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A Systematic Review of Known Mechanisms of Hydroxyurea-induced Foetal Haemoglobin for Treatment of Sickle Cell Disease

Gift D. Pule¹, Shaheen Mowla², Nicolas Novitzky², Charles S. Wiysonge³, and Ambroise Wonkam^{1,*}

¹Division of Human Genetics, Department of medicine, Faculty of Health Sciences, University of Cape Town, Cape Town, Republic of South Africa

²Division of Hematology, Department of Clinical Laboratory Sciences, Faculty of Health Sciences, University of Cape Town, Cape Town, Republic of South Africa

³Centre of Evidence-based Health Care, Stellenbosch University, Cape Town, Republic of South Africa

Abstract

Aims—To report on molecular mechanisms of foetal haemoglobin (HbF) induction by hydroxyurea (HU) for the treatment of Sickle Cell Disease (SCD).

Study Design—Systematic review.

Results—Studies have provided consistent associations between genomic variations in HbF-promoting loci and variable HbF level in response to HU. Numerous signal transduction pathways have been implicated, through the identification of key genomic variants in *BCL11A*, *HBSIL-MYB*, *SAR1* or *XmnI* polymorphism that predispose the response to the treatment, and signal transduction pathways, that modulate γ -globin expression (cAMP/cGMP; Gi α /JNK/Jun; methylation and microRNA). Three main molecular pathways have been reported: 1) Epigenetic modifications, transcriptional events and signalling pathways involved in HU-mediated response, 2) Signalling pathways involving HU-mediated response and 3) Post-transcriptional pathways (regulation by microRNAs).

Conclusions—The complete picture of HU-mediated mechanisms of HbF production in SCD remains elusive. Research on post-transcriptional mechanisms could lead to therapeutic targets that may minimize alterations to the cellular transcriptome.

Keywords

Sickle cell disease; hydroxyurea; foetal haemoglobin; Molecular mechanism; *BCL11A*; *HBSIL-MYB* and *SAR1*

Corresponding author: Associate Professor Ambroise Wonkam, Division of Human Genetics, Department of Medicine, Faculty of Health Sciences, University of Cape Town, Anzio Road, Observatory, 7925, Cape Town, Republic of South Africa. Tel: +27214066307; Fax: +27214066826; ambroise.wonkam@uct.ac.za.

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INTRODUCTION

Sickle Cell Disease (SCD) is a monogenic, haematological and multi-organ disorder associated with progressive organ damage and chronic and acute illness (Wheatherall et.al. 2005). SCD is caused by a point mutation (A>T) in the sixth codon of the β -globin gene on chromosome 11, resulting in the substitution of the amino acid glutamic acid to valine. The resulting haemoglobin S (HbS) leads to polymerization and precipitation of haemoglobin during deoxygenation or dehydration resulting in sickling, abnormal adhesion of leukocytes and platelets, inflammation, hypercoagulation, haemolysis and hypoxia, in addition to microvascular obstruction and ultimately organ damage [1].

There is strong correlation between the frequency of the HbS gene and the historical distribution and incidences of malaria [2]. It is estimated that more than 300 000 births with SCD occur annually, nearly two-third of which take place in Africa [3]. SCD is relatively common in other continents such as North America and Europe with 2 600 and 1 300 affected new-borns annually, respectively [4]. It is well accepted that the sickle mutation exists in Africa on diverse genetic haplotype backgrounds [5]. Five typical haplotypes have been described across the β -globin gene cluster based upon the pattern of specific restriction fragment-length polymorphisms across the region. Four haplotypes are associated with HbS in Africa (Benin, Bantu/Central African Republic (CAR), Senegal and Cameroon) and the fifth is thought to have arisen in India and/or the Arabian Peninsula (Arab/Hindu) [6,7]. It has been suggested that these haplotypes also have an effect on the severity of the disease through their genetically-determined effect on HbF level [8,9].

