months, respectively, were induced in 2 patients relapsing after allogeneic SCT using CD19-redirected chimeric antigen receptor–modified T cells.[9] The future is thus open to developing more effective single and multitargeted therapies that could eventually relegate chemotherapy and transplants to a secondary role.

However, imatinib has proven powerful, starting a season of progress and allowing many Ph+ ALL patients to grow older along with it. This is a long sought after turning point, which was well caught by this study.

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

REFERENCES

1. Fielding AK, Rowe JM, Buck G, et al. UKALLXII/ECOG2993: addition of imatinib to a standard treatment regimen enhances long-term outcomes in Philadelphia positive acute lymphoblastic leukemia. *Blood*. 2014;123(6):843-850.

2. Chalandon Y, Thomas X, Hayette S, et al. Is less chemotherapy detrimental in adults with Philadelphia chromosome (Ph)-positive acute lymphoblastic leukemia (ALL) treated with high-dose imatinib? Results of the prospective randomized Graaph-2005 study. *Blood*. 2012; 120:Abstract 138.

3. Pfeifer H, Goekbuget N, Volp C, et al. Long-term outcome of 335 adult patients receiving different schedules of imatinib and chemotherapy as front-line treatment for Philadelphia-positive acute lymphoblastic leukemia (Ph+ ALL). *Blood*. 2010;116: Abstract 173.

4. Intermesoli T, Cattaneo C, Pogliani EM, et al. In Philadelphia chromosome-positive acute lymphoblastic leukemia (Ph+ ALL) imatinib (IM) and chemotherapy in combination improve remission rate, stem cell transplantation rate and long-term outcome. *Blood*. 2012;120:Abstract 662.

5. Rousselot P, Coude MM, Huguet F, et al. Dasatinib (Sprycel(R)) and low intensity chemotherapy for first-line treatment in patients with de novo Philadelphia positive ALL aged 55 and over: final results of the EWALL-Ph-01 study. *Blood*. 2012;120:Abstract 666.

6. Ravandi F, Jorgensen JL, Thomas DA, et al. Detection of MRD may predict the outcome of patients with Philadelphia chromosome-positive ALL treated with tyrosine kinase inhibitors plus chemotherapy. *Blood*. 2013; 122(7):1214-1221.

7. Wetzler M, Watson D, Stock W, et al. Autologous transplantation for Philadelphia chromosome-positive acute lymphoblastic leukemia achieves outcomes similar to allogeneic transplantation - results of CALGB Study 10001 (Alliance) (published online ahead of print September 27, 2013). *Haematologica*. doi:10.3324/haematol.2013.085811.

8. Kantarjian H, Thomas D, Wayne AS, O'Brien S. Monoclonal antibody-based therapies: a new dawn in the treatment of acute lymphoblastic leukemia. *J Clin Oncol*. 2012;30(31):3876-3883.

9. Cruz CRY, Micklethwaite KP, Savoldo B, et al. Infusion of donor-derived CD19-redirected virus-specific T cells for B-cell malignancies relapsed after allogeneic stem cell transplant: a phase 1 study. *Blood*. 2013;122(17): 2965-2973.

Comment on Eckly et al, page 921

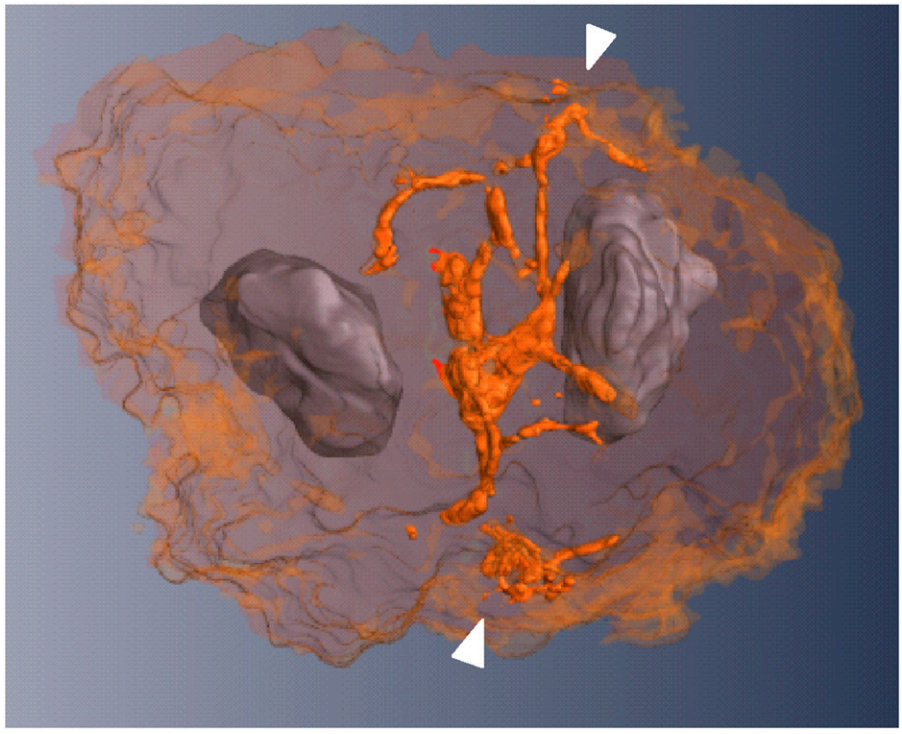
New roads to a megakaryocyte inner territory

