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Gene expression analysis and pharmacologic simulation of HbF expression an approach for the treatment of beta thalassemia

Soumya Khare [1, 2], Tanushree Chatterjee [2] and Ashish Patel [1]
[1]Chhattisgarh Swami Vivekanand Technical University, Bhilai (C.G), India.
[2] Raipur Institute of Technology Raipur (C.G), India.

(Corresponding author: Soumya Khare)*
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ABSTRACT: *Beta thalassemia* is a globin gene synthesis condition in which the beta globin chain is not produced in red blood cells. With the development of biotechnologies, more research will be conducted to examine and improve novel therapies and natural cures to restart fetal hemoglobin (HbF) production for β-thalassemia patients. It is anticipated that more HbF inducing substances will eventually be discovered in traditional medicines and natural therapies around the world. Further research is necessary in this regard to explore further natural herbal remedies and to examine the effectiveness and safety of transitioning from laboratory to clinical use for those with β-hemoglobinopathies. The development of a new class of therapeutic agent consisting of some bioactive compounds such as flavanone for the treatment of beta thalassemia and the search for complementary and alternative medicine (CAM) that could prevent the regulated switch from fetal to adult globin gene expression. Flavones, for example, are a plant-derived chemical that has been shown to inhibit the HDAC2 enzyme and increase acetylation, thereby restoring cell homeostasis via the p38 MAPK pathway. HDAC2 could be involved in a complicated gamma globin suppression mechanism. In beta thalassemia, inducing HbF expression in erythroid cells is an important and additional treatment method. Dietary flavones can regulate HDAC activity, which could be useful in developing epigenetic treatment to control cell gene expression. As a result, it can be utilized to reactivate gamma globin expression through pharmaceutical means.

Keywords: Fetal Hemoglobin, Beta thalassemia, Histone Deacetylase, Flavanone, MicroRNA.

INTRODUCTION

Two α globin and two β globin polypeptide chains make up hemoglobin. It's an iron-containing tetramer oxygen transport protein. The embryonic two distinct genes α_1 and α_2 globin, HBA1 (α_1), HBA2 (α_2) are encoded by the alpha globin chain locus on chromosomal number 16 in humans (Pace and Sima 2006). The β globin chain locus on chromosome 11 encodes the five distinct genes HBE1 (ε), HBG1 (γA), HBG2 (γG), HBD (δ), HBB (β). Beta Thalassemia is one of the most common types of this disease; Hemoglobinopathies are the most prevalent type of blood ailment caused by a mutation in the human beta globin gene HBB, which causes a change in hemoglobin structure (Ahmadvand *et al*., 2014). The global yearly incidence of symptomatic cases and carriers of HBB at birth is estimated to be 1/100000, with a high prevalence in poor countries (Stamatoy 2005). Early embryonic globin (ζ_2 ε_2, HbF) converts to fetal hemoglobin ($\alpha_2\gamma_2$, HbF) during fetal development, and subsequently fetal hemoglobin ($\alpha_2\gamma_2$, HbF) begins to be replaced by adult hemoglobin ($\alpha_2\beta_2$) after birth (Cavazzana 2018). According to the World Health Organization (WHO), 7% of the population is afflicted by these illnesses, and current studies estimate that between 300.000 and 400.000 infants are affected each year, with the prevalence expected to rise in the future (Forget 2013). Over 1000 haemoglobinopathies have been found and classified to date (Goonasekera *et al*., 2018). The high prevalence of hemoglobinopathies is assumed to be attributable to the carrier selective advantage against malaria. As a result of this positive selection, these disorders are quite common in the tropics and subtropics (Sub-Saharan Africa, the Mediterranean, and Southeast Asia), where malaria is still endemic. They have, however, become more common in non-endemic locations as a result of population migrations, making it a global health issue (Mabaera *et al*., 2008). In normal conditions, HbF comprises less than 5% of the total hemoglobin at six month and features label by two years of age. HbF switching is regulated by epigenetically with include histone modification, DNA methylation alternation of higher order of chromatin structure and cytosine methylation increase with HBG association loss of surrounding on activation of histone modification and fetal erythroid cells influencing by the decrease in chromatin level. HbF is mostly produced in the liver and spleen, whereas HbA is produced in the bone marrow beginning during the 12th week of pregnancy and persisting throughout life. HbF consisting predominantly of γ chain, during the first 10 months after birth, the HbF level declines, it's limited to a fraction of erythrocytes called F-cells, and it's dispersed in a hepatocellular pattern (Stamatoy 2005). The synthesis of HbF and the expression of the fetal γ-globin gene have recently been increased by the

