An interesting case in point is the TP53 gene, which is frequently mutated in AEL. GATA1 and p53 have been reported to interact directly in murine erythroid cells, inhibiting each other's transactivating activities.[7] It is presently unclear how TP53 mutations may have an impact on GATA1 functions, or how GATA1short (GATA1s) mutations may affect the repression of p53 functions in erythroid cells. It is also interesting to note that the present study identified a patient with a TET2 loss-of-function mutation and a GATA1 mutation predicted to give rise to the GATA1s isoform that cannot support erythropoiesis.[8] This is reminiscent of GATA1s mutations associated with transient myeloproliferative disorder in Down syndrome, progressing to acute megakaryoblastic leukemia as a result of the acquisition of secondary mutations.[9] It would also be of interest to investigate whether and how GATA1 functions are impaired in AEL caused by alterations in genes that are not readily linked to GATA1.

Last, in another set of informative experiments, Fagnan et al showed that the hematopoietic stage at which an oncogenic transcription factor is aberrantly expressed is important for the development of disease. Specifically, the aberrant expression of SKI in hematopoietic stem cells or in megakaryocytic-erythroid progenitors, but not in granulocyte-macrophage progenitors, resulted in AEL-like disease in transplanted lethally irradiated recipients.

Taken together, the present study (i) identified a transcriptional space for refining AEL, MDS, and AML classification; (ii) showed that the aberrant expression of oncogenic driver transcription factors result in erythroleukemia by downregulating

the GATA1-regulated erythroid epigenome; and (iii) showed in vivo that the erythroid and/or myeloid outcomes of disease depend on the driving oncogene and the hematopoietic target cell in which it is aberrantly expressed. In the longer term, it will be of interest to establish whether a direct impact on GATA1 functions is a unifying underlying feature in all AELs.

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RED CELLS, IRON, AND ERYTHROPOIESIS

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Iron turns to wild when the transferrin is away

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In this issue of *Blood*, Yu et al report that hepatic transferrin (Trf) has a pivotal role in the regulation of systematic iron metabolism, erythropoiesis, and in the protection of liver from iron overload-evoked ferroptosis, fibrosis, and cirrhosis.[1] Based on their novel findings, the authors propose a potential therapeutic strategy for mitigating a spectrum of iron overload disorders by targeting iron-evoked ferroptosis and/or the divalent metal transporter Slc39a14.

It is well known that the trace element iron is required for the normal function of many tissues and that abnormalities of iron metabolism contribute to disease in humans, but the underlying mechanisms remain incompletely understood. Trf, encoded by the *Trf* gene, is a blood plasma glycoprotein that controls free iron (Fe) levels by binding to ferric ion and delivering it to a variety of tissues, including the liver, bone marrow, and spleen. Trf was first identified in 1947.[2] Since then, 2 of the enduring enigmas in the field of iron biology are Which tissues produce Trf? and What is the role of tissue-specific Trf? Historically, much of our knowledge of Trf was derived from studies of atransferrinemia, a rare autosomal recessive metabolic disorder that reduces Trf to undetectable levels and causes severe anemia, liver iron overload, and fibrosis.[3] More recently, much has been learned from the hypotransferrinemic (*Hpx*) murine model of atransferrinemia, attained by a point mutation in the *Trf* gene yielding less than 1% of the normal Trf expression. *Hpx* mice cannot survive unless exogenous Trf is supplemented during the first 2 weeks after birth.[3,4] *Trf* mutations have been shown to attenuate iron binding at either the N lobe and/or C lobe of Trf in mice. Affected mutants display hepatocellular iron overload with decreased hepcidin expression.[5] However, the liver-specific role of Trf has not been described in detail.

To gain insight into the role of hepatic Trf in iron metabolism, Yu et al generated and characterized the hepatocyte-specific *Trf* knockout mice, *Trf*-LKO. The *Trf*-LKO mutants developed hypoferric anemia as a result of iron-restricted erythropoiesis, which was associated with dampened erythropoietin responsiveness, elevated erythroferrone expression, and dysregulation of iron metabolism. There was a marked reduction of Trf-bound iron (TBI) in serum and an overload of non–Trf-binding iron (NTBI) in various tissues, particularly the liver. The hepatic pathology worsened with elevated dietary iron absorption and subsequent tissue iron accumulation as the consequence of hepcidin suppression by the erythropoietin-dependent induction of erythroferrone (see figure). In contrast to the well-studied *Hpx* mice, this liver-specific *Trf* knockout mouse model is genetically and phenotypically distinct. Notably, the *Trf*-LKO mouse model allows for the study of Trf in a tissue-specific manner, in particular its intra- vs extra-hepatic functions. The findings from this study indicate that hepatic Trf is indispensable for the maintenance of iron homeostasis and hematopoiesis and that extra-hepatic Trf can partially compensate for the loss of hepatic Trf. Conceivably, further exploration of the extra-hepatic Trf function using these genetically modified animals would be of great interest.

Insights from this study greatly inform our understanding of the role of Trf in the pathology of iron overload-evoked hepatic injury, and other iron-related organ disorders. The liver, as the primary Trf-producing organ, is crucial to iron metabolism and homeostasis. However, whether hepatic Trf has a relationship with liver fibrosis was formerly unknown. Using multiple linear regression analysis, Yu et al found that patients with liver fibrosis had significantly reduced serum Trf, which was inversely correlated with the level of hepatic fibrosis markers.