Katya Ravid[1] [1]BOSTON UNIVERSITY SCHOOL OF MEDICINE

In this issue of *Blood*, Eckly et al describe megakaryocyte inner membranes' origin and territories, implying an additional role they might play.[1]

Intracellular demarcation membranes have been long recognized in mature megakaryocytes and proposed as a reservoir for platelet biogenesis. A convoluted internal system of membranes within the megakaryocyte, termed demarcation membranes, was observed decades ago, using electron microscopy.[2] An early study,[3] as well as subsequent ones, proposed that this system forms the plasma membrane of newly generated platelets[2,3] and that thrombopoietin promotes the development of these membranes.[4] Live cell imaging of mature megakaryocytes showed that the intracellular demarcation membranes extend from peripheral plasma membranes.[5] However, it has not been clear whether the demarcation membrane system is formed intracellularly and then directed to the plasma membrane or whether it is first delivered to the plasma membrane, followed by rapid invagination. Considering these possible mechanisms, the demarcation membrane system has been also recently referred to as the invaginated membrane system.[6]

Eckly et al use glycoprotein-Ib as a membrane tracer and an array of methods, including confocal microscopy, pulse-chase experiments, and correlative light and electron microscopy to show that the biogenesis of the



Focused ion beam-scanning electron microscopy 3D reconstruction of the predemarcation membrane system (orange) located between the 2 lobes of the nucleus (gray) of an immature megakaryocyte. The white arrows point to the inner membrane system. See Figure 2 in the article by Eckly et al that begins on page 921.

demarcation membrane system starts at focal points of the cell surface. They could capture the very first moments of demarcation membrane formation, which they named the predemarcation membrane system, and determined their 3-dimensional (3D) architecture using dual axis electron tomography and large volume focused ion beam-scanning electron microscopy. Further, the authors found that a growing demarcation membrane system requires, besides invagination of the plasma membrane, insertion of Golgi-derived membrane vesicles and endoplasmic reticulum–demarcation membrane system tethering. Earlier reports showed numerous Golgi stacks targeted to the region of furrow formation during anaphase in mitotic cells, thereby contributing to active membrane delivery. This suggested that as the megakaryocyte increases in size and ploidy, it is also prepared to augment the mass of intracellular membranous territories.

Past studies proposed that the demarcation membranes define platelet territories.[7] Do these membranes play yet unidentified additional roles? In the current study, Eckly et al mapped the location of the predemarcation membranes that start by plasma membrane invaginations, in relation to nuclear material. These membrane structures were observed between the nuclear lobes of polyploid megakaryocytes (see figure), with an intriguing correlation between the number of lobes and the plasma membrane connections. During normal mitosis, the Golgi complexes disassemble and reform during telophase. This process, however, was never studied during megakaryocyte endomitosis. Considering the origin and dynamics of demarcation membrane formation and its localization between nuclear lobes, the authors discuss the interesting hypothesis that these membranes are extended from the Golgi in consortium with endomitosis to aid in control of megakaryocyte endomitosis and polyploidy. What might argue against this contention is the uncoupling reported earlier between the development of demarcation membranes and ploidy acquisition, such as in the case of targeted expression of cyclin D3 to megakaryocytes in vivo,[8] resulting in ploidy level similar to thrombopoietin administration, despite poorer development of demarcation membranes. Further, mutant gunmetal mice exhibit abnormal megakaryocyte demarcation membranes but also an increase in ploidy level.[9] Naturally, each of these cases represents abnormal gene expression that might not mirror the situation in normally developing megakaryocytes. Whether or not the demarcation membranes take part in controlling megakaryocyte endomitosis warrants future examination.

