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MYELOID NEOPLASIA

Comment on Toledo et al, page 2070

"Mast"ering drug discovery with iPSCs

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In this issue of *Blood*, Toledo et al describe the generation of KIT D186V–induced pluripotent stem cells (iPSCs) from patients with aggressive systemic mastocytosis (SM) to use as patient-specific models for mechanistic studies and drug discovery. Using these iPSCs, the authors identified nintedanib, a US Food and Drug Administration–approved angiokinase inhibitor, as a potential new therapy for SM.[1]

SM is a rare disease involving expansion and organ infiltration of neoplastic mast cells. Initially, SM was thought to be a homogenous disease; however, clinical differences in disease progression and response to therapy, along with the identification of mutations supports the modern premise that SM is a complex heterogenous disease. According to the World Health Organization classification of SM there are several subcategories, including: indolent SM, smoldering SM, aggressive SM (ASM), SM with an associated hematologic neoplasm (involving cell lineages other than mast cells), and mast cell leukemia (MCL). ASM and MCL subgroups have poor outcomes with a median survival for MCL being <1 year.[1-3] Therefore, there is an unmet need for novel therapies for these patients. Although the KIT D816V mutation is found in >80% of patients with SM, it does not appear that the KIT D816V mutation alone is sufficient to induce malignant transformation of mast cells. This mutation contributes to increased proliferation and survival of neoplastic MCs, making it an important therapeutic target for SM.[2,3] The US Food and Drug Administration recently approved midostaurin for the treatment of adults with ASM, SM with an associated hematologic neoplasm, and MCL based on response rates and duration of responses. However, in patients with advanced SM, midostaurin does not induce complete remission.[4]

Advances in our understanding of the biology and treatment of SM have been limited not only by the rarity of the disease, but also by the relatively low number of primary patient cells recovered from patients with SM. Unlike acute myeloid leukemia, where leukemic cells become the dominant cell type within the blood and bone marrow, ASM and MCL present with relatively low levels of MCs in peripheral blood and bone marrow.[2,3] Although human MCL cell lines exist, they do not adequately recapitulate the full spectrum of the human disease. To overcome these limitations, Toledo et al generated iPSCs from patients with ASM and MCL. Importantly, KIT D816V iPSCs derived from patients with SM had important features of the human disease. Specifically, these iPSCs showed increased activation of KIT in the absence of cytokine stimulation and increased proliferation and survival of hematopoietic cells upon iPSC differentiation, compared with KIT unmutated iPSCs. Importantly, iPSCs also displayed patient-specific differences in hematopoietic differentiation capacity, demonstrating that patient heterogeneity is maintained when generating iPSCs. The poor prognosis of SM has also been attributed to the presence of mutations commonly found in other hematologic malignancies such as RUNX1, SRSF2, and TET2 mutations, among others.[5] These cooccurring mutations have not been found in MCL cell lines, but were present in patient-derived SM iPSCs with those additional mutations. This is an important advance because this model reflects more accurately the human disease and its complex biology. Although the authors state that 1 limitation of the model is the persistence of the KIT D816V and associated mutations in the iPSCs, the use of CRISPR/Cas9n would allow for deletion of these mutations within the patient-derived iPSCs to further dissect the contribution of each these mutations in disease progression, similar to what have been done in MDS/acute myeloid leukemia iPSCs.[6]

No longer being limited by cell numbers, Toledo et al used patient-derived iPSCs to perform a drug screen to identify novel SM inhibitors. Nintedanib was found to be a highly potent KIT D816V inhibitor, resulting in decreases in cell viability and KIT activation. Induced fit molecular docking studies confirmed preferential targeting of nintedanib for KIT D816V compared with wild-type KIT, thus possibly eliminating some of the negative off-target drug effects associated with TKIs.[1]

Using patient-derived SM iPSCs, Toledo et al have eliminated critical barriers that prevented large-scale drug screens in a rare subset of hematologic malignancy and identified a potential new treatment of patients with SM with a poor prognosis. This study also demonstrates that the use of patient-specific iPSCs is a valuable tool to investigate other hematologic diseases where primary samples are limited to identify novel targets for therapy.

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

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RED CELLS, IRON, AND ERYTHROPOIESIS

