lead to optimized antibody selection. In addition, CD38 can be further developed as a target for novel antibody drug conjugates and CD38-directed CAR T-cell therapy for T-ALL. Clinical trials testing the efficacy of anti-CD38 antibodies in T-ALL are currently being planned, and we eagerly await the results.

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REFERENCES

1. Bride KL, Vincent TL, Im S-Y, et al. Preclinical efficacy of daratumumab in T-cell acute lymphoblastic leukemia. *Blood*. 2018;131(9):995-999.
2. Hunger SP, Lu X, Devidas M, et al. Improved survival for children and adolescents with acute lymphoblastic leukemia between 1990 and 2005: a report from the children's oncology group. *J Clin Oncol*. 2012;30(14):1663-1669.
3. Coustan-Smith E, Mullighan CG, Onciu M, et al. Early T-cell precursor leukaemia: a subtype of very high-risk acute lymphoblastic leukaemia. *Lancet Oncol*. 2009;10(2):147-156.
4. Marks DI, Rowntree C. Management of adults with T-cell lymphoblastic leukemia. *Blood*. 2017;129(9):1134-1142.
5. Leonard J, Stock W. Progress in adult ALL: incorporation of new agents to frontline treatment. *Hematology Am Soc Hematol Educ Program*. 2017;2017:28-36.
6. van de Donk NWCJ, Richardson PG, Malavasi F. CD38 antibodies in multiple myeloma: back to the future. *Blood*. 2018;131(1):13-29.
7. Patrick K, Wade R, Goulden N, et al. Outcome for children and young people with Early T-cell precursor acute lymphoblastic leukaemia treated on a contemporary protocol, UKALL 2003. *Br J Haematol*. 2014;166(3):421-424.
8. Jain N, Lamb AV, O'Brien S, et al. Early T-cell precursor acute lymphoblastic leukemia/lymphoma (ETP-ALL/LBL) in adolescents and adults: a high-risk subtype. *Blood*. 2016;127(15):1863-1869.
9. de Weers M, Tai YT, van der Veer MS, et al. Daratumumab, a novel therapeutic human CD38 monoclonal antibody, induces killing of multiple myeloma and other hematological tumors. *J Immunol*. 2011;186(3):1840-1848.
10. Ito M, Hiramatsu H, Kobayashi K, et al. NOD/SCID/gamma(c)(null) mouse: an excellent recipient mouse model for engraftment of human cells. *Blood*. 2002;100(9):3175-3182.

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PLATELETS AND THROMBOPOIESIS

Comment on Mayer et al, page 1000

BEACHcombing for α-granules

Sidney W. Whiteheart | University of Kentucky College of Medicine

In this issue of *Blood*, Mayer et al illuminate the molecular mechanisms of platelet α-granule biogenesis and the molecular defects of gray platelet syndrome (GPS) through the identification of the Nbeal2 interactome.[1]

GPS (MIM 139090) is an autosomal recessive disorder associated with macrothrombocytopenia, splenomegaly, myelofibrosis, heterogeneous bleeding, and defective platelet α-granules.[2] The gene mutated in GPS encodes the neurobeachin-like 2 (Nbeal2) protein, which is 1 of 9 Beige and Chediak-Higashi (BEACH) domain–containing proteins (BDCPs).[3] The BEACH domain (~300 amino acids) was named for the charter family member lysosomal trafficking regulatory/Chediak-Higashi syndrome 1 (LYST/CHS1) protein, which is defective in the Beige mouse and in human Chediak-Higashi syndrome. Most BDCPs also contain membrane-binding domains (eg, pleckstrin homology [PH] or Fab1, YOTB, Vac1, and EEA1 [FYVE] zinc fingers) and domains that support multiprotein complex formation (WD40 repeats). BDCPs are physiologically critical, because mutations in 4 of them cause autosomal recessive diseases. They are linked to several membrane-related processes (eg, lysosome size regulation, synaptosome formation, cilium maintenance, and autophagy). In platelets, LYST and Nbeal2 are needed for dense and α-granule biogenesis, respectively. Despite our wealth of knowledge, the identity of specific BDCP-interacting partners and their mechanisms of action remain unclear.

