Seminars in Hematology ▮ (2018) ▮▮▮–▮▮▮

Contents lists available at ScienceDirect

Seminars in Hematology

journal homepage: www.elsevier.com/locate/enganabound

Review

Fetal Hemoglobin Induction by Epigenetic Drugs

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ARTICLE INFO

Keywords:
Sickle cell disease
Fetal hemoglobin
Epigenetic
Therapy

ABSTRACT

Fetal hemoglobin (HbF) inhibits the root cause of sickle pathophysiology, sickle hemoglobin polymerization. Individuals who naturally express high levels of HbF beyond infancy thus receive some protection from sickle complications. To mimic this natural genetic experiment using drugs, one guiding observation was that HbF is increased during recovery of bone marrow from extreme stress. This led to evaluation and approval of the cytotoxic (cell killing) drug hydroxyurea to treat sickle cell disease. Cytotoxic approaches are limited in potency and sustainability, however, since they require hematopoietic reserves sufficient to repeatedly mount recoveries from stress that destroys their counterparts, and such reserves are finite. HbF induction even by stress ultimately involves chromatin remodeling of the gene for HbF (*HBG*), therefore, a logical alternative approach is to directly inhibit epigenetic enzymes that repress *HBG*—implicated enzymes include DNA methyltransferase 1, histone deacetylases, lysine demethylase 1, protein arginine methyltransferase 5, euchromatic histone lysine methyltransferase 2 and chromodomain helicase DNA-binding protein 4. Clinical proof-of-principle that this alternative, noncytotoxic approach can generate substantial HbF and total hemoglobin increases has already been generated. Thus, with continued careful attention to fundamental biological and pharmacologic considerations (reviewed herein), there is potential that rational, molecular-targeted, safe and highly potent disease-modifying therapy can be realized for patients with sickle cell disease, with the accessibility and cost-effective properties needed for world-wide effect.

Introduction

The global scope of the sickle cell disease (SCD) problem, with its especially high prevalence in pediatric populations in low income countries, demands a practical, accessible oral medication solution. There are natural models for drug development to emulate—some patients with SCD express high levels of fetal hemoglobin (HbF) beyond infancy, because of genetic traits separate from, and co-inherited with, the sickle mutation in the β-globin gene (HBB^S), and consequently have, in the best cases, asymptomatic and normal life-spans [1-3]. Analyses of these HbF-augmenting genetic traits, coupled with biochemical studies of the β-globin locus, have identified specific molecular targets for drug development efforts that aim to recapitulate these natural exemplars of powerful disease modification.

HbF Interdicts Sickle Pathophysiology at Its Inception

Red blood cells (RBCs) at the fetal stage of life contain HbF ($\alpha_2\gamma_2$). HbF intercalates with but does not polymerize with sickle hemoglobin (HbS) (normal adult hemoglobin [HbA and $\alpha_2\beta_2$] can) [4-6]. HbF thus interrupts SCD pathophysiology at its inception [4,5] (Fig. 1), and higher HbF correlates continuously with fewer vaso-occlusive pain crises, less renal damage, less pulmonary hypertension, fewer strokes, and longer survival [7-14] (reviewed in [6]). Those few patients with SCD who inherit HbF at 20%-30% of total hemoglobin (hereditary persistence of HbF and HPFH) have essentially normal life-spans [1-3].

The First Attempts at Pharmacologic Induction of HbF Were Based on Creating Bone Marrow Stress

Thus, several decades of translational research have been directed toward pharmacologic recapitulation of this naturally selected protective state of persistent high HbF expression (reviewed in [6]). The

Conflict of interest statement: Yogen Saunthararajah has issued patents around tetrahydrouridine and decitabine and ISWI family inhibition, and is a consultant to EpiDestiny, that has licensed oral tetrahydrouridine-decitabine

Funding source: National Heart, Lung and Blood Institute UO1 HL117658.

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https://doi.org/10.1053/j.seminhematol.2018.04.008
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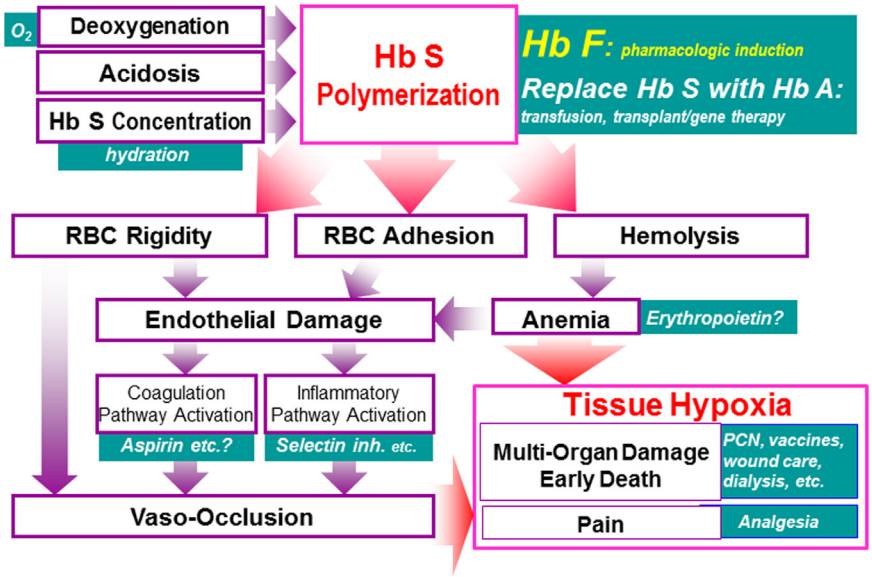

