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RED CELLS, IRON, AND ERYTHROPOIESIS

Comment on Ling et al, page 1619

The long and the short of it

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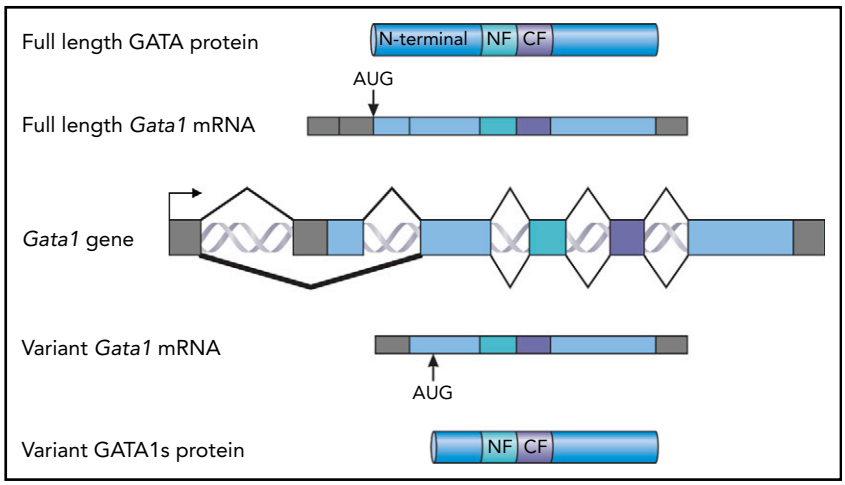
In this issue of *Blood*, Ling et al have compared the function of 2 forms of the transcription factor GATA1 (the full-length protein and a shorter form). Their study shows that the N-terminal domain of GATA1 is critical to normal red cell differentiation (erythropoiesis).[1]

For those who study erythropoiesis, the foundational role of GATA1 is well known.[2] Originally, the GATA1 protein was identified solely by its ability to bind DNA. After the gene encoding the protein was cloned, it was quickly shown that deletion of the *Gata1* gene in transgenic mice caused a lethal anemia, resulting in death at the fetal liver stage.[3] GATA1 is a member of a family of zinc finger proteins that share a similar structure. The C-terminal zinc finger is primarily responsible for the binding of GATA1 to the sequence WGATAR. The N-terminal finger stabilizes the binding to DNA and also facilitates the interaction with FOG1, which is 1 of several proteins that interact with GATA1. The N-terminal domain of GATA1 interacts with other proteins, including RUNX1 and RB[4] (see figure).

Mutations in GATA1 are associated with many hematologic diseases.[5] In particular, mutations in the second exon lead to an alternative splicing event or altered translation that essentially deletes the N-terminal domain of GATA1, which results in a shorter protein, GATA1 short (GATA1s). Germline mutations in exon 2 result in a form of Diamond-Blackfan anemia,[6] and somatic mutations are seen in Down syndrome acute megakaryocytic leukemia.[5]



Full-length forms and short forms of the GATA1 protein. A map of the Gata1 locus is shown in the center (*Gata1* gene). The exons are shown as blocks. Gray indicates noncoding sequence; blue indicates coding sequence. Above the gene, a spliced, full-length Gata1 messenger RNA (mRNA) is shown with the AUG initiation codon indicated. This RNA is translated into full-length GATA1 protein (top). The N-terminal domain, the N-terminal zinc finger (NF), and the C-terminal zinc finger (CF) are indicated. Below the gene, a spliced variant Gata1 mRNA encoding the variant GATA1s protein is shown, with the alternative GATA1s AUG initiation codon indicated. This RNA is translated as the GATA1s protein. The N-terminal zinc finger and the C-terminal zinc finger are indicated.

These observations lead to the question: What is the functional role of GATA1s? To address this question, Ling et al used a transgenic mouse that was engineered to exclusively express GATA1s.[7,8] These mice show an inhibition of erythropoiesis and a transient expansion of megakaryocyte progenitors at the fetal liver stage of development, similar to what is seen in Down syndrome patients. Ling et al performed multi-omics profiling of GATA1s and wild-type fetal liver cells. They found that although the transcriptional profiles, the GATA1 occupancy, histone modification, and chromatin accessibility profiles were largely overlapping between the GATA1s and wild-type fetal liver cells, the loss of the N terminus of GATA1 specifically altered the expression of a subset of genes, which was accompanied by changes in GATA1s occupancy and/or chromatin modifications. These data clearly demonstrate a specific role for the N- terminal domain of GATA1 in normal erythropoiesis. Ling et al provide a number of targets and pathways that will be important to investigate in the future.

In normal hematopoiesis, GATA2 occupies WGATAR sites in the genome in primitive cells before being replaced by GATA1 as cells differentiate down the erythroid lineage.[2] GATA1s mice expressed higher levels of *Gata2* and *Runx1* messenger RNA, leading to the hypothesis that the lingering expression of GATA2 was inhibiting erythropoiesis by preventing the binding of GATA1s. To test this, Ling et al crossed their *Gata1s* mice to mice carrying a *Gata2* knockout allele. They found that erythropoiesis was rescued in *Gata1s* mice carrying a *Gata2* null allele, demonstrating that Gata1-mutant embryos can be rescued by haploinsufficiency for *GATA2*.