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

REFERENCES

1. Eckly A, Heijnen H, Pertuy F, et al. Biogenesis of the demarcation membrane system (DMS) in megakaryocytes. *Blood*. 2014;123(6):921-930.
2. Behnke O. An electron microscope study of the megacaryocyte of the rat bone marrow. I. The development of the demarcation membrane system and the platelet surface coat. *J Ultrastruct Res*. 1968;24(5):412-433.
3. Yamada E. The fine structure of the megakaryocyte in the mouse spleen. *Acta Anat (Basel)*. 1957;29(3):267-290.
4. Zucker-Franklin D, Kaushansky K. Effect of thrombopoietin on the development of megakaryocytes and platelets: an ultrastructural analysis. *Blood*. 1996;88(5):1632-1638.
5. Mahaut-Smith MP, Thomas D, Higham AB, et al. Properties of the demarcation membrane system in living rat megakaryocytes. *Biophys J*. 2003;84(4):2646-2654.
6. Machlus KR, Italiano JE Jr. The incredible journey: From megakaryocyte development to platelet formation. *J Cell Biol*. 2013;201(6):785-796.
7. Stenberg PE, Levin J. Mechanisms of platelet production. *Blood Cells*. 1989;15(1):23-47.
8. Zimmet JM, Ladd D, Jackson CW, Stenberg PE, Ravid K. A role for cyclin D3 in the endomitotic cell cycle. *Mol Cell Biol*. 1997;17(12):7248-7259.
9. Novak EK, Reddington M, Zhen L, et al. Inherited thrombocytopenia caused by reduced platelet production in mice with the gunmetal pigment gene mutation. *Blood*. 1995;85(7):1781-1789.

● ● ● VASCULAR BIOLOGY

Comment on Dinarvand et al, page 935

PolyP and APC fight a RAGEing battle

Laurent O. Mosnier[1] [1]THE SCRIPPS RESEARCH INSTITUTE

In this issue of *Blood*, Dinarvand and colleagues identify polyphosphate (polyP) as a potent mediator of proinflammatory effects induced by nuclear proteins such as histone H4 and high mobility group box 1 (HMGB1). Coagulation, platelet activation, and inflammation are intricately linked and regulatory mechanisms ensure a balanced response to infection and inflammation. Not only do these observations promote polyP to the ranks of an all-round proinflammatory and procoagulant agent, but also protection by activated protein C (APC) against these proinflammatory effects reveals an intricate battle between polyP and APC that is fought on multiple fronts.[1]

Nuclear proteins such as histones and HMGB1 are increasingly recognized to play an important role in infection and inflammation. Released in the circulation via neutrophil extracellular traps, leaking from necrotic cells, or secreted in response to lipopolysaccharide, these nuclear proteins, or nuclear cytokines, are generally proinflammatory and often cytotoxic to cells as they are recognized by pattern-recognition receptors (PRRs) such as the various Toll-like receptors (TLRs) and the receptor for advanced glycation end products (RAGE).[2,3] In a striking example, histones cause profound platelet activation mediated at least in part by platelet TLRs, resulting in the release of platelet-derived polyP with prohemostatic, prothrombotic, and proinflammatory effects.[4-6]

Previously, the pathophysiologically achievable concentrations of nuclear proteins in the circulation and the concentrations required for cytotoxicity in experimental models seemed to overlap narrowly at best. However, polyP changes this picture. As proposed by Dinarvand et al, polyP promotes binding of HMGB1 (and histone H4) to RAGE, facilitates clustering of oligomeric receptor complexes to initiate signaling, and amplifies their proinflammatory signaling via ligation with polyP-activated $P2Y_1$ receptors.[1] Consequently, polyP reduces the concentrations of HMGB1 or histone H4 that are required to elicit proinflammatory signaling, suggesting that the contributions of these nuclear cytokines to proinflammatory effects may be much more intricate than thus far appreciated.