mature HGBL. HGBLs include BL, double-hit lymphomas, HGBL with 11q aberration, and the rare HGBL, not otherwise specified, as well as perhaps 10% to 15% of tumors currently diagnosed as DLBCL. Despite the confusing range of histologic patterns, these aggressive lymphomas seem to use consistent sets of mutated transcription factors to hijack mechanisms that operate within the germinal center dark zone. The emerging GEP and genomic classifiers need translation into trials that would separate the HGBL-like tumors from DLBCL to identify more efficacious treatments and mitigate their refractoriness to the standard-intensity chemotherapy.

The study by Thomas et al does not paint an easily interpretable picture of prognostic relevance for the discovered BL subgroups. The overall survival of the pediatric and adult patients included in the study exceeded 80%, much higher than observed in clinical practice in either North America or Europe.[4] In aggregate, outcomes did not differ between DGG-BL and IC-BL, and borderline (and contradictory) associations from the pediatric and adult cohorts are hard to interpret with as few as 5 to 8 patients in the subgroups and the lack of stratification in baseline variables or treatment. Nevertheless, the notable prognostic disadvantage for adult BL carrying *TP53* mutations provides a hint that sequencing data might add value to clinical prognosis. The constellation of *MYC* rearrangement with *TP53* mutation, regardless of histology, may define one of the worst categories of HGBL.[8] Future research should include more cases of disseminated, extranodal BL, which is often diagnosed using blood, bone marrow, or cerebrospinal fluid. Such tumors carry significantly worse prognosis in both experimental and observational cohorts[4,9,10] but may be underrepresented in sequencing studies due to unavailability of archival paraffin-embedded nodal tissue. Analysis of prospectively collected samples from patients who are uniformly treated on clinical trials will be the next critical step to examine the prognostic impact of specific genomic alterations in BL and the practical relevance of the subtypes.

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MYELOID NEOPLASIA

Comment on Pecquet et al, page 917

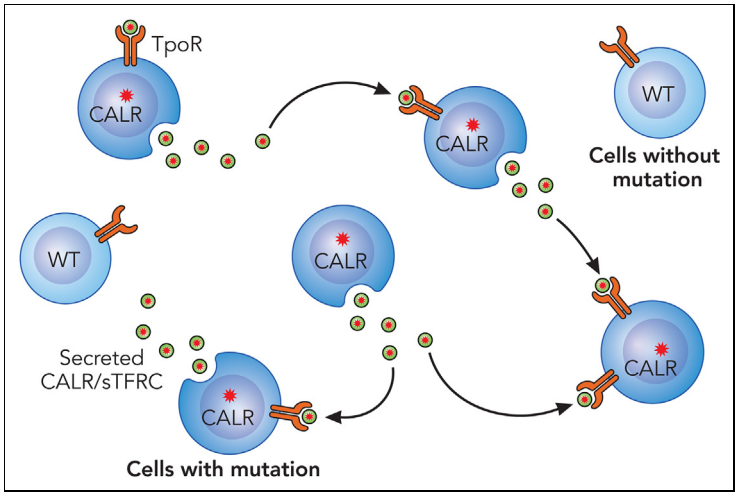
CALR goes rogue

Johanna Melo-Cardenas and John D. Crispino | St. Jude Children's Research Hospital

In this issue of *Blood*, Pecquet et al[1] report that hematopoietic cells with a mutation in calreticulin (*CALR*) secrete a soluble form of the protein that acts in paracrine fashion to enhance the growth of surrounding tumor cells.

The BCR-ABL-negative myeloproliferative neoplasms (MPNs) are caused by mutations in the kinase JAK2, the thrombopoietin receptor MPL (also known as TpoR), and the endoplasmic reticulum (ER) chaperone CALR.[2] Although it was readily apparent that *JAK2* and *MPL* mutations promote cytokine independent growth through activation of the JAK/STAT signaling pathway, how mutations in *CALR* contribute to the MPNs was not obvious. CALR is involved in the quality control of newly synthesized proteins and glycoproteins that prevent misfolded proteins from leaving the ER prematurely. CALR is also involved in the regulation of intracellular calcium levels, integrin signaling, and loading of antigens onto the major histocompatibility complex. CALR can be found on the cell surface where it initiates prophagocytic signals and mediates immunostimulatory effects. Mutations of *CALR* in the MPNs result in an altered C terminus that selectively binds TpoR in the ER and leads to its activation independent of its ligand thrombopoietin.[3] Mutant CALR is then transported to the cell surface along with TpoR, where it leads to JAK/STAT pathway activation and promotes malignant growth. Glycosylation of TpoR is necessary for the CALR binding through its lectin binding domain, and this former modification was recently shown to be a therapeutic vulnerability in CALR mutant MPNs.[4] In addition to binding TpoR, previous reports showed that mutant CALR is also found as a soluble form.[5-7]

