 sequenced the exomes of 4 patients with the familial form of the disease and identified exactly the same mutations found by Hsu and colleagues. Given the different geographic origin of the patients, it is unlikely that the patients analyzed in the 2 studies are related. Therefore, overall these 2 papers establish a strong correlation between the presence of mutations in the GATA2 gene and disease manifestation. The fact that these 2 studies independently identified the same gene also provides strong validation for the efficacy of the exome sequencing approach as tool for gene mutation discovery.

As far as a mechanism that could subsequently lead to development of MDS, it can be argued that hyperstimulation of a stem cell clone that produces hematopoietic progenitor cells with reduced expansion potential (after haploinsufficient reduction in levels and/or optimal activity of GATA2 as described in the present studies) may lead to stem cell exhaustion (see figure). MonoMAC/DCML patients described in these 2 studies have had to deal with an extended onslaught of infection from the immunodeficiency, yielding a highly stressed bone marrow with a low capacity for mounting an adequate response in the absence of additional acquired mutation(s).

Of interest, the mutations associated with congenital anemia and thrombocytopenias are located in the amino-terminal zinc finger domain of GATA1 while those found in MonoMAC and in DCML deficiency are located in the carboxy-terminal zinc finger of GATA2. This suggests that, in spite of the stringent similarities of the primary structure of the 2 proteins, there must be subtle but important differences in the tertiary structure of GATA1 and GATA2 zinc fingers that determine the unique biologic functions of the 2 proteins. Related to this point, the 2 zinc fingers of all GATA proteins are critical for establishing proper DNA binding and protein interactions. As a result, the nonhaploinsufficient category of GATA2 mutations described in both studies (R398W and T354M) suggests that at least one, if not both, of these critical functions are likely altered. Structural modeling suggests how the point mutants can alter GATA2 interaction with DNA, either by loss of stabilizing hydrogen bonds or steric issues with large side chains. Related to this, a more extreme case is the del 340–381 mutation (again seen in patients in both studies), which removes a large portion of zinc finger 2. It will be of interest to quantitatively verify the predicted lower DNA binding affinity of these GATA2 variants. By analogy to GATA1, other regions of the GATA2 protein are likely associated with positive cofactors. Given that these GATA2 variants are expressed in the presence of a normal allele, dominant negative effects may be operant as a result of nonproductive interactions, a suggestion supported by the recent abstract linking the T354M mutation to MDS/AML.

The work of Hsu et al and Dickinson et al breaks ground on numerous fronts, as they (1) identify a previously nonrecognized correlation between GATA2 and development of bone marrow derived immune cells, including macrophages (see figure); (2) suggest there are important functional differences between 2 factors (GATA1 and GATA2) whose biologic activity have typically been considered to be almost identical; and (3) establish a link between the stress response of donors with a genetically impaired ability to expand hematopoietic/stem progenitor cells (based on reduced GATA2 activity) and development of MDS. In this regard, it would be informative to evaluate whether hypomorphic GATA2 mice will develop a myelodysplastic-like phenotype when challenged with mycobacteria (or other stimuli) and would therefore represent an animal model for this mysterious disease.

In conclusion, these papers represent a satisfying conclusion to the long scientific journey that began with the recognition of a human genetic disease and ends with identification of the genetic lesion that caused the disease. Conflict-of-interest disclosure: The authors declare no competing financial interests.

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Comment on Zhang et al, page 2868

Can erythroblasts donate iron?

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In this issue of Blood, Zhang and colleagues propose the novel concept that when serum hepcidin is low, it increases ferroportin at the erythroblast membrane, thereby shutting down erythropoiesis when iron is scarce to favor its usage by cells sensitive to iron deprivation.
Iron transporter 1 (FPN1), the only known iron exporter in mammalian cells, is highly expressed in macrophages, duodenal enterocytes, and hepatocytes. Hepcidin acts as an iron gatekeeper by regulating the amount of FPN1 present at the cell surface of target cells. The high serum hepcidin levels that are found when transferrin saturation or tissue iron stores are increased, or in inflammatory conditions, induce FPN1 internalization and degradation, thereby limiting iron availability. On the reverse, iron deficiency or expansion of the erythropoietic activity of the bone marrow will induce a drastic reduction in serum hepcidin levels and subsequent increase in FPN1, stimulation of intestinal iron absorption, and increased mobilization of iron stores.