To gain insights into BDCPs, Mayer et al identified several new proteins that interact with Nbeal2. By using the tandem affinity purification method with a truncated form of Nbeal2 (consisting of PH, BEACH, and WD40 domains) as bait in HEK293 cells, they purified Nbeal2-interacting proteins and identified them by mass spectrometry. This interactome was further characterized by using bioinformatic methods, and 129 potential Nbeal2 interactors were defined. Ten were chosen for further validation by reverse immunoprecipitation and ligation proximity assays in a megakaryoblastic leukemia cell line. Three proteins, Sec16a (a protein involved in endoplasmic reticulum [ER] exit sites), Dock7 (a guanine nucleotide exchange factor [GEF] for Rac/Cdc42), and Vac14 (a regulator of phosphatidylinositol (3,5) bisphosphate [PI(3,5)P_2] metabolism), were confirmed as interactors and were further analyzed. Binding by Sec16a required the WD40 domain of Nbeal2 whereas Dock7 and Vac14 binding required only the PH and BEACH domains. Five GPS-causing mutations in the BEACH domain negatively affected Vac14 and Dock7 binding. Intriguingly, when NBEAL2$^{-/-}$ platelets were examined, there was a significant reduction in Dock7, although its messenger RNA levels were unchanged. These data suggest that Nbeal2 is required for Dock7 stability and/or targeting. Consistent with the role of Dock7 as an Rac/Cdc42 GEF, NBEAL2$^{-/-}$ platelets showed dysregulation of cofilin phosphorylation, F-actin formation, and spreading.

What do these new interactions tell us about how Nbeal2 works? Originally identified in yeast as being required for constitutive vesicular transport, Sec16a is thought to organize ER exit sites for proteins transported from the ER to the Golgi apparatus. Sec16a is found in cup-like structures in the transitional ER adjacent to coat protein II (COP II) formation sites. It interacts with COP II coat components Sec23A, and Sec13.[4] Given the localization of Sec16a, its interaction with Nbeal2 implies an early role for the BDCP, perhaps in granule cargo sorting at the ER of megakaryocytes. Because Nbeal2 is largely cytoplasmic, it is possible that it alternates between compartments selecting cargo at the ER and subsequently guiding its transport to nascent granules. Studies with Nbeal2 that lacks WD40 might be informative to probe the role of the Sec16a/Nbeal2 interaction.

Dedicator of cytokinesis 7 (DOCK 7) is a member of the DOCK180 superfamily

of Rac/Cdc42 GEFs. Rac/Cdc42 proteins are small guanosine-5′-triphosphate–binding proteins known to be important for platelet function but are mainly important for their roles in controlling the cytoskeleton. Dock7 is a member of the DOCK-C subfamily and contains 2 family-defining domains (DHR-1 and DHR-2).[5] DHR-1 mediates binding to PI(3,5)P_2 and localization to membranes. DHR-2 is important for GEF activity. Interestingly, misty and moonlight hypopigmentation mice are both the result of mutations in Dock7 that cause truncations either inside (misty) or downstream (moonlight) of DHR-1.[5,6] Although these animals have been studied for their defects in neurogenesis, misty mice have a bleeding diathesis, which mimics the NBEAL2$^{-/-}$ phenotype.[6] Moonlight mice do not have that phenotype.[5] This convergence of phenotypes supports the physiological relevance of the Nbeal2/Dock7 interaction; however, it is unclear how this Rac/Cdc42 GEF affects granule biogenesis. The interaction could simply mediate Dock7 transport to platelets to affect Rac/Cdc42 signaling and play no role in cargo sorting. Alternatively, Dock7 could mediate correct localization of Nbeal2. Further analysis of platelets and megakaryocytes from misty and moonlight mice may help clarify how or whether Dock7 affects α-granules.

The third confirmed Nbeal2 interactor, Vac14 (also known as associated regulator of PIKfyve [ArPIKfyve]), is important for regulating PI(3,5)P_2 metabolism. Its interactome is linked to endosomal and autophagic pathways.[7] With the lipid phosphatase Fig4, Vac14 regulates PIKfyve (phosphoinositide kinase for five position containing a fyve finger) activity and thus the levels of PI(3,5)P_2.[8] Although the global knockout of Vac14 is lethal at the perinatal stage,[9] an Leu156Arg mutant is viable with reduced lifespan, hypopigmentation, and altered PI(3,5)P_2 levels. Cells that lack Vac14 show increased vacuolization, suggesting some defect in organelle biogenesis.[8] Mutations in Vac14 and Fig4 are causative of Yunis-Varón syndrome, an autosomal recessive disorder characterized by intracytoplasmic vacuolization.[10] Platelet function has not been studied in patients with this disease. Because the control of phosphatidylinositide lipids and their localization is very important for endosomal trafficking, the interaction of Vac14 with Nbeal2 may yield mechanistic insights on how phosphatidylinositides contribute to α-granule cargo sorting.

