Noncoding RNA-Mediated Control of HbF Switching: From Molecular Mechanisms to Clinical Translation

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Abstract

Noncoding RNAs (ncRNAs) act as critical epigenetic regulators of fetal hemoglobin (HbF) switching, offering new therapeutic possibilities for treating β-hemoglobinopathies, including sickle cell disease and β-thalassemia. Despite significant advances, the exact role of ncRNA-mediated regulatory networks within existing models of transcriptional and epigenetic regulation of γ-globin expression remains unclear. This review summarizes the roles and interplay of microRNAs (miRNAs), long noncoding RNAs (lncRNAs), circular RNAs (circRNAs), and PIWI-interacting RNAs (piRNAs) in controlling γ-globin expression, along with their interactions with key regulators such as BCL11A, KLF1, and various epigenetic modifiers. miRNAs, including miR-15a, miR-16-1, miR-486-3p, and miR-210, have been found to regulate key transcription factors that act as either enhancers or suppressors of HbF production. lncRNAs, such as BGLT3 and NR_120526, exert diverse regulatory functions through chromatin remodeling, transcriptional regulation, and protein interactions. circRNAs, including hsa-circRNA-100466 and hsa-circRNA-0008102, exert an indirect effect on HbF, as they are thought to act as molecular sponges for miRNAs. The precise mechanism by which piRNAs contribute to HbF switching remains unclear; future studies should aim to map the complete regulatory networks of ncRNAs, elucidate their molecular interactions, and test therapeutic approaches in advanced preclinical and clinical settings for the treatment of β-hemoglobinopathies. Together, this review moves beyond the descriptive classification of ncRNA-mediated switching mechanisms toward an integrated regulatory model of HbF switching, and examines the therapeutic potential of targeting these networks to develop novel, next-generation treatment approaches for β-hemoglobinopathies.

Keywords: noncoding RNAs; fetal hemoglobin (HbF); β-hemoglobinopathies; microRNAs (miRNAs); long noncoding RNAs (lncRNAs); circular RNAs (circRNAs); piwiRNA (piRNA)

Highlights

- Noncoding RNAs are key regulators that regulate fetal hemoglobin switching.
- MiRNAs, lncRNAs, and circRNAs regulate the expression of γ-globin by an epigenetic mechanism.
- NcRNA networks bind with major HbF repressors and transcription factors.
- New therapeutic approaches to hemoglobinopathies exist in the form of emerging ncRNA-based approaches.

- The discussion focuses on the translational challenges and future directions for ncRNA-targeted HbF induction.

1. Introduction

Human hemoglobin (Hb) is a tetrameric protein composed of two pairs of globin polypeptide chains, with each pair encoded by genes belonging to distinct globin gene family. The human alpha-like globin genes (ζ, α_1, and α_2) are found on chromosome 16, whereas the beta-like globin genes (ε, Gγ, Aγ, δ, and β) are found on chromosome 11 (1). Six distinct hemoglobin types are formed during development as a result of successive changes in the alpha and beta like globin clusters (2). In the early stages of embryonic development, hemoglobin production starts in the yolk sac: this results in the formation of three types of hemoglobins: Hb Gower 1 ($\zeta_2\varepsilon_2$), Hb Portland ($\zeta_2\gamma_2$), and Hb Gower 2 ($\alpha_2\varepsilon_2$) (3). In the thirteenth week of pregnancy, there is a shift of erythropoiesis from the yolk sac of the embryo to the fetal liver, and fetal hemoglobin (HbF; $\alpha_2\gamma_2$) becomes the most abundant type of hemoglobin throughout pregnancy due to erythroid precursor cells from 10-12 weeks of pregnancy to the first six months after birth (4). HbF include two alpha and two gamma subunits ($\alpha_2\gamma_2$), differing from adult hemoglobin in its oxygen delivery characteristics, which are crucial for fetal circulation (5). Throughout development, a complex hemoglobin switch takes place, where HbF is progressively replaced by adult hemoglobin (HbA, $\alpha_2\beta_2$) postnatally (6). This transition is significant since the persistence of HbF can benefit patients with β-globin disorders, like β-thalassemia.

1.1 Clinical significance of HbF in β-hemoglobinopathies (SCD, β-thalassemia)

The role of HbF in β-thalassemia is associated with the constant actuation of the γ-globin gene, which helps to correct the imbalance of globin chains by helping the produced γ-globin to be connected with the remaining unbound α-globin chain (7). Similarly, in sickle cell disease (SCD), elevated HbF levels are associated with less severe symptoms because deoxygenated red blood cells take a longer time to sickle (8). The underlying pathological process of SCD involves the polymerization of deoxygenated sickle hemoglobin in hypoxia due to changed physical characteristics, which is inhibited by the increase of HbF (9). Both HbF and its hybrid tetramer ($\alpha_2\gamma\beta S$) cannot engage in the polymerization process of deoxygenated sickle hemoglobin (HbS); the consequent and detrimental damage to cellular structures due to the polymerization of HbS is thus prevented (10). In both forms of hemoglobinopathy, the enhanced γ-globin regulation gene is recognized as a modulating factor on the phenotype of

the disease. Thus, knowledge of the intricate mechanisms associated with γ-globin gene regulation can be anticipated to have direct clinical implications, as well as to find potential application in therapeutic development of β-hemoglobinopathies (11).

1.2. Noncoding RNAs as emerging epigenetic regulators

Non-coding RNAs (ncRNAs) are known for their significant role in regulating the expression of the β-globin gene and the production of HbF, making them promising targets for the treatment of β-hemoglobinopathies (12). MicroRNAs (miRNAs) are especially crucial in particularly important in globin regulation because of their post-transcriptional gene silencing activities (13). Specific miRNAs including miR-15a, miR-16-1, miR-23a, miR-26b, miR-27a, and miR-451, are capable of targeting key transcription factors, including Myeloblastosis Oncogene (MYB), B Cell Lymphoma 11A (BCL11A), GATA binding protein 1 (GATA1), Kruppel-Like Factor 3 (KLF3), and Sp1 (Specificity Protein 1) and thus cause increased γ-globin synthesis and production of HbF (14). These ncRNAs serve as protein decoys, they interact with many aspects of the biological processes and serving as markers for hematological disorders Understanding these molecular mechanisms is key for development ncRNA-based therapy for β-thalassemia and SCD (15).

Besides numerous miRNAs, a few long noncoding RNAs (lncRNAs) have been discovered that are associate with HbF regulation; however, the whole regulatory network of these ncRNAs involved in hemoglobin switching remains incomplete. Furthermore, the functions of lncRNAs, circular RNAs (circRNAs), and piwi interacting RNAs (piRNAs) have not been widely explored, and there is a need for more thorough and advanced research on these topics as well as the development of new treatment approaches. This review addresses the gap by summarizing recent findings on how ncRNA regulates HbF, emphasizing the intertwined regulatory networks that regulate γ-globin expression, and exploring the potential for ncRNA-based therapeutic strategies. Furthermore, we highlight existing challenges, limitations, and future research focuses necessary for the clinical implementation of ncRNA-targeted therapies for β-hemoglobinopathies.

2. Molecular Basis of HbF Switching

The transition from fetal to adult hemoglobin is regulated by complex transcriptional mechanisms, which are governed by negative regulation of the fetal γ-globin genes and positive regulation of the adult β-globin genes during development (16). This transcriptional regulator

is important for the regulation of the γ-globin gene. With the advancement of biological technologies and numerous factors have been found and studied extensively (17, 18). One of the key components now well-established in the modulate the activation of the γ-globin protein is the zinc-finger protein, BCL11A, which is a strong repressor of the expression of the γ-globin protein (19). Research indicates that BCL11A has been shown to interact with DNA methyltransferase 1 (DNMT1), the nucleosome remodeling and deacetylase complex (NuRD), GATA1, and SRY-box 6 (SOX6) to inhibit the expression of γ-globin by binding to its distal and proximal promoters (20, 18).

Another important repressor, ZNF410, a zinc-finger protein that attaches to and consequently upregulates the transcription of chromodomain helicase DNA binding protein 4 (CHD4), coding for the NuRD complex, which in turn represses the expression of the γ-globins (21). Studies have revealed that MYB attaches to the promoter of the β-globin activator Kruppel-Like Factor 1 (KLF1), increases KLF1 expression, which subsequently triggers the activation of the γ-globin repressor GATA-1, making MYB a potent γ-globin repressor. Each globin promoter is associated with a locus control region (LCR) that is able to loop this region back to the promoter to enhance transcription (22).

Recent preclinical and clinical studies suggest that epigenetic changes involving DNA methyltransferases (DNMTs), histone methyltransferases (HMT), demethylases, histone deacetylases (HDACs), and histone acetyltransferases (HATs), and changes in the levels of miRNAs, have been proposed to influence the transition from γ-globin to β-globin synthesis (23). After birth, changes take place in which the γ-globin genes are suppressed. Since epigenetic marks are reversible, they offer promising targets for therapies aimed at reactivating fetal hemoglobin genes (24). Earlier methods for inducing HbF were mostly empirical and not very effective, but identifying specific molecular targets opens up new possibilities for developing more effective, rationally designed treatments (25).

DNA methyltransferases (DNMT) are enzymes that catalyse the methylation of the cytosine residues on DNA. Three functional DNMTs have been discovered in mammalian cells: DNMT1 for maintenance methylation, and DNA methyltransferases 3A (DNMT3A) and DNA methyltransferases 3B (DNMT3B) for de novo methylation (26). These enzymes facilitate the attachment of a methyl group on the 5th position of cytosine in CpG dinucleotides, using an S-adenosyl-methionine donor. 5-methylcytosine in the regulatory regions of certain genes may influence the interaction of transcription complexes to the DNA (27). Moreover, 5-

methylcytosine can associate with silencing proteins, such as methyl-DNA-binding proteins, HDAC, and HMT, which modify chromatin architecture. Both mechanisms lead to the suppression of genes with methylated regulatory elements (28).

Another epigenetic mechanism is histone modification. Histones have numerous post-translational modifications, such as acetylation, methylation, phosphorylation, ADP-ribosylation, and ubiquitination, which can change their chromatin structure due to their high content in lysine residues of the N-terminal tail regions (16, 29). Increased acetylation of histones is highly correlated with increased gamma globin expression. In particular, acetylation of lysine residues within histones H3 and H4 and binding of RNA polymerase II to the γ-globin enhancer leads to the increase of HbF (30). The acetylation of lysine residues in the N-terminal tail region of the histones H3 and H4 has been demonstrated to modulate the expression of γ-globin gene, inducing its silencing (31).

Methyltransferases are also responsible for methylating histone amino acid residues, including lysine methyltransferase (KMT), protein arginine methyltransferase (PRMT), may result in an open chromatin configuration which is responsible for activating genes, or a more condensed chromatin state that represses gene expression (32). These investigations suggest that the interaction between histone modifications and the recruitment of factors with ultimate control on globin gene expression at the HBB locus is complex (32).

