and telomere attrition.[1] Through an initial screening of 835 known genes in 57 discovery cases, followed by focused sequencing of detected targets in 93 extended cases, a total of 32 somatic mutations typically seen in MDS and other myeloid malignancies were detected in 29 of 150 patients (19%), where the predominant mutational targets included *ASXL1*, *DNMT3A*, and *BCOR*. Transformation to MDS occurred in 17 cases, which included 11 of the 29 mutation (+) cases, or 7 of 12 *ASXL1*-mutated, 3 of 8 *DNMT3A*-mutated, and 1 of 6 *BCOR*-mutated cases. Importantly, somatic mutations were significantly associated with longer disease duration, that is, time from diagnosis to sample collection (37 vs 8 months, $P < .04$), shorter telomere lengths (median telomere-to-single copy gene ratio length, 0.9 vs 1.1, $P < .001$), and a higher rate of progression to MDS/AML (38% [11 of 29] vs 5.0% [6 of 121], $P < .001$).

Clonal hematopoiesis in AA has long been discussed based on the presence of cytogenetic abnormalities,[6] skewed X-chromosome inactivation in female patients,[7] appearance of varying degrees of blood cells having paroxysmal nocturnal hemoglobinuria phenotypes,[2] and more recently, recurrent uniparental disomy (UPD) in the 6p arm commonly involving the class I HLA locus.[8] It has been speculated that the clonal hematopoiesis may be derived from some "bottleneck" effect caused by hematopoietic repopulation from a severely reduced number of hematopoietic stem cells,[2] or represent escaped hematopoiesis from autoimmunity, especially in 6pUPD(+) cases[8] and/or premalignant hematopoiesis as has been demonstrated recently in AML.[9] The current study, together with a previous report by Lane et al,[10] demonstrated that clonal hematopoiesis in AA frequently accompanies somatic mutations commonly seen in MDS/AML and also presented the first implication that these "MDS/AML-like" somatic mutations predict a substantial risk for progression to MDS/AML (~40%). The findings not only provide intriguing insight into the relationship between clonal hematopoiesis and malignant transformation in AA, but also have significant clinical relevance in terms of choice of therapies, such as early use of allo-HSCT for mutation (+) cases. On the other hand, a number of important issues are raised with regard to the origin and chronological behavior of these mutated clones that frequently heralded clinically relevant MDS. The higher frequency of mutations in patients with longer disease duration and the trend of larger clone sizes and shorter telomere lengths thereof may indicate clonal dominance of the mutated clones over time. However, to identify the exact origin of mutations and their temporal behavior, analysis of carefully fractionated cells using serially collected samples from the diagnosis to overt MDS would be needed. Also, the entire picture of clonal hematopoiesis in AA and its pathogeneic/clinical significance could be better delineated through more exhaustive detection of somatic mutations in an unbiased way, using whole genome/exome sequencing.

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

REFERENCES

1. Kulasekararaj AG, Jiang J, Smith AE, et al. Somatic mutations identify a subgroup of aplastic anemia patients who progress to myelodysplastic syndrome. *Blood*. 2014;124(17):2698-2704.

2. Young NS, Maciejewski J. The pathophysiology of acquired aplastic anemia. *N Engl J Med*. 1997;336(19): 1365-1372.

3. Doney K, Pepe M, Storb R, et al. Immunosuppressive therapy of aplastic anemia: results of a prospective, randomized trial of antithymocyte globulin (ATG), methylprednisolone, and oxymetholone to ATG, very high-dose methylprednisolone, and oxymetholone. *Blood*. 1992;79(10):2566-2571.

4. Socié G, Henry-Amar M, Bacigalupo A, et al; European Bone Marrow Transplantation-Severe Aplastic Anaemia Working Party. Malignant tumors occurring after treatment of aplastic anemia. *N Engl J Med*. 1993;329(16):1152-1157.

5. Calado RT. Immunologic aspects of hypoplastic myelodysplastic syndrome. *Semin Oncol*. 2011;38(5):667-672.

6. Mikhailova N, Sessarego M, Fugazza G, et al. Cytogenetic abnormalities in patients with severe aplastic anemia. *Haematologica*. 1996;81(5):418-422.