discovery of naturally occurring inducers and medication therapies. It has been noted that some chemotherapy drugs, like 5-azacytidine and hydroxyurea (HU), can increase the production of HbF (Li *et al*., 2011). However, the majority of these HbF-inducing drugs currently known to science have limited efficacy and specificity, myelotoxicity, and carcinogenesis, as well as modest responses to therapy, all of which significantly reduce their usefulness in clinical practice (Mabaera *et al*., 2008). Due to this, developing new screening techniques for accurately and efficiently identifying prospective inducers, and finding new HbF-inducing substances in nature that combine efficiency, safety, and convenience of use.

A. Beta Thalassemia

Thalassemia is a type of chronic hemolytic anemia caused by a mutation in the β globin chain, which causes inefficient erythropoiesis. Patients with continuous blood transfusions are at danger of iron overload, which is the cause of their mortality (Trachtenberg *et al.*, 2014). Thalassemia patients are largely reliant on blood transfusions to sustain and balance their normal lives. The most severe form of beta thalassemia requires, current and future alternative therapy is divided into four parts such as conventional therapy in which regular blood transfusion and chelating therapies, second gene therapy curative strategy under clinical validation, third pharmaceutical induction of HbF and fourth allogeneic transplantation in which bone marrow transplantation from cord blood unit (Cao and Galanello 2010). In this contest discovery of novel HBF induces can be important in identifying and their target involving thalassemia. Beta-thalassemia is characterized by decreased (β^+ thalassemia) or non-existent (β^0 thalassemia) synthesis of β-globin subunits, resulting in β globin chain number imbalances that cause hemolysis and impair erythropoiesis (Thein 2005). The clinical signs of β-thalassemia are quite varied, ranging from severe anemia to a variety of other conditions with transfusion dependency when patients inherit deleterious mutations in both β-globin genes from both parents to asymptomatic state with a mutation affecting only one nucleotide substitutions, deletions, or insertions in the β-globin gene (Thein 2017). The molecular foundation of β thalassemia is quite diverse; more than 300 β-thalassemia alleles have been identified in the database of human hemoglobin variations and β thalassemia, resulting in a wide range of symptoms (Higgs *et al*., 2012). The degree of imbalance between α globin and non-globin β chains, which is caused by insufficient synthesis of chains to mate with α globin chains to generate adult hemoglobin, determines the severity of β thalassemia. Chronic hemolytic anemia, splenomegaly, marrow expansion (by stimulating erythropoietin synthesis), bone deformities (of the skull and face), a variety of growth and metabolic abnormalities, hypermetabolic state, and iron accumulation result from excess α-globin precipitation in red-cell precursors, resulting in abnormal maturation and inefficient erythropoiesis resulting in chronic hemolytic anemia, splenomegaly and marrow expansion (Galenello, 2014). Hematologic and molecular genetic testing can be used to diagnose β-thalassemia, however the presence of HbA2 (> 3.5%) in combination with lowered erythrocyte indices is the first factor to consider. Major, intermedia, and mild thalassemia is the three main forms of thalassemia (Fard *et al*., 2013). Individuals with β thalassemia major (also known as Mediterranean anemia or Cooley's Anemia) typically develop severe anemia within the first two years of life, necessitating regular red blood cell (RBC) transfusions to survive (Akinsheye *et al*., 2011).

B. Regulation of Gamma Globin Gene Expression

HbF increasing is a significant and important therapeutic tool to overcome the problem of hemolysis and anemia during the first trimester fetal hemoglobin has high level in the fetus and then gradually down to adult stage by maintaining the ratio of alpha and beta chain the accumulation of alpha globin chain is a thyroid precursor is reduced and as a result, by boosting the oxygen supply to tissue and reducing clinical symptoms, the inefficient withdrawal process is inhibited.