3. Overview of Noncoding RNAs

NcRNAs comprise a large class of regulatory RNA molecules that don't specify any protein but are playing a critical and more appreciated role in gene expression regulation in eukaryotic cells (33, 34). These are crucial regulators of HbF switching, thereby influencing the change over from fetal to adult globin gene expression during erythropoiesis (35). They mediate environmental responses, chromatin remodeling, transcriptional regulation, and post-transcriptional modulation of the transcript to new therapeutic possibilities in hemoglobinopathies like SCD and β-thalassemia (36). NcRNAs are divided into two broad groups: short ncRNAs, which contain miRNAs, small interfering RNAs (siRNAs), and piRNAs; and lncRNAs, which include long intergenic ncRNAs (lincRNAs) and circRNAs (37). The interplay of ncRNAs with DNA as well as other RNA and proteins can regulate gene activity to adjust the expression of genes in several biological pathways, making them important regulators of gene expression (38). The regulatory functions of ncRNAs are manifested at different levels: transcriptional, often post-transcriptional, or even epigenetic

regulation, related to functions of chromatin-modifying complexes, for activation or repression of target genes (39).

3.1. MicroRNAs in HbF Switching

MiRNAs are small noncoding RNAs, typically 19 to 25 nucleotides long, that regulate expression of gene at the post-transcriptional level. They typically achieve this by forming either perfect or partial base-pairing with the 3′ UTR of the target mRNA (40). When miRNAs bind to target mRNA, they can influence gene expression through several mechanisms. One common method is the suppression of translation, where the miRNA-RISC complex obstructs ribosome attachment to the mRNA (41). Moreover, miRNAs may mediate mRNA degradation through the promotion of deadenylation, decapping and exonucleolytic decay. They may also play a role in stability of mRNA by either safeguarding target mRNAs from degradation or boosting their stability (42). Increasing evidence shows that miRNAs are important in erythropoiesis. Some of the miRNAs are directed at major transcriptional repressors, including BCL11A and MYB, or epigenetic regulators including DNMT, can relieve the repression of γ-globin, resulting in enhanced HbF synthesis. Transcription factors of fetal to adult hemoglobin switch are important regulators of BCL11A, SOX6, MYB, KLF1 and KLF3, and in normal conditions, these factors are inhibitory in adult erythropoiesis on HbF levels (43).

Reactivation of the switch and a rise in HbF production, together with the developmental activation of globin gene expression, have been associated with several miRNAs (44). In particular, Bianchi et al. have found that high levels of HbF in an erythroid stem cell patient with thalassemia are associated with increased levels of miR-210; mithramycin was shown to target miR-210 and induce γ-globin activation in K562 cells in this study (45). In one study investigating SCD patients receiving HU, the activity of miR-26b and miR-151-3p were shown to be linked with HbF levels at the maximum tolerated dose (46). Another study shown that miR-34a suppressed Signal Transducer and Activator of Transcription 3 (STAT3), a recognized inhibitor of γ-globin, to cause HbF production in K562 cells (47).

Further functional studies revealed that, upon the introduction of both normal and sickle erythrocytes, miR-144 silenced the transcription of Nuclear Factor Erythroid 2-Related Factor 2 (NRF2) and consequently, the transcription of γ-globin. However, this suppression was inhibited in a dose-dependent manner by the treatment with miR-144 antagomir (48). In cultured adult primary erythroid cells, the overexpression of the tumor promoting LIN28B led to a decrease in miR-Let7 levels and an increase in the expression of the γ-globin gene and

HbF levels, which was caused partly by its effect on the expression of BCL11A (49). Let-7 directly targets and represses HIC2, which in turn functions as a transcriptional repressor of BCL11A (50). Furthermore, miR-486-3p facilitated HbF synthesis in adult erythroid progenitors by suppressing BCL11A expression (51).

The heightened expression of miR-15a and miR-16-1 in human erythroid cells is linked to the repression of MYB and the up-regulation of fetal and embryonic Hb genes, as a mechanism of delayed switching (52). Both miR-221 and miR-222 have been demonstrated to modulate the expression of the kit receptor in the erythroid cells and to influence HbF expression (53). MiR-29b induces HbF by directly targeting DNMT3A/3B mRNAs, resulting in decreased DNA methyltransferase expression, DNA hypomethylation at the γ-globin gene, and reactivation of fetal globin. miR-29b mimics enhance the γ/β globin mRNA ratio and increase HbF-positive cell populations both in vitro (human CD34+ and SCD erythroid progenitors) and in vivo (SCD mouse models), with significant reductions in pathological sickling (54). An intriguing in vitro study, miR-96 was found to be an immediate suppressor of γ-globin gene expression, and there was an inverse relationship between the amount of HbF and the amount of miR-96 in the umbilical cord blood and adult blood reticulocytes (55). The **Figure 1** depicts miRNA-mediated transcriptional and epigenetic regulation of fetal-to-adult hemoglobin switching.

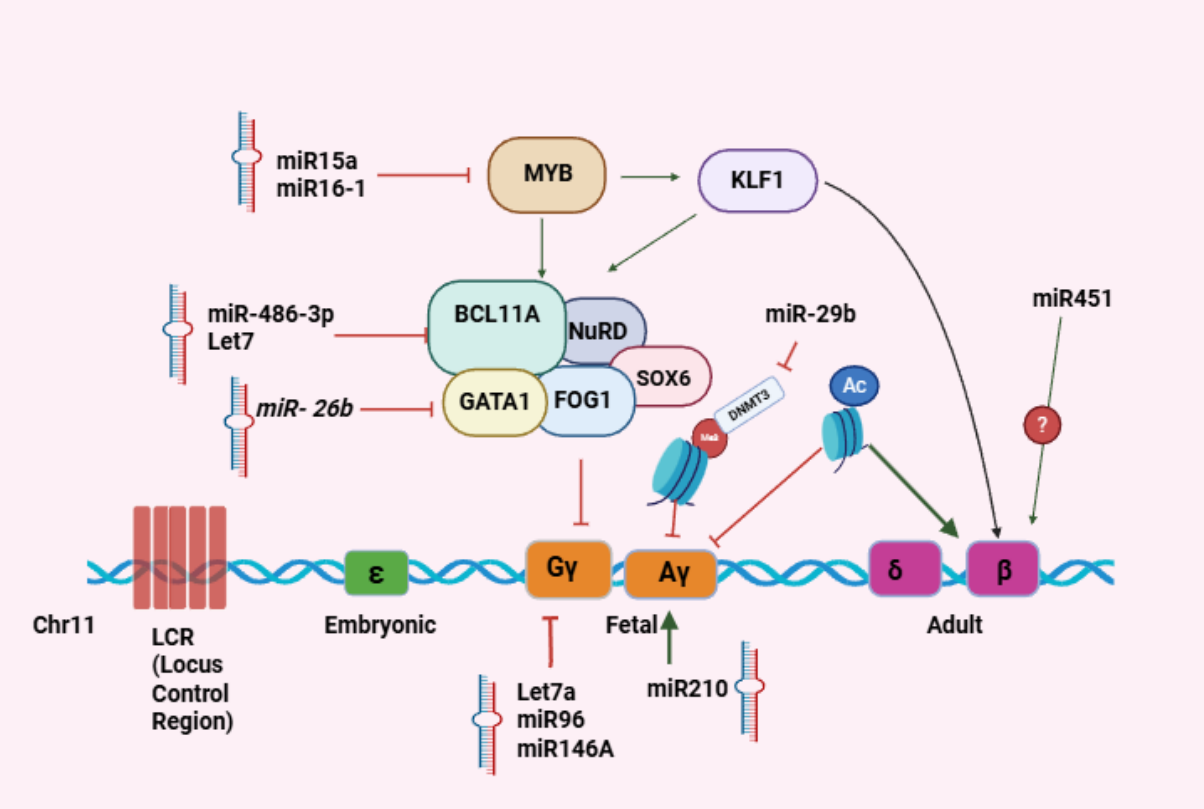

Fig. 1- miRNA-mediated transcriptional and epigenetic regulation of fetal-to-adult hemoglobin switching. This image is created using https://BioRender.com

Schematic representation of the miRNA-transcription factor-epigenetic network involved in switching the globin genes at the β-globin locus on chromosome 11 is presented. miR-15a/16-1 inhibits MYB, which results in decreased KLF1-driven activation of BCL11A, a crucial suppressor of γ-globin. miR-486-3p and Let-7 directly target BCL11A, whereas miR-26b influences GATA1 activity. BCL11A is involved in establishing a repressive chromatin state at the fetal gamma globin genes (Gγ and Aγ) in association with the NuRD complex, GATA1, FOG1, and SOX6. Conversely, miR-29b and miR-210 facilitate chromatin acetylation and the reactivation of γ-globin. This comprehensive regulatory network controls the suppression of HbF and the adult β-globin expression.

3.2. Long Noncoding RNAs in HbF Switching

LncRNAs are the most prevalent and variable members of the ncRNA family. It represents the majority of ncRNAs transcripts and their lengths are greater than 200 nucleotides (56, 57). Some lncRNAs act as guides to lead chromatin remodelers (including SWI/SNF (Switch/Sucrose Non-Fermentable) family members and NuRD complex components) as well as histone-modifying enzymes (including HATs, HDACs, methyltransferases, and demethylases) to the β-globin locus, facilitating the creation or alteration of repressive chromatin structures (58). Additionally, they can also act as competing endogenous RNAs (ceRNAs) to indirectly control the expression of other genes (59, 60). Besides, certain lncRNAs have been observed to be able to inhibit miRNAs, both intronic and exonic sequences during miRNA maturation (61). With their central function in biological processes, lncRNAs are expected to play a role in the pathogenesis of many diseases, such as malignancies and hematologic disorders such as hematopoietic stem cell disorder (62). The most likely mechanisms suggested so far are: (i) activation of histone protein in the epigenetic modulation; (ii) scaffolding of transcription factors; and (iii) activation of the interaction between activator and promoters (63). High levels of HbF have been linked to dysregulated gene expression of lncRNAs and miRNAs in carriers of β-thalassemia and hereditary persistence of fetal Hb (HPFH) (64). High levels of HbF could be induced by the activation of haemopoietic cell lineage-inducible molecules and Hemoglobin Subunit Epsilon 1 (HbE1) by lncRNA as one of the possible mechanisms (44). At a mechanistic level, this lncRNA interacts directly with the promoter of the ERF (Ets2 Repressor Factor) gene and brings DNMT3A into action to promote

hypermethylation of the ERF promoter, effectively silencing ERF, which is recognized as a repressor of γ-globin (65).

H19 is an abundant lncRNA and plays a critical role in controlling hemoglobin expression. It affects the cell more at critical developmental periods, such as the transition from fetal to adult hemoglobin and embryogenesis (66, 67). H19 showed characteristic locations in the cell, in K562 cells it was principally in the nucleus and in HUDEP-2 cells it was principally in the cytoplasm (66). The presence of H19 in the cytoplasm of erythroid precursor-2 (HUDEP-2) cells from human umbilical cord blood opens the interesting possibility that it may be a competitive endogenous RNA (ceRNA) which may mediate the interaction of microRNA or mRNAs in regulating the expression of the γ-globin gene (15, 66).

The intergenic long noncoding RNA HBS1L-MYB (HMI-lncRNA) is a possible regulator of the γ-globin gene. This lncRNA is investigated to be enhancer RNA (eRNA) involved in the association between the remote activator and promoter of MYB (68). A reduction of this lncRNA's expression will lead to a marked rise in HbF expression due to increased expression (68).

Recent studies have drawn attention to the role of Beta globin locus transcript 3 (BGLT3) lncRNA that is transcriptionally activated by hypoxia-inducible factor 1 (HIF1) under hypoxia or following von Hippel-Lindau (VHL) gene disruption (69). HIF1 attach to hypoxia response elements (HREs) in the BGLT3 locus inducing chromatin changes to stimulate expression of γ-globin gene. This is achieved through recruitment of GATA1, p300, and increased H3K27ac, an enhancer mark resulting in long-range looping between the BGLT3 region and globin loci ultimately leading to HbF upregulation (70).