In macrophages, FPN1 is regulated at the transcriptional level by heme derived from phagocytosed red blood cells and at the translational level by iron released from heme by heme oxygenase. This posttranslational regulation is mediated by an iron regulatory element (IRE) present in the 5′ untranslated region (UTR) of the FPN1 mRNA. Iron regulatory proteins (IRPs), acting as intracellular sensors, will bind to this IRE in conditions of limited iron supply and repress mRNA translation. Iron entry into cells or release of iron from heme degradation will inactivate the IRPs and recruit FPN1 mRNA to the polyribosomes, thereby rapidly increasing FPN1 synthesis.

Cianetti et al first identified a non-IRE FPN1 mRNA in human erythroblasts and Zhang et al subsequently identified the mouse FPN1B mRNA, generated by transcription from an upstream promoter and alternative splicing of the 5′ UTR. This FPN1B mRNA is also highly expressed in duodenum, escaping translational repression in conditions of iron deficiency.

Using the human erythroleukemia cell line K562 and cultured erythroid progenitors from mouse fetal liver, Zhang and colleagues show that FPN1B mRNA is highly expressed at the early stages of differentiation and that culturing the cells in the presence of hepcidin induces degradation of the FPN normally present at the cell surface of erythroblasts. The evidence that erythroblasts express an important amount of FPN at their membrane initially came as a surprise because these cells have a high iron demand to reach a sufficient rate of heme synthesis to allow hemoglobin formation. However, FPN1B is expressed at the early stages of differentiation, before the onset of high rate of heme and globin synthesis. This might be considered as a safety valve mechanism, allowing the erythroid progenitors to eliminate the iron incorporated in excess, to avoid its toxicity. A similar mechanism has already been proposed for the elimination of excess heme by FLVCR, a heme exporter. Inactivation of this gene induced early death of pro-erythroblasts, probably because of heme toxicity.

Interestingly, Zhang et al propose the challenging hypothesis that in conditions of severe iron deficiency, the high FPN1 expression associated with low heme levels would favor iron export from erythroblasts. In a way, these cells would “sacrifice” themselves for the benefit of other cells more sensitive to iron deprivation such as neurons or cardiomyocytes. The negative impact of hepcidin on erythroblast FPN1 would also account for an observation that has remained unexplained so far. In anemia of inflammation, iron–restricted erythropoiesis results in iron retention in macrophages, because of the high levels of serum hepcidin. However, the anemia is usually normocytic, suggesting that erythroid iron stores are sufficient to insure normal hemoglobin formation. Shuttling down FPN1 expression at the erythroblast membrane could be one way of achieving this (see figure).

There are a number of unresolved issues concerning the role of FPN in erythropoiesis. First, there is some discrepancy regarding the subcellular localization of erythroblast FPN1, which has been found either intracellular or at the cell membrane. The demonstration that it can interact with plasma hepcidin is more in favor of a trafficking to the plasma membrane.

Second, all the evidence provided so far has been obtained with cultured erythroid progenitors either from mouse fetal liver or from human circulating progenitors. In vivo, erythroid precursors proliferate, differentiate, and enucleate within the erythroblastic islands where they surround a central macrophage. Cell–cell and cell–extracellular matrix interactions play a major role in positive and negative regulatory feedback, and the FPN1–hepcidin interactions might operate very differently in this context than in vitro. Finally, from which pool is iron exported to the plasma? Ferritin must be degraded for iron to be exported by FPN1 and erythroblasts do not have high amounts of ferritin–associated iron. Alternately, iron available for export could also derive from iron released from heme degradation by heme oxygenase highly expressed at the early stages of differentiation, a mechanism reminiscent of the macrophage iron pathway. Iron exported by FPN1 could also derive from iron released into the cytosol after endocytosis of the Fe(III)–transferrin complex by transferrin receptors 1, to be eliminated only when taken up in excess of porphyrin availability. However, it is not clear whether this iron is directly targeted to the mitochondria or whether it transits by a cytosolic pool of labile Fe(II) iron. In favor of this direct mitochondrial targeting is the observation that the synthesis of ferritin subunits, encoded by IRE-containing mRNAs, is not stimulated during erythroid differentiation despite the high iron influx into these cells. If this is true, iron would not be available for export by FPN1.

Clearly, a precise analysis of the timing of onset of iron uptake, storage, and efflux during erythropoietic differentiation is required to get a better understanding of the role of this
FPN1–hepcidin interaction and of its implications in pathologic situations.

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