Pecquet et al sought to understand the biological function of soluble mutant



CALR acts as a rogue cytokine in the MPNs. Mutant CALR protein is secreted from *CALR* mutant MPN cells where it binds the TpoR of surrounding cells to enhance downstream JAK/STAT activation. The mutant protein has a much lesser effect on wild-type (WT) hematopoietic cells. Note that it is also possible that this secreted CALR rogue cytokine may also act in an autocrine fashion. sTFRC, soluble TFRC. Professional illustration by Patrick Lane, ScEYEnce Studios.

CALR in MPNs. They began by demonstrating that MPN patients with *CALR* mutations have elevated levels of soluble mutant CALR in the plasma and that these levels correlate with the mutational burden. Next, by immunoelectron microscopy, they demonstrated that mutant CALR follows a secretory pathway, as it was detected in the Golgi apparatus and plasma membrane, whereas wild-type CALR was mainly localized to the ER. Further analysis demonstrated that the secretion of mutant CALR is independent of TpoR expression, since mice lacking TpoR had comparable levels of soluble mutant CALR in the plasma to that of control mice. The authors then generated recombinant mutant CALR protein to investigate whether it could act as a stimulatory factor in hematopoietic cells. They discovered that the recombinant protein has a half-life 10-fold shorter compared with mutant CALR isolated from the plasma of MPN patients. This led them to investigate the factors that increased mutant CALR stability in the plasma and to their findings that transferrin receptor 1 (TFRC) forms a complex with mutant CALR and that adding recombinant TFRC increases its half-life in vitro. Furthermore, the authors observed that mutant CALR and TFRC colocalize in intracellular vesicles and that mutant CALR increases the cleavage of TFRC, the soluble form. Next, by using Nano-BRET, they showed that exogenous soluble mutant CALR can bind to TpoR on the cell surface and induce JAK/STAT pathway activation. The major advance in the Pecquet et al study is the finding that secreted mutant CALR acts as a "rogue cytokine" to enhance the megakaryocytic differentiation of MPN progenitors (see figure). Intriguingly, the exogenous mutant CALR had a selective effect on hematopoietic cells also harboring the *CALR* mutation.

An important unresolved question is the degree to which the secreted CALR protein contributes to the disease. Although CALR in the plasma is readily detectable and correlates with allele burden, how much this drives expansion of the tumor cell population is not clear. Studies to impair its secretion in an in vivo setting would shed light on this point. Another question is why the CALR cytokine selectively promotes growth and megakaryocytic differentiation of CALR mutant progenitors but not healthy hematopoietic cells that express TpoR. The authors found that mutant CALR binds the transferrin receptor, which mediates iron uptake. Previous reports have shown that low iron levels promote megakaryopoiesis,[8] raising the possibility that modulation of iron homeostasis, together with TpoR activation, contributes to the prominent effect of the CALR mutant on megakaryocytes. Finally, how much of the activity involves autocrine signaling where the CALR mutant cytokine reinforces enhanced JAK/STAT activation in the secreting cell itself is unclear.

The observation that secreted mutant CALR has a positive effect on growth of MPN cells raises the possibility that targeting this factor in the microenvironment may ameliorate the disease. Consistent with this prediction, a recent study demonstrated that treatment of *Calr* mutant mice with an antibody targeting CALR mutant protein decreased circulating platelet counts.[9] Another recent study reported that secreted CALR has an immunosuppressive effect, providing further rationale for targeting this protein.[10]

In summary, the Pecquet et al study provides exciting new insights into the pathogenesis of CALR mutant MPNs and supports targeting CALR as a therapeutic axis in this malignancy. This discovery therefore represents a major advance in our understanding of how mutations in *CALR* contribute to the MPNs.

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THROMBOSIS AND HEMOSTASIS

Comment on El-Mansi et al, page 930

CSI: Weibel-Palade bodies

Ruben Bierings[1] and Jan Voorberg[2,3] | [1]Erasmus MC University Medical Center; [2]Sanquin Research; and [3]Amsterdam UMC

In this issue of *Blood*, El-Mansi et al have used proximity biotinylation proteomics, together with a high-throughput dual loss-of-function screening, to find new factors involved in Weibel-Palade body (WPB) biology.[1] Via the experimental setup designed to focus on the events that take place around WPBs during secretion, their study provides a new inventory of potential regulators of WPB exocytosis and identifies several new components that contribute to WPB cargo expulsion.

