powerful ameliorative effects of HbF in patients with sickle cell anemia: ie, the percentage of erythrocytes containing ≥10 pg of HbF. This amount has been shown to inhibit deoxy sickle hemoglobin (HbS) polymer formation when the oxygen saturation is less than the 40% to 70% encountered in the microcirculation. By preventing polymerization, the beneficial effects for the patient include reduction in intravascular hemolysis, vaso-occlusion–induced pain and tissue damage, and endothelial injury.

The authors note the experiment of nature exemplified by the phenotype resulting from the compound heterozygous state of HbS and pancellular hereditary persistence of fetal hemoglobin (S–HPFH). In this condition, every erythrocyte remarkably contains not only HbS but also approximately 10 pg of HbF. The result is nearly complete inhibition of deoxyHbS polymerization so that such individuals have a normal phenotype or only very minimal hemolysis. The authors thus posit that a therapeutic goal of achieving 10 pg of HbF per cell in persons with sickle cell anemia could represent a pharmacologic cure.

Hydroxyurea therapy is often beneficial (and correlates roughly with the percentage rise in HbF), but unfortunately the delterious clinical manifestations of intravascular hemolysis and vaso-occlusion frequently continue. In this article, the authors describe examples from the clinical sickle cell literature of diverse β globin haplotypes that are associated with widely ranging HbF values and clinical sequelae. Yet, because it is uncommon for HbF levels to exceed 20% to 25% (with or without hydroxyurea), most persons with sickle cell anemia have pain, organ damage, and a shortened lifespan. Nevertheless, the authors depict in various hypothetical examples how persons with specific concentrations of HbF or percentages of F cells can have widely differing percentages of circulating erythrocytes that exceed the protective threshold of 10 pg/cell, ranging from virtually none to 70% or more (see figure).

A clinical correlate of the observation by Steinberg et al is the use of a chronic hypertransfusion program for primary or secondary stroke prevention or as a means of reducing the frequency or severity of vaso-occlusive crisis and acute chest syndrome. Suppressing the proportion of cells containing HbS to <30%, a common goal of chronic transfusions, assures that sickle cell–related complications are reduced or prevented. The result would be similar when 70% HbS-containing cells also have >10 pg of HbF/cell (as in persons with HbS–HPFH).

So how do we achieve the postulated objective of increasing HbF to >10 pg in most or nearly all of these patients’ cells? Clearly hydroxyurea alone is not capable of that goal without risks of excessive toxicity. The authors point out that even the promising approach of blocking the BCL11A pathway may not result in the desired pharmacologic cure unless the result is a pancellular increase in HbF as observed in compound heterozygotes with HbS–HPFH.

The authors’ characterization of packaging HbF in the majority of cells to achieve a 10-pg level and render them incapable of sickling is an admirable goal. What an achievement it would be if each individual in the world with sickle cell anemia could someday be presented with such a therapeutic package as a gift of life.

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

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Comment on Alvarez-Dominguez et al, page 570

Long noncoding RNAs in erythropoiesis

Patrick G. Gallagher

In this issue of Blood, Alvarez-Dominguez et al use a combination of genomics technology, bioinformatics, and functional analyses to provide new insights into our understanding of the role of long noncoding RNAs (lncRNAs) in erythropoiesis. This is an initial step forward in our understanding of the many roles of lncRNAs in normal and perturbed erythropoiesis. lncRNAs have recently emerged as critical, multifunctional regulators of cellular gene expression.

Traditionally, the regulatory functions of RNA have been thought to be limited to their roles as ribosomal, messenger, and transfer RNAs. Development of high-throughput sequencing methodology and its application to transcriptome analyses has led to the realization that there are many more RNAs, termed noncoding RNAs (ncRNA), produced within the cell. We now know that the majority of the mammalian genome (~2/3) is transcribed, with <2% of the genome yielding transcripts with protein coding potential.

Thus, huge amounts of ncRNA are produced within the cell. ncRNAs have been classified as housekeeping RNAs, microRNAs, small interfering RNAs, PIWI-interacting RNAs, small ncRNAs (<200 nucleotides [nt] in length), and long ncRNAs (lncRNAs, >200 nt in length).