The presence of NR_120526 was found to be strongly associated with HbF levels, and a variety of different lncRNAs were found to be differentially expressed between the individuals with high HbF levels. NR_120526 is hypothesized to negatively regulate the expression of γ-globin gene, and thus presents an attractive therapeutic target for increasing HbF in β-thalassemia patients, as it is believed to be suppressed in the presence of NR_120526. The therapeutic approach of targeting such negative regulators could be useful to enhance HbF levels in β-thalassemia and other disorders (71).

Seven β-thalassaemia patients with high HbF levels underwent microarray profiling analysis, and there was a considerably greater average gene expression of the lncRNA uc002fcj.1 and

the lncRNA NR_001589. This evidence proves the positive correlation of the lncRNAs and HbF. Further functional studies for the lncRNA uc002fcj.1 and lncRNA NR_001589 would help shed light on the molecular pathways involved in β-thalassaemia. It is hypothesized that the lncRNAs have the potential to work as ceRNAs, where it regulates the up-regulation of γ-globin gene through the sequestration of certain miRNAs, thus promoting HbF production in β-thalassaemia (72). The role of ncRNAs in the regulation fetal hemoglobin is summarized in **Table 1** (23, 44- 48, 51, 52, 55, 69, 73-78).

Table 1. The roles and mechanisms of noncoding RNAs in the fetal hemoglobin regulation

Noncoding RNAs	Target	Effect of γ-globin	Molecular mechanism	References
miRNAs				
Let-7a	γ globin	γ globin gene switching	Indirectly modulating erythroid maturation and γ-globin repression pathways	(73)
miR-486-3p	BCL11A	Increase expression of γ globin gene	Modulates erythroid differentiation signaling pathways, indirectly affecting γ-globin expression	(51)
miR-96	γ globin (CDSregion)	Suppress γ globin gene expression	Directly targets γ-globin mRNA, suppressing its translation	(55)
miR-29b	DNMT, MYB	Increase expression of γ globin gene	leading to hypomethylation of the γ-globin promoter	(23)
miR-144	NRF2	Decrease HbF (negative regulator)	Targets NRF2 and oxidative stress response pathways, indirectly influencing HbF regulation	(48)
miR-26b	GATA1	Increase γ globin gene expression	Modulates chromatin regulators and erythroid transcriptional programs	(46)

miR-210	γ globin	γ globin gene switching	Hypoxia-induced miRNA influencing erythroid differentiation and metabolic adaptation	(45)
miR-23a	SP1	Increases γ and ε globin expression	Targets components of the γ-globin repressor network, including erythroid transcription regulators	(74)
miR-27a	KLF3	Regulate HbF expression	Regulates erythroid differentiation pathways affecting globin gene expression	(74)
miR-34a	STAT3	γ-globin activation	Indirectly activating γ-globin through silencing of the repressor STAT3	(47)
miR-15a/-16-1	MYB	Increase γ globin gene expression	Suppress MYB expression, resulting in γ-globin de-repression	(52)
LncRNAs				
BGLT3	γ globin	Increase HbF	Promotes chromatin accessibility and LCR–γ-globin interaction	(69)
AlncRNA-EC7	γ-globin regulatory network	Increase γ-globin gene expression	Supports erythroid transcriptional program	(75)
HMI-lncRNA	MYB → BCL11A	Increases γ-globin gene expression and HbF	Suppresses MYB, reducing BCL11A-mediated repression	(76)

NR_001589, NR_120526 and T315543	γ-globin regulatory regions Potential	Increase γ-globin gene expression	Associated with epigenetic regulation in HbF-high states	(44)
CircRNAs				
has_circ RNA_100466	miR-19b-3p SOX6	Associated with increased γ-globin / HbF	Acts as a miRNA sponge, modulating erythroid regulatory pathways linked to γ-globin expression	(77)
has_circ RNA_0008102	MiR-425-3p GATA2 (GATA-binding factor 2)	Associated with increase γ-globin / HbF	Regulates erythroid gene expression through miRNA sequestration and transcriptional network modulation	(78)

3.3. Circular RNAs in HbF Switching

CircRNAs are a category of endogenous non-coding RNA molecules that have been recognized for their role in regulating gene expression (78). These circRNAs are innovative, endogenous, single-stranded, ncRNAs produced by a process called back-splicing. In contrast to their linear counterparts, circRNAs possess a covalently closed loop structure that is missing both a 5' cap and a 3' tail, and they vary in size from 100 nucleotides to over 4 kilobases (79). Covalently closed loop structure and superb stability with an absence of 5' cap and 3' tail render circRNAs less sensitive to degradation by nucleic acid exonucleases. Predominantly found in the cytoplasm and not the nucleus, circRNAs act like a sponge for miRNAs, thereby regulating the target genes expression and reducing the suppressive effects of miRNAs on target RNAs (80). It serves as competitive endogenous RNAs (ceRNAs), through the presence of miRNA binding, this miRNA-sequestering activity of circRNAs can affect target gene expression (81). CircRNAs can also interact with RNA binding proteins (RBPs) and modulate their functions. This interaction may alter the stability, localization and function of RBPs and subsequently influence gene expression (76).

CircRNAs have become important regulators in the fetal to adult hemoglobin switch, mainly via what is known as their interaction with miRNA and RNA-binding proteins in the erythroid cells. They bind the miRNAs like sponges and regulate the gene CircRNAs can sequester miRNAs and prevent them from binding to their target mRNAs, resulting in the enhanced stability and expression of target mRNAs (81). CircRNAs can also attach to RNA binding proteins (RBPs) and influence their activity. This interaction may alter the stability, localization and/or function of RBPs and subsequently influence gene expression involved in the HbF to HbA transition (82, 83). For instance, hsa-circRNA-100466 is downregulated in individuals who are carriers of β-thalassemia with high levels of HbF and promotes HbF levels by sponging miR-19b-3p, which in turn represses SOX6, a repressor of γ-globin (77). Another circRNA, circ-0008102, is upregulated in pediatric patients with β-thalassemia and is linked to the activation of γ-globin expression via the regulation of miRNAs (78). The interaction between miRNAs, lncRNAs, and circRNAs in fetal hemoglobin regulations is described in below **Figure 2.**

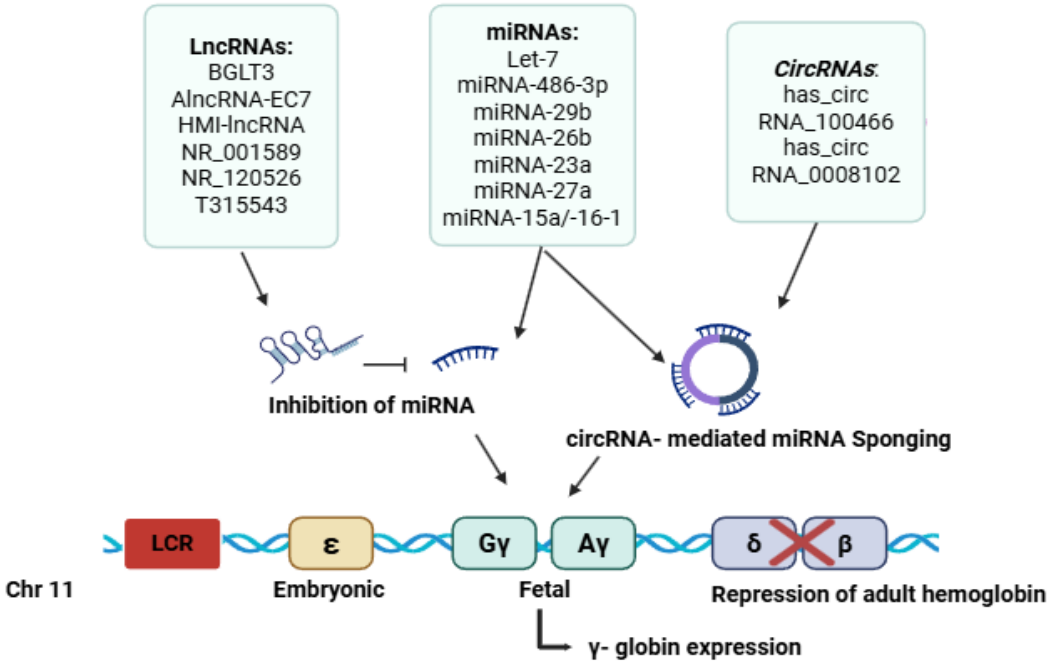

Fig. 2- Cross-talk between miRNAs, lncRNAs, or circRNAs in fetal hemoglobin regulation. This image is created using https://BioRender.com)

The schematic diagram (Fig. 2) illustrates how non-coding RNAs work together to regulate the globin genes expression at the β-globin locus on chromosome 11. Various microRNAs

(miRNAs) including Let-7, miR-486-3p, miR-29b, miR-26b, miR-23a, miR-27a, and miR-15a/16-1, along with long non-coding RNAs (lncRNAs) like BGLT3, AlncRNA-EC7, HMI-lncRNA, lncRNA-GT, NR_001589, NR_120526, and T315543, and circular RNAs (circRNAs) including hsa_circRNA_100466 and hsa_circRNA_0008102, engage in miRNA–lncRNA–circRNA interactions to influence globin gene switching. This network affects the locus control region (LCR) and increases the transcriptional activation of fetal γ-globin genes (Gγ and Aγ) while suppressing the expression of adult β-globin. This ncRNA-driven regulation supports the continued or renewed expression of γ-globin, resulting in elevated levels of fetal hemoglobin. This process provides therapeutic potential for use in the treatment of hemoglobinopathies including SCD and β- thalassemia.

3.4 Piwi-Interacting RNAs in Hemoglobin Switching

Piwi-interacting RNAs (piRNAs) are a unique type of ncRNAs that range from 24 to 31 bp long and are associated with members of the PIWI (P-element Induced Wimpy Testis) protein family (PIWIL1-PIWIL4) (84). They may play a role in preserving genome stability and regulating chromatin organization by interacting with PIWI proteins (85). There is no direct experimental evidence that links piRNAs with the regulation of HbF or the hemoglobin switching process, although piRNAs have been identified in somatic tissues and are believed to play a role in epigenetic gene silencing (86). Nevertheless, indirect evidence points to a possible connection, as piRNA-PIWI complexes are known to be engaged in chromatin remodeling, histone modifications, and interactions with epigenetic regulators like the NuRD complex, which is recognized for repressing γ-globin expression. These findings suggest that piRNAs could potentially affect HbF expression via epigenetic mechanisms. As a result, the function of piRNAs in regulating HbF is still a relatively unstudied field, and additional mechanistic and functional research is necessary to determine if there is a direct connection between piRNAs and the induction of HbF.

4. Noncoding RNA–Based Therapeutic Strategies for HbF Switching

Reactivating HbF in adults can have the potential to treat patients with all hemoglobinopathies. Clinical strategies for reactivating HbF through pharmacological means have been used to treat β-hemoglobinopathies (62). This positive association between elevated HbF production and the amelioration of severe cases of β-hemoglobinopathies is demonstrated by the existence of HPFH syndrome with an asymptomatic phenotype (86). Therefore, understanding of the HbF

switch is of critical importance for successful attempts to express the γ-globin gene in human expression systems (87). Despite the extensive investigations of the switching of hemoglobin, some of the regulatory mechanisms, particularly those involving epigenetic regulation, still remain obscure.

