

effective than standard factor-based therapy in >90% of simulations, consistent with findings of cost-effectiveness analyses for hemophilia A gene therapy.<sup>5</sup> In general, although the price of gene therapy is steep, it is not as costly as factor, orthopedic surgery, bleeding management, and hospitalization over an adult lifetime.

Although these are encouraging findings, much work remains. Novel approaches are needed to adapt current vector-gene technology for those excluded from gene therapy: those with inhibitor alloantibodies, natural AAV antibody,<sup>6</sup> and active hepatitis B or hepatitis C liver disease.

Approaches to hepatocyte rescue may be needed in individuals who no longer express the transgene because of concomitant drug toxicity, nonalcoholic steatohepatitis, or viral cirrhosis, which is not uncommon in the hemophilia population.<sup>7</sup> Efforts to develop steroid-sparing immunosuppression or vector decoys to reduce the host anti-AAV capsid T-cell response that limits gene expression<sup>8</sup> is critical. Finally, careful surveillance is important for determining long-term risks, such as AAV integration and hepatocellular cancer (HCC),<sup>9</sup> given the continuing risk for HCC in those with hemophilia<sup>10</sup> (see figure).

In summary, it is exciting to consider that after years of complications of hemophilia factor therapy, including inhibitor formation, hepatitis, and HIV, it is now possible to receive a single dose of gene therapy and achieve therapeutic factor levels sufficient to prevent bleeding episodes and avoid factor use and its complications, while providing hope for those globally affected and achieving an improved quality of life.

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

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## HEMATOPOIESIS AND STEM CELLS

Comment on Yu et al, page 1691

# Epigenetic plasticity of erythroid progenitors

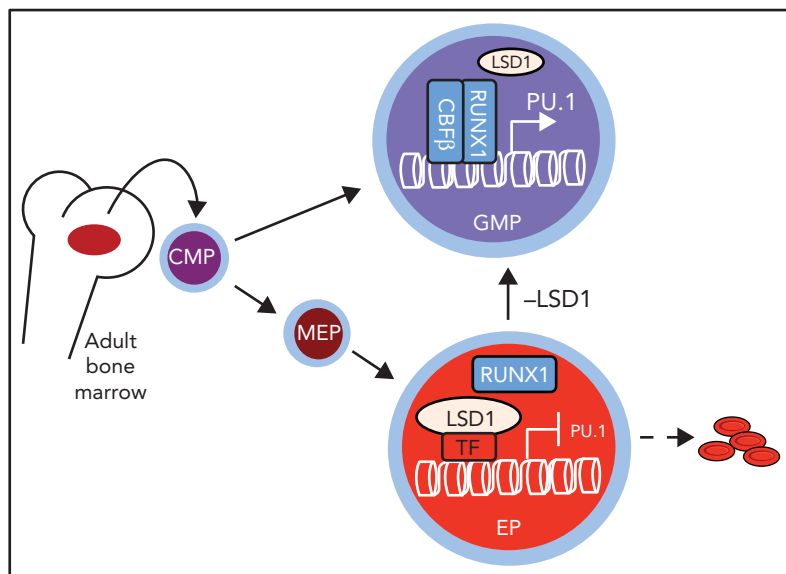
Marjorie Brand | Ottawa Hospital Research Institute

**In this issue of *Blood*, Yu et al,<sup>1</sup> report unexpected cell fate plasticity in erythroid progenitors (EPs) whereby removal or inhibition of the chromatin modifier LSD1 (also called KDM1a) triggers lineage conversion toward a myeloid fate, both in vitro and in vivo. This finding is important because it shows that progenitors that are typically thought of as being committed to the erythroid lineage have in fact retained the potential to differentiate toward other hematopoietic lineages. Furthermore, these findings provide strong support for the idea that active lineage restriction through epigenetic mechanisms, rather than irreversible loss of cell fate potential, underlies cell differentiation in hematopoiesis.**

The search for strategies to reactivate fetal  $\gamma$ -globin genes expression in adult erythroid cells dates back to the discovery that even a small increase in the level of fetal hemoglobin is enough to significantly alleviate symptoms of  $\beta$ -globinopathies, including sickle cell disease and  $\beta$ -thalassemia.<sup>2</sup> This early finding set the stage for decades-long research that led to the identification of multiple factors that inhibit fetal  $\gamma$ -globin genes expression, including transcriptional repressors (eg, BCL11A<sup>3</sup>) and chromatin-modifying enzymes. Although recent gene therapy trials targeting BCL11A have shown great promise for patients with sickle cell disease or  $\beta$ -thalassemia,<sup>4,5</sup> the development of pharmacological agents to reactivate fetal hemoglobin

remains a priority. A promising target for pharmacological induction of fetal hemoglobin is LSD1, an epigenetic enzyme that interacts with BCL11A and represses  $\gamma$ -globin genes transcription through the removal of active histone marks H3K4me1 and H3K4me2.<sup>6</sup> Although previous studies showed that LSD1 inhibitors increase fetal hemoglobin levels in animal models,<sup>7</sup> these chemicals also partly block erythroid differentiation, and the effects of prolonged loss of LSD1 activity in erythroid cells remain unknown.