Indeed, despite the fact that there are several key phenotypes of SCD (anaemia, stroke, infections), foetal haemoglobin (HbF) has emerged as a central disease modifier [10], that is amenable to therapeutic manipulation [11]. Genetic variants at three principal loci, *BCL11A*, *HBS1L-MYB* and *HBB* cluster account for 10–20% of HbF variation [12–14]; among SCD patients in USA and Brazil [15], Tanzania [16] and Cameroon [17]. Currently, Hydroxyurea (HU) is the only FDA-approved pharmacologic treatment for induction of HbF in patients with SCD. The major HU benefit is directly related to its HbF-producing effect [18,19] that leads to significant reduction of pain, acute chest episodes, the need for blood transfusions and mortality [8,20–22].

A few studies have shown that individual responses to HU treatment are highly variable, with induced HbF levels ranging from 10% to greater than 30% HbF [23–25]; and sibling pairs analysis have shown a that strong genetic component could influence this variable response to HU [20]. Some authors have hypothesized that the effect of HU on HbF level could act through HbF- promoting loci like *BCL11A* [26]. A recent GWAS studies have revealed further sequence variants that could influence the response to HU [27]. However, the precise molecular mode of action of HU, though these genetic variants, remained to be comprehensively determined.

In this paper, it is our aim to provide a comprehensive and systematic review of the current literature on HU's mode of action at the molecular levels and postulate on future work in this field. It is anticipated that adequate knowledge of the mode of action of HU will result

in the exploration of alternative therapeutic agents that may minimize alterations to the cellular transcriptome.

METHODS

A review of the current literature on reported mechanisms of HbF induction by HU treatment was conducted from October 2014 – December 2014 using Pubmed (National Library of Medicine), Medline and Google scholar. Key words included individual use or a combination of the following: “Hydroxyurea or HU or hydroxycarbamide”, “Foetal hemoglobin or HbF or gamma globin”, “hemoglobin-induction”, “Sickle Cell treatment”, “erythroid treatment” and specific author names were also used. Prior knowledge of research groups working on HU and SCD Africa and globally further facilitated the identification and selection of research articles. Only available full-length articles, in English, with the use of HU on erythroid cells (cell lines and/or primary cells) were selected. In cases where multiple studies reported a similar pathway, the most recent with the most detailed mechanisms of HbF induction was included. The main search was conducted by a PhD student in Human Genetics and reviewed by a Medical/Human Geneticist with expertise in SCD and a Hematologist/Cell biologist with expertise in signal transduction pathways. A total of 563 articles were consulted after the search, eliminating on the basis of the article title and its relevance to the scope of the review. Subsequently, 361 papers were retrieved and their abstracts and results sections perused for further elimination, of which a final total of 129 papers were selected for inclusion in the review.

RESULTS

SCD is highly heterogeneous in clinical manifestation and disease severity even though patients may be diagnosed with the same genetic mutation resulting in the disease. HbF is a major modifier of the SCD disease phenotype that has been associated with reduced risk for vaso-occlusive complications, organ damages and increased life expectancy [10,21,28–30]. During gestation, the γ -globin genes are the predominantly transcribed genes from the β -cluster. After birth the expression of the γ -globin genes is replaced by the adult β -globin genes; this process is known as the “Fetal Switch” [31]. However, some HbF remains to be heterogeneously transcribed with variable expression levels across HbF red blood cells (F cells) [32]. Initial observations suggested that *HBB* haplotypes also have an effect on the severity of SCD [8,9]. The Bantu haplotype being the less favourable and the Indian-Arab haplotype associated to the least severity; as the latter is associated with higher HbF levels [33], due to a single nucleotide polymorphism (SNP) upstream of the β -globin gene (rs7482144 or Xmn- γ), characteristically present in Indian Arab and Senegal haplotypes [34]. The degree of HbF expression and the blood percentage of F cells at adulthood are heritable quantitative trait loci (QTL) [35]. Normal individuals have < 0.6% HbF distributed among 1–7% F cells in their peripheral blood. However, approximately 2% of the population produce up to 5% HbF and 25% F cells, a trait known as heterocellular Hereditary Persistence of Foetal Haemoglobin (hHPFH) [36]. hHPFH is characterized by a persistent high level of foetal haemoglobin in adults [32] and a genome-wide SNP scan of 10 members of a Maltese family with hHPFH revealed a nonsense mutation in the *KLF-1* gene, a critical activator of *BCL11A* [32]. This mutation was characterized and shown to cause an ablation