Hydroxyurea is a U.S.-approved pharmacological HbF inducer for moderate to severe SCD (88). While the precise mechanisms by which hydroxyurea promotes HbF remain unclear, several studies have noted its effect on reducing BCL11A, MYB, and KLF1, underscoring these genes are significant in the study of β-thalassemia as therapeutic targets (89). 5-azacytidine is a DNA hypomethylating agent which was initially identified to stimulate production of HbF in SCD and β-thalassemia patients; however, due to its carcinogenic activity it has limited applications for HbF therapies (90). Recent clinical trials have demonstrated that the combination of decitabine, a less toxic chemical derivative of 5-azacytidine and tetrahydrouridine (THU), stabilizes decitabine, showing its potential to be used in treatment of sickle cell disease (90). DNMT1 is highly familiar for maintaining methylation on DNA during cell division. Moreover, DNMT1 is a common repressor that can be recruited by sequence-specific DNA binding factors, such as DRED (TR2/TR4) and BCL11A that regulate epigenetic silencing of γ-globin (91). Additionally, recent investigations have revealed a HPFH-associated germline missense mutation in DNMT1, which directly demonstrated the role of DNMT1 as an important epigenetic co-repressor of the γ-globin gene, and also indicated that DNMT1 could be a therapeutic target for hemoglobinopathies (92, 93).

Among the naturally occurring short chain fatty acids, a popular HDAC inhibitor, butyrate, was reported to induce long-term HbF production in the majority of patients with SCD (94). The upregulation of the γ-globin gene induced by butyrate has typically been considered a result of enhanced acetylation at the promoters of this gene. Additionally, butyrate can activate the soluble guanylate cyclase and p38 MAP kinase pathways (95). Nevertheless, the exact mechanisms through which these signaling pathways stimulate the activation of γ-globin genes remain unclear. Butyrate was more recently reported to stimulate production of HbF in SCD patients via increase in translation of γ-globin mRNA (96). Although using butyrate can lead to notable improvements in anemia for individuals with β-thalassemia, most patients receiving treatment continue to experience considerable anemic symptoms (97).

The erythroid-specific enhancer and transcript provide a benefit to the erythroid-specific transcription factor BCL11A, which is associated with HbF, compared to other HbF

transcription factors. The long form of the BCL11A gene i.e. BCL11A-XL is connected to functional studies highlighting its crucial role in the silencing of erythroid γ-globin and the repression of HbF during erythroid maturation (98). MYB is autoregulated by enhancers for erythroid activation in the distal region of the HBS1L-MYB intergenic region, which influences HbF levels and BCL11A is expressed only in its erythroid-specific isoform compared to MYB which is far more accessible to miRNA (99). Genome-wide association studies have identified BCL11A as the primary regulator of HbF production, and various studies have shown that reducing BCL11A expression positively impacts HbF levels (24). Nevertheless, targeting only the coding site of BCL11A for genetic editing may not be an effective therapeutic approach, as this site is also crucial for the function of the haematopoietic stem cell reservoir and the progression of lymphoid development. In K562 cells where BCL11A was disrupted using the CRISPR/Cas9 system, there was a notable reduction in BCL11A levels along with an increase in HbF expression (100). KLF1 (EKLF) is a vital transcription factor necessary for the expression of β-globin, the process of definitive erythropoiesis, and the transition from HbF to HbA (101). Reduced KLF1 levels lead to decreased BCL11A expression, resulting in higher HbF levels (102).

Lentiviral gene therapy has become a potential curative approach, and it is now possible to introduce functional variants of β-globin into autologous hematopoietic stem cells (HSCs) with high fidelity, allowing production of normal hemoglobin levels (103). Patients become transfusion independent and have lowered hemolysis markers when treated with lentiviral β-globin transduced autologous hematopoietic stem and progenitor cells (HSPCs) in Sickle Cell Anemia (SCA) (104).

The induction of the globin gene by thalidomide and the production of HbF, both the activation of p38 MAPK and the acetylation of histone H4 are correlated with protein. Additionally, one study suggested that thalidomide triggers to induce Reactive Oxygen Species (ROS) production in erythroid progenitor cells, leading to the expression of later globin genes through the involvement of p38 MAPK and the acetylation of histone H4 (105). This is the initial report that clarifies the role of ROS in the expression of the thalidomide-induced globin expression (105).

Emerging research emphasizes the potential of the ncRNAs from being important biomarkers to diagnose human diseases to being promising therapeutic targets to prevent and treat hemoglobinopathies (12). A rapidly growing area of biotherapeutics is RNA therapeutics,

which has several advantages over conventional drugs. Therapies based upon antisense RNA modifiers of targeted mRNA or protein expression or function, post within a cell or organ, either transcriptionally or translationally for the treatment of specific clinical conditions (39).

As mentioned earlier, miRNAs are single-stranded RNA molecules, and the therapeutic application of miRNAs consists of modifying and affecting pathological changes of miRNA expression using miRNA mimics and anti-miRs, respectively (105). MiRNA-based therapeutics have two main advantages. Firstly, miRNAs are not synthetic RNAs such as ASOs, but are naturally occurring in human cells, thus their processing and target selection are naturally occurring processes. Secondly, miRNA can regulate a wide variety of targets or there can be a variety of targets that can be regulated by miRNAs in various ways, including by complementary base pairs binding mechanisms (40). Consequently, miRNA may be alternative to current siRNA/ASO RNA therapeutics and may offer enhanced therapeutic benefits than synthetic siRNAs/ASOs specific to one target gene (44). The therapeutic potential of lncRNAs is evident due to their multifaceted functions and accordingly, their targeting strategies must be tailored to their mode of action (106). The inhibition of lncRNAs might occur at both the transcriptional and post-transcriptional levels through secondary structure variation or interactions of protein, this can happen with the by introducing synthetic lncRNAs and by discovering key elements regulating lncRNA transcription, such as lncRNA-targeted miRNA sites, which can be edited in the transcription of these proteins by the CRISPR- Cas9 technique currently under investigation for the treatment of diseases of the β-hemoglobin chain, i.e., β-hemoglobinopathies (107). MiR-29b mimics or inhibitors of suppressive lncRNAs (e.g., NR_120526 antisense oligos) are the perfect candidate as next-generation epigenetic drugs for SCD and thalassemia because of its low toxicity demonstrated in preclinical models (108).

The high stability of circRNAs and their contribution to hemoglobin switching pathways render them excellent biomarkers and therapeutic targets in hemoglobinopathies including SCD and β-thalassemia (109). They introduce a novel regulatory dimension by interacting with miRNAs and RNA-binding proteins, which may have an impact on developmental gene switches in erythroid lineage cells. Beyond diagnostics, there has been a limited amount of research on circRNAs as therapeutic formulations for clinical trials, particularly for RNA therapeutics based on circRNA (110, 78). Additionally, engineered circRNAs have been demonstrated to act as stable mRNA templates for protein synthesis, which could serve as a potential alternative to conventional RNA-based therapies (107).

At the current stage, no direct evidence at the present stage to connect piRNA with the switch of HbF, as most studies have been done on miRNAs instead of piRNAs (111). PiRNAs are widely known for their function in the silencing of genes, controlling the activity of transposons and genome stability, particularly in germline cells, while also playing regulatory roles in somatic cells (112-114). In theory, piRNAs may influence HbF switching by altering epigenetic status of globin gene loci or silencing transposons near key regulatory regions (113, 114). More focused research is needed to conclusively demonstrate whether piRNAs have direct or indirect impact on HbF switching and regulation of globin genes in human erythroid cells. The **Table 2** describes some therapeutic approaches that regulates HbF expression (43, 56, 93,100, 102, 103, 110, 115-120).

Table 2. Therapeutic approaches that modulate fetal hemoglobin (HbF) expression

Therapy/ molecule	Molecular target/pathway	Mechanism of action	Key clinical findings	References
Hydroxyurea	γ-globin gene regulation; NO–cGMP (Nitric oxide–cyclic guanosine monophospha te)signaling	Stimulates γ-globin expression via stress erythropoiesis and nitric oxide signaling; reduces BCL11A expression indirectly	Increases HbF (up to ~20–30%); reduces vaso-occlusive crises and mortality in SCD	(115,116)
BCL11A inhibition (CRISPR/Cas 9 editing)	BCL11A erythroid enhancer	Destabilizes BCL11A binding → derepression of γ-globin gene	HbF induction (>30–40%); transfusion independence in β-thalassemia; reduced VOC in SCD	(100)
Lentiviral β-globin gene therapy	β-globin gene addition	Introduces functional β-globin or γ-globin–like variants → compensates defective hemoglobin	Sustained hemoglobin production; reduced transfusion dependence	(103)

Histone deacetylase (HDAC) inhibitors	Epigenetic chromatin remodeling	Enhanced histone acetylation → opens γ-globin gene locus for transcription	Induces HbF in preclinical and early clinical studies	(117)
DNA methyltransferase (DNMT) inhibitors (e.g., Decitabine, Azacitidine)	DNA methylation at γ-globin promoters	Hypomethylation of γ-globin gene promoters → reactivation of HbF	Significant HbF induction in SCD patients; improved erythropoiesis	(93)
KLF1 modulation	KLF1 transcription factor	Downregulation of KLF1 reduces BCL11A expression → enhances HbF	Genetic and experimental evidence supports HbF induction	(102)
MicroRNAs (e.g., miR-15a/16-1, miR-486-3p)	BCL11A, MYB, KLF1	Post-transcriptional repression of HbF repressors	Enhances γ-globin expression; potential therapeutic targets	(43)
Long non-coding RNAs (e.g., BGLT3, HMI-LNCRNA)	γ-globin locus regulation	Regulates chromatin looping and transcription factor recruitment	Emerging regulators of HbF switching; therapeutic potential under investigation	(56)

Circular RNAs (circRNAs)	miRNA sponging networks (BCL11A/MYB axis)	Act as miRNA sponges → relieve repression of γ-globin activators or inhibit repressors	Emerging evidence: circRNA-mediated networks regulate erythroid differentiation and HbF levels; potential therapeutic targets	(110)
Short-chain fatty acids (e.g., Butyrate derivatives)	Epigenetic modifiers	Histone acetylation → activation of γ-globin gene	Moderate HbF induction; limited clinical use due to pharmacokinetics	(118)
Thalidomide	Transcriptional regulators	Modulates erythroid differentiation and γ-globin expression	Increases HbF; reduces transfusion requirement in β-thalassemia	(119,120)

5. Challenges and Future Directions

The role of ncRNAs in the transition of HbF is becoming increasingly recognized, but there are still some barriers to get out of them so that they can be fully utilized for therapeutic purposes. Firstly, the regulatory mechanisms of ncRNAs are not fully known, especially lncRNAs and circRNAs, regarding their interaction with transcriptional repressors and chromatin modifiers that is under current investigation. Secondly, most insights are made from in vitro systems or small-scale animal research, which do not have significant preclinical and clinical validation. Thirdly, finding an effective and safe way to deliver ncRNA-based treatments to hematopoietic cells remains a great challenge, that is complicated by issues of stability, off-target effects and the complexity of many of the ncRNAs. These challenges highlight the necessity of good optimization before its use in the clinic. Among the key challenges, there is the detection and confirmation of particular biomarkers of ncRNAs and establishment of effective and reliable ways to detect them. Furthermore, the preclinical and clinical trials must consider in detail the off-target effects of agents against ncRNA and find out the safety concern of these agents in their long-term use.