To address this question, Yu et al began by generating a new transgenic mouse model whereby a specific knockout can be induced exclusively in the erythroid lineage starting at the megakaryocyte-EP



In committed adult EPs, differentiation toward the myeloid lineage is actively repressed by the chromatin-modifying enzyme LSD1. If LSD1 is depleted or inactivated pharmacologically, the cells convert to GMPs characterized by increased expression of the myeloid-specifying gene *Pu.1*. CMP, common myeloid progenitor; MEP, megakaryocyte-erythroid progenitor. See Figure 5F in the article by Yu et al that begins on page 1691.

stage. By crossing this mouse with an LSD1 homozygous floxed mouse, they could then study the long-term consequences of LSD1 ablation in the adult erythroid lineage. First, they found that the mice were anemic, with significantly reduced numbers of early (blast-forming unit-erythroid [BFU-e]) and late (colony-forming unit-erythroid [CFU-e]) EPs. This result was expected, given the block of erythroid differentiation observed after LSD1 inhibition in cell culture. However, no increase in cell death was observed. In contrast, there was a significant increase in the number of granulocyte-macrophage progenitors (GMPs), suggesting that erythroid cells without LSD1 have converted to the myeloid lineage. This result was surprising, because “committed” EPs, as their name indicates, are thought to be incapable of a change in cell fate. To determine whether GMP-like cells are derived from LSD1-depleted EPs, Yu et al designed an elegant lineage-tracing experiment with a triple-mutant transgenic mouse, wherein only cells from the erythroid lineage were fluorescently marked with tandem tomato (TdT). Using this approach, they observed that GMP-like cells that arise in vivo upon LSD1 knockout are TdT<sup>+</sup>, which confirms that those cells originate from EPs. Importantly, they also showed that erythroid-derived GMP-like cells give

rise to mature myeloid cells, which further supports the idea of an erythroid-to-myeloid switch upon LSD1 ablation. Further supporting this observation, they detected a reversal of the ratio between lineage-specifying transcription factors that control the erythroid (eg, GATA1) vs myeloid (eg, *Pu.1*) cell fate transition and found increased binding of the myeloid transcription factor RUNX1 to the *Pu.1* gene accompanied by enhanced H3K4me2 signal. Finally, they provided evidence that the erythroid-to-myeloid lineage conversion can be recapitulated in vitro using purified CFU-e cells exposed to an LSD1 inhibitor, which confirms that LSD1 acts through its enzymatic activity to restrict lineage potential in EPs (see figure).

Overall, the finding by Yu et al that cell fate decisions in committed EPs can be reversed by pharmacological inhibition of a chromatin-modifying enzyme has major implications for our understanding of the principles governing cell fate decisions and lineage commitment. Indeed, even though lineage commitment has been proposed to occur early in the hematopoietic stem/progenitor compartment (eg, Paul et al<sup>8</sup> and reviewed in Watcham et al<sup>9</sup>), quantitative mass spectrometry experiments have indicated that transcription factors from

nonerythroid lineages continue to be expressed in late EPs,<sup>10</sup> which suggests that these cells still have the potential to differentiate toward other hematopoietic lineages. Consistent with this finding, Yu et al showed that EPs have not lost their potential to differentiate toward the myeloid lineage but rather that this potential is being actively suppressed by LSD1, at least partly through demethylation of H3K4me2. This finding implies that chromatin modifiers have an active role in restricting lineage potential and that, contrary to current beliefs, this process is reversible through epigenetic mechanisms. Another important finding is the decisive role played by myeloid transcription factors that continue to be expressed in EPs, in driving differentiation. Indeed, the deletion or inactivation of *Pu.1* or *RUNX1* was sufficient to prevent myeloid conversion upon LSD1 inhibition. Overall, Yu et al provide important new evidence that lineage choice results from close cooperation between transcription factors that provide cells with the potential to differentiate toward multiple lineages and epigenetic modifications of chromatin that restrict this potential by regulating transcription factor activity and/or binding to lineage-determining genes.

Going forward, it is important to establish the full extent of chromatin changes (eg, H3K4me 1/2) and changes in transcription factors binding that occur upon LSD1 inhibition. Furthermore, the possibility that LSD1’s function in maintaining erythroid identity is also mediated through demethylation of nonhistone proteins remains to be addressed. Finally, the observation of cell fate plasticity upon LSD1 inhibition raises the possibility of increased susceptibility to malignant transformation by spurious activation of oncogenes, either through stochastic changes in gene expression or in response to environmental fluctuations. Regardless of the mechanism, malignant transformation should be carefully monitored when using pharmacological agents that can potentially alter cell fate decisions in the context of  $\beta$ -globinopathies or other diseases.

