Comment on Ito et al, page 1499

The (miR)e of CTCL

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In this issue of *Blood*, Ito et al demonstrate pathogenic implications of microRNA-150 (miR-150) repression in an aggressive form of cutaneous T-cell lymphoma (CTCL).[1] Noncoding RNAs, such as microRNA, profoundly influence gene transcription and protein translation machinery to change hematopoietic cell fate in physiologic and pathologic conditions.

Among the hematopoietic cells, miR-150 is predominantly expressed in B, T, and natural killer cells through their development and maturation, except during the differentiation of naïve T cells into the effector Th1 and Th2 cells.[2-4] Importantly, miR-150–deficient mice lack lymphoid cell maturation and effector functions.[2,4] In nonlymphoid lineages, miR-150 favors differentiation of megakaryocyte-erythrocyte progenitors to megakaryocytes at the expense of erythrocytes.[5] In determining cell fate, miR-150 targets multiple downstream targets, including *MYB*, *FLT3*, *CBL*, *EGR2*, *DKC1*, *AKT2*, *Myb* and *Notch3*.[6]

While miR-150 functions as a tumor suppressor in acute leukemia and lymphoma, its role in altering the behavior of the malignant CTCL cells is largely unknown.[6] Similar to data previously published by other groups,[7,8] data in this study showed that miR-150 was significantly reduced in patients with advanced-stage CTCL who exhibited extensive nodal or visceral involvement. Ito et al[1] report an interesting series of events initiated by miR-150 repression in $CD4^+$ CTCL cells. By using CTCL cell lines, Ito et al identify chemokine receptor 6 (CCR6) as a novel target for miR-150, as evidenced by direct binding of miR-150 within the CCR6 regulatory region. Of note, $CCR6^+$ cells migrate toward a chemokine ligand 20 (CCL20) gradient, and their activation by interleukin-22 (IL-22) causes cell proliferation and migration. Through comprehensive gain- and loss-of-function approaches, Ito et al show that miR-150 negatively regulates an IL-22-CCL20-CCR6 autocrine pathway in CTCL cells. These findings uncover a previously unknown miR-150-chemokine receptor pathway that may act widely to control metastatic potential of CTCL.

What determines whether malignant cells migrate to the skin is a riddle that has baffled scientists for long time. Since skin produces CCL20 during inflammation,[9] these novel observations fit well in a model in which the cutaneous tissue provides chemotactic signals for migrating cells as well as a fertile niche in which the neoplastic cells can grow and survive. Previous studies have linked both chemokines and chemokine receptors in the migration of malignant T cells to epidermal keratinocytes.[10] Although the mechanisms controlling cell migration to the skin are poorly understood, the study by Ito et al hints at the possibility that they may be due to intrinsic defects in T cells, specifically from diminished expression of noncoding RNA such as miR-150. Since an increase in tumor cell migration is not enough to fuel metastasis, induction of IL-22 for cell proliferation represents a significant step in dissemination of lymphoma in vivo. Further work is required to resolve how miR-150 is regulated in malignant T cells in CTCL.

Overall, Ito et al present a compelling study of the importance of miR-150 in CTCL metastasis. Their work also paves the way for the therapeutic strategies that can be used to restore miR-150 levels in malignant cells by either pharmacologic inhibitors that target miR-150 repression or by miR replacement therapy for CTCL treatment. Undertaking the challenges of dissecting signaling mechanisms upstream of miR-150 provides an invaluable insight on pathogenic signals and furthers our understanding of complex oncogenic pathways in T cells that can be extrapolated beyond CTCL. ■

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Comment on Rumi et al, page 1544, and on Rotunno et al, page 1552

Two faces of ET: *CALR* and *JAK2*

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In this issue of *Blood*, Rumi et al and Rotunno et al demonstrate that essential thrombocythemia (ET) patients with *calreticulin* mutations exhibit lower leukocyte and hemoglobin values, higher platelet counts, and a lower thrombosis risk vs *JAK2*-mutated ET. *Calreticulin*-mutated ET appears to be a distinct entity with a more indolent course.[1,2]

