in Inaba et al). This represents an intriguing new paradigm in malignant transformation and also adds additional complexity to dissecting the disease pathogenesis in −7/del(7q).

Based on their literature review, Inaba et al hypothesize that −7/del(7q) can be an early event in HSPCs. They picture 2 scenarios: (1) an unperturbed environment and (2) a perturbed environment. They reason that in scenario 1 (in a normal HSPC and normal bone marrow), the −7/del(7q) clone has a relative growth advantage over normal HSPCs in the bone marrow and sequentially acquires secondary genetic or epigenetic events. In scenario 2, during bone marrow failure, hematopoietic stem cells are embedded in an inflammatory environment (cytokines). Here, aneuploid stem cells with haploinsufficiency of multiple genes implicated in the regulation of DNA damage checkpoints, the cell cycle, and apoptosis facilitate the accumulation of additional mutations and aberrant expansion, ultimately leading to leukemogenesis.

Notably, data obtained so far are correlative. Dissecting how abnormalities affecting large chromosomal regions mechanistically give rise to distinct cancers is challenging. Future efforts need to focus on validating findings in greater numbers of patients, in addition to identifying more definitive causal relationships between genes and function. Additional single-cell studies and gene editing using CRISPR/Cas9 in HSPCs will be instrumental in delineating how distinct chromosomal abnormalities interact with additional gene mutations to determine the stepwise transformation to leukemia. These studies will also help in dissecting gene targets for targeted therapies.

Conflict-of-interest disclosure: The authors declare no competing financial interests. ■

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DOI 10.1182/blood-2018-04-844746

RED CELLS, IRON, AND ERYTHROPOIESIS

Comment on Huang et al, page 2955

Lipid metabolism in terminal erythropoiesis

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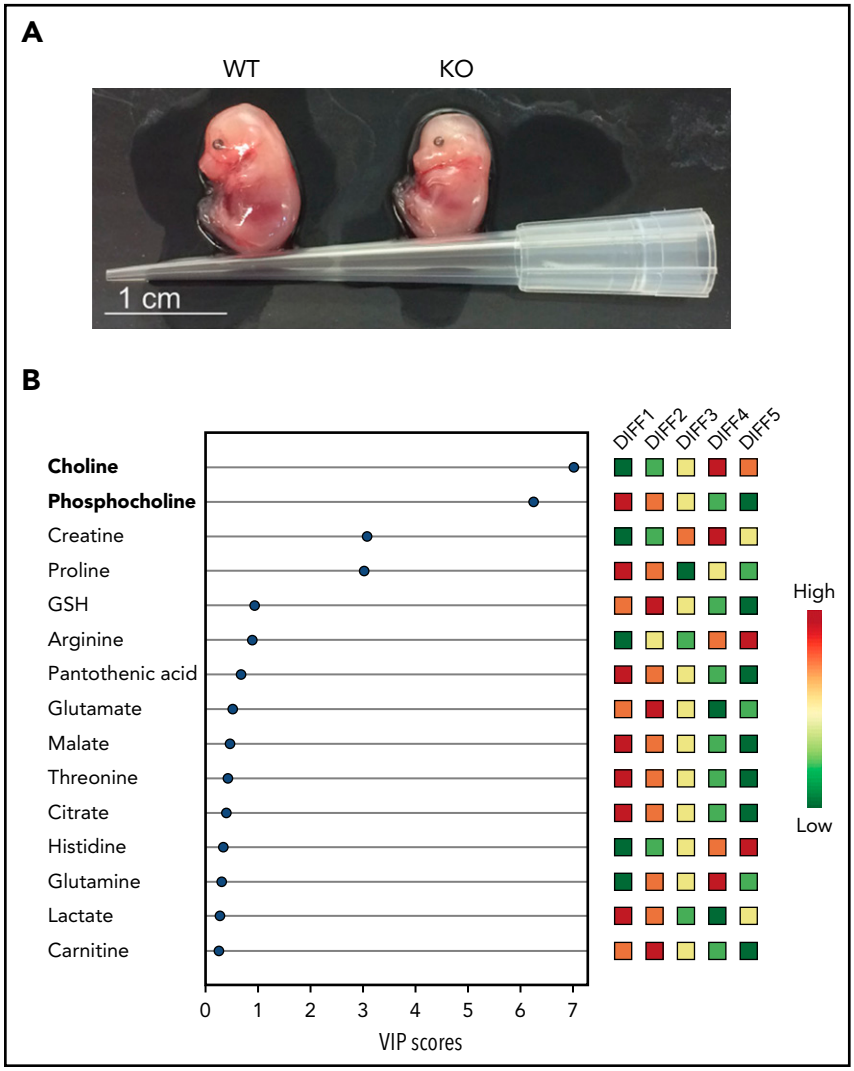
In this issue of *Blood*, Huang et al have provided evidence that altered lipid metabolism is critical for terminal erythropoiesis. A key role is proposed for the *PHOSPHO1* gene product, a phosphocholine phosphatase. *PHOSPHO1* knockouts (KOs) showed reduced erythroblast proliferation and enucleation in both mice and human erythroid tissues, apparently through energy depletion mediated via inhibition of oxidative phosphorylation of fatty acids and reduced adenosine triphosphate (ATP) production in late glycolysis. This work emphasizes that altered expression of genes involving lipid metabolism are important during late red cell maturation.[1]