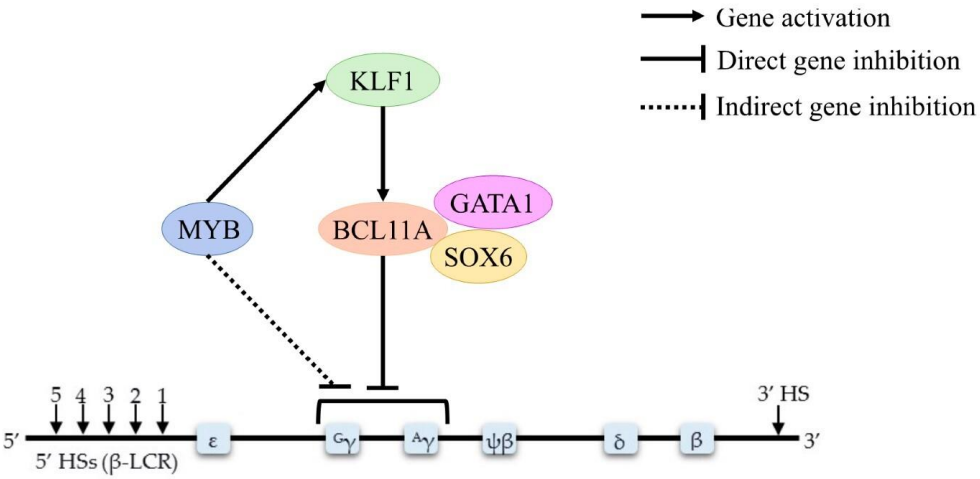

Fig. 1. HbF production can be triggered by globin gene regulators. BCL11A is a globin silencer that KLF1 favorably regulates. MYB affects erythroid differentiation rates, which regulates HbF expression indirectly, and it also activates KLF1 and other repressors directly.

In recent research, the regulation of the fetal hemoglobin gene brings the developing process is a complex process involving many different regulators. (Akinsheye *et al*., 2011). When connecting to the β globin locus, which lies between the fetal and adult genes, the BCL11A pathway, which generally cooperates with other repressors (e.g., Sox6), silences the β-globin genes. The transcription factor KLF1 (a β-globin gene promoter) also regulates BCL11A expression.

BCL11A - BCL11A or its companion proteins may provide useful clues for creating targeted therapy techniques to reactivate HbF in people with hemoglobinopathies, changing the clinical features of both illnesses. BCL11A expression inhibition could be useful strategies (Ginder 2015).

KLF1- KLF1 was assumed to be a particular factor that enables HbF to HbA flipping in this way at first. However, investigations have shown that KLF1 is important in controlling both definitive and primitive erythropoiesis, in addition to regulating globin expression. By attracting Sin3A and HDAC1, KLF1 also acts as a transcriptional repressor. KLF1 plays a more direct involvement in γ-globin gene silencing by stimulating BCL11A production by binding to its promoter. As a result, KLF1 has a dual impact switching by directly activating the γ-globin gene while also silencing it. As a result, KLF1 has a dual impact, activating the γ-globin gene directly while inhibiting the αglobin gene indirectly via activating BCL11A (Sankaran *et al*., 2010).

SOX6 - Another important protein involved in the expression of the γ-globin gene is SOX6. When it interacts with BCL11A, it aids in the suppression of the γ-globin gene, but it also protects cells from apoptosis by positively regulating BCL11 (an anti-apoptotic protein) in the last stages of erythropoiesis (Sankaran *et al*., 2010). SOX6 serves as a compensating factor activating the BCL11 gene, sparing cells from apoptosis, when the effects of erythropoietin (EPO) on boosting BCL11 expression decrease (Mahdavi *et al*., 2017).

MYB-The enhanced HbF impact is mediated by MYB is down-modulated by microRNA targeting its 3′ UTR. MYB is clearly critical in erythropoiesis, and current research suggests that it does so in part via trans activating KLF1 expression and other repressors of β-globin genes (e.g., nuclear receptors TRF2/TRF4) (Modell 2008).

GATA1- GATA binding protein 1 (GATA1) is a zinc-finger transcription factor with a GATA binding domain that may both activate and inhibit target genes (Sankaran 2013). This motif can be present in a variety of places in erythroid-expressed genes, but especially in the γ-globin locus. GATA1 is a protein that binds to the γ-globin locus and necessary for the formation of erythroid cells (Sankaran 2013). GATA1 appears to aid hemoglobin switch by facilitating chromatin loop formation at γ-globin sites, and it has been shown that in a FOG1-dependent way, GATA1 binds to a region upstream of the promoter (needed for HbF silencing) of γ_1 and γ_2-globin, resulting in recruitment of the repressive NuRD complex (Sankaran 2013).