of the DNA binding domain of this erythroid transcription factor and thus provided a rationale for the effect of *KLF-1* haploinsufficiency on HbF levels. In total, genetic variants at three principal loci, *BCL11A*, *HBS1L-MYB* and *HBB* cluster account for 10–20% of HbF variation [13,14,37]. *BCL11A* loci have been shown to be amenable to therapeutic manipulation with the aim to increase HbF level [38,39], that have major implications for research on new treatments of SCD.

Hydroxyurea treatment and foetal haemoglobin induction in SCD

Currently, Hydroxyurea (HU) is the only pharmacologic treatment for induction of HbF in patients with SCD both approved by the FDA in 1998 and by the European Medicines Agency in 2007. In addition, the National Institutes of Health Office of Medical Applications of Research (NIH-OMAR) and the Agency of Healthcare Research and Quality (AHRQ) both declared HU as an effective drug treatment for adults and children with SCD [40–42]). HU is an oral, S-phase specific cytotoxic, anti-metabolic and anti-neoplastic drug treatment; it is a potent inhibitor of a ubiquitous enzyme called ribonucleotide reductase [43]. In 1984, the first clinical application of HU in haemoglobinopathies demonstrated swift and vivid increases in HbF concentration within reticulocytes and insignificant toxicity to bone marrow [44]. Initial trial showed that HU was associated to decreases in the frequencies of painful episodes, acute chest syndrome, hospitalization and transfusions [28]. Subsequent trials in SCD have demonstrated clinical efficacy and increase in survival rates and life expectancy [20,21], protection against cerebrovascular disease [45], long term drug safety, capacity to prevent organ damage, reduced morbidity and mortality in school-age children [24], toddlers [46,47] and infants [48]. HU has also been associated with clinical drift, where physicians use the drug for related complications of SCD such as stroke prevention, priapism and pulmonary hypertension [49].

However potential short and long term potential adverse effects such as male impotence [49–51], susceptibility to infections [52–55], potential teratogenic effect [28,56] and cutaneous adverse reactions [57] have also been associated with HU. Although sparse and in the majority unsupported, some studies have demonstrated recovery of spermatogenesis, normal counts and motility after the cessation of HU treatment [58]. The fear of such side-effects has been a subject of concern to some professionals [59,60], parents as well as patients [40,61–64] and a potential barrier to compliance in some settings [65,66]. As consequences, HU is still underutilized [40,61], despite the studies that have reported on the overall drug safety [67] and limited evidence regarding potential HU-induced leukemogenic [68] or teratogenic effects [69]. It is however noteworthy that there is no present data validating fertility and teratogenic effects of HU nor on the incidence of abnormal pregnancies as a result of HU treated father. The decision for continuation (or otherwise) of HU in instances of pregnant SCD mothers on HU should thus done on a case-to-case basis where the risk of cessation is carefully weighed against potential effects of terminating an effective treatment [21,70]. Furthermore, less evidence is currently available on the persistence over time of responses to HU. Long term studies such as the Hydroxyurea Study of Long-Term Effects (HUSTLE), Hydroxyurea Safety and Organ Toxicity (HUSOFT) together with BABY HUG follow-up studies continue to reveal more pharmacogenetics data to help define long term risk of early-initiation of HU, potential adverse effects and persistence of response.