As with most good experiments, the data presented by Mayer et al generate more questions than answers. Their initial Nbeal2 interactome suggests a host of new experiments for probing platelet granule biogenesis and for better understanding granulopathies. There are also questions about the approach used that need clarification. Were other interactors missed because the tandem affinity purification bait lacked the concanavalin A-like domain of Nbeal2? Could the purification approach be used in megakaryocytes instead of HEK293 cells and would that yield a different interactome? Are the interacting proteins part of larger complexes much like the BLOC complexes, which control dense granule biogenesis? Is Nbeal2 needed for Dock7 sorting or is Dock7 mediating a Rac/Cdc42-dependent step required for granule biogenesis or both? Given that Vac14 is so critical to endosomes and autophagosomes, how important are these organelles to α-granule biogenesis? Finally, what role does PI(3,5)P_2 play in granule cargo sorting? Both Dock7 and Vac14 have connections to this phospholipid. The data of Mayer et al have given us a valuable cast of new characters whose next act in the GPS story is about to begin.

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REFERENCES

1. Mayer L, Jasztal M, Pardo M, et al. Nbeal2 interacts with Dock7, Sec16a, and Vac14. *Blood*. 2018;131(9):1000-1011.
2. Gunay-Aygun M, Zivony-Elboum Y, Gumruk F, et al. Gray platelet syndrome: natural history of a large patient cohort and locus assignment to chromosome 3p. *Blood*. 2010;116(23):4990-5001.
3. Cullinane AR, Schäffer AA, Huizing M. The BEACH is hot: a LYST of emerging roles for BEACH-domain containing proteins in human disease. *Traffic*. 2013;14(7):749-766.
4. Iinuma T, Shiga A, Nakamoto K, et al. Mammalian Sec16/p250 plays a role in membrane traffic from the endoplasmic reticulum. *J Biol Chem*. 2007;282(24):17632-17639.
5. Blasius AL, Brandl K, Crozat K, et al. Mice with mutations of Dock7 have generalized hypopigmentation and white-spotting but show normal neurological function. *Proc Natl Acad Sci USA*. 2009;106(8):2706-2711.
6. Sviderskaya EV, Novak EK, Swank RT, Bennett DC. The murine misty mutation: phenotypic effects on melanocytes, platelets and brown fat. *Genetics*. 1998;148(1):381-390.
7. Schulze U, Vollenbröker B, Braun DA, et al. The Vac14-interaction network is linked to regulators of the endolysosomal and autophagic pathway. *Mol Cell Proteomics*. 2014;13(6):1397-1411.
8. Jin N, Chow CY, Liu L, et al. VAC14 nucleates a protein complex essential for the acute interconversion of PI3P and PI(3,5)P(2) in yeast and mouse. *EMBO J*. 2008;27(24):3221-3234.
9. Zhang Y, Zolov SN, Chow CY, et al. Loss of Vac14, a regulator of the signaling lipid phosphatidylinositol 3,5-bisphosphate, results in neurodegeneration in mice. *Proc Natl Acad Sci USA*. 2007;104(44):17518-17523.
10. Lines MA, Ito Y, Kernohan KD, et al; Care4Rare Consortium. Yunis-Varón syndrome caused by biallelic VAC14 mutations. *Eur J Hum Genet*. 2017;25(9):1049-1054.

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RED CELLS, IRON, AND ERYTHROPOIESIS

Comment on Guilliams et al, page 1012

Unwinding the path from anemia to stroke

John C. Wood | Children's Hospital of Los Angeles

In this issue of *Blood*, Guilliams et al demonstrate that white matter is chronically hypoxic in sickle cell disease and that transfusions acutely lower the volume of brain tissue at risk for stroke.[1]

In the past 2 decades, regular transcranial Doppler screening, chronic transfusion, and liberal hydroxyurea utilization have dramatically reduced cerebral vasculopathy and childhood ischemic stroke. However, the mechanisms by which transfusions affect stroke risk are largely unknown. The authors use a novel magnetic resonance imaging (MRI) technique known as asymmetric spin echo (ASE) to probe brain oxygenation in 3 cohorts of patients with sickle cell disease (SCD). ASE exploits the different magnetic properties of oxygenated and deoxygenated