C. Epigenetic Regulation of the Expression of the Fetal Globin Gene

The ideal target for treating hemoglobin diseases would be one that mimics and amplifies the effect of genetic variations that govern HbF levels at loci like MYB and BCL11A without interfering with other biological pathways. MYB, on the other hand, performs a pleiotropic role in hematopoiesis, while BCL11A is important for neuronal and B-lymphocyte development (Thein 2013). There has been a growing recognition of the involvement of epigenetic mechanisms in gene regulation, particularly gene silencing and in addition to the role of transcription factors in controlling γ-globin expression.

Epigenetics refers to changes in chromosomal DNA and histone proteins that affect gene expression and can be passed on through somatic replication. A deeper knowledge of the molecular mechanisms controlling HbF expression epigenetic silencing could aid in the development of more effective treatments for hemoglobinopathies, and several researchers have focused their research on epigenetic induction of HbF expression in clinical and laboratory settings. Methylation, histone deacetylation, and chromosomal looping have all been proposed as epigenetic mechanisms for HbF control (Thein 2013). MicroRNAs (miRNAs), by binding sequence-specific mRNAs a family of 19–25 nucleotide noncoding RNAs that regulate gene expression and may lead to HbF activation (Dreuzy *et al*., 2016).

DNA Methylation- The insertion of a methyl group in the 5′ position of cytosine residues in a cytosine-phosphate-guanosine dinucleotide (CpG) by DNA methyltransferases is the most important epigenetic approach for transcriptional repression (DNMTs). Genes with hypermethylated CpG islands are often dormant, whereas genes with hypomethylated CpG islands are active. The γ-globin promoter is hypo-methylated during fetal life, while β -globin is hyper-methylated, according to this theory. However, with low HbF expression in adulthood, the γ-globin promoter becomes hyper-methylated, whereas with increased HbA expression, the β -globin promoter becomes hypo-methylated (Ginder 2015).

Histone acetylation/methylation- Another epigenetic process is histone modification. Histones N-terminal tails are rich in lysine residues, which are sensitive to a variety of post-transcriptional changes like as acetylation and methylation, which can alter chromatin structure. Histone acetyltransferases (HATs) regulate the acetylation of lysine residues, which causes an open conformation of chromatin and activation of gene expression. Histone deacetylases (HDACs) regulate the deacetylation of lysine residues, which causes a closed conformation and repression of gene expression. HDAC1 and HDAC2 inhibitors have been discovered as inducers of γ-globin gene expression in recent large-scale genetic research (Das *et al*., 2019).

D. Pharmacologic Agents Reactivation of Fetal Hemoglobin

In β-thalassemia, boosting γ-globin chain synthesis compensates for the β-globin deficiency by improving the balance between α globin and non- α globin chains. The γ-globin chains join with an excess of mismatched accumulative chains. The combination provides usable hemoglobin (HbF), but it also lessens the burden of chains that cause the majority of the β-thalassemia pathophysiology. Several agents, including cytotoxic chemicals and epigenetic regulators, have been explored 5-azacytidine was the first drug to show that epigenetic silencing might promote γ-globin expression. By blocking histone deacetylation, small chain fatty acid derivatives have also been shown to enhance the production of γ-globin (Feriotto *et al*., 2018). For its proliferative and anti-apoptotic properties, erythropoietin (EPO) is also referred to as a potentiality. Hydroxyurea is currently the only medicine licensed for inducing HbF in thalassemia patients. It has a number of effects, but its cytotoxic impact is expected to hasten the process of differentiation and activate cell stress response pathways, resulting in an overall increase in HbF levels. Currently, transcriptional γ-globin repressors such as BCL11A, MYB, KLF1, and other repressors in the same family are the emerging targets for HbF induction techniques. As well as regulators like Mi2, which binds directly to the KLF1 and BCL11A genes and controls them, making these genes interesting targets for HbF inducing therapy. Although no medication exists to target these molecules or related pathways at the moment, based on current research potential candidates for therapeutic HbF inducers could be discovered soon (Feriotto *et al*., 2018).