It is evident that concomitant to more HU clinical trials, predictive drug-responder indexes, efficacy and toxicity studies, educating physicians and/or care-providers as well as the global healthcare system is necessary to overcome other barriers such as access to specialty care, insurance coverage as well as further provider-level aspects such as care-giver ambivalence. It is also urgent to fully understand HU molecular mode of action, in order to explore alternative and potential less toxic and more acceptable agent that could equally increase the level of HbF.

Genomic variants associated to Hydroxyurea-induced HbF level

Patients' response to HU is highly variable, with induced HbF levels ranging from as low as 2% to greater than 30% HbF [23–25], potentially due to the pharmacogenomic interactions [67]; but our understanding of the reasons for the wide spectrum of clinical responses to HU treatment is incomplete [71]. It has been initially suggested that haplotypes in the *HBB* gene cluster possibly affect the possibly the clinical response to HU, likely mediated by their genetically-determined effect on HbF level [34,72,73] (Table 1). There is a positive association between the *XmnI* polymorphism (rs7482144) in the γ -globin promoter and HbF levels in numerous populations [15,16,74–76], as well as accounting for more than 2% in HbF variation in patients and 13 – 32% in F cell variation in non-anaemic European population [77]. This polymorphism has also been associated with the less severe disease course of patients with Senegal and/or Indian-Arab haplotype backgrounds as well as improved response to HU treatment [74,75,77–81]. Subsequent research provides some evidence that the effect of HU on HbF level could act on other HbF-promoting loci like *BCL11A* [26]. Indeed, *BCL11A* is central to the 'fetal switch', *BCL11A* is co-expressed, directly interacts and co-occupies the β -globin loci with *SOX-6* in association with the Mi-2/ nucleosome remodelling and deacetylase (NuRD) complex for long-range re-conformation of the β -globin cluster for the transcriptional silencing of γ -globin [38]. Besides *BCL11A*, from DNA structural alteration to sequence modification, the secretion-associated and ras-related protein (SAR-1) has been shown to play a significant role in γ -globin regulation [82] and three SNPs in the SAR-1a in its promoter sequence have been associated with level of HbF in peripheral blood of SCD patients on HU [83]. Variants in regulatory sequences of *SAR-1* was shown in vitro to be associated to HU induced HbF production, underlying *SAR-1* as an alternative therapeutic target for β -globin disorders [82]. A recent GWAS studies have also shown that a coding variant in Spalt-like transcription factor, or *SALL2*, was associated with higher final HbF in response to Hu treatment, a new insight into the pharmacological HbF upregulation by hydroxyurea in patients with SCD [27] that deserve further functional studies.

Molecular pathway of action of HU

Various molecular pathways have been reported to explain the mode of action of hydroxyurea (Tables 2 and Figure 1): 1) Epigenetic modifications, transcriptional events and signalling pathways involved in HU-mediated response, 2) Signalling pathways involving HU-mediated response and 3) Post-transcriptional pathways (regulation by microRNAs).

Epigenetic and Transcriptional Events regulating HU response

BCL11A, SOX-6 and the Mi-2/NuRD complex co-occupy the β -globin loci for long-range re-conformation of the β -globin cluster for the transcriptional silencing of γ -globin [38]. Chromosomal looping mediates the long range interaction between methylation-sensitive regulatory sites in the locus control region and the β -globin gene cluster. It has also been shown that *BCL11A* occupies the LCR HS3 and the intergenic region between $G_{\gamma A}$ and δ -globin genes [31]. Acetylation-based transcriptional activity has been observed in the LCR, δ - and β -globin genes as well as the intergenic region between A_{γ} and δ -globin genes, whereas methylation was most observed in the CpG islands of the γ -globin proximal promoter [38,84]. Furthermore, HU-induced HbF has previously been inversely correlated to G_{γ} -globin methylation in SCD patients [84]. Albeit, the changes in methylation did not reach statistical significance, decreases in methylated CpG islands in the G_{γ} -globin promoter were identified and associated with increased γ -globin expression. Such epigenetic modifications have proven to play a significant role in the transcriptional regulation of the β -globin gene cluster and thus may require further investigation to find target-specific chromatin remodelling agents for the re-activation of γ -globin expression to ameliorate SCD symptoms.