Comment on Petit et al, page 2090

Iron, FRDA, and intermediary metabolism

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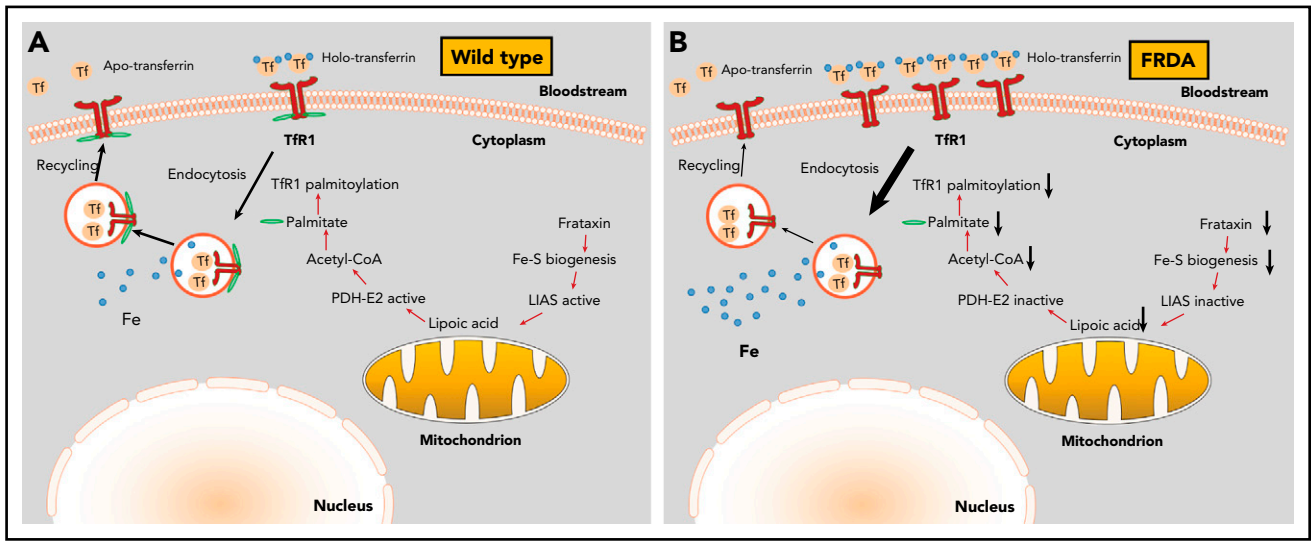
In this issue of *Blood*, Petit et al identify a mechanism that promotes cellular iron overload in Friedreich's ataxia (FRDA), which is a result of a defect that prevents appropriate palmitoylation of transferrin receptor 1 (TfR1).[1]

FRDA is an autosomal recessive neurodegenerative disease that is mainly caused by expansions of a GAA trinucleotide repeat within the first intron of the *FXN* gene.[2] This impairs expression of the gene product frataxin, a mitochondrial protein involved in iron-sulfur (Fe-S) cluster (ISC) biogenesis. Frataxin deficiency is associated with mitochondrial iron overload, oxidative stress, and inactivation of ISC proteins such as mitochondrial aconitase and respiratory chain complexes I to III. These biochemical manifestations primarily affect the central nervous system and the heart and lead to sensory neuron dysfunction and cardiomyopathy.

The impact of frataxin deficiency on overall cellular iron metabolism is not well understood. Moreover, assessment of cytosolic iron status in cells and tissues from FRDA patients and mouse models has yielded conflicting results. It is generally believed that accumulation of mitochondrial iron is the result of an increased metabolic need for the metal because iron has not been incorporated into ISCs. This in turn drives iron flux from the cytosol into mitochondria. The ensuing cytosolic iron depletion is sensed by iron regulatory proteins IRP1 and IRP2, which stabilize the TfR1-encoding *TFRC* messenger RNA (mRNA) to increase iron uptake in a vicious cycle. At the same time, IRPs inhibit translation of the *FTH*, *FTL*, and *SLC40A1* mRNAs that encode H-ferritin, L-ferritin, and ferroportin, respectively, to inhibit iron storage and export.[3] Of note, proper regulation of IRPs requires functional ISC assembly machinery. Thus, in iron-replete cells, IRP1 acquires a 4Fe-4S cluster and operates as cytosolic aconitase at the expense of its RNA-binding activity. Conversely, IRP2 undergoes proteasomal degradation after ubiquitination by the E3 ubiquitin ligase FBXL5, which was recently shown to act by using a 2Fe-2S cluster.[4] There is evidence that frataxin deficiency triggers activation of both IRP1 and IRP2 for RNA binding,[5] which is consistent with cytosolic iron deficiency and defective ISC biogenesis.

Petit et al used skin fibroblasts from FRDA patients and showed that these cells have a higher iron content compared with controls in both cytosolic and mitochondrial compartments. Moreover, they fail to mount a negative feedback homeostatic response to exogenous holotransferrin or ferric ammonium citrate,



Mechanism for cellular iron overload in FRDA. (A) In wild-type cells, the presence of intact Fe-S assembly machinery allows proper TfR1 palmitoylation, plasma membrane expression, and iron uptake function. (B) In FRDA cells, frataxin deficiency impairs Fe-S biogenesis and inactivates LIAS, inhibiting lipoic acid synthesis. Because lipoic acid is an essential cofactor of the pyruvate dehydrogenase E2 subunit (PDH-E2), these responses limit acetyl-CoA production by PDH and subsequent fatty acid biosynthesis. In the absence of sufficient palmitate, TfR1 fails to undergo palmitoylation and accumulates on the plasma membrane. Moreover, the lack of TfR1 palmitoylation increases endocytosis of transferrin-TfR1 complexes and delays recycling of transferrin, which results in iron overload.