The mature red cell is unique. Although highly specialized for gas transport, it is much more than an inert receptacle for hemoglobin, with many surprisingly sophisticated properties. Among these, the subtle control of glucose metabolism, cytoskeletal integrity, and membrane permeability by oxygen tension has recently been elucidated.[2] During maturation, the developing red cell must both proliferate and undergo considerable modifications to acquire the necessary properties to survive in the circulatory system, where it lacks the ability to synthesize proteins de novo while experiencing profound challenges such as repeated episodes of shear and oxidative stress. Many important changes occur during later erythrogenesis, including loss of the nucleus, shedding of surface markers, establishment of the final surface area/volume ratio, and establishment of a robust but malleable cytoskeleton.[3]

Our understanding of the processes occurring during erythropoiesis remains partial. Much is known about globin gene switching, which is of particular relevance to a number of the common hemoglobinopathies.[4] Some other nonglobin protein changes have also been well studied. These include accumulation of cytoskeletal elements with condensation of spectrin, increased expression of band 3, and acquisition of other requisite membrane transporters.[3] Mutations in these proteins are relatively rare, but are sometimes associated with hemolytic anemia and irregularities in red cell shape or volume (such as stomatocytes and spherocytes).[5] Elucidation of their molecular causes continues to improve our understanding of red cell physiology.

Diseases involving altered lipid metabolism are arguably less well characterized. As for those involving protein transporters, they can be secondary (ie, subsequent to other diseases). An obvious example here is loss of aminophospholipid asymmetry in a number of hemoglobinopathies such as sickle cell disease.



Changes in lipid metabolism are essential for terminal erythropoiesis in mice and humans. (A) Photographs of E14.5 WT and *PHOSPHO1* KO mice showing impaired growth and pallor of the mutants, highlighting the importance of phosphocholine metabolism in later red cell maturation. (B) Human CD34$^+$ cells differentiated using a 5-stage in vitro culture, following which lipid metabolites were extracted and analyzed. Relative amounts of polar metabolites (colored boxes, right) with VIP scores (bottom) indicate loss of phosphocholine and increase in choline toward the end of erythroid differentiation. VIP, variable importance in projection; WT, wild-type. See Figures 2B and 6A in the article by Huang et al that begins on page 2955.

Primary disturbances involving specific gene mutations directly involved in lipid metabolism have been described but are much rarer. Examples include neuroacanthocytosis and phytosterolemia, which result in abnormalities of erythropoiesis and in acanthocytosis and stomatocytic hemolysis, respectively, in addition to their more obvious presenting clinical complications.[6,7]

Lodish and colleagues have a long track record in cellular and developmental biology, including erythropoiesis. Their current work identifies an additional lipid pathway important for normal red cell development. Subpopulations of developing mouse erythroblasts were separated into 4 terminal stages and were analyzed individually, showing marked variation in lipid content and metabolism. In particular, phosphocholine and its precursor phosphatidylcholine were downregulated during terminal differentiation, whereas sphingomyelin and choline were upregulated and catabolic end products of phosphatidylcholine metabolism became enriched.

By analyzing genes upregulated during terminal differentiation, only 1 pertinent to phosphatidylcholine metabolism was identified, *PHOSPHO1*. Using short hairpin RNA, Huang et al reduced expression of *PHOSPHO1* in mouse fetal erythroid progenitors and found deficiencies in terminal erythroblast differentiation. Cell proliferation and enucleation were reduced, fetal livers showed fewer mature red cells, late embryos were smaller and paler while reticulocytosis was increased (see figure panel A). When early red cells from fetal livers of KO mice were induced to differentiate into erythrocytes in vitro, genes usually upregulated showed reduced expression levels. The number of enucleation events was smaller. Surprisingly, red cell count and volume in postpartum mice were largely normal. However, reticulocytosis suggested that this was probably subsequent to a compensatory stress erythropoiesis. *PHOSPHO1* KO mice were also less able to respond to pharmacologically induced stress erythropoiesis using phenylhydrazine. Taken together, these findings suggest that *PHOSPHO1* is important for both normal erythropoiesis and response to anemia.