Despite growing mechanistic interest, ncRNA-mediated regulation of HbF switching remains largely confined to preclinical and in vitro systems, and this stage gap is the central translational barrier facing the field. Unlike genome-editing approaches targeting BCL11A, which have already reached regulatory approval, none of the miRNA mimics, antagomirs, lncRNA-targeting antisense oligonucleotides, or circRNA-based constructs discussed in this review have advanced into clinical trials, reflecting unresolved challenges in delivering RNA therapeutics specifically to hematopoietic stem and progenitor cells rather than the liver, where most lipid nanoparticle systems are naturally tropic. Compounding this, synthetic RNA constructs carry inherent risks of immunogenicity and off-target silencing that require extensive toxicology work before first-in-human studies, while GMP-grade manufacturing of RNA therapeutics at clinically relevant scale remains costly and technically demanding. Trial design is further hampered by the absence of validated, mechanism-specific biomarkers to track ncRNA target engagement independent of HbF percentage itself, making it difficult to establish dose-response relationships or predict durability of effect. Finally, even if these scientific and regulatory hurdles are overcome, the populations bearing the greatest burden of sickle cell disease and β-thalassemia are concentrated in low- and middle-income regions of Africa and South Asia, where the cost and infrastructure demand of RNA-based or cell-based therapies pose a separate, equally consequential barrier to equitable access. Bridging these gaps mechanistic validation, targeted delivery, safety profiling, and affordable manufacturing will determine whether ncRNA-targeted HbF induction moves from a promising conceptual framework to a clinically deployable therapy.

In the future, comprehensive mapping of ncRNA regulatory networks by multi-omics and single-cell techniques will be crucial to understand the role of these networks in erythropoiesis and HbF reactivation. Expanding studies beyond the area of miRNAs to examine new lncRNAs and circRNAs, could reveal new mechanistic insights and therapeutic opportunities. Advances in delivery systems, such as lipid nanoparticles and exosomes-based delivery systems, are showing promising approaches to increase specific and stability.

Future clinical studies should investigate combination and sequential strategies for reactivating HbF that target complementary aspects of γ-globin silencing. For example, using epigenetic priming (inhibiting HDAC to enhance chromatin accessibility) followed by genome editing (disruption of BCL11A using CRISPR) could lower the CRISPR dosage needed and minimize off-target side effects, while also achieving greater HbF elevation. Implementing sequential

miRNA inhibition (to decrease the most prevalent miRNAs that silence γ-globin) followed by lncRNA elevation (to sequester remaining miRNA pools) could exploit mechanistic synergies. These approaches require well-planned clinical trial designs with defined efficacy endpoints, safety monitoring procedures, and assessments of mechanistic biomarkers to facilitate real-time optimization.

The ability to adapt platforms for the application of proven HbF-reactivation methods across various therapeutic avenues (such as gene therapy, base editing, prime editing, and ncRNA modulation) would allow different regions and healthcare systems to adopt strategies that align with their technological capabilities and financial limitations. Achieving this necessitates standardized evaluation of HbF-reactivation effectiveness and safety across different platforms, enabling regulatory bodies to compare new methods against recognized benchmarks instead of needing separate approvals for each new approach.

6. Conclusion

Noncoding RNAs have identified as important epigenetic controllers in the switch-over of HbF and fill in a role played by the known functions of transcription factors and chromatin modifiers. Studies about miRNAs, lncRNAs and circRNAs have shown the role of these molecules in modulation of γ-globin expression and influence the clinical progression of β-hemoglobinopathies. Although promising, current understanding is far from complete, the mechanistic understanding of many ncRNAs such as piRNA is still incomplete and the amount of translational data is limited. The clinical advancement of ncRNA-targeted HbF reactivation has achieved significant successes: therapies involving CRISPR-Cas9 editing of BCL11A have received approval from the FDA and demonstrate lasting positive effects in patients. To progress the field, we need to: (1) finalize the exploration of ncRNA networks that regulate hemoglobin switching, particularly focusing on the less understood piRNA pathways and their feedback systems; (2) create in vivo methods for ncRNA modulation that eliminate the need for ex vivo manufacturing and lessen the toxicity associated with conditioning treatments; (3) adopt personalized profiling of ncRNA to enable treatment choices tailored to individual patients; (4) establish manufacturing systems and regulatory frameworks that ensure worldwide accessibility; (5) conduct long-term studies on safety and durability to provide data-driven assessments of risk and benefit. Achieving these goals will turn HbF reactivation from

an expensive, specialized academic treatment into a practical, effective option for millions of patients with hemoglobin disorders around the world.

List of Abbreviations

ADP - Adenosine Diphosphate

ASO - Antisense Oligonucleotide

BCL11A - B-cell Lymphoma/Leukemia 11A

BCL11A-XL - BCL11A Extra-Long Isoform

BGLT3 - Beta-Globin Locus Transcript 3

CDS - Coding Sequence

ceRNA - Competing Endogenous RNA

cGMP - Cyclic guanosine monophosphate

CHD4 - Chromodomain Helicase DNA-binding Protein 4

CircRNA - Circular RNA

CpG - Cytosine-phosphate-Guanine

CRISPR - Clustered Regularly Interspaced Short Palindromic Repeats

DNMT - DNA Methyltransferase

DNMT1 - DNA Methyltransferase 1

DNMT3A - DNA Methyltransferase 3A

DNMT3B - DNA Methyltransferase 3B

DRED - Direct Repeat Erythroid-Definitive Complex

EKLF - Erythroid Kruppel-Like Factor (KLF1)

eRNA - Enhancer RNA

ERF - ETS2 Repressor Factor

FDA - Food and Drug Administration

FOG1 - Friend of GATA1

GATA1 - GATA Binding Protein 1

GATA2 - GATA-binding factor 2

Hb - Hemoglobin

HbA - Adult Hemoglobin

HbF - Fetal Hemoglobin

HBB - β-Globin Gene

HBE1 - Hemoglobin Subunit Epsilon 1

HBG - Gamma-Globin Gene

HBG1/HBG2 - Gamma-Globin Genes 1 and 2

HIF1 - Hypoxia-Inducible Factor 1

HDAC - Histone Deacetylase

HMI-lncRNA - HBS1L-MYB Intergenic Long Noncoding RNA

HPFH - Hereditary Persistence of Fetal Hemoglobin

HRE - Hypoxia Response Element

HSC - Hematopoietic Stem Cell

HSPC - Hematopoietic Stem and Progenitor Cell

HU - Hydroxyurea

HUDEP-2 - Human Umbilical Cord Blood-Derived Erythroid Progenitor-2 Cells

KLF1 -Kruppel-Like Factor 1

KLF3 - Kruppel-Like Factor 3

KMT - Lysine Methyltransferase

LCR - Locus Control Region

LincRNA - Long Intergenic Noncoding RNA

LncRNA - Long Noncoding RNA

MAPK - Mitogen-Activated Protein Kinase

MiRNA - MicroRNA

mRNA - Messenger RNA

MYB - Myeloblastosis Oncogene

NcRNA - Noncoding RNA

NO - Nitric Oxide

NO–cGMP - Nitric oxide–cyclic guanosine monophosphate

NRF2 - Nuclear Factor Erythroid 2-Related Factor 2

NuRD - Nucleosome Remodeling and Deacetylase Complex

piRNA - PIWI-Interacting RNA

PIWI - P-element Induced Wimpy Testis

PRMT - Protein Arginine Methyltransferase

RBP - RNA-Binding Protein

RISC - RNA-Induced Silencing Complex

ROS - Reactive Oxygen Species

SCA - Sickle Cell Anemia

SCD - Sickle Cell Disease

siRNA - Small Interfering RNA

SOX6 - SRY-Box Transcription Factor 6

Sp1 - Specificity Protein 1

STAT3 - Signal Transducer and Activator of Transcription 3

SWI/SNF - Switch/Sucrose Non-Fermentable

THU - Tetrahydrouridine

TR2/TR4 - Testicular Receptor 2 / Testicular Receptor 4

UTR - Untranslated Region

VHL - Von Hippel–Lindau

VOC - Vaso-Occlusive Crisis

ZNF410 - Zinc Finger Protein 410

Author Contributions: Conceptualization, methodology, software, Investigation, resources, data curation, writing—original draft preparation, writing—review and editing, visualization: S.M.; Validation, writing—review and editing: A.N.; Supervision, project administration: S.S.N. All authors have read and agreed to the final version of the manuscript.

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AI-declaration

During the preparation of this work the authors used Open AI to provide grammar suggestions. After using this tool, the authors carefully reviewed and edited the content as required and takes full responsibility for the content of the published article.

References

1. Mukherjee M, Rahaman M, Ray SK, Shukla PC, Dolai TK, Chakravorty N. Revisiting fetal hemoglobin inducers in beta-hemoglobinopathies: a review of natural products, conventional and combinatorial therapies. Molecular Biology Reports. Springer Science and Business Media B.V.; 2022. p. 2359–73. doi:10.1007/s11033-021-06977-8 PubMed PMID: 34822068.
2. Steinberg MH. Fetal hemoglobin in sickle cell anemia. Blood. 2020 Nov 19;136(21):2392–400. doi:10.1182/BLOOD.2020007645 PubMed PMID: 32808012.
3. Ahmed MH, Ghatge MS, Safo MK. Hemoglobin: Structure, Function and Allostery. In: Subcellular Biochemistry. Springer; 2020. p. 345–82. doi:10.1007/978-3-030-41769-7_14 PubMed PMID: 32189307.
4. Mack R, Zhang L, Breslin, SJ P, Zhang J. The Fetal-to-Adult Hematopoietic Stem Cell Transition and its Role in Childhood Hematopoietic Malignancies. Stem Cell Reviews and Reports. Springer; 2021. p. 2059–80. doi:10.1007/s12015-021-10230-x PubMed PMID: 34424480.
5. Khandros E, Blobel GA. Elevating fetal hemoglobin: recently discovered regulators and mechanisms.
6. de Souza MM, Pavan AR, de Faria Pastre VG, Rodriguez Gini AL, de Araújo Silva R, Lanaro C, et al. Epigenetic strategies for fetal hemoglobin induction in sickle cell disease. European Journal of Medicinal Chemistry. Elsevier Masson s.r.l.; 2026. doi:10.1016/j.ejmech.2026.119186
7. Lu HY, Orkin SH, Sankaran VG. Fetal Hemoglobin Regulation in Beta-Thalassemia. Hematol Oncol Clin North Am. 2023 Apr 1;37(2):301–12. doi:10.1016/J.HOC.2022.12.002 PubMed PMID: 36907604.

8. Paikari A, Sheehan VA. Fetal haemoglobin induction in sickle cell disease. British Journal of Haematology. Blackwell Publishing Ltd; 2018. p. 189–200. doi:10.1111/bjh.15021 PubMed PMID: 29143315.

9. El Hoss S, Cochet S, Godard A, Yan H, Dussiot M, Frati G, et al. Fetal hemoglobin rescues ineffective erythropoiesis in sickle cell disease. Haematologica. 2021 Oct 1;106(10):2707–19. doi:10.3324/haematol.2020.265462 PubMed PMID: 32855279.