7. Josten KM, Tooze JA, Borthwick-Clarke C, Gordon-Smith EC, Rutherford TR. Acquired aplastic anemia and paroxysmal nocturnal hemoglobinuria: studies on clonality. *Blood*. 1991;78(12):3162-3167.

8. Katagiri T, Sato-Otsubo A, Kashiwase K, et al; Japan Marrow Donor Program. Frequent loss of HLA alleles associated with copy number-neutral 6pLOH in acquired aplastic anemia. *Blood*. 2011;118(25): 6601-6609.

9. Shlush LI, Zandi S, Mitchell A, et al; HALT Pan-Leukemia Gene Panel Consortium. Identification of pre-leukaemic haematopoietic stem cells in acute leukaemia [published correction appears in *Nature*. 2014;508(7496):420]. *Nature*. 2014;506(7488): 328-333.

10. Lane AA, Odejide O, Kopp N, et al. Low frequency clonal mutations recoverable by deep sequencing in patients with aplastic anemia. *Leukemia*. 2013;27(4): 968-971.

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● ● ● PLATELETS & THROMBOPOIESIS

Comment on Potts et al, page 2725

Diploid, not polyploid: new platelet producers

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In this issue of *Blood*, Potts et al have identified a unique cell population in the yolk sac (YS) as the source of the first platelet-forming cells in mouse embryos. These cells are diploid and are produced via a pathway independent of hematopoietic progenitor cells (HPCs) generating polyploid megakaryocytes (MKs).[1]

When and how hematopoiesis emerges and proceeds are still intriguing questions. Despite remarkable advances in this field,[2] details of blood cell development are still largely veiled in mystery. Understanding the mechanism by which hematopoietic cells are generated is critical and could lead to novel strategies for the making of functional hematopoietic cells in vitro for regenerative medicine using not only cord blood hematopoietic stem and progenitor cells but also pluripotent stem cells such as embryonic stem (ES) and induced pluripotent stem (iPS) cells.[3,4]

Mature MKs derived from HPCs, which are characterized by polyploid nuclei, have been thought to generate platelets from the beginning throughout life. However, Potts et al have discovered previously unrecognized diploid cells in the YS as the first platelet-forming cells, which are distinct from polyploid MKs. These unexpected findings provide novel insights into our understanding of the

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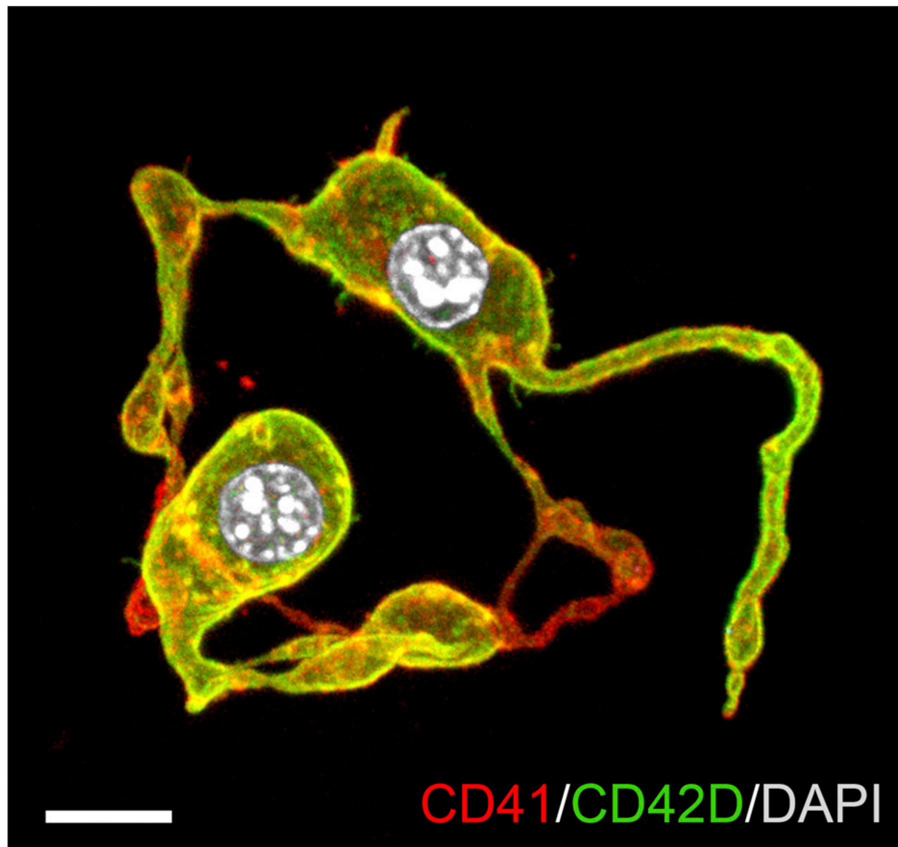