Huang et al went on to investigate the function of *PHOSPHO1* in terminal erythropoiesis. In KO mice, accumulation of lipid, reduction in ATP/adenosine 5′-monophosphate ratio, AMPK activation, with reduced levels of oxidative respiration and increased glycolytic flux, were all observed, which indicatives energy depletion, probably through reduction in the supply of lipids for oxidative phosphorylation, together with a greater dependence on anaerobic metabolism. The authors hypothesized that inhibition of phosphatidylcholine and phosphocholine metabolism following *PHOSPHO1* KO would lower levels of amino acids glycine and serine, which are essential to normal protein synthesis and are partly supplied through lipid metabolism. Cells would therefore be more dependent on a glycolytic shunt from 3-phosphoglycerate for synthesis of these amino acids. The net effect would be a concurrent reduction in ATP formation from the terminal steps of glycolysis (via pyruvate kinase). Elevated expression of the genes involved in this glycolytic shunt, encoding phosphoserine phosphatase and serine hydroxymethyltransferase, was consistent with their postulate, while glycine or serine supplementation to KO cells, to support/protect glycolysis, largely corrected the falls in enucleation and proliferation.

In the final series of experiments, Huang et al examined the behavior of human CD 34$^+$ stem/progenitor cells induced to proliferate and differentiate into enucleated erythrocytes. The fall in phosphocholine

and rise in choline during terminal differentiation paralleled the situation in mice cells (see figure panel B). *PHOSPHO1* gene expression was concurrently increased, whereas knockdown of *PHOSPHO1* caused marked reduction in cell proliferation and enucleation. Finally, glycine or serine supplementation corrected proliferation rate. These findings suggest a conserved function of *PHOSPHO1* in terminal erythropoiesis from mice to humans.

The work of Huang et al serves to emphasize that studies of red cell metabolism during erythropoiesis should not be restricted to that of proteins. It suggests a potential new cause of hemolytic anemia in humans. Recent advances in erythroid culture will provide an excellent opportunity to study in detail changes in lipid metabolism and other key events during late erythroid generation.[8] Findings will increase our understanding of normal red cell development and may provide new molecular tools for diagnosis, to correct abnormalities, or to facilitate in vitro erythrogenesis.

Conflict-of-interest disclosure: The authors declare no competing financial interests. ■

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DOI 10.1182/blood-2018-05-850255

TRANSPLANTATION

Comment on Haak et al, page 2978

The microbiome: more than a gut reaction

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In this issue of *Blood*, Haak et al describe an association between the abundance of butyrate-producing intestinal bacteria early after allogeneic hematopoietic cell transplantation (HCT) and the subsequent occurrence of lower respiratory tract viral infections during the first 6 months after transplant.[1]

Is this a chance association or is there a causal link? There are numerous endogenous and exogenous risk factors for infections after HCT and often there are multiple contributing factors that make causal relationships difficult to elucidate. The authors appropriately performed multivariate analyses of multiple pertinent clinical risk factors to exclude confounding clinical factors and were successful in concluding that gut microbiota at time of engraftment was an independent risk factor.

There are several limitations of the analyses. One could argue about the validity of the case definition of lower tract infection. The case definition has been a point of argument in other studies of viral respiratory infection: pulmonary infiltrates are not accurate in proving a particular microbiological etiology and even detection of an organism from a lower tract specimen, such as bronchoalveolar lavage, does not exclude contamination from the upper tract. One could inquire about studying gut microbiota when an assessment of the upper respiratory microbiota might have been more germane to study because of its proximity to the portal of entry and the site of infection; other studies suggest loss of diversity of upper respiratory microbiota diversity is associated with lower tract infection.[2] One could ask why the microbiota pattern at engraftment would have long-lasting effects affecting the risk for events months later. Moreover, does restoration of butyrate-producing populations at a later time after engraftment occur in some patients and, if so, does restoration abrogate the risk? One could ask if the associations found at this single center are unique because specific institutional transplant or antimicrobial practices differ from other centers, or is this finding generalizable for other transplant centers? Not explored in this study and relatively unexplored in other studies are the roles of the mycobiome and virome on immunity; they are likely to have important roles as well. All are issues that future studies should probe.

Notwithstanding those limitations, there is a sound scientific basis to believe that the link may be real. As the authors note, animal models have shown that gut microbiota can protect against both bacterial and viral respiratory infections and can be manipulated. The authors focused on butyrate-producing bacteria, reasoning from findings in an earlier study that this bacterial metabolite can reduce lung inflammation and injury during pneumonia by reduction of tumor necrosis factor and maintenance of interleukin-10.[3] The authors argue that engraftment is a crucial time for immune reconstitution and thus the microbiota at that time could have pivotal and lasting effects in shaping protective immunity. Thus, there is a scientific rationale to support this finding to be something more than merely an association only. Associations of gut dysbiosis with multiple other clinical complications after HCT have been described, including serious bacterial infections, graft-versus-host disease, relapse, and survival.[4] Finally, a recent presentation at the 2018 Bone and Marrow Transplantation Tandem Meetings indicates that the microbiota changes in patients treated at 3 centers were similar, providing some reassurance that the observations are likely not merely center specific.[5]

The evidence mounts for important interactions between the host and its microbiome that lead to health or disease.