10. Finotti A, Gambari R. Combined approaches for increasing fetal hemoglobin (HbF) and de novo production of adult hemoglobin (HbA) in erythroid cells from β-thalassemia patients: treatment with HbF inducers and CRISPR-Cas9 based genome editing. Frontiers in Genome Editing. Frontiers Media SA; 2023. doi:10.3389/fgeed.2023.1204536

11. Li L, Mandal PK. Recent advancements in gene therapy for sickle cell disease and β-thalassemia. Frontiers in Hematology. Frontiers Media SA; 2024. doi:10.3389/frhem.2024.1468952

12. Nappi F. Non-Coding RNA-Targeted Therapy: A State-of-the-Art Review. International Journal of Molecular Sciences. Multidisciplinary Digital Publishing Institute (MDPI); 2024. doi:10.3390/ijms25073630 PubMed PMID: 38612441.

13. Shang W, Geng X, Sun X, Fan X, Li A, Zhang C, et al. Non-coding RNAs modulate pyroptosis in diabetic cardiomyopathy: A comprehensive review. International Journal of Biological Macromolecules. Elsevier B.V.; 2025. doi:10.1016/j.ijbiomac.2025.142865 PubMed PMID: 40188918.

14. Pang B, van Weerd JH, Hamoen FL, Snyder MP. Identification of non-coding silencer elements and their regulation of gene expression. Nat Rev Mol Cell Biol. 2023 Jun 1;24(6):383–95. doi:10.1038/S41580-022-00549-9 PubMed PMID: 36344659.

15. Kausar S. Kalaigar S, Vishwanath P, Prashant A. Exploring Long Non-Coding RNAs as Therapeutic Targets for Foetal Haemoglobin Induction in β-Thalassaemia. In. 2025. doi:10.5772/intechopen.1010178

16. Hariharan P, Nadkarni A. Insight of fetal to adult hemoglobin switch: Genetic modulators and therapeutic targets. Blood Reviews. Churchill Livingstone; 2021. doi:10.1016/j.blre.2021.100823 PubMed PMID: 33726930.

17. Butterfield GL, Reisman SJ, Iglesias N, Gersbach CA. Gene regulation technologies for gene and cell therapy. Molecular Therapy. Cell Press; 2025. p. 2104–22. doi:10.1016/j.ymthe.2025.04.004 PubMed PMID: 40195118.

18. Diamantidis MD, Ikonomou G, Argyrakouli I, Pantelidou D, Delicou S. Genetic Modifiers of Hemoglobin Expression from a Clinical Perspective in Hemoglobinopathy Patients with Beta Thalassemia and Sickle Cell Disease. International Journal of Molecular Sciences. Multidisciplinary Digital Publishing Institute (MDPI); 2024. doi:10.3390/ijms252211886 PubMed PMID: 39595957.

19. Janoudi T, Jagdale M, Wu M, Gorlla S, Zhang P, Shao Y, et al. Nonclinical evaluation of HBG1/2 and BCL11A as genome-editing targets for the treatment of β-

hemoglobinopathies. Blood Advances. American Society of Hematology; 2025. p. 808–13. doi:10.1182/bloodadvances.2024014040 PubMed PMID: 39637306.

20. Vasiloudis NI, Paschoudi K, Beta C, Georgolopoulos G, Psatha N. Epigenetic Regulation of Erythropoiesis: From Developmental Programs to Therapeutic Targets. International Journal of Molecular Sciences. Multidisciplinary Digital Publishing Institute (MDPI); 2025. doi:10.3390/ijms26136342 PubMed PMID: 40650116.

21. Vinjamur DS, Yao Q, Cole MA, McGuckin C, Ren C, Zeng J, et al. ZNF410 represses fetal globin by singular control of CHD4. Nat Genet. 2021 May 1;53(5):719–28. doi:10.1038/s41588-021-00843-w PubMed PMID: 33859416.

22. Fontana L, Alahouzou Z, Miccio A, Antoniou P. Epigenetic Regulation of β-Globin Genes and the Potential to Treat Hemoglobinopathies through Epigenome Editing. Genes. MDPI; 2023. doi:10.3390/genes14030577 PubMed PMID: 36980849.

23. Starlard-Davenport A, Fitzgerald A, Pace BS. Exploring epigenetic and microRNA approaches for γ-globin gene regulation. Experimental Biology and Medicine. SAGE Publications Inc.; 2021. p. 2347–57. doi:10.1177/15353702211028195 PubMed PMID: 34292080.

24. Demirci S, Leonard A, Tisdale JF. Genome editing strategies for fetal hemoglobin induction in beta-hemoglobinopathies. Human Molecular Genetics. Oxford University Press; 2020. p. R100–6. doi:10.1093/hmg/ddaa088 PubMed PMID: 32406490.

25. Kargutkar N, Nadkarni A. Decoding HbF reactivation by hydroxyurea in hemoglobinopathy patients through microRNA signatures. Ann Hematol. 2025 Apr 1;104(4):2159–69. doi:10.1007/s00277-025-06252-x PubMed PMID: 40278921.

26. Chera A, Stancu-Cretu M, Zabet NR, Bucur O. Shedding light on DNA methylation and its clinical implications: the impact of long-read-based nanopore technology. Epigenetics and Chromatin . BioMed Central Ltd; 2024. doi:10.1186/s13072-024-00558-2 PubMed PMID: 39734197.

27. Shen Y, Wei J, Tang S, Wu D, Zong L, Ma S, et al. Discovery of a Novel DNMT1 Inhibitor with Improved Efficacy in Treating β-Thalassemia. Advanced Science. 2025 Feb 23. doi:10.1002/advs.202513469

28. Gilmartin AG, Groy A, Gore ER, Atkins C, Long ER, Montoute MN, et al. In vitro and in vivo induction of fetal hemoglobin with a reversible and selective DNMT1 inhibitor. Haematologica. 2021 Jul 1;106(7):1979–87. doi:10.3324/haematol.2020.248658 PubMed PMID: 32586904.

29. Ginder G. DNA methylation matters: methylation of the γ-globin (HBG) gene promoters is required for postnatal silencing of HbF [Internet]. doi:10.1182/blood.2025031153/2500469/blood.2025031153.pdf

30. Ye D, Chen M, Zhu Y, Feng XY, Xu L, Huang H. Comprehensive regulation of γ-globin expression by epigenetic modifications and protein post-translational modifications. Clinical Epigenetics. BioMed Central Ltd; 2025. doi:10.1186/s13148-025-01962-5 PubMed PMID: 41107978.

31. Ayuba S, Bashar F, Abbas A. Targeting fetal hemoglobin induction in sickle cell anemia: Epigenetic and gene-modifying therapeutic strategies (Review). International Journal of Epigenetics. 2025 Nov 11;5(1):1–6. doi:10.3892/ije.2025.29

32. Chauhan W, Zennadi R. Protein Arginine Methyltransferases in γ-Globin Regulation and Sickle Cell Disease: Emerging Connections to Oxidative Stress. Antioxidants. Multidisciplinary Digital Publishing Institute (MDPI); 2026. doi:10.3390/antiox15030324

33. Uppaluri KR, Challa HJ, Gaur A, Jain R, Krishna Vardhani K, Geddam A, et al. Unlocking the potential of non-coding RNAs in cancer research and therapy. Transl Oncol. 2023 Sep 1;35. doi:10.1016/j.tranon.2023.101730

34. Slack FJ, Chinnaiyan AM. The Role of Non-coding RNAs in Oncology. Cell. Cell Press; 2019. p. 1033–55. doi:10.1016/j.cell.2019.10.017 PubMed PMID: 31730848.

35. Sebastiani P, Steinberg MH. Fetal hemoglobin per erythrocyte (HbF/F-cell) after gene therapy for sickle cell anemia. American Journal of Hematology. John Wiley and Sons Inc; 2023. p. E32–4. doi:10.1002/ajh.26791 PubMed PMID: 36420999.

36. Kaikkonen MU, Lam MTY, Glass CK. Non-coding RNAs as regulators of gene expression and epigenetics. Cardiovascular Research. 2011. p. 430–40. doi:10.1093/cvr/cvr097 PubMed PMID: 21558279.

37. Toden S, Zumwalt TJ, Goel A. Non-coding RNAs and potential therapeutic targeting in cancer. Biochimica et Biophysica Acta - Reviews on Cancer. Elsevier B.V.; 2021. doi:10.1016/j.bbcan.2020.188491 PubMed PMID: 33316377.

38. Ali H, Khan F. Non-Coding RNA: An Emerging Modulator of β-Globin Regulation and β-Hemoglobinopathies. Molecular Medicine Communications. 2022 Dec 31;2(02):173–89. doi:10.55627/mmc.002.002.0147

39. Winkle M, El-Daly SM, Fabbri M, Calin GA. Noncoding RNA therapeutics — challenges and potential solutions. Nature Reviews Drug Discovery. Nature Research; 2021. p. 629–51. doi:10.1038/s41573-021-00219-z PubMed PMID: 34145432.

40. Karpagavalli M, Sindal MD, Arunachalam JP, Chidambaram S. miRNAs, piRNAs, and lncRNAs: A triad of non-coding RNAs regulating the neurovascular unit in diabetic retinopathy and their therapeutic potentials. Experimental Eye Research. Academic Press; 2025. doi:10.1016/j.exer.2025.110236 PubMed PMID: 39800284.

41. Cyrus C. The role of mirnas as therapeutic tools in sickle cell disease. Medicina (Lithuania). MDPI; 2021. doi:10.3390/medicina57101106 PubMed PMID: 34684143.

42. Athanasopoulou K, Chondrou V, Xiropotamos P, Psarias G, Vasilopoulos Y, Georgakilas GK, et al. Transcriptional repression of lncRNA and miRNA subsets mediated by LRF during erythropoiesis. J Mol Med. 2023 Sep 1;101(9):1097–112. doi:10.1007/s00109-023-02352-1 PubMed PMID: 37486375.

43. Alotaibi D, Aldagdog F, Alramadhan S, Almuhaidib B, Asiri N, Almodhi L, et al. miRNA-Mediated Regulation of γ-Globin to β-Globin Switching: Therapeutic Potential in β-Hemoglobinopathies. International Journal of Molecular Sciences.

Multidisciplinary Digital Publishing Institute (MDPI); 2026. doi:10.3390/ijms27031203

44. Lai K, Jia S, Yu S, Luo J, He Y. Genome-wide analysis of aberrantly expressed lncRNAs and miRNAs with associated co-expression and ceRNA networks in β-thalassemia and hereditary persistence of fetal hemoglobin [Internet]. 2017. Available from: www.impactjournals.com/oncotarget

45. Bianchi N. MicroRNA 210 and human erythroid differentiation [Internet]. Available from: http://bmbreports.org

46. Starlard-Davenport A, Gu Q, Pace BS. Targeting Genetic Modifiers of HBG Gene Expression in Sickle Cell Disease: The miRNA Option. Molecular Diagnosis and Therapy. Adis; 2022. p. 497–509. doi:10.1007/s40291-022-00589-z PubMed PMID: 35553407.

47. Ward CM, Li B, Pace BS. Original Research: Stable expression of miR-34a mediates fetal hemoglobin induction in K562 cells. Exp Biol Med. 2016 Apr 1;241(7):719–29. doi:10.1177/1535370216636725 PubMed PMID: 26940952.

48. Li B, Zhu X, Ward CM, Starlard-Davenport A, Takezaki M, Berry A, et al. MIR-144-mediated NRF2 gene silencing inhibits fetal hemoglobin expression in sickle cell disease. Exp Hematol. 2018;70:85-96.e5. doi:10.1016/j.exphem.2018.11

49. Lee YT, De Vasconcellos JF, Yuan J, Byrnes C, Noh SJ, Meier ER, et al. LIN28B-mediated expression of fetal hemoglobin and production of fetal-like erythrocytes from adult human erythroblasts ex vivo. Blood. 2013 Aug 8;122(6):1034–41. doi:10.1182/BLOOD-2012-12-472308 PubMed PMID: 23798711.

