MEDICINE

Developing a pill to treat sickle cell disease

A newly identified epigenetic modifier increases fetal hemoglobin in preclinical studies

By **Douglas Higgs** *and* **Mira Kassouf**

The red blood cell pigment hemoglobin (Hb) is a tetrameric protein comprising two α- and two γ-globin chains in fetal life [fetal hemoglobin (HbF): $\alpha 2\gamma 2$] and two α- and two β-globin chains in postnatal life [adult hemoglobin (HbA): $\alpha 2\beta 2$]. Sickle cell disease (SCD) is an inherited blood disorder affecting millions of individuals worldwide that is caused by a point mutation in the adult β-globin gene, which changes a single amino acid ($Val^6 \rightarrow Glu$). On page 91 of this issue, Ting *et al.* (*1*) report a screen of cereblon (CRBN)–dependent protein degraders, identifying a small molecule that degrades a transcription factor, widely interspaced zinc finger (WIZ). Preclinical studies in mice and monkeys showed that the optimized molecule, dWIZ-2, degraded WIZ and substantially increased the expression of HbF through epigenetic mechanisms. This reveals a promising direction to develop an oral drug for SCD.

In homozygotes, the SCD mutation causes sickle-cell hemoglobin (HbS) to polymerize under low-oxygen conditions, and this in turn causes red blood cells to adopt a rigid, sickle-like shape rather than the flexible, biconcave disc of normal red blood cells. Normal red blood cells can negotiate even the smallest blood vessels, whereas sickle cells get stuck in capillaries, which causes inflammation, oxidative stress, activation of clotting pathways, and altered nitric oxide metabolism. Sickling also intermittently and unpredictably cuts off blood supply—so-called vaso-occlusive episodes—to many different tissues, causing pain and chronic damage. Although many therapies address these secondary effects, a curative therapy would ideally target the primary abnormality by reducing polymerization of HbS.

The most effective way of reducing HbS polymerization was originally highlighted in 1948 by the observations of pediatrician Janet Watson, who noted that infants who later develop SCD have few sickled red blood cells or clinical problems as newborns. She attributed this to the high levels of HbF present at that early stage of life and proposed that this prevents HbS from polymerizing (*2*). Family studies in the 1960s and 1970s showed that rare individuals with hereditary persistence of fetal hemoglobin (HPFH) and SCD were often free from all complications and symptoms (*3*).

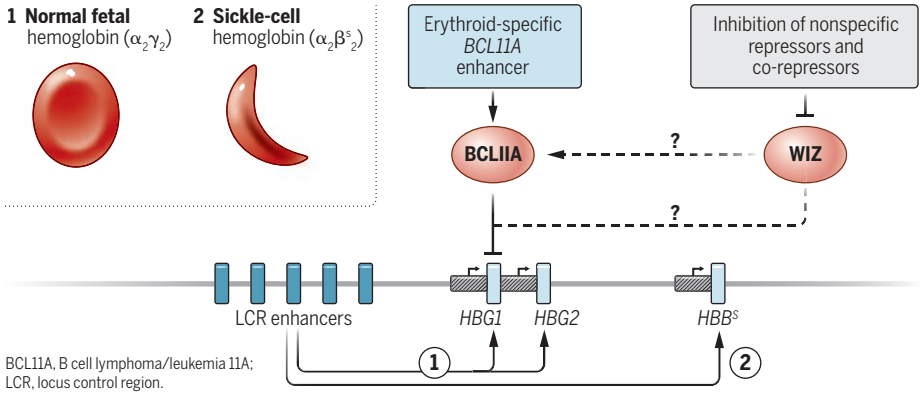
Within the hemoglobin (*HB*) locus, the fetal genes (*HBG1* and *HBG2*) that encode the γ-globins and the adult gene (*HBB*) that encodes β-globin are regulated by a cluster of enhancers, called the locus control region (LCR) (*4*). In fetal life, the LCR predominantly interacts with the *HBG1* and *HBG2* genes and in adult life with *HBB*, up-regulating its expression to eventually fill each red blood cell with more than 250 million molecules of HbA ($\alpha 2\beta 2$). In adults, the *HBG1* and *HBG2* promoters become silenced, predominantly through the transcription factor B cell lymphoma/leukemia 11A (BCL11A). BCL11A is activated in adult erythroid cells by its own erythroid-specific enhancer (*5*, *6*). Together with another transcription factor, leukemia/lymphoma-related factor (LRF) (*7*), this normally represses the expression of *HBG1* and *HBG2*. Switching off BCL11A in adult erythroid cells thereby raises the expression level of HbF. Several recent clinical trials using gene and base editing to down-regulate BCL11A expression or to interfere with the BCL11A or LRF binding sites in the *HBG1* and *HBG2* promoters have shown that it is possible to raise levels of HbF to substantially improve and possibly cure patients with SCD (*8*). However, such treatments are estimated to cost ~$2 million per patient, which precludes these approaches from becoming a feasible way to treat the millions of affected individuals.