Fig. 1. Polymerization of deoxy sickle hemoglobin (HbS) drives the multiorgan cascade of SCD pathophysiology. Fetal hemoglobin (HbF), by blocking this polymerization, can interdict essentially all manifestations of disease, illustrated by the natural genetic experiment of hereditary persistence of fetal hemoglobin expression, and a model for drug development efforts to emulate. By contrast to HbF, normal adult hemoglobin (HbA, β-chains) can participate in polymerization. (We published a variation of this figure in Molokie et al, PLoS Medicine 2017.)

earliest efforts built on the observation that HbF is enriched during recovery of the bone marrow from severe insults or stress [15-20]. One way of creating such stress is to administer cytotoxic (cell killing) drugs, leading to clinical evaluation in SCD of the oral ribonucleotide reductase inhibitor hydroxyurea [19-21]. In the pivotal trial, hydroxyurea (15-35 mg/kg) increased HbF for 2 years in ~50% of the adult patients with SCD treated [21,22]. As predicted, HbF increases with hydroxyurea correlated strongly with increased RBC half-life [23,24], fewer pain crises [22], and better quality of life [25]. Trial patients with HbF levels >0.5 g/dL survived longer [10] (a caveat being that these analyses did not determine whether the higher HbF levels were intrinsic to the patients or a result of hydroxyurea therapy). Of significance, however, average HbF increases at 2 years were modest (3.6%) [19-22,26]. Moreover, HbF increases were particularly unlikely in patients with the lowest baseline HbF levels and thus at highest risk of morbidity and mortality [22,24,27,28], and HbF increases diminished over time, even in patients with excellent initial HbF inductions [22,29]. A reason for these properties of hydroxyurea therapy was suggested by the correlation between lower and less durable HbF increases and fewer reticulocytes ($<300{,}000 \times 10^9$/L) and neutrophils ($<7.5 \times 10^9$/L) at baseline: this correlation underscored that HbF induction by cytotoxicity requires reserves of hematopoietic precursors sufficient to mount repeated recoveries from stress that destroys their counterparts [20,22]. Such reserves are circumscribed, and moreover subject to attrition via vaso-occlusion in the marrow and to the kidneys, and from the aging process [22,24,27,28]. This is a problem even separate from considerations of sustainable HbF induction via cytotoxicity: patients with SCD require erythropoiesis at >10-fold the normal rate to barely sustain hemoglobin levels compatible with life, and dwindling compensatory capacity is a major cause of early death [10,22,30,31]. Therefore, alternative, noncytotoxic, durable, and more potent methods of inducing HbF are needed.

Directly Targeting the Enzymes That Silence the γ-Globin Gene Instead

DNA in nuclei is packaged together with RNA and structural proteins—histones—to form chromatin. These structures regulate accessibility of genes to the massive multiprotein machinery (~150 proteins) that executes gene transcription. Reorganization or remodeling of chromatin, to facilitate or hinder gene transcription, is signaled via posttranslational modifications to histones—methylation and acetylation of lysine residues, phosphorylation of threonines and serines—and by modifications to DNA, mainly, methylation of cytosines that precede guanines (CpG). These signals motivate shifting of histones in relation to gene transcription start sites, repositioning these substantial physical barriers to either welcome or obstruct the gene transcribing basal transcription factor machinery.

Thus, whether a stimulus for *HBG* activation is natural or from without, HbF induction requires chromatin remodeling of the β-globin gene locus (*HBG*) [32]. Specifically, there is decreased operation at the locus of the epigenetic enzymes that create epigenetic "off" marks, or that reposition histones toward the transcription start site, and increased function of the enzymes that create epigenetic "on" marks, and that reposition histones away from the transcription start site. Cytotoxic methods of inducing HbF generate these necessary epigenetic changes crudely and indirectly, via bone marrow stress [20,32,33] (Fig. 2). So why not identify repressing epigenetic enzymes and inhibit them directly? This notion has been supported by extensive preclinical work and even clinical proof-of-principle [34,35], reviewed here.

Fundamentals Worth Keeping in Mind

The wealth of preclinical and clinical experience to date has underscored biological and clinical considerations fundamental to the success or failure of attempts at epigenetic induction of HbF. Here are five such fundamentals:

(1) *Epigenetic enzymes by themselves do not dictate activation of genes*: This is illustrated with a thought experiment: imagine rendering all the DNA accessible (opening all the chromatin) in a cell devoid of DNA-binding transcription factors—no change in gene expression would be expected—gene activation requires a cellular context primed to express the gene, with localization of a cooperating complement of sequence-specific and basal DNA-binding transcription factors, and their requisite, massive, multiprotein coregulator apparatus, and exceeding more than 150