50. Huang P, Peslak SA, Shehu V, Keller CA, Giardine B, Shi J, et al. let-7 miRNAs repress HIC2 to regulate BCL11A transcription and hemoglobin switching [Internet]. Available from: https://www.encodeproject.

51. Lulli V, Romania P, Morsilli O, Cianciulli P, Gabbianelli M, Testa U, et al. MicroRNA-486-3p Regulates γ-Globin Expression in Human Erythroid Cells by Directly Modulating BCL11A. PLoS One. 2013 Apr 4;8(4). doi:10.1371/journal.pone.0060436 PubMed PMID: 23593217.

52. Sankaran VG, Menne TF, Šćepanović D, Vergilio JA, Ji P, Kim J, et al. MicroRNA-15a and -16-1 act via MYB to elevate fetal hemoglobin expression in human trisomy 13. Proc Natl Acad Sci U S A. 2011 Jan 25;108(4):1519–24. doi:10.1073/pnas.1018384108 PubMed PMID: 21205891.

53. Gabbianelli M, Testa U, Morsilli O, Pelosi E, Saulle E, Petrucci E, et al. Mechanism of human Hb switching: A possible role of the kit receptor/miR 221-222 complex. Haematologica. 2010;95(8):1253–60. doi:10.3324/haematol.2009.018259 PubMed PMID: 20305142.

54. Gu Q, Palani CD, Smith A, Li B, Amos-Abanyie EK, Ogu U, et al. MicroRNA29B induces fetal hemoglobin via inhibition of the HBG repressor protein MYB in vitro

and in humanized sickle cell mice. Front Med (Lausanne). 2022 Nov 25;9. doi:10.3389/fmed.2022.1043686

55. Azzouzi I, Moest H, Winkler J, Fauchère JC, Gerber AP, Wollscheid B, et al. Microrna-96 directly inhibits γ-Globin expression in human erythropoiesis. PLoS One. 2011;6(7). doi:10.1371/journal.pone.0022838 PubMed PMID: 21829531.

56. Rahaman M, Bhowmick C, Komanapalli J, Mukherjee M, Byram PK, Shukla PC, et al. Debunking the "junk": Unraveling the role of lncRNA–miRNA–mRNA networks in fetal hemoglobin regulation [Internet]. 2021. Available from: http://biorxiv.org/lookup/doi/10.1101/2021.10.13.464339 doi:10.1101/2021.10.13.464339

57. Asadabadi T, Mokhtari MJ, Bayat M, Safari A, Borhani-Haghighi A. Association between Genetic Polymorphism of The lncRNA MIAT rs1894720 with Ischemic Stroke Risk and lncRNA MIAT Expression Levels in The Blood after An Ischemic Stroke: A Case-Control Study. Cell J. 2023 Dec 1;25(12):863–73. doi:10.22074/CELLJ.2023.2003573.1315

58. Tang Y, Wang J, Lian Y, Fan C, Zhang P, Wu Y, et al. Linking long non-coding RNAs and SWI/SNF complexes to chromatin remodeling in cancer. Molecular Cancer. BioMed Central Ltd.; 2017. doi:10.1186/s12943-017-0612-0 PubMed PMID: 28212646.

59. Keshavarz F, Mokhtari MJ, Poursadeghfard M. Increased level of GATA3-AS1 long non-coding RNA is correlated with the upregulation of GATA3 and IL-4 genes in multiple sclerosis patients. Mol Biol Rep. 2024 Dec 1;51(1). doi:10.1007/S11033-024-09818-6 PubMed PMID: 39080124.

60. Hosseini M, Mokhtari MJ. Up-regulation of HOXA-AS2 and MEG3 long non-coding RNAs acts as a potential peripheral biomarker for bipolar disorder. J Cell Mol Med. 2024 Nov 1;28(21). doi:10.1111/JCMM.70150 PubMed PMID: 39482996.

61. Zhang X, Wang W, Zhu W, Dong J, Cheng Y, Yin Z, et al. Mechanisms and functions of long non-coding RNAs at multiple regulatory levels. International Journal of Molecular Sciences. MDPI AG; 2019. doi:10.3390/ijms20225573 PubMed PMID: 31717266.

62. Rivers A, Molokie R, Lavelle D. A new target for fetal hemoglobin reactivation. Haematologica. Ferrata Storti Foundation; 2019. p. 2325–7. doi:10.3324/haematol.2019.230904 PubMed PMID: 31787612.

63. Bhat SA, Ahmad SM, Mumtaz PT, Malik AA, Dar MA, Urwat U, et al. Long non-coding RNAs: Mechanism of action and functional utility. Non-coding RNA Research. KeAi Communications Co.; 2016. p. 43–50. doi:10.1016/j.ncrna.2016.11.002

64. Morceau F, Chateauvieux S, Gaigneaux A, Dicato M, Diederich M. Long and short non-coding RNAs as regulators of hematopoietic differentiation. International Journal of Molecular Sciences. 2013. p. 14744–70. doi:10.3390/ijms140714744 PubMed PMID: 23860209.

65. Yang Z, Xu F, Teschendorff AE, Zhao Y, Yao L, Li J, et al. Insights into the role of long non-coding RNAs in DNA methylation mediated transcriptional regulation. Frontiers in Molecular Biosciences. Frontiers Media S.A.; 2022. doi:10.3389/fmolb.2022.1067406

66. Xie D, Han Y, Zhang W, Wu J, An B, Huang S, et al. Long Non-Coding RNA H19 Leads to Upregulation of γ-Globin Gene Expression during Erythroid Differentiation. Hemoglobin. 2024;48(1):4–14. doi:10.1080/03630269.2023.2284950 PubMed PMID: 38419555.

67. Ghapanchi J, Mokhtari MJ, Zahed M, Ardekani ST, Fattahi MJ, Khademi B, et al. Genetic analysis of lncRNA H19 (rs217727) and MIAT (rs1894720) polymorphisms in patients with salivary gland tumors. Gene Rep. 2023 Mar 1;30. doi:10.1016/j.genrep.2022.101724

68. Morrison TA, Wilcox I, Luo HY, Farrell JJ, Kurita R, Nakamura Y, et al. A long noncoding RNA from the HBS1L-MYB intergenic region on chr6q23 regulates human fetal hemoglobin expression. Blood Cells Mol Dis. 2018 Mar 1;69:1–9. doi:10.1016/j.bcmd.2017.11.003 PubMed PMID: 29227829.

69. Ivaldi MS, Diaz LF, Chakalova L, Lee J, Krivega I, Dean A. Fetal g-globin genes are regulated by the BGLT3 long noncoding RNA locus. Blood [Internet]. 2018. Available from: http://ashpublications.org/blood/article-pdf/132/18/1963/1469486/blood862003.pdf

70. Chondrou V, Shaukat AN, Psarias G, Athanasopoulou K, Iliopoulou E, Damanaki A, et al. LRF Promotes Indirectly Advantageous Chromatin Conformation via BGLT3-lncRNA Expression and Switch from Fetal to Adult Hemoglobin. Int J Mol Sci. 2022 Jul 1;23(13). doi:10.3390/ijms23137025 PubMed PMID: 35806029.

71. Jia W, Wu X, Chen Z, Lin W, He Y. Negative regulation of HBG1/2 expression through S6K by long noncoding RNA NR_120526. Transl Pediatr. 2023 May 30;12(5):907–17. doi:10.21037/tp-23-174

72. Jia W, Jia S, Chen P, He Y. Construction and analysis of a long non-coding RNA (lncRNA)-associated ceRNA network in β-thalassemia and hereditary persistence of fetal hemoglobin. Medical Science Monitor. 2019 Sep 21;25:7079–86. doi:10.12659/MSM.915946 PubMed PMID: 31541070.

73. de Vasconcellos JF, Byrnes C, Lee YT, Kaushal M, Allwardt JM, Rabel A, et al. Targeted Reduction of Let-7a miRNA Increases Fetal Hemoglobin in Human Adult Erythroblasts. Blood. 2014 Dec 6;124(21):451–451. doi:10.1182/blood.v124.21.451.451

74. Ma Y, Wang B, Jiang F, Wang D, Liu H, Yan Y, et al. A Feedback Loop Consisting of MicroRNA 23a/27a and the β-Like Globin Suppressors KLF3 and SP1 Regulates Globin Gene Expression. Mol Cell Biol. 2013 Oct 1;33(20):3994–4007. doi:10.1128/mcb.00623-13 PubMed PMID: 23918807.

75. Alvarez-Dominguez JR, Knoll M, Gromatzky AA, Lodish HF. The Super-Enhancer-Derived AlncRNA-EC7/Bloodlinc Potentiates Red Blood Cell Development in trans.

Cell Rep. 2017 Jun 20;19(12):2503–14. doi:10.1016/j.celrep.2017.05.082 PubMed PMID: 28636939.

76. Morrison TA, Wilcox I, Luo HY, Farrell JJ, Kurita R, Nakamura Y, et al. A long noncoding RNA from the HBS1L-MYB intergenic region on chr6q23 regulates human fetal hemoglobin expression. Blood Cells Mol Dis. 2018 Mar 1;69:1–9. doi:10.1016/j.bcmd.2017.11.003 PubMed PMID: 29227829.

77. Yang F, Ruan H, Li S, Hou W, Qiu Y, Deng L, et al. Analysis of circRNAs and circRNA-associated competing endogenous RNA networks in β-thalassemia. Sci Rep. 2022 Dec 1;12(1). doi:10.1038/s41598-022-12002-0

78. Chen M, Lv A, Zhang S, Zheng J, Lin N, Xu L, et al. Peripheral blood circular RNA circ-0008102 may serve as a novel clinical biomarker in beta-thalassemia patients. Eur J Pediatr. 2024 Mar 1;183(3):1367–79. doi:10.1007/s00431-023-05398-y PubMed PMID: 38165465.

79. Greene J, Baird AM, Brady L, Lim M, Gray SG, McDermott R, et al. Circular RNAs: Biogenesis, function and role in human diseases. Frontiers in Molecular Biosciences. Frontiers Media S.A.; 2017. doi:10.3389/fmolb.2017.00038

80. Yu CY, Kuo HC. The emerging roles and functions of circular RNAs and their generation. Journal of Biomedical Science. BioMed Central Ltd.; 2019. doi:10.1186/s12929-019-0523-z PubMed PMID: 31027496.

81. Ran Y, Chen R, Huang D, Qin Y, Liu Z, He J, et al. The landscape of circular RNA in preterm birth. Front Immunol. 2022 Aug 22;13. doi:10.3389/fimmu.2022.879487 PubMed PMID: 36072601.

82. Babin L, Andraos E, Fuchs S, Pyronnet S, Brunet E, Meggetto F. From circRNAs to fusion circRNAs in hematological malignancies. JCI Insight. American Society for Clinical Investigation; 2021. doi:10.1172/jci.insight.151513 PubMed PMID: 34747369.

83. Habara A. Comparative circRNA Profiling in Human Erythroblasts Derived from Fetal Liver and Bone Marrow Hematopoietic Stem Cells Using Public RNA-Seq Data. Int J Mol Sci. 2025 Sep 1;26(17). doi:10.3390/ijms26178397 PubMed PMID: 40943320.