Representative confocal z-stack of diploid platelet-forming cells (DPFCs) showing proplatelet formation. E10.5 YS CD45$^-$CD41^{high} cells were cultured in serum-free medium with thrombopoietin for 72 hours. Cultures were stained with anti-CD41 and anti-CD42D antibodies. Nuclei were stained with 4,6-diamidino-2-phenylindole (DAPI). Scale bar represents 10 μm. See Figure 1H in the article by Potts et al that begins on page 2725.

development of platelet-producing cells and therapeutic control of them.

Primitive hematopoiesis occurs in the YS on embryonic day (E) 7 in mice. First MK progenitors, which are immunophenotypically CD45$^+$CD41^{low}, could be detected as early as E7.5 in the YS.[5] These progenitors are derived from conventional HPCs and differentiate into mature polyploid MKs, which undergo proplatelet formation. Because MKs are first detected at E8.5 to E9.5 YS,[5,6] these MK progenitors have been thought to mature rapidly and give rise to platelets until definitive hematopoiesis begins to produce platelets. However, Potts et al found that these conventionally identified MK progenitors on as early as E8.5 YS do not mature into proplatelet-forming cells within 72 hours in culture. They also found that platelets are released into the bloodstream as early as E9.5, then dramatically increase in number by E10.5.[1] Based on these findings, they hypothesized that an alternative cell population is responsible for initial platelet formation.

To this end, the investigators compared the transcriptional profiles of E10.5 YS cell fractions with E13.5 fetal liver reference fractions. Unexpectedly, the profile of previously uncharacterized CD45$^-$CD41^{high} cells in the YS most resembled that of E13.5 fetal liver MKs. This population indeed expressed MK-associated proteins including myeloproliferative leukemia protein, CD42D (glycoprotein V), and acetylcholinesterase. Although the majority of CD45$^-$CD41^{high} cells remained diploid and did not exhibit the high ploidy range associated with MKs, half of them formed proplatelets within 72 hours in culture (see figure) and, surprisingly, also did so in vivo while in a diploid state. They therefore defined this fraction as DPFCs.

CD41$^+$ cells in the YS at E7.75 to E8.25 express vascular endothelial cadherin (VECAD) and then by E8.5 diverge into VECAD$^+$ and VECAD$^-$ fractions. Potts et al clarified that at E8.5 VECAD$^+$CD41^{high} cells contain all HPC progeny (myeloid/erythroid and MK progenitors), whereas VECAD$^-$CD41^{high} cells contain immature DPFC precursors (pre-DPFCs) capable of acute proplatelet formation. They finally performed proplatelet formation assays using *Runx1*-null YS cells, in which HPC formation is completely blocked[7] to address the next question of whether HPCs and DPFCs both progress via VECAD-positive precursors, that is, if HPCs give rise to DPFCs. VECAD$^-$CD41^{high} pre-DPFCs capable of generating proplatelets in vitro were detected even in *Runx1*-null E8.5 YS. Furthermore, although fewer than wild type, there existed pre-DPFCs and DPFCs producing proplatelets in the E8.5 and E10.5 $Runx1^{\Delta/\Delta}$ YS, respectively. All these data summarily suggested that DPFCs develop independently of HPCs.