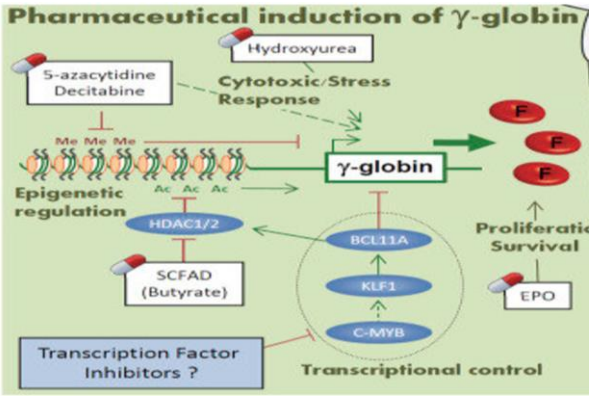

Fig. 2. γ–globin induction by pharmaceutical agents adopted by Edouard de Dreuzy, current and future alternative therapies for beta-thalassemia major (Dreuzy *et al.*, 2016).

Various treatments, including cytotoxic chemicals and epigenetic regulators, have been studied. The first medicine to be shown to boost γ-globin expression was 5-azacytidine and arginine, a demethylating chemical. This was most likely due to preventing histone deacetylation. Currently, the only medicine license for γ-globin induction is hydroxyurea. Its cytotoxic effects are hypothesized to hasten differentiation and promote cellular stress response pathways, resulting in an increase in the number of F cells. Proliferative and anti-apoptotic characteristics of erythropoietin (EPO). For those with low baseline EPO levels, combining recombinant EPO injections with cytotoxic medicines can be useful. Future therapeutics could target transcription factors like BCL11a and KLF1 that have been linked to β-globin suppression. The protein BCL11a is a transcription factor involved in the down regulation of γ-globin. It binds to the HBB locus intergenic regions, encouraging long-range interactions with the LCR that boost γ-globin synthesis. To suppress γ-globin, it recruits histone deacetylase. When generated in significant numbers in adult cells, KLF1 is a powerful inducer of γ -globin expression and promoter of BCL11A transcription. C-Myb has been shown to stimulate the expression of KLF1.

HDAC Inhibitor: Flavones are small plant-derived chemicals that have been demonstrated to inhibit class I histone deacetylase (HDAC) enzymes and restore cell homeostasis by increasing acetylation. We investigated the possible physical interactions of flavones with human HDAC1 and HDAC2 using in silico molecular docking simulations. Our findings suggest that flavone, along with the other two flavones investigated previously, apigenin and luteolin; can act as ligands in the active site binding pocket of HDAC1 and HDAC2. The potential of dietary flavones to alter HDAC activity could be significant in developing epigenetic treatments to control cell gene expression. Docking simulations were used to see if these three drugs could occupy the same binding site. The fact that flavones have binding energy values that are equivalent to or better than those of phenols shows that it is possible. Flavones can engage with HDAC1 and HDAC2 with energies similar to the known inhibitor vorinostat (Bernardina *et al*., 2020) by occupying the catalytic site and forming connections with the Zn^{2+} ion and amino acids in the binding pocket. The current work adds to our knowledge of the molecular basis of flavones' pharmacological potential as naturally occurring chemicals that lack traditional HDACi. These plant secondary metabolites could be a strong weapon against a range of illnesses if epigenetic treatment is used.

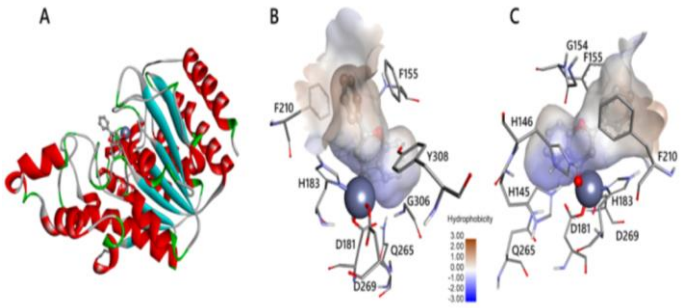

Fig. 3. Flavone interaction with HDAC2 adopted by Bernardina Scafuri, molecular docking simulations on histone deacetylases HDAC1 and HDAC2 to investigate the flavone binding, (Bernardina *et al.,* 2020).