84. Luteijn MJ, Ketting RF. PIWI-interacting RNAs: From generation to transgenerational epigenetics. Nature Reviews Genetics. 2013. p. 523–34. doi:10.1038/nrg3495 PubMed PMID: 23797853.

85. Iwasaki YW, Murano K, Ishizu H, Shibuya A, Iyoda Y, Siomi MC, et al. Piwi Modulates Chromatin Accessibility by Regulating Multiple Factors Including Histone H1 to Repress Transposons. Mol Cell. 2016 Aug 4;63(3):408–19. doi:10.1016/j.molcel.2016.06.008 PubMed PMID: 27425411.

86. Sienski G, Dönertas D, Brennecke J. Transcriptional silencing of transposons by Piwi and maelstrom and its impact on chromatin state and gene expression. Cell. 2012 Nov 21;151(5):964–80. doi:10.1016/j.cell.2012.10.040 PubMed PMID: 23159368.

87. Lettre G, Bauer DE. Fetal haemoglobin in sickle-cell disease: From genetic epidemiology to new therapeutic strategies. The Lancet. 2016 Jun 18;387(10037):2554–64. doi:10.1016/S0140-6736(15)01341-0 PubMed PMID: 27353686.

88. Deshpande S V., Bhatwadekar SS, Desai P, Bhavsar T, Patel A, Koranne A, et al. Hydroxyurea in Sickle Cell Disease: Our Experience in Western India. Indian Journal of Hematology and Blood Transfusion. 2016 Jun 1;32(2):215–20. doi:10.1007/S12288-015-0542-1

89. Bank A. Regulation of human fetal hemoglobin: New players, new complexities. Blood. American Society of Hematology; 2006. p. 435–43. doi:10.1182/blood-2005-05-2113 PubMed PMID: 16109777.

90. Venkatesan V, Christopher AC, Rhiel M, Azhagiri MKK, Babu P, Walavalkar K, et al. Editing the core region in HPFH deletions alters fetal and adult globin expression for treatment of β-hemoglobinopathies. Mol Ther Nucleic Acids. 2023 Jun 13;32:671–88. doi:10.1016/j.omtn.2023.04.024

91. Molokie R, Lavelle D, Gowhari M, Pacini M, Krauz L, Hassan J, et al. Oral tetrahydrouridine and decitabine for non-cytotoxic epigenetic gene regulation in sickle cell disease: A randomized phase 1 study. PLoS Med. 2017 Sep 1;14(9). doi:10.1371/JOURNAL.PMED.1002382 PubMed PMID: 28880867.

92. Xu J, Bauer DE, Kerenyi MA, Vo TD, Hou S, Hsu YJ, et al. Corepressor-dependent silencing of fetal hemoglobin expression by BCL11A. Proc Natl Acad Sci U S A. 2013 Apr 16;110(16):6518–23. doi:10.1073/pnas.1303976110 PubMed PMID: 23576758.

93. Gong Y, Zhang X, Zhang Q, Zhang Y, Ye Y, Yu W, et al. A natural DNMT1 mutation elevates the fetal hemoglobin level via epigenetic derepression of the g-globin gene in b-thalassemia [Internet]. 2021. Available from: www.mbiobank.com

94. Atweh GF, Sutton M, Nassif I, Boosalis V, Dover GJ, Wallenstein S, et al. Sustained Induction of Fetal Hemoglobin by Pulse Butyrate Therapy in Sickle Cell Disease. 1999.

95. Pace BS, Qian XH, Sangerman J, Ofori-Acquah SF, Surendra Baliga B, Han J, et al. p38 MAP kinase activation mediates γ-globin gene induction in erythroid progenitors. Experimental Hematology. 2003.

96. Perrine SP, Castaneda SA, Boosalis MS, White GL, Jones BM, Bohacek R. Induction of fetal globin in β-thalassemia: Cellular obstacles and molecular progress. In: Annals of the New York Academy of Sciences. New York Academy of Sciences; 2005. p. 257–65. doi:10.1196/annals.1345.033 PubMed PMID: 16339673.

97. Weinberg RS, Ji X, Sutton M, Perrine S, Galperin Y, Li Q, et al. Butyrate increases the efficiency of translation of γ-globin mRNA. Blood. 2005 Feb 15;105(4):1807–9. doi:10.1182/blood-2004-02-0454 PubMed PMID: 15479724.

98. Trakarnsanga K, Wilson MC, Lau W, Singleton BK, Parsons SF, Sakuntanaga P, et al. Induction of adult levels of β-globin in human erythroid cells that intrinsically express

embryonic or fetal globin by transduction with KLF1 and BCL11A-XL. Haematologica. 2014 Nov 1;99(11):1677–85. doi:10.3324/haematol.2014.110155 PubMed PMID: 25107887.

99. Hu J, Chen H, Gong W, Feng M, Fu S, Xu W, et al. Association Between KLF1, BCL11A and HBS1L-MYB Polymorphisms and Phenotypes With β-Thalassemia Patients in Hainan. Molecular Genetics and Genomic Medicine . 2025 Sep 1;13(9). doi:10.1002/mgg3.70142 PubMed PMID: 40990146.

100. Motlagh FM, Soleimanpour-Lichaei HR, Shamsara M, Etemadzadeh A, Modarressi MH. CRISPR/Cas9 Ablated BCL11A Unveils the Genes with Possible Role of Globin Switching. Adv Pharm Bull. 2023 Nov 1;13(4):799–805. doi:10.34172/apb.2023.074

101. Hodge D, Coghill E, Keys J, Maguire T, Hartmann B, Mcdowall A, et al. A global role for EKLF in definitive and primitive erythropoiesis. Blood. 2006;107:3359–70. doi:10.1182/blood-2005-07

102. Borg J, Papadopoulos P, Georgitsi M, Gutiérrez L, Grech G, Fanis P, et al. Haploinsufficiency for the erythroid transcription factor KLF1 causes hereditary persistence of fetal hemoglobin. Nature Genetics. 2010. p. 801–7. doi:10.1038/ng.630 PubMed PMID: 20676099.

103. Roy P, Mitra S, Bose S, Basu S, Ghosh A. Novel advances in lentiviral gene therapy for sickle cell anemia. J Stem Cell Res Ther (Edmond). 2025;10(1):239–47. doi:10.15406/jsrt.2025.10.00211

104. Kanter J, Walters MC, Krishnamurti L, Mapara MY, Kwiatkowski JL, Rifkin-Zenenberg S, et al. Biologic and Clinical Efficacy of LentiGlobin for Sickle Cell Disease. New England Journal of Medicine. 2022 Feb 17;386(7):617–28. doi:10.1056/nejmoa2117175 PubMed PMID: 34898139.

105. Aerbajinai W, Zhu J, Gao Z, Chin K, Rodgers GP. Thalidomide induces γ-globin gene expression through increased reactive oxygen species-mediated p38 MAPK signaling and histone H4 acetylation in adult erythropoiesis. Blood. 2007 Oct 15;110(8):2864–71. doi:10.1182/blood-2007-01-065201 PubMed PMID: 17620452.

106. Diener C, Keller A, Meese E. Emerging concepts of miRNA therapeutics: from cells to clinic. Trends in Genetics. 2022 Jun 1;38(6):613–26. doi:10.1016/J.TIG.2022.02.006 PubMed PMID: 35303998.

107. Bao X, Gao Y, Wang Z, Ye Y, Chen D, Zuo Y, et al. Activation of γ-globin expression by LncRNA-mediated ERF promoter hypermethylation in β-thalassemia. Clin Epigenetics. 2024 Dec 1;16(1). doi:10.1186/s13148-023-01614-6 PubMed PMID: 38218889.

108. Frangoul H, Altshuler D, Cappellini MD, Chen YS, Domm J, Eustace BK, et al. CRISPR-Cas9 Gene Editing for Sickle Cell Disease and β-Thalassemia. New England Journal of Medicine. 2021 Jan 21;384(3):252–60. doi:10.1056/nejmoa2031054 PubMed PMID: 33283989.

109. Kollinerova S, Vassanelli S, Modriansky M. The role of miR-29 family members in malignant hematopoiesis. Biomedical Papers. 2014;158(4):489–501. doi:10.5507/bp.2014.029 PubMed PMID: 24993745.

110. Habara A. Exploratory Review and In Silico Insights into circRNA and RNA-Binding Protein Roles in γ-Globin to β-Globin Switching. Cells. Multidisciplinary Digital Publishing Institute (MDPI); 2025. doi:10.3390/cells14040312 PubMed PMID: 39996784.

111. O'Leary E, Jiang Y, Kristensen LS, Hansen TB, Kjems J. The therapeutic potential of circular RNAs. Nature Reviews Genetics. Nature Research; 2025. p. 230–44. doi:10.1038/s41576-024-00806-x PubMed PMID: 39789148.

112. Simonelig M. PiRNAs, master regulators of gene expression. Cell Res. 2014;24(7):779–80. doi:10.1038/cr.2014.78 PubMed PMID: 24946739.

113. Gupta G, Afzal M, Goyal A, M RM, Sharma GC, Jayabalan K, et al. piRNAs in leukemogenesis: Mechanisms, biomarkers, and therapeutic implications. Clinica Chimica Acta. Elsevier B.V.; 2025. doi:10.1016/j.cca.2025.120220 PubMed PMID: 40044105.

114. Ramat A, Simonelig M. Functions of PIWI Proteins in Gene Regulation: New Arrows Added to the piRNA Quiver. Trends in Genetics. Elsevier Ltd; 2021. p. 188–200. doi:10.1016/j.tig.2020.08.011 PubMed PMID: 32951946.

115. Martyn GE, Doerfler PA, Yao Y, Quinlan KGR, Weiss MJ, Crossley M. Hydroxyurea reduces the levels of the fetal globin gene repressors ZBTB7A/LRF and BCL11A in erythroid cells in vitro . Journal of Sickle Cell Disease. 2024 Nov 7;1(1). doi:10.1093/jscdis/yoae008

116. Sauntharajah Y, Desimone J. Clinical studies with fetal hemoglobin—enhancing agents in sickle cell disease. Semin Hematol. 2004 Oct 1;41(SUPPL. 6):11–6. doi:10.1053/J.SEMINHEMATOL.2004.08.003 PubMed PMID: 15534852.

117. Esrick EB, Mcconkey M, Lin K, Frisbee A, Ebert BL. Inactivation of HDAC1 or HDAC2 induces gamma globin expression without altering cell cycle or proliferation. Am J Hematol. 2015 Jul 1;90(7):624–8. doi:10.1002/ajh.24019 PubMed PMID: 25808664.

118. Pace BS, White GL, Dover GJ, Boosalis MS, Faller D V., Perrine SP. Short-chain fatty acid derivatives induce fetal globin expression and erythropoiesis in vivo. Blood. 2002 Dec 15;100(13):4640–8. doi:10.1182/blood-2002-02-0353 PubMed PMID: 12393583.

119. Lu Y, Wei Z, Yang G, Lai Y, Liu R. Investigating the Efficacy and Safety of Thalidomide for Treating Patients With ß-Thalassemia: A Meta-Analysis. Frontiers in Pharmacology. Frontiers Media S.A.; 2022. doi:10.3389/fphar.2021.814302

120. Ju W, Din G, Huang J, Zheng M, Wang X, Liu L, et al. Efficacy and safety of thalidomide in patients with β-thalassemia intermedia and major. Medicine (United States). 2024 Oct 25;103(43):e40328. doi:10.1097/MD.0000000000040328 PubMed PMID: 39470504.