Now, newly identified DPFCs have been proved to play a role in hematopoietic development. DPFCs are quite unique in their rapid terminal differentiation, which is achieved by skipping endomitosis, a critical process for MKs to increase ploidy. Given the requirement of acute platelet production to supply platelets timely upon initiation of embryonic circulation, rapid platelet production at the expense of the amount of platelet production per single producer cell seems reasonable. However, we do not know when and how HPCs and DPFCs diverge and how the platelet production by DPFCs is regulated. The investigators showed that DPFCs produce platelets in a thrombopoietin-independent manner. What are the alternative regulators? Furthermore, when do conventional MKs take over platelet production instead of DPFCs in fetus? Many exciting questions remain to be answered. Future studies will undoubtedly deepen our understanding of embryonic hematopoiesis, especially the mechanisms of how the first hematopoiesis arises.

Finally, what kind of impact do DPFCs have on ex vivo expansion of platelets? Extensive efforts have been taken to improve the efficiency of platelet production, particularly from ES/iPS cells.[3,4,8] One of the rate-limiting steps on ex vivo generation of platelets is MK maturation. The degree of MK polyploidization is correlated with the amount of platelet production. However, hematopoietic progenies derived from ES/iPS cells largely exhibit characters of primitive but not definitive blood cells and often recapitulate YS hematopoiesis. It will therefore be very interesting to examine

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whether platelet production by ES/iPS cells follows the DPFC pathway or HPC-derived MK pathway or both switching in a time-dependent manner. These approaches should give a hint to improve the therapeutic manipulation of ES/iPS cells for platelet production.

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REFERENCES

1. Potts KS, Sargeant TJ, Markham JF, et al. A lineage of diploid platelet-forming cells precedes polyploid megakaryocyte formation in the mouse embryo. *Blood*. 2014;124(17):2725-2729.
2. Ciau-Uitz A, Monteiro R, Kirmizitas A, Patient R. Developmental hematopoiesis: ontogeny, genetic programming and conservation. *Exp Hematol*. 2014;42(8):669-683.
3. Avanzi MP, Mitchell WB. Ex vivo production of platelets from stem cells. *Br J Haematol*. 2014;165(2):237-247.
4. Togarrati PP, Suknuntha K. Generation of mature hematopoietic cells from human pluripotent stem cells. *Int J Hematol*. 2012;95(6):617-623.
5. Xu M, Matsuoka S, Yang FC, et al. Evidence for the presence of murine primitive megakaryocytopoiesis in the early yolk sac. *Blood*. 2001;97(7):2016-2022.
6. Tober J, Koniski A, McGrath KE, et al. The megakaryocyte lineage originates from hemangioblast precursors and is an integral component both of primitive and of definitive hematopoiesis. *Blood*. 2007;109(4):1433-1441.
7. North TE, de Bruijn MF, Stacy T, et al. Runx1 expression marks long-term repopulating hematopoietic stem cells in the midgestation mouse embryo. *Immunity*. 2002;16(5):661-672.
8. Nakamura S, Takayama N, Hirata S, et al. Expandable megakaryocyte cell lines enable clinically applicable generation of platelets from human induced pluripotent stem cells. *Cell Stem Cell*. 2014;14(4):535-548.

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● ● ● RED CELLS, IRON, & ERYTHROPOIESIS

Comment on Hahn and Lowrey, page 2730

Stress-enhanced translation of γ-globin mRNA

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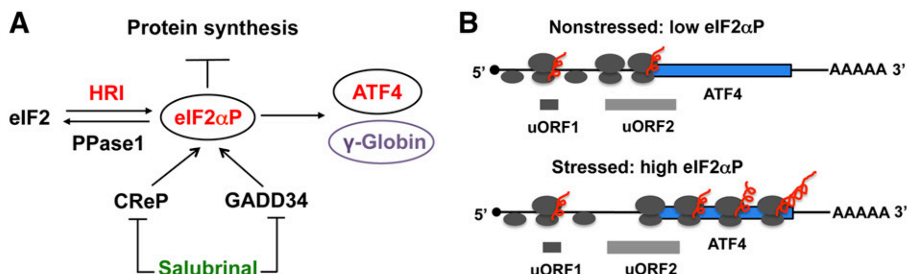
In this issue of *Blood*, Hahn and Lowrey demonstrate enhanced translation of γ-globin messenger RNA (mRNA) upon activation of eukaryotic initiation factor 2αP (eIF2αP) stress signaling. This finding underscores translational control as a novel mechanism in regulating fetal hemoglobin production (HbF).[1]