WPBs are endothelial cell–specific secretory organelles that, together with a long list of inflammatory and angiogenic mediators, store the hemostatic protein von Willebrand factor (VWF) as their main cargo.[2] In response to vascular injury, WPBs are rapidly deployed to the vascular lumen, where they deliver their cocktail of procoagulant and vasoactive mediators, including the release of long platelet-adhesive strings of VWF multimers. Balanced VWF release is important, as shown by the pivotal role of VWF: low levels of VWF can lead to a bleeding tendency, such as in von Willebrand disease, and elevated levels of VWF are associated with an increased risk for thrombosis and associated cardiovascular disease.[3]

In the past, interactomic screens based on affinity purification mass spectrometry have been used to identify new components of the WPB exocytic machinery. But an important limitation of earlier work was the focus on only interactors of previously identified regulators and the requirement that the interactions survive the pull-down purification procedure; thus, weak and transient partnerships were missed. Proximity biotinylation does not depend on protein–protein interactions but rather uses the enzymatic activity of a biotin ligase-tag to biotinylate proteins surrounding the tagged protein of interest. The list of biotinylated proteins thus obtained is a log list of all the proteins that have been in the immediate vicinity, not all of which were in physical contact but are nevertheless potentially relevant for the biological process of interest.

For their screen for new exocytotic regulators, El-Mansi et al tagged the biotin ligase APEX2 to Rab27A, a small Rab GTPase that is recruited to mature WPBs and thereby focuses APEX2 biotinylation activity in a small zone around these organelles. The same APEX2 Rab27A–based strategy has been used before by Holthenrich and coworkers in resting endothelial cells, which identified Munc13-2 as a novel WPB-associated regulator of secretion.[4] Notably, that study identified many proteins that are present on organelles other than WPBs—which probably reflects the rubbing up of WPBs against other organelles as they make their way through the cell—but lacked many of the known late-phase mediators of WPB exocytosis, such as SNARE proteins.[5]

To specifically capture exocytotic regulators, El-Mansi et al took a forensic approach that combined APEX2–Rab27A proximity proteomes from endothelial cells in resting conditions with those from cells stimulated with agonists that induce WPB exocytosis. This cunning plan generates a snapshot of biotinylated proteins around WPBs during exocytosis, akin to a recording of a crime scene with perpetrators, accomplices, witnesses, and other bystanders all on tape. The gathered evidence implicates proteins and pathways caught in the act of exocytosis, such as microtubule motors that are involved in moving WPBs into the crime scene, SNARE proteins that pull the trigger on membrane fusion, and regulators of the actin cytoskeleton that keep WPBs in a headlock during release (see figure). The resulting list represents a major leap toward an understanding of the regulation of distinct phases of WPB exocytosis.

A class of proteins that so far had remained elusive are the plus-end directed motor proteins that are responsible for anterograde transport of WPBs along the microtubule cytoskeleton. From their screen in resting endothelial cells, the authors identify the kinesin heavy chain KIF5B, a novel component of WPBs that appears remarkably concentrated at one end of the organelle at contact points with microtubule filaments. A particularly interesting observation from their data sets is that the kinesins that were identified differed in the unstimulated (KIF5B) vs the agonist-induced conditions (KIF11), suggesting that during the final stages of the WPB lifecycle, a dynamic exchange of distinct kinesins occurs that may have specialized contributions to the exocytotic process. Whether, and exactly how, these or other kinesins participate in steady-state WPB transport or fast mobilization of WPBs during exocytosis cannot yet be determined, but the proteomic data sets reported here will help in directing future studies on this topic.

VWF is densely packed in tubules that are arranged in parallel along the length of the WPB; the VWF tubules unwind into long strings as they emerge from the fusing WPB. The high state of condensation and large size of VWF (in the range of tens of MDa per multimer) represent significant hurdles to efficient release and may require an extra push to get out. Contractile rings consisting of actomyosin can form around fused WPBs and contribute to the expulsion of VWF into strings,[6] but an actomyosin-independent mechanism for VWF expulsion also operates on a subsecond timescale well before the appearance of actomyosin rings.[7] The relative contributions of each of the 2 pathways to VWF release have yet to be determined, and conceivably, they could work in tandem on the same