Molecular Mechanisms of Hemoglobin F Induction

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Abstract

Hemoglobin F (HbF, $\alpha_2\gamma_2$) is a major contributor to the clinical heterogeneity and ameliorating agent observed in patients with the β-globin disorders including β-thalassemia and sickle cell disease (SCD). During fetal life, HbF is the major hemoglobin but is largely substituted by adult hemoglobin (HbA, $\alpha_2\beta_2$) following a globin expression switch after birth. Increased γ-globin expression can reduce the clinical severity of β-thalassemia and SCD. Therefore, increase in HbF production has served as a longstanding goal. The progression of target-based therapeutics has been confused by limited comprehension of molecular mechanisms of gamma-globin gene expression. However, recent discoveries of regulators of HbF level represent a major development and provide new opportunities in employing novel rational therapeutic strategies. In this review, molecular mechanisms of hemoglobin F induction will be discussed.

Keywords: Hemoglobin F, β-thalassemia, Expression, Induction

Introduction

Hemoglobinopathies are genetic disorders that result in abnormal structure or underproduction of the globin chains specialy α and β globin chains. Hemoglobinopathies include monogenic blood disorders with autosomal recessive inheritance, which cause high mortality in the world. β-thalassemia and sickle cell disease (SCD) are the most common forms of β-hemoglobinopathies.(1) β-thalassemia is caused by a defect in hemoglobin production due to absence or decreased expression of β-globin gene. In the absence γ or β- globin chains, α-globin chains are not able to participate in the structure of normal hemoglobin and therefore precipitate in erythroid precursors and lead to ineffective erythropoiesis. In fact, lack of balance in α- and β-globin chains is the major factor in pathology of β-thalassemia.(2, 3) In SCD, hemoglobin S (HbS) polymerization due to lack of oxygen causes symptoms such as ischemia, vascular occlusion, tissue injury and other acute and chronic complications in patients. SCD is caused by a point mutation in the β-globin gene in amino acid at position 6 (β6Glu→Val) that results in the production of defective β-globin chain and formation of HbS.(4) Currently, the main treatment protocol in patients with β-thalassemia is a regular blood transfusion scheme and prescription of iron chelating drugs. The allogeneic hematopoietic stem cells transplantation is regarded as the main therapeutic approach in certain treatment of Thalassemia. In thalassemic patients in class 1 and 2 that are under the age of 17 years, it has been observed that bone marrow transplantation (BMT) from HLA-compatible donors can improve the disease in 82-93% of patients. However, BMT is associated with problems including finding HLA compatible donors and the need for long-term use

of immunosuppressive drugs to prevent or treat graft versus host disease (GVHD).(1, 5) Due to these limitations and severe complications of routine therapeutic strategies, the use of novel therapeutic approaches such as prescription of drugs inducing the expression of gamma globin gene expression appears to be a suitable approach in β-thalassemia.

Genetic mechanisms in Hemoglobin F induction: Several genetic mechanisms have been suggested for HbF induction. So far, three major quantitative trait loci (QTL) have been introduced for 20-50% of the common variations in HbF levels in patients with SCD and major thalassemias as well as in normal adults. These three major QTLs include: C/T polymorphism at position -158 of HBG2 termed Xmn1-HBG2 or rs7482144, HBS1L-MYB intergenic region on chromosome 6q23 and BCL11A on chromosome 2p16.

Xmn1-HBG2 polymorphism was identified in 1985 by Gilman et al. They re-sequenced the HBG genes and showed that Xmn1-HBG2 polymorphism promotes the expression of HBG2 and results in the expression of HbF. Other independent studies have confirmed the association between Xmn1-HBG2 polymorphism and increased the HbF levels. Increase in the HbF level ameliorates anemia symptoms in patients with sickle cell anemia and β-thalassemia.(6) In addition, Xmn1-HBG2 polymorphism was shown in Swiss-type hereditary persistence of fetal hemoglobin (HPFH).(7) Garner et al. evaluated Xmn1-HBG2 polymorphism frequency in a non-anemic North European population. They showed that Xmn1-HBG2 polymorphism is responsible for approximately 13-32% of F-cell variance in normal non-anemic population.(8) Also, Xmn1-HBG2 polymorphism is associated with normal HbF levels both in normal adults and β-thalassemia heterozygotes, but this condition is not consistent. Therefore, presence of Xmn1-HBG2 polymorphism is not always associated with high HbF levels.

Another QTL that controls HbF variation is HBS1L-MYB intergenic region on chromosome 6p23. The HBS1L-MYB intergenic region accounts for 20% of the overall trait variance of F cells in normal European whites.(9, 10) However, this QTL accounts for 3-7% of F cells variance in African-American and Brazilian patients with sickle cell anemia.(11) The control mechanism of HBS1L-MYB intergenic region on HbF increase is not still clear. Jiang et al. showed that expression of MYB and HBS1L was significantly reduced in erythroid colonies of in vitro cultures in individuals with high HbF levels, whereas K562 cells with over-expression of MYB inhibited γ-globin expression. This revealed that MYB regulates HbF expression.(12)

Recent advances in genome analysis tools discovered another QTL controlling HbF variation. Menzel et al. showed that the BCL11A locus accounts for 15.1%, gamma globulin for 10.2% and HBS1L-MYB for 19.4% of F cell variability in northern Europeans(10). Of the three major QTLs, single nucleotide polymorphisms within the 14 kb intron 2 of BCL11A are most strongly associated with HbF expression.(11, 13, 14) This genotype of BCL11A is associated with both reduced BCL11A expression and high expression of HbF.(15) More recent studies showed that in the absence of BCL11A, developmental silencing of the human γ-globin genes is markedly impaired in the definitive erythroid lineage.(16) Recent studies in K562 cells suggest that BCL11A binds to a core motif in the HBG promoter to form a repressor complex.(17)