The focus of the report by Alvarez-Dominguez et al is on lncRNAs in erythropoiesis. Defined as RNA transcripts >200 nt in length that lack coding potential, lncRNAs are a large and diverse group of transcripts. Thousands and thousands of lncRNAs have been identified in cells from diverse organisms. Although the role of most lncRNAs is unknown, functions of several lncRNAs have been identified, including regulation of cellular processes such as

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development, differentiation, division, survival, and death. In the nucleus, lncRNAs have been shown to participate in critical epigenetic processes controlling the nuclear organization that influences gene expression. In erythroid cells, an lncRNA called LincRNA-erythrocyte prosurvival participates in regulation of cellular differentiation and programmed cell death.

In the first part of the study by Alvarez-Dominguez et al., a well-defined set of bioinformatic criteria is used to identify and comprehensively catalog lncRNAs expressed during erythropoiesis. These data were further refined to identify erythroid-specific lncRNAs. Determined by examining patterns of expression in burst forming units-erythroid, colony forming units-erythroid, and erythroblasts, erythroid-specific lncRNAs were found to demonstrate dynamic patterns of expression throughout erythropoiesis. Integration of lncRNA expression data with genomewide data sets of chromatin state revealed that changes in lncRNA expression during erythropoiesis were reflected at the chromatin level, with patterns of gene expression correlated with patterns of histone architecture reflecting active and repressive chromatin.

Genomewide maps of the erythroid transcription factors GATA1, KLF1, and TAL1 were intersected with the promoter regions of differentially expressed erythroid lncRNAs. Individual and combinations of the transcription factors were found in the proximal promoters of most differentially expressed lncRNAs, with co-occupancy of GATA1 and TAL1 found at lncRNAs induced during erythropoiesis. These studies fit well with initial observations of lncRNAs that indicate that their transcription is tightly regulated by cellular developmental or differentiation stage, external environment, etc.

The second half of this study is a series of functional analyses of 12 carefully chosen erythroid-specific lncRNAs. Knockdown studies of these 12 candidates using short hairpin RNA technology were performed in erythroid cells. Varying phenotypic effects on erythroid differentiation, including influence on TER119 expression, cell size, and enucleation, were observed in knockdown cells.

Detailed analyses of lncRNAs have revealed that there is poor conservation of lncRNA sequence across species. Orthologs have been identified between species, leading to “syntelogs” with minimal or no sequence similarity, but with conserved positions relative to neighboring protein coding genes through evolution. Based on these observations, the authors also examined the expression of neighboring genes of knocked down erythroid lncRNAs. Nine of the knocked down lncRNAs had no influence on neighboring gene expression, 1 knocked down lncRNA was associated with an increase in neighboring gene expression, and 2 knocked down lncRNAs were associated with decreased neighboring gene expression. Further analysis of one of the lncRNAs associated with decreased expression, alncRNA-EC7, revealed that it is an enhancer for SLC4A1, the gene encoding band 3, the primary anion exchanger of the erythrocyte. Knockdown of alncRNA-EC7, which is located 10 kb away from the band 3 gene locus, was associated with an 80% decrease in band 3 gene mRNA expression. Experimental data suggested a model for looping of the alncRNA-EC7 enhancer to the SLC4A1 gene locus with subsequent activation of band 3 gene expression in erythroid cells.

The field of lncRNA biology is exploding. The list of lncRNA transcripts continues to expand across differing cell types, with functional understanding limited to a few well-characterized examples. Varying cell types express varying patterns and amounts of lncRNAs suggesting that complex patterns of lncRNA expression regulate cells in many different ways. Despite their apparent importance, to date, there has been no unifying structural, biochemical, or functional characteristic that define a transcript as an lncRNA.

There is much more to be learned about lncRNAs. What are their roles in regulating cellular gene expression? What are their roles in normal and perturbed hematopoiesis? Based on studies of inherited neuromuscular diseases and select malignancies, we know they make important contributions to the pathogenesis of inherited and acquired disease. What are the mechanisms of these contributions? Can lncRNAs be used as biomarkers of disease presence or progression? Can we exploit them as potential therapeutic targets in disease? We are at the beginning of a long journey into the new field of ncRNA biology. Expect many surprises along the way.

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Comment on Scheer and Shea, page 590

PAI at breakfast (whether you like it or not)

Martin E. Young

In this issue of Blood, Scheer and Shea report that the morning surge of the prothrombotic factor plasminogen activator inhibitor-1 (PAI-1) observed in
Long noncoding RNAs in erythropoiesis

Patrick G. Gallagher