An alternative approach is to develop an oral agent that can raise HbF expression. In 1998, hydroxyurea became the first oral agent to be approved for the treatment of SCD. Hydroxyurea causes variable and modest increases in HbF levels and reduces many of the vascular complications seen in patients with SCD, extending their life expectancy. However, the mechanism(s) by which it does so are still unclear. Many other oral agents targeting epigenetic modifiers (e.g., histone deacetylases, histone demethylases, and inhibitors of DNA methylation) have been shown to activate the production of HbF in preclinical studies (*9*). However, none has successfully made it to the clinic either because they fail to increase HbF to clinically effective levels or they have unacceptable off-target effects.

In their screen, Ting *et al.* identified a mo-

Increasing fetal hemoglobin expression in sickle cell disease

A primary aim of ameliorating sickle cell disease (SCD) is to prevent or reverse the switch from fetal hemoglobin ($\alpha_2\gamma_2$) to adult hemoglobin ($\alpha_2\beta^s_2$). The major pathway that represses the γ-globin genes (*HBG1* and *HBG2*) involves the transcription factors BCL11A and leukemia/lymphoma-related factor (LRF; not shown). One targeted therapeutic strategy is to engineer the *BCL11A* enhancer to prevent BCL11A expression. Epigenetic modifiers, including widely interspersed zinc finger (WIZ), may also act on the BCL11A pathway and/or other pathways to repress expression of the γ-globin genes. The epigenetic modifiers, such as dWIZ-2, may restore γ-globin gene expression to alleviate vaso-occlusive episodes, but they may also cause off-target effects.



BCL11A, B cell lymphoma/leukemia 11A; LCR, locus control region.

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lecular glue degrader (dWIZ-1), which they found targets WIZ. Subsequent optimization identified a chemical analog with improved pharmacokinetic properties, called dWIZ-2. They show that dWIZ-2 induces substantially increased expression of HbF in human erythroid cells ex vivo, which displayed normal maturation after treatment. They also observed increased expression of HbF in two of three cynomolgus monkeys treated with dWIZ-2 in vivo; there were no off-target effects during the 28 days of treatment. A key issue is whether dWIZ-2 or a related molecule can be translated into an effective and safe oral therapeutic agent.

The preclinical data suggest that downregulation of WIZ might produce high levels of HbF in vivo. Notably, how WIZ regulates expression of HbF is far from clear. WIZ is a core component of the repressive G9a–G9a-like protein (GLP) histone methyltransferase complex (also called the EHMT1-EHMT2 complex). The authors propose that WIZ thereby acts as a negative regulator of HbF expression by reducing the repressive histone H3 Lys^9 (H3K9) dimethylation at the promoters of the *HBG1* and *HBG2* genes. Thus, degrading WIZ removes this repression of HbF expression (see the figure).