Hemoglobin F Inducer drugs mechanisms: In recent years, the use of fetal hemoglobin (HbF) inducing drugs has been highly regarded as the best treatment approach in the treatment of β-hemoglobinopathies. Hydroxyurea (ribonucleotide reductase inhibitor) is one of the drugs that induce HbF production. Since this drug has both increasing effect on γ-globin expression and decreasing effect on β-globin gene expression, prescription of this drug is appropriate in the treatment of SCD because of its impact in reducing the production of defective β-globin chains and its anti-sickling effect. In fact, this drug induces HbF expression probably through cytotoxic effects, erythroid regeneration and also by increasing nitric oxide (NO) production.(18, 19)

The histone deacetylase (HDAC) inhibitor drugs including butyrate, azacitidine (inhibitor of methyltransferases), decitabine (5-aza-2'-deoxycytidine, a DNA demethylating agent), and trichostatine (histone deacetylase inhibitor) act in switching β- to γ-globin genes through increased histone acetylation especially of H3 and reduced DNA methylation of γ-globin gene. In fact, the method in which HDAC inhibitor can cause changes in DNA methylation it is not clear; probably HDAC inhibitor reduces the amount of DNA methyl transferase type 3B (DNMT3B) enzyme.(18, 20, 21) Decitabine, an analog drug of azacitidine, has a higher potential in inhibiting DNMT, and on the other hand it can cause activation of tumor suppressor genes.(4, 22, 23)

Butyrate can increase histone acetylation in ε gene, but this is not followed by increased expression of ε gene. This is because ε-globin chain production requires other factors such as specific transcription factors and epigenetic changes in the LCR.(18, 24) Pomalidomide (TNF-α inhibitor) and lenalidomide (an immunomodulator) both increase γ-globin gene expression without cytotoxic effects on other inducing agents. Pomalidomide is a stronger inducer of HbF production compared with other inducing drugs. Indeed, this drug increases the expression of HbF through H3K9 and H3K14 acetylation in DNAse I Hyper sensitive Sites 1,2 (DHS1, DHS2) in the LCR. Note that this pattern is locus specific, and does not change the H3 acetylation pattern globally. Both drugs have the synergistic effect with HU in inducing HbF.(25) In this drug family, thalidomide has proper effects in inducing HbF with a similar mechanism to the other two drugs of this family.(26) Thalidomide is a stronger inducer of β-globin and γ-globin genes. Thalidomide and sodium butyrate have synergistic effect in the induction of β- and γ-globin genes.(27) The molecular mechanism of thalidomide in induction of HbF expression may be related to suppression of cytokine-induced nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB), tumor necrosis factor (TNF), vascular endothelial growth factor (VEGF) and prostaglandin E2 synthesis (PGE2) along with production of reactive oxygen species (ROS). Reactive oxygen species can suppress or induce the expression of several genes and can also affect signal transduction pathways in cells by changing the activity of some protein kinases and transcription factors. The reason for teratogenic effect of thalidomide is still debatable, although the teratogenic effect of thalidomide may be related to reactive oxygen species causing DNA damage.(26, 28) Also, our investigations have showed that thalidomide can decrease H3K27 methylation of γ-globin gene following upregulation of HbF induction, as this marker considers as heterochromatin marker (accepted paper).(29) In a case report published in 2010, treatment of a patient with β-thalassemia using thalidomide was achieved without neurotoxic effects.(30)

In other investigations, a number of cytokines such as Stem Cell Factor (SCF) and TGF-β have been mentioned as HbF inducers. SCF has been found to increase the expression of HbF by activation of Mitogen-activated Protein Kinases (MAPK) signaling pathways. This signaling pathway can affect the regulatory region in the β-globin gene cluster through increased production of Nuclear Factor Erythroid-derived 2 (NF-E2) transcription factor. TGF-β induces the expression of γ-globin gene by activating TGF- β inducible early response gene 2 (TIEG2) transcription factor, renamed to FKLF (Kruppel-type zinc finger protein) by determining its direct effect on expression of γ-globin gene. In a study, the effect of cytokine combination of SCF, TGF-β and erythropoietin (EPO) in the induction of HbF has been evaluated. The results indicated the high capacity of this cytokine combination compared with the combination of EPO and TGF-β and also EPO and SCF in induction of HbF in $CD133^{+}$ derived erythroid cells in cord blood.(31)

Conclusion

In spite of intensive research in the past decade in HbF-inducing agents, only some drugs have been approved in hemoglobinopathies treatment. Therefore, there are excessive needs for more effective therapies. New strategies for targeting of HbF genetic regulators (such as BCL11A and HBS1L-MYB) may be consider as future therapy. Studying molecular and epigenetic mechanisms of HbF expression may lead to identifying effective mechanisms in the induction of gene expression. It also appears that the simultaneous use of two HbF inducing drugs acting with two different molecular mechanisms can effectively increase the HbF level. Therefore, careful choose of effective low-risk drugs in inducing HbF is important as a proper therapeutic strategy on one hand, and the use of effective drug combination in different molecular and epigenetic mechanisms in the level of γ-globin gene on the other hand.

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