The Ling et al study is an example of both hypothesis-testing (role of the

GATA1 N-terminal domain) and hypothesis-generating (identification of GATA1 N-terminal domain regulated pathways) results. It will be important to determine the features of the N-terminal domain of GATA1 that are required for normal erythropoiesis. Identifying the proteins that interact with the N-terminal domain of GATA1 will be critical to our understanding of how GATA1 mutations that result in the deletion of the N-terminal domain cause hematologic diseases. Ling et al have made an impressive start in answering this question.

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

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THROMBOSIS AND HEMOSTASIS

Comment on Aymonnier et al, page 1632, and de Maat et al, page 1658

Serpin targets in hemostasis/kinin formation

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In this issue of *Blood*, the papers of Aymonnier et al[1] on the use of protease nexin 1 and de Maat et al[2] on the use of altered α-1-antitrypsin propose therapeutic uses of these serpins for the management of coagulation and contact system disorders, respectively. Serpins are a family of serine protease inhibitors that regulate proteases of plasma, mostly enzymes of blood coagulation, complement, and inflammatory systems.

Interest in serpins as possible therapeutic tools began in 1978, when Lewis et al published a paper about a 10-year-old boy who had an unknown life-long hemorrhagic disorder.[3] After his death, an abnormal plasma α-1-antitrypsin (α-1-antitrypsin Pittsburgh [α_1AT-Pitt]) was characterized that has a reactive center Met358Arg polymorphism, converting this elastase inhibitor to a thrombin inhibitor (see figure panel A).[4] α_1AT-Pitt is a tragic event of nature, but therapeutic serpin modification, this commentary's focus, as in the papers of Aymonnier et al and de Maat et al, has clinical importance. α_1AT-Pitt also is a potent inhibitor of plasma kallikrein (PKa), αFXIIa, and βFXIIa.[5,6] It anticoagulates simulated cardiopulmonary bypass circuits, but its infusion into septic baboons did not prevent the associated coagulopathy due to in vivo inactivation.

Much recent effort has been made to derive novel treatments for hemophilia by rebalancing the reduced coagulation in hemophilia patients with designer immunoglobulin made to function like the missing factors or immunoglobulin or other inhibitors to regulate hemostasis (see figure panel B). Emicizumab is a monoclonal antibody that binds FIXa/IX and FX/Xa to function like FVIII/FVIIIa in patients with hemophilia A or inhibitors to FVIII.[7] Monoclonal antibodies to TFPI (eg, concizumab, PF-06741086, Bayer 1093884) rebalance hemostasis by allowing more factor VIIa–tissue factor complex to activate factor X directly.[8] In addition, forms of factor X less susceptible to antithrombin inhibition (zymogen-like FXa [I16L]) have been produced to bypass a hemophilia block or coagulation protein inhibitor.[9]

Lessons learned from α_1AT-Pitt also have been applied to hemophilia rebalancing. If one has FVIII or FIX deficiency, lowering antithrombin with a siRNA (fitusiran) rebalances the hemostatic defect (see figure panel B).[10] Altering α_1AT by substituting a Lys and Arg in the P2 and P1 positions, respectively, and a Lys in the P1′ spot (see figure panel A) changes α_1AT into an improved activated protein C inhibitor that reduces thrombin inhibition and, presumably, improves hemostasis.[11] Like α_1AT-Pitt, the Arg in the P1 position makes it a serine protease target, but the Lys in the P2 and P1′ position prevents the reactive center from thrombin interference.

In this issue of *Blood*, a novel approach to hemophilia rebalancing via a serpin is presented. Protease nexin-1 (PN-1) has specificity toward thrombin. Very early PN-1 was recognized on the activated platelet surface in complex with thrombin.[12] Aymonnier and colleagues observed that platelet PN-1 deficiency in activated *f8*$^{-/-}$ platelets or PN-1 inhibition in platelet-rich plasma from severe hemophilia patients significantly improved thrombin generation. In murine models, combined *f8*$^{-/-}$/*Serpine2*$^{-/-}$ (PN-1 knockouts) mice have reduced blood loss and bleeding times compared with *f8*$^{-/-}$ mice alone.[1] Neutralizing antibody to PN-1 enhances clot stability and lengthens clot lysis time on thromboelastometry in blood from *f8*$^{-/-}$/*Serpine2*$^{-/-}$ mice and hemophilia A patients. These data indicate that removal of PN-1 from plasma or platelet-rich plasma produces increased thrombin generation. Because PN-1's target is thrombin, an end point in the hemostatic pathway, its inhibition additionally may be applicable to correct hemostasis in hemophilia B and factor XI deficiency (hemophilia C). Development of a single form of rebalancing therapy for hemophilias A