It should be noted that like other epigenetic modifiers, WIZ binds widely throughout the genome, including at enhancers, promoters, and CTCF-bound insulators, which predicts its involvement in the expression of many genes. It is also widely expressed in a variety of tissues: Mutation of WIZ and interference with the G9a-GLP complex in mice causes widespread developmental abnormalities and is lethal in mid- to late gestation (*10*). In humans, mutations in WIZ and the G9a-GLP complex are associated with a range of genetic diseases. In addition, G9a and GLP have important roles in neurological disorders, cancer progression, immune cell diversification, and the heart (*11*). Thus, it is hoped that dWIZ-2 will provide a new avenue to safely raise HbF expression and that it does not go the way of so many other initially promising epigenetic modifiers that have been proposed for this purpose. ■

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NEUROSCIENCE

A new path to migraine

Cerebrospinal fluid influx directly activates trigeminal neurons in a migraine model

By **Andrew F. Russo**[1,2] *and* **Jeffrey J. Iliff**[3,4,5]

Migraine pain is believed to result from the activation of pain receptors (nociceptors) after the cortical spreading depression (CSD) that is associated with the aura phase of migraine (*1*). Previous studies have demonstrated that preclinical CSD events release small molecules through the cerebrospinal fluid (CSF) that activate and sensitize afferent trigeminal fibers within the meninges (*2*, *3*). However, it has been thought that trigeminal ganglia reside "outside" the blood-brain barrier and hence are not directly exposed to CSF (*4*). On page 80 of this issue, Rasmussen *et al.* (*5*) show in a mouse model of migraine that after CSD, subarachnoid CSF carries signals from the cortex directly to cell bodies in the trigeminal ganglia, where they activate nociceptors through a pathway that bypasses meningeal trigeminal afferents. The demonstration that the trigeminal ganglia lies within the blood-brain barrier and the identification of the signals that connect aura and headache may provide a new path for preventing and treating migraine.

"...a new mechanism that links the central and peripheral nervous systems by an intercranial humoral pathway..."

Through a combination of proteomic, histological, imaging, and functional approaches, Rasmussen *et al.* used a mouse model of classical migraine to show that CSD leads to changes in the subarachnoid CSF content, including elevated levels of calcitonin gene-related peptide (CGRP), a neuropeptide whose link to migraine is well documented (*6*). Accordingly, CSD-conditioned CSF can cause trigeminal ganglia neural activation. They also found that CSF can enter the proximal regions of the trigeminal ganglia, owing to the lack of a tight nerve barrier. Together, these findings provide a new mechanism that links the central and peripheral nervous systems by an intercranial humoral pathway that can in turn modulate neurochemical crosstalk between neurons, glia, and immune cells within the trigeminal ganglia (*7*) (see the figure).

The humoral pathway from the central nervous system (CNS) to the trigeminal ganglia after CSD that is described by Rasmussen *et al.* differs from the long-established meningeal pathway in several respects (*1*, *2*). Most notably, in mice, the two pathways appear to have different kinetics, with the movement of cortical interstitial solutes to the trigeminal ganglia in the skull base being more rapid than their movement to the meningeal site of trigeminal afferents. This finding should be interpreted carefully, however, because the anatomical distances that this communication travels in the brain differ substantially between human and mouse, and imaging studies suggest that glymphatic CSF and solute transport are markedly slower in the human brain than in the rodent brain (*8*). Nonetheless, it seems likely that communication via both the direct CSF-borne and the classic trigeminal afferent pathways co-occur and contribute to peripheral sensitization in migraine with aura. A key implication of these observations is that the trigeminal ganglia as well as the meninges should be considered as a target for peripherally restricted drugs that are used to attenuate pathologies initiated in the cortex.

The study by Rasmussen *et al.* provides important new insights for the understanding of cranial fluid and solute transport. Just over a decade ago, the role of perivascular fluid and solute transport via the glymphatic system in the clearance of cerebral wastes began to be defined (*9*). The characterization of functional lym-

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