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## RED CELLS, IRON, AND ERYTHROPOIESIS

Comment on Murphy et al, page 1740

# When Pol II sees red

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**In this issue of *Blood*, Murphy et al report changes in histone posttranslational modifications (PTMs) and genome-wide RNA polymerase II occupancy during differentiation of primary human erythroid cells.<sup>1</sup>**

Previous studies have shown that during erythropoiesis, RNA polymerase II (Pol II) transcription becomes more and more restricted to genes essential for the function of red blood cells.<sup>2</sup> This is thought to be primarily the result of an overall decrease in chromatin accessibility and restricted recruitment of Pol II to erythroid genes by cell-type-specific transcription factors, including GATA1. Murphy et al demonstrate that although histone marks associated with active transcription (eg, H3 lysine 36 di- and trimethylation [H3K36me2/3] and H3 lysine 79 di-methylation [H3K79me2]) decrease, heterochromatin-associated histone modifications (eg, H3 lysine 27 di- and tri-methylation [H3K27me2/3] and histone H3 lysine 9 di- and trimethylation [H3K9me2/3]) do not change during maturation of erythroid cells (see figure). Moreover, the authors demonstrate that elongation competent Pol II becomes limited during differentiation, which leads to an increased pausing index at genes not essential for the development of red blood cells. Erythroid-specific genes

maintain efficient recruitment of elongation competent transcription complexes despite the marked reduction in Pol II. This study highlights an as yet underappreciated aspect of transcription regulation during erythropoiesis: the repression of transcription elongation at nonerythroid genes during a transient phase of erythroid maturation.

The authors used primary human CD34<sup>+</sup> hematopoietic progenitor cells and induced differentiation along the erythroid lineage. At day 7, representing intermediate erythroblasts, and at day 10, representing mature erythroblasts, the cells were analyzed by mass spectrometry focusing on histone PTMs. The data show that histone PTMs associated with Pol II transcription elongation declined whereas PTMs typically associated with heterochromatin remained unchanged. ChIP-seq, ATAC-seq, and Cut and Tag experiments verified that decline in transcription activity was not associated with heterochromatin formation. Instead, the authors found that the decline in

transcription of nonerythroid genes was the result of a defect in transcription elongation (at day 7) and eventually was the result of the lack of recruitment of Pol II (day 10). Remarkably, erythroid genes maintained high levels of transcribing Pol II. The finding that intermediate erythroblasts repress nonerythroid genes at the level of transcription elongation rather than at the level of chromatin accessibility or Pol II recruitment is unexpected and suggests a novel mechanism involved in erythroid cell specification. Intriguingly, the levels of activities that inhibit transcription elongation factor P-TEFb, HEXIM1, and the noncoding RNA 7SK, increase during erythroid maturation, with HEXIM1 exhibiting the highest levels in intermediate erythroblasts, at which stage the Pol II pausing index at nonerythroid genes is increased. The levels of P-TEFb decrease during erythropoiesis. Murphy et al also show that overexpression of HEXIM1 or HEXIM1 haploinsufficiency causes erythroid differentiation defects.

How do P-TEFb and HEXIM1 fit into the process of cell-specific gene regulation during erythropoiesis? After transcription initiation, Pol II pauses to allow capping of the 5' end of the RNA.<sup>3</sup> This pause is mediated by negative elongation factors. P-TEFb phosphorylates these negative elongation factors as well as the Pol II C-terminal domain at serine 2, leading to the formation of an elongation competent transcription complex. How the reduced pool of transcriptionally active P-TEFb and elongation competent Pol II is preferentially recruited to erythroid genes becomes an important question, as emphasized by the Murphy et al study. P-TEFb consists of the CDK9 kinase and cyclin T1 or T2.<sup>4</sup> HEXIM1 and 7SK RNA interact with the cyclin T1 subunit of P-TEFb. This renders P-TEFb unable to function as a transcription elongation factor. Several transcription factors have been shown to interact with cyclin T1 thereby dissociating HEXIM1 and allowing P-TEFb to activate transcription.<sup>4</sup> For example, the HIV-encoded Tat protein efficiently competes with HEXIM1 for interactions with cyclin T and recruits active P-TEFb to paused Pol II in the HIV genome.<sup>5</sup> With respect to hematopoietic cells, previous studies have shown that GATA1 and Ikaros transcription factors recruit P-TEFb to megakaryocytic and erythroid genes.<sup>6-8</sup> This is consistent with the study by Murphy et al, which