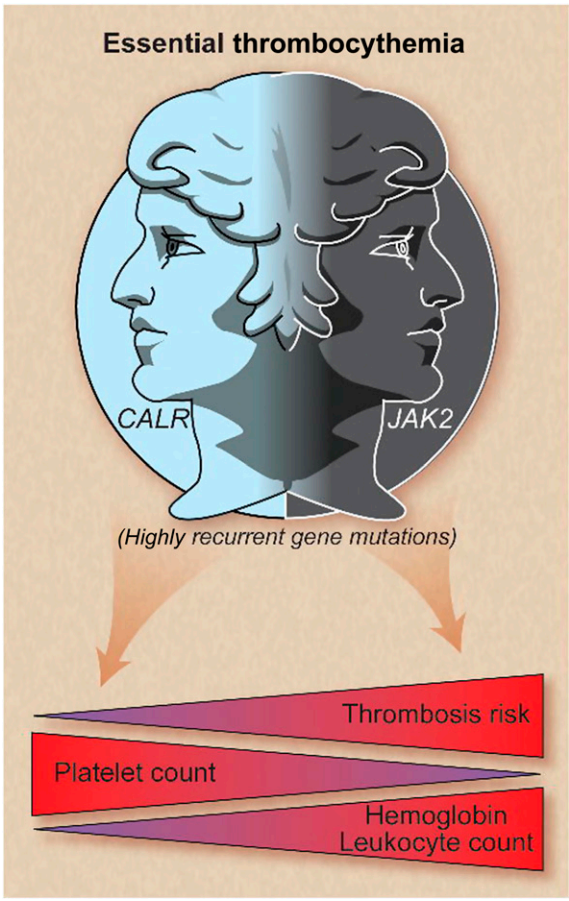
Calreticulin (CALR) is a highly conserved multifunctional endoplasmic reticulum (ER) protein. Inside the ER, CALR plays an integral role in calcium homeostasis and protein folding. Outside the ER, CALR is found in the cytosol and on the cell surface, where it regulates integrin-mediated cell adhesion, gene nuclear transport, programmed cell removal, and immunogenic cell death. The CALR protein structure contains 3 distinct domains with specific functions. The N- and P-domains are mainly responsible for the protein's chaperone function, whereas the C-domain is principally involved in calcium regulation in the ER.[3] The C-domain also contains the KDEL sequence, which is responsible for preventing secretion of the protein from the ER.

Recently, recurrent *CALR* indel mutations were discovered in ET and primary myelofibrosis (PMF) patients with nonmutated *JAK2* and *MPL*.[4,5] All *CALR* mutations were localized to exon 9 and uniformly led to loss of most of the C-terminal acidic domain, multiple calcium-binding sites, and the KDEL sequence. These mutations were an early event in myeloproliferative neoplasm (MPN) tumorigenesis.[5]

CALR has been implicated in human cancers by playing roles in macrophage-mediated immune evasion,[6] activation of the unfolded protein response (UPR),[7] and calcium signaling. The consequence of these *CALR* mutations in MPNs is unknown but may be related to one or more of the aforementioned functions. First, *CALR* mutations may lead to evasion of macrophage phagocytosis. We demonstrated that many cancers constitutively express cell surface CALR and that increased CALR mRNA expression is associated with a poor prognosis.[6] Cell surface CALR serves as a pro-phagocytic signal that facilitates clearance of damaged cells.[8] Although one might expect increased CALR cell surface expression to lead to increased cell removal due to its pro-phagocytic function, this effect is counterbalanced by overexpression of the antiphagocytic signal CD47. In MPNs, it is possible that *CALR* mutations, due to loss of the KDEL ER retention sequence, disrupt CALR cell surface expression, leading to decreased susceptibility to phagocytosis. Although initial reports of the *CALR*-mutated MPN patients are mixed in demonstrating abnormal localization of CALR in the cytosol[4]/cell membrane,[5] further investigation is needed. Second, *CALR* mutations may cause disruption of the UPR, leading to a competitive growth advantage. In response to ER stress, the UPR is activated to prevent abnormal cell proliferation by stopping protein translation through activation of ER protein folding chaperones. Indeed, perturbation of the UPR has been implicated in human cancers.[9] Mutations may impair CALR protein chaperone function in the ER and disrupt the homeostatic UPR response, leading to cell hyperproliferation. Last, it is very likely that *CALR* mutations play a role in altered signal transducer and activator of transcription (STAT) signaling, similar to *JAK2* V617F. This hypothesis is supported by several observations. First, *CALR* mutations are mutually exclusive to *JAK2* and *MPL* mutations in ET and PMF patients,[4,5] suggesting a redundant role of these genes. Second, increased STAT5 phosphorylation was observed in *CALR* mutant transfected cell lines compared with wild type.[4] Third, the Janus kinase (JAK) inhibitor ruxolitinib elicited responses in *CALR*-mutated, hydroxyurea-refractory ET patients, similar to those observed in *JAK2*-mutated patients.[1]

To establish the clinical implications of these molecular findings, Rotunno et al compared mutational subgroups of ET,[2] and Rumi and colleagues compared cohorts of *CALR*- and *JAK2*-mutated ET to patients with polycythemia vera (PV).[1] Compared with *JAK2*-mutated ET, both groups found that *CALR*-mutated ET patients were more commonly male, demonstrated higher platelet counts, and lower leukocyte and hemoglobin levels (see figure).[1,2] Individuals with PV exhibited higher leukocyte and hemoglobin values compared with *CALR*- and *JAK2*-mutated ET patients but lower platelet counts and serum erythropoietin levels.[1]

Both analyses identified a lower incidence of thrombosis in *CALR* vs *JAK2*-mutated ET patients (see figure),[1,2] but thrombosis rates were not different between PV and



CALR and *JAK2* mutations represent 2 disease spectrums in essential thrombocythemia whereby cases with mutated *CALR* are characterized by higher platelet levels, lower hemoglobin and leukocyte counts, and lower thrombosis risk compared with *JAK2*-mutated patients. Professional illustration by Debra T. Dartez.