Selective enhancement of translation of activating transcription factor 4 (ATF4) and γ-globin mRNAs by eIF2αP in erythroid precursors. eIF2 is a heterotrimeric protein complex, which binds initiating methionyl transfer RNA and the 40S ribosomal subunit to start nascent polypeptide synthesis. HRI is the major eIF2α kinase in the erythroid lineage, and is indispensable in coordinating heme and globin synthesis as well as in combating oxidative stress. Phosphorylation of the α-subunit of eIF2 by HRI impairs the recycling of eIF2 for another round of initiation and thus inhibits translation of the vast majority of mRNAs. However, eIF2αP also selectively increases the translation of ATF4 mRNA. (A) In the 5′UTR of ATF4 mRNA, there are 2 uORFs that are preferentially translated under nonstressed conditions and prevent the downstream translational initiation in the coding sequence of ATF4 mRNA. As initiating 40S ribosomal subunits scan from the cap structure, translation starts at the uORF1. After termination of translation, the 40S subunit remains associated with mRNA and reinitiates efficiently at uORF2 under nonstressed conditions. Upon stress, elevated eIF2αP impairs the reinitiation of 40S at uORF2 due to limiting functional eIF2. Thus, 40S continues to scan downstream and initiates at the AUG codon of the coding sequence of ATF4 mRNA permitting the synthesis of ATF4 protein. (B) The homeostasis of the cellular eIF2αP level is controlled not only by the activation of HRI, but also by dephosphorylation of eIF2αP by PPase1 in order to regenerate active eIF2. CreP and GADD34 are the 2 regulatory proteins that recruit eIF2αP to PPase1 for dephosphorylation. Salubrinal, a small chemical molecule, is a selective inhibitor of dephosphorylation of eIF2αP by interfering with the recruitment of eIF2αP to PPase1. Thus, treatment of cells with salubrinal results in an increased eIF2αP level. In differentiating human erythroid cells, γ-globin translation is increased upon salubrinal treatment as shown in this *Blood* article.

Persistent HbF expression is known to lessen the severity of β-thalassemia and sickle cell anemia (SCA),[2,3] 2 of the most prevalent β-hemoglobinopathies. Reactivation of HbF has since been an active area of research. These research efforts have led to the discovery of hydroxyurea (HU) as a successful treatment of some, but not all, patients with SCA and β-thalassemia.[4] Recent discoveries of Bcl11A as a transcription repressor of HbF expression and a silencer of γ-globin expression during development[5] have reinvigorated the field of globin switching and reactivation of HbF. To date, the majority of these studies have been focused on the transcriptional regulation with anticipation of discovering new therapeutic targets for treating β-thalassemia and SCA.[6] Last year, Hahn and Lowrey reported that induction of γ-globin expression could also be achieved posttranscriptionally via phosphorylation of eIF2α.[7] In the current article, they have extended this study further and demonstrated that the activation of fetal globin occurs at the level of translation.

During late-stage erythroid differentiation, heme-regulated eIF2α kinase (HRI) is predominant and responsible for >90% of eIF2α phosphorylation (see figure, panel A).[8] The steady state of eIF2αP in vivo is regulated by the equilibrium of eIF2α kinases and eIF2αP phosphatase (PPase1). Salubrinal is a selective inhibitor of eIF2αP dephosphorylation by interfering with the recruitment of eIF2αP to PPase1 through growth arrest and DNA damage–inducible protein 34 (GADD34) and constitutive repressor of eIF2α phosphorylation (CReP). Hahn and Lowrey found that treatment of differentiating human CD34$^+$ cells with salubrinal did not affect either stability or the cytoplasmic-to-nuclear ratio of γ-globin or β-globin mRNA. They further investigated the effect of salubrinal on translation of globin mRNAs by polysome profiling, which measures the loading of ribosomes onto specific mRNAs and thus the translational efficiency of each mRNA. At 24 hours of salubrinal treatment, eIF2αP returned to near basal level of untreated controls. During this time, it was observed that both γ-globin and β-globin mRNAs from salubrinal-treated cells were translated more efficiently relative to actin mRNA. This was revealed by the shift of globin mRNAs toward the heavier polyribosomes after salubrinal treatment. By comparing the ratios of

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