Signalling pathways involved in HU-mediated response

Cyclic AMP and GMP pathways—The cGMP pathway has been implicated as a role player in the induction of HbF *in vitro*, in K562 cells and human erythroblasts, as well as *in vivo*, in SCD patients [85–87]. The pivotal role of the cGMP-dependent pathway in γ -globin expression regulation has previously been described [85] with sGC-PKG being involved in γ -globin induction in response to HU [86]. A humanized SCD mouse model of tumor necrosis factor α (TNF- α)-induced acute vaso-occlusion has been used to delineate an HU-induced nitric oxide (NO)-cGMP pathway, where HU-induced NO stimulates intracellular sGC thus increasing cGMP and thus PKG [88]. The observed effects of increased PKG included induction of γ -globin expression in erythroid cells and decreased leukocyte recruitment and adhesion. Although still a mouse model, this pathway is considered true also in humans and provides numerous therapeutic targets for HbF production.

There is cross-talk between the cGMP- and cAMP-dependent pathways and it has been demonstrated in several cell types [89]. Central to the cross-talk is the cAMP-specific phosphodiesterase 3 (PDE3) that is inhibited by cGMP and results in the activation of cAMP-dependent protein kinase (PKA) through the increase of intracellular cAMP levels [85]. The role of the cAMP pathway and the mechanisms of HbF regulation have been described previously with c-Myb identified as a key role player in the cAMP-mediated inhibition of γ -globin expression, particularly in K562 cells that significantly express c-Myb as compared to adult erythroblasts [90], possibly suggesting the absence of c-Myb/cAMP regulation of HbF in adult erythroblasts. Although c-Myb reduces the transcriptional activity in the γ -globin promoter, which is concomitant with the low expression of c-Myb in γ -globin expressing-adult erythroblasts, induction of the cAMP pathway is central to erythroid differentiation in K562 cells [90]. The role of the cAMP pathway in γ -globin regulation in erythroblasts appears to be transient and developmental stage-specific, with significantly higher intracellular cAMP levels in fetal liver erythroblasts as compared to the low-to-

undetectable levels in adult erythroblasts. These data advocate for further investigation of the cAMP pathway and identification of the “cAMP-switch” during erythropoiesis as a potential therapeutic target for cAMP-mediated HbF induction in erythroblasts. Furthermore, the polar role of the cAMP pathway in K562, where it inhibits γ -globin expression, and adult erythroblasts, where it induces HbF production, suggests that the role of the cAMP pathway in the regulation of HbF may depend on the global cellular context of erythroid cells.

MAP Kinases—Mitogen-activated protein kinases (MAPKs) such as extracellular signal-regulated protein kinase (ERK), p38 and c-Jun N-terminal kinase (JNK) appear to be independent of cAMP-dependent pathways regulating HbF [91], even though cross-talk between cAMP-dependent and MAPK pathways has been described in non-erythroid cells [92–96]. Several studies have investigated the role of MAPKs in HbF production using erythroid cells [97–100]. HU has been shown to increase phosphorylation of MAPK p38, while simultaneously causing dephosphorylation of ERK and JNK [97], effects which are known to affect erythroid differentiation and induce γ -globin expression. Furthermore, specific inhibition of p38 in K562 cells without affecting ERK and JNK, resulted in decreased expression of γ -globin, contrary to inhibition of ERK.