JAK2-mutated ET.[1] Rotunno et al identified a 10-year cumulative incidence of thrombosis of 5.1% vs 14.5% in *CALR*- vs *JAK2*-mutated patients, respectively,[2] paralleling the 15-year cumulative thrombosis incidence of 10.5% vs 25.1% identified by Rumi et al.[1] The higher thrombosis rate in *JAK2*-mutated ET patients may be partly attributable to the hyperviscosity associated with higher hematocrit and leukocyte levels, the latter being a putative risk factor for thrombosis in MPNs. Evaluation of leukocyte and platelet activation, as well as hypercoagulability profiles, will lend further insight into this differential propensity for thrombosis.

The effect of mutational status on the clinical course of ET was evaluated in both studies and was also compared with PV. Although ET patients carrying a *CALR* mutation exhibited a better overall survival than PV patients, a nonsignificant trend[1] or no difference in survival[2] was found between *CALR* and *JAK2*-mutated ET patients. In contrast, a significant difference ($P = .04$) was observed in 10-year overall survival between these ET subgroups by Klampfl et al.[4] The 15-year cumulative incidence of secondary myelofibrosis was similar between *CALR*- and *JAK2*-mutated ET and PV,[1] but evolution to leukemia was lower for *CALR*-mutated ET patients compared with the other groups.[1] Whereas a significant fraction of *JAK2*-mutated ET patients progressed to PV, none with a *CALR* mutation did so.[1] Consistent with prior data, the median *JAK2* mutant allele burden was higher in ET patients who progressed to PV, as well as in post-PV/ET MF compared with the initial MPN.[1] These data reinforce that PV and *JAK2*-mutated ET form a continuum wherein phenotype and natural history are substantially influenced by *JAK2* allele burden.

While investigators decipher how *CALR* mutations contribute to MPN pathogenesis, it is clear that *CALR* will be quickly assimilated into World Health Organization diagnostic criteria for ET and PMF. Less clear is whether *CALR* mutation status will provide independent prognostic utility in scoring systems[10] used to estimate vascular risk and survival. Also, the effect of *CALR* mutant allele burden on clinical correlates remains untested. Looking forward, these observational studies provide a framework for assessing whether conventional therapies used broadly for ET (eg, aspirin for low-risk patients and hydroxyurea for high-risk individuals) are also appropriate for *CALR*-mutated patients who may exhibit lower-risk features.

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● ● ● RED CELLS, IRON, & ERYTHROPOIESIS

Comment on Poli et al, page 1564

BuMPing iron with modified heparins

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In this issue of *Blood*, Poli et al demonstrate that heparin analogs engineered to minimize their anticoagulant properties can potently downregulate hepcidin production in vitro and in vivo, and may potentially be used to treat the anemia of inflammation.[1]

Hepcidin is the key iron hormone that regulates iron availability from 3 main sources: dietary absorption, red blood cell recycling, and body iron stores.[2] Hepcidin acts by inducing degradation of the principal iron exporter ferroportin, thereby blocking iron release into the bloodstream and leading to iron sequestration in enterocytes, macrophages, and hepatocytes.[2] Increases in iron levels stimulate hepcidin production to "turn off" further iron release into the circulation and to prevent iron excess. Inflammation also stimulates hepcidin production to limit iron accessibility to infectious organisms. Alternatively, when erythropoiesis is stimulated by anemia or hypoxia, hepcidin production is inhibited to "turn on" iron release into the circulation to support red blood cell production. Diseases of dysregulated hepcidin include hemochromatosis, in which too little hepcidin leads to iron overload, and the anemia of inflammation (also called anemia of chronic disease), in which too much hepcidin leads to iron-restricted erythropoiesis.[2]

Hepcidin is mainly secreted by hepatocytes in the liver, where its expression is controlled by a variety of signals. One central signaling pathway that controls hepcidin production is the bone morphogenetic protein (BMP)-sons of mothers against decapentaplegic (SMAD) signaling pathway through the coreceptor hemojuvelin[3] and the ligand BMP6.[4] This pathway is not only essential for hepcidin regulation by iron, but its activity is also