HDAC2 docking simulation with flavone in the zinc ion interaction. Flavone in the catalytic channel of HDAC2. Fig. 3 depicts the labelling of amino acids. The zinc ion, which is depicted as a sphere, is coordinated by the carbonyl oxygen of flavone rings C and D181, H183, D269, which is shown as a sphere.

In the opposite view of panel B, the position of amino acids not visible in the other panel is shown. Histone deacetylase is inhibited by butyrate, a short-chain fatty acid (HDAC). It has been discovered that it increases the expression of embryonic and fetal globin genes in mice however, to avoid cytotoxicity, a pulsed, or intermittent, dose schedule was required (Bunn 1997). Despite the drug's enormous potential in the treatment of beta thalassemia, the difficulties of giving large amounts of it via venous catheters are a significant therapeutic obstacle. Butyrate's full potential will not be achieved until an oral molecule with the same potency as butyrate is discovered (Bunn 1997).

Natural Strategies for the fetal hemoglobin induction-Several HbF inducers have been studied in clinical trials, but only hydroxyurea has been approved by the FDA, and the benefits of this treatment strategy have sparked interest in finding more compounds that can induce HbF. Natural remedies are widely employed in traditional medicine and have long been thought to be a natural reservoir for future medicinal medications (Cappellin *et al.,* 2014). Natural techniques for the treatment of β-hemoglobinopathies have recently received a lot of attention. Natural inducers are hoped to boost HbF levels and reduce iron overload, but most importantly, they must be made available to all people (Frenette and Atweh 2013). Natural substances have been used to induce HbF and have been shown to be successful in some investigations. Various natural compounds have been found to induce HbF levels, including *Angelicin, Rapamycin, Bergamot, Triticum aestivum, Curcuma comosa, Astragalus, Apicidin, Curcuminoid, Piceatannol, and Resveratrol.* However, more investigation into their biological function is required. The most promising natural therapeutic drug that may successfully increase HbF production and reduce iron overload, consequently prolonging the life duration of ill patients, must be identified. More information on the bioavailability of these natural chemicals as well as their effects on people is required (Kukreja *et al*., 2013).

Hydroxyurea- Hydroxyurea is a cytotoxic, antimetabolic, and antineoplastic medication that has been used to treat human immunodeficiency virus infection by inhibiting ribonucleotide reductase, a key enzyme in DNA synthesis and repair (Musallam *et al*., 2013). In addition to these physiological effects, hydroxyurea increases HbF expression via stimulating the γ-globin gene and decreasing β-globin gene expression, lowering total leukocyte count and avoiding activation, and therefore reducing the likelihood of vaso-occlusive crises. Hydroxyurea thus reduces hospitalizations and deaths while maintaining a high safety profile; however, some patients do not respond well, primarily because it was discovered that hydroxyurea increases total intracellular hemoglobin while preferentially increasing mRNA levels of γ-globin in the human K562 erythroleukemia cell line. The quantities of γ-globin and HbF mRNA are greatly increased by hydroxyurea. Hydroxyurea considerably boosts γ-globin and HbF mRNA levels and has a minor stimulating effect on mRNA expression of γ -globin in two-phase mixed cultures of healthy individuals' peripheral blood erythroid progenitor cells (Koury *et al*., 2002).

E. Signalling Pathways- P38MAPK

The erythropoiesis process includes the lineage commitment, maturity, and final differentiation of a hematopoietic stem cell (HSC) into a mature red blood cell (RBC) (Ney 2006; Goh *et al*., 2007 and Cokic *et al*., 2003). Ineffective erythropoiesis induced by intramedullary apoptosis and delayed maturation of erythroid progenitor cells has long been thought to be the etiology of β -thalassemia. According to Mabaera *et al*., 2008, the p38 MAPK signaling pathway plays a crucial role for increasing HbF production. Activating the p38 MAPK signal pathway in response to numerous environmental stressors can result in apoptosis, cell proliferation, and erythroid differentiation. Many HbF-inducing medications have been connected to the p38 MAPK signaling pathway in diverse studies, including butyrate (Pace *et al*., 2003) apicidin (Mabaera *et al*., 2008), and trichostatin A (Pace *et al*., 2003). As a result, they both concluded that the p38 MAPK pathway is essential for increasing γ -globin gene expression. Several mechanistic models of HbF induction have been proposed in recent years, with the majority of them based on what are thought to be the primary actions of HbF-inducing agents, such as global DNA hypomethylation caused by DNA methyltransferase inhibitors (DNMT inhibitors) or