JNK/Jun pathway—Recently the role of *SAR1*, a small guanosine triphosphate-binding protein in adult erythroid cells [101], in HU-induced HbF has been shown in bone marrow CD34⁺ and K562 cells [82]. Nuclear factor κ B (NF κ B) was shown to be central to the expression of *SAR1* through direct binding to the promoter sequence after induction by HU. Furthermore, the involvement of c-Jun NH₂ (N)-terminal kinase (JNK)/Jun phosphorylation and *Gia* pathways in γ -globin expression were demonstrated through inhibition and silencing experiments in both CD34⁺ and K562 cells, resulting in the reduction of HbF and thus demonstrating that activation of the *Gia/JNK/Jun* pathway proteins is necessary for NF κ B-dependent SAR1 expression in the production of HU-mediated HbF.

Other molecular pathways—GATA erythroid transcription factors are widely considered key modulators of γ -globin expression [101]. Both *GATA-1* and *GATA-2* proteins have been associated with HU treatment in K562 cells prompting the possibility of a HU-induced *GATA-1* and/or -2/p21-dependent signal transduction pathway toward γ -globin expression [101]. Reactive oxygen species (ROS) such as superoxide, hydrogen peroxide (H₂O₂) and NO have been shown to mediate the phosphorylation of p38 MAPK [102,103], whose association with HbF production was discussed above. Inducers of γ -globin such like sodium butyrate and trichostatin-A have demonstrated an H₂O₂-dependent activation of p38 through phosphorylation and subsequent HbF production in erythroid progenitors and K562 cells [104]. However, HU-induced γ -globin expression is independent of H₂O₂ formation, albeit other ROS such as NO, superoxide and hydroxyl radicals are known to activate MAPKs and cGMP [105,106].

Nitric oxide—HU treatment is known to improve blood flow through reperfusion and decreased sickling; however the reasons for the improved circulation are not completely understood. Nitric oxide (NO) release has had a long-standing association with HU-induced improved blood flow through vasodilation [107–113]. Recently, a rabbit erythrocyte model

was used to demonstrate that HU regulates the production of NO by the enzyme endothelial nitric oxide synthase (eNOS) [114], and further showing that the released NO induces blood flow-induced ATP, which is a known mediator of vasodilation through endothelial cell purinergic receptor binding [115–118] and subsequent release of endothelial NO [119–121]. The improved blood flow results from the NO-induced increase in oxygen affinity of sickle erythrocytes in SCD patients because NO exhibits anti-sickling properties and inhibits HbS polymerization [85,122]. NO may be released by endothelial cells and/or erythrocytes, establishing a feedback loop with flow-derived ATP release from erythrocytes and provide rationale for some of the immediate reported benefits of HU treatment (improved blood flow and attenuated sickling events in SCD patients) [123].

Post-transcriptional pathways: Regulation by microRNAs

Transcriptional regulation through short non-coding RNA oligonucleotides (miRNAs) holds significant promise in explaining variances in HbF induction in SCD patients of similar genomic background and environmental influence. Recently, a study demonstrated significant HU-induced miRNAs expression in SCD patients and associated with HbF at both baseline (miR-494) and maximum dose tolerated (miR-26b and miR-151–3p). Furthermore, HU has also been shown to up-regulate miR-15a and miR-16–1, whose direct target is *MYB*, a critical regulator of γ -globin expression [124]. Down-regulation of miR-148b has also exhibited tumor suppressor properties through inhibition of cell proliferation in gastric cancer [125]. Reticulocytes have also been shown to contain miRNAs, although enucleated and previously thought not to contain any nucleic acids [126]. Furthermore, it was shown that the miRNA profile of terminally differentiated erythrocytes from SCD patients was significantly different from unaffected individuals, thereby prompting an association with disease physiology. Defective terminal differentiation during sickle cell erythropoiesis was supported by an association with poor expression of miR-320 [126]. The role of miRNAs in haematopoiesis, specifically erythropoiesis and erythrocyte physiology has also been previously reviewed [127].

The field of post-transcriptional regulation of HbF has made a recent surge to scientific interest as a potential level along the pathway to γ -globin expression for therapeutic targets. In a recent study, inhibition of dephosphorylation of the eukaryotic initiation factor 2 α (eIF2 α), a critical regulator of protein translation, enhanced production of HbF and was devoid of any changes at the levels of gene transcription, cellular proliferation and differentiation [128]. The use of salubrinal, a known inhibitor of eIF2 α phosphorylation, improved γ -globin mRNA turnover to HbF without a globin preference shift (the ratio of $\gamma/[\gamma+\beta]$ remained unchanged), and thereby elucidating another level for potential therapeutic targets. In support of these findings, salubrinal was further shown to increase translation efficiency during the recovery phase of cellular stress response through an increase in the number of selective translating ribosomes on the γ -globin mRNA [129]. Further investigation of translation-altering agents and the global disease-specific miRNA profile in understudied and highly heterogeneous populations such as in Africa promises to yield mechanistic insight into the disease phenotype and differential response to HU treatment.

Discussion and Conclusion

Hydroxyurea (HU) treatment has demonstrated success in several settings, both in children and adults with SCD. The current dilemma stems from the various clinical response to the treatment and often the lack thereof. There has been great progress made towards understanding the various mechanisms by which HU induces HbF production through identifying key genomic variants that predispose the response to the treatment, various environmental contributors as well as specific signal transduction pathways that modulate γ -globin expression. However the complete picture of HU-mediated HbF production remains elusive. While the majority of HU benefit on SCD is directly related to the amount of HbF produced, it is also suspected that HU have some disease modulating effects outside of HbF induction, such as stress haematopoiesis, endothelial nitric oxide release or the reduction of leucocytes counts that the present paper have not fully addressed.

In recent times, there has been a shift in the search for therapeutic targets toward the post-transcriptional and post-translational mechanisms that regulate HbF production as a way of minimizing alterations to the global transcriptional system through stress. There is an urgent need to investigate the role of miRNAs in HU-induced HbF production in SCD patients and further explore other potent negative regulators of γ -globin expression and target of miRNAs, with the aim of elucidating a miRNA-mediated signalling transduction pathway induced by HU treatment. The outcomes of such studies would firstly, yield post-transcriptional population-specific variants and provide potential explanations for the vast inter-patient variation in response to HU treatment. And secondly, delineate another regulatory level of gene expression to known pathways and therefore possibly reveal therapeutic targets that may minimize alterations to the cellular transcriptome.

Expert commentary

Hydroxyurea (HU) treatment has demonstrated success in several settings, both in children and adults with SCD. Negating the sparse evidence on the potential treatment-related adverse effect and the barriers to use, as the only available drug treatment for SCD, HU has a potential role in ameliorating the global problem of SCD, particular in high disease burden locales such as Africa. The current dilemma stems from the various clinical response to the treatment and often the lack thereof. This challenge could be overcome in the future with 3 clinical trials underway in Africa, spanning Angola, Congo, Kenya and Nigeria, as well as North America and Europe, with 21 and 3 open trials respectively [130]. Furthermore, there has been great progress made towards understanding the various mechanisms by which HU induces HbF production through identifying key genomic variants that predispose the response to the treatment, various environmental contributors as well as specific signal transduction pathways that modulate γ -globin expression. However the complete picture of HU-mediated HbF production remains elusive. The present study represents, to our knowledge, the first systematic review on known molecular mechanism of action of hydroxyurea-induced HbF in SCD, the only medication available for this condition of global importance. It is anticipated that adequate knowledge of the mode of action of HU will result in the exploration of alternative therapeutic agents with possibly less toxicity.