global histone hyperacetylation caused by histone deacetylase inhibitors (HDAC inhibitors), including SCFA derivatives. During adult erythropoiesis, most HbF inducers work by activating cell stress signaling pathways, which leads to globin gene activation and HbF synthesis, they hypothesized. Among additional HbF-inducing drugs, hydroxyurea, butyrate (SCFA), thalidomide, trichostatin A (HDAC Inhibitor), and azinomycin have been reported to activate nitric oxide, oxidative stress (ROS), osmotic shock, and protein synthesis inhibition (Wei *et al*., 2007). The p38 MAPK signaling pathway, which includes downstream kinases and transcription factors, will eventually be active, leading the γ-globin gene to be activated and HbF to be generated. In addition to the p38 MAPK signaling system, the cAMP signaling pathway has been identified as having potential in HbF synthesis in the cell stress signaling model. In early erythroid cell cultures, a rather than the p38 MAPK pathway (Witt *et al*., 2000). The phosphorylated CREB subsequently activates downstream transcription factors, causing the γ-globin gene to be activated. As a result, the cell stress signaling model is not only relevant to the majority of HbF inducing drugs, but it also explains the fundamental findings of certain prior investigations (Wei *et al*., 2007).Because the majority of the β-globin producing substances so far reported are cytotoxic and several of them stimulate the cell stress response, it's possible that p38 MAPK pathway activation could be the target of new therapeutic approaches, and that this and other stress-related pathways could be the key to understanding β-globin expression. In the search for new γ-globin inducing drugs, molecules that target the same signaling pathway are of particular relevance (Poitou *et al*., 2001).

CONCLUSION

Patients with beta thalassemia frequently use complementary and alternative medicine (CAM), and a better understanding of healthcare education in this field is critical to minimizing any difficulties that may arise from the use of both conventional and CAM therapies. Complementary and alternative medicine (CAM) therapies are used by thalassemia patients to improve their health and quality of life. This research reveals how small groups of thalassemia patients were treated. Patients were willing to try complementary and alternative medicine (CAM), but not at the expense of conventional care. Combining complementary and alternative medicine with orthodox medicine, on the other hand, could pose a significant public health danger. As a result, there is a clear need for greater research in this area. Epigenetic pathways play a significant role in fetal globin gene silencing, both alone and in combination with specific transcription factor silencers such BCL11A and KLF1. The first proof-of-concept experiments in patients with hemoglobinopathies focused on DNA methylation and histone acetylation, two important epigenetic indicators of globin gene transcriptional activity. Gaining a better understanding of the specificity of epigenetic fetal globin gene silencing processes should lead to more effective globin disease treatment by raising HbF levels, according to the researchers. Methylation status, which limits β-globin synthesis, gene polymorphisms, which boost γ-globin production, and β thalassemia coinheritance, which lessens β thalassemia severity, is the most studied β thalassemia genetic modifiers. More genetic modifiers may be researched in the future in order to fully appreciate the clinical heterogeneity of β thalassemia and to identify therapeutic strategies to effectively treat the sickness. The current work adds to our knowledge of the molecular basis of flavones pharmacological potential as naturally occurring chemicals that lack traditional HDACi. These plant secondary metabolites could be a strong weapon against a range of illnesses if epigenetic treatment is used.

FUTURE PERSPECTIVES

Beta-thalassemia is a blood condition characterized by a lack of hemoglobin synthesis. There are certain drawbacks to the existing beta-thalassemia therapy options. For patients with beta-thalassemia, employing natural substances to induce HbF is a viable treatment option. Various natural compounds have been observed to induce HbF levels in beta-thalassemic patients including microRNA-pathway-drug connections to find repurposing candidates for HbF induction curcumin, ginsenoside, valproate, vorinostat, HDACi and Hydroxyurea. Because these natural agents have no negative effects, greater investigation into their biological function is required. The most promising natural therapeutic drug that may successfully increase HbB production and reduce iron overload, consequently prolonging the life duration of ill patients, must be identified. More information on the bioavailability of these natural chemicals as well as their effects on humans is needed.

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