Five-year view

The study has provided some specific perspectives in future research, specifically on post-transcriptional regulations by microRNAs that could lead to possible therapeutic targets that minimize alterations to the cellular transcriptome. There is an urgent need to investigate the role of miRNAs in HU-induced HbF production in SCD patients and further explore other potent negative regulators of γ -globin expression and target of miRNAs, with the aim of elucidating a miRNA-mediated signalling transduction pathway induced by HU treatment. The outcomes of such studies would firstly, yield post-transcriptional population-specific variants and provide potential explanations for the vast inter-patient variation in response to HU treatment. And secondly, delineate another regulatory level of gene expression to known pathways and therefore possibly reveal therapeutic targets that may minimize alterations to the cellular transcriptome.

While the majority of HU benefit on SCD is directly related to the amount of HbF produced, it is also suspected that HU have some disease modulating effects outside of HbF induction, such as stress haematopoiesis, endothelial nitric oxide release or the reduction of leucocytes counts; this paper did not explore those mechanisms. It is anticipated more research on other HU-modulating effects on SCD outside of HbF induction will evolve from these areas to complete a comprehensive profile of the mechanism of action of HU in SCD.

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Reference annotations:

Of particular interest = **

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Key issues

- The study have summarised the various molecular pathways that have been reported to explain the mode of action of HU.
- Numerous signal transduction pathways have been implicated, through the identification of key genomic variants in *BCL11A*, *HBS1L-MYB* or *SAR1* that predispose the response to the HU treatment in SCD.
- Additional signal transduction pathways modulate γ -globin expression (cAMP/cGMP; Gi α /JNK/Jun; methylation and microRNA).
- Three main molecular pathways have been reported:
 1. Epigenetic modifications, transcriptional events and signalling pathways involved in HU-mediated response,
 2. Signalling pathways involving HU-mediated response
 3. Post-transcriptional pathways (regulation by microRNAs).

Fig.1. Various HU-activated signal transduction pathways for γ -globin expression
Summarized pathways previously associated and/or induced by HU treatment in primary erythroid and K562 cells.

Table 1

Genomic variants associated to Hydroxyurea-induced HbF level

Gene	SNPs	Chromosome: locus	References
<i>HBB</i> gene cluster haplotype	rs7482144	11:5254939	[5,34,72–81,131]
<i>BCL11A</i>	rs1427407	2:60490908	[132]
	rs4671393	2:60491212	
	rs7606173	3:60493111	
	rs7557939	2:60494212	
	rs1186868	2:61764103	
<i>ARG1/2</i>	rs2295644	14:67599842	
	rs17599586	6:131583579	
	rs28384513	6:135055071	
<i>HBS1L-MYB</i>	rs9399137	6:135097880	
<i>SARI</i>	rs2310991	3:142444839	[82,83]
	rs4282891	10:70171890	
	rs76901216	10:70170313	
<i>SALL2</i>	rs61743453	14:21523209	[27]

Table 2

Summary of Mechanisms of HbF production in response to hydroxyurea

Signal level	Pathway	Mechanism of γ -globin induction	References
Epigenetic & Transcriptional Events	DNA remodelling DNA methylation	<i>Mi-2/NuRD</i> ; Acetylation and methylation <i>DNMT-1</i>	[31,38,84,133,134] [131, 132]
Signalling pathways	<i>Gia/JNK/Jun</i>	<i>NFκB</i> induction of <i>SAR1</i>	[82]
	cGMP	sGC-PKG and NO-induced sGC-PKG	[86,88,90,91]
	cAMP	<i>MYB</i> transcriptional control	[90,124,135]
	MAPKs	<i>Erk1-2/p38/JNK/CREB1</i>	[91,97–100,136]
	Nitric oxide	eNOS, flow-induced ATP and anti-sickling	[85,123]
	Phosphorylation	<i>EIF2α</i>	[128]
	Transcription factors	<i>GATA-1/2; p21; EPO; KLF1</i>	[32,101,137]
	Stress signals	ROS (superoxide and hydroxyl radicals)	[104]
Post-transcription	Translational Regulation	miR-494; 15a; 16-1; 151-3p; 148b	[84,124–126,138–141]