

REVIEW ARTICLE

Biological Roles of Induction of Fetal hemoglobin Production by Genetic Switching In Medical Practice: A Mini Review

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Abstract

Fetal hemoglobin switching is a good model for application of reversing silenced genes in the treatment of hemoglobin diseases. By understanding of the mechanisms and targeting the silenced genes to reactivate the fetal hemoglobin by transcription factors, in future it will be possible to control many hemoglobinopathies in human. From this aspect it is interesting to know about major molecular axis about negative regulators and positive regulators of fetal hemoglobin genes and also this field especially by using of CRISPR, siRNA and shRNA can be used for the treatment of considerable spectrum of diseases.

KeyWords: Fetal Hemoglobin, Gene Silencing, Hemoglobinopathies

Introduction

Hemoglobin synthesis results from an orderly evolution of a series of embryonic, fetal, and adult hemoglobin. Fetal hemoglobin is made from alpha and gamma globin. The gamma globin gene is expressed during fetal development. The alpha₂ gamma₂ globin is the main oxygen transport protein in the human fetus during last seven months. The gamma subunit is encoded on chromosome 11 as is the beta globin, two similar copies of the gamma subunit globin genes exist, gamma G (glycine) position 136 and gamma A (alanine). At birth HbF constitutes 60% to 90% of total hemoglobin. It declines from 90% at 30 weeks to 7% after 12 weeks post birth. The gene that codes for alpha subunit is located on chromosome 16 and present in duplicate. Mutations in the Beta-globin locus give rise to the hemoglobinopathies such as beta thalassemia and sickle cell disease. Beta thalassemia has 200 different mutations but sickle cell disease has one point mutation. Starting from birth, the molecular defects in beta thalassemia result in absent or reduced Beta Globin synthesis, hemoglobin A, then anemia and excess imbalanced alpha globin deposition and ineffective erythropoiesis, iron loading with increased iron absorption with repeated blood transfusions, in contrast, in the sickle cell disease, the problem is abnormal beta globin, substitution of valine for the glutamic acid and subsequent sickle phenomenon with high morbidity and mortality due to hemolytic anemia and vascular catastrophe in many organs, from these views with making to increase the gamma globin synthesis and subsequent rise in fetal

hemoglobin concentration, we can reduce fatal diseases theoretically by reactivation of the silenced gamma gene, increased hemoglobin F decreases the sickle cells and inhibits polymerization of sickle hemoglobin, to reach at least twenty percent of HbF concentration, and at that level the symptoms of severe hemoglobinopathies can be ameliorated.^{1,2,3}

Gamma Globin Synthesis

Globin synthesis is highly regulated at transcription level by complex interaction of DNA sequence to cis-promoters and enhancers and silencers soluble transcription factors. Globin synthesis is also regulated during translation when the mRNA coding for the globin chain associates with ribosomes to produce the polypeptides. Many protein factors are required to control initiation, elongation, and termination steps of translation. Fetal to adult hemoglobin switching is due to two stem cell lineage with different globin gene expression program. HbF is an interesting molecule and binds oxygen more strongly than adult hemoglobin enabling for the transfer of oxygen from the mother to fetus.^{4,5,6} Sequences involved in gamma gene activation are located both in LCR and gamma promoter region. Elevating expression of the developmentally silenced gamma globin gene can supplant mutant or inadequate levels by beta globin in human disease state thereby suppressing the associated symptoms. Change from fetal hemoglobin to adult hemoglobin from the aspect of genetic system is interesting in the field of biology and the gene silencing in postnatal state is a major genetic event. Fetal

hemoglobin silencing genes is a model for the gene manipulation and reactivation genes in adults.^{7,8,9}

History of HbF Induction

The only HbF inducing agents that is currently approved by the United States Food and Drug Administration (FDA), is hydroxyurea, for the treatment of sickle cell anemia, but only half of the cases respond with elevation of HbF. 5-Azacytidine can induce HbF induction in human and primates, it can reverse methylation of gamma globin gene with this mechanism; but the carcinogenicity of this product is a problem. Like 5-azacytidine, short chain fatty acids, erythropoietin stimulating agents and thalidomide derivatives have been evaluated in thalassemia major to increase fetal hemoglobin, because of their ability to switch on the gamma globin genes, to produce more gamma chains, the gamma chains then combine with excess alpha chains to form hemoglobin F, thus correcting alpha-beta imbalance. Hydroxyurea can be helpful for few thalassemic patients but not for majority of them. Another compound, Beta hydroxybutyrate is increased in the infants with high HbF from the diabetic mothers and can be a therapeutic model in hemoglobinopathies because of interfering in switching off the fetal hemoglobin to adult globin; their mechanism of action is inhibiting histone deacetylase. Erythropoietin is another agent which can increase HbF¹⁰. Erythropoietin and hydroxyurea can elevate hemoglobin F synergistically.

Myb

This gene encodes a transcription factor that is critical for hematopoietic stem cells and progenitors, also is involved in fetal globin synthesis¹¹.

BCL11A

Is a potent silencer of gamma globin. BCL11A can be a target of for therapeutic intervention of hemoglobinopathies using genome editing approaches. It is required for the function of several cell types and the ablation of BCL11 function can cause abnormal side effects such as leukemia or lymphomas besides, it is better to restrict target to only erythroid lineage.¹²

LCR

Locus control region (LCR), has emerged as an extremely important constituent in the regulation of hemoglobin switching, it is a good target for therapeutic modulation. During the fetal stage of development there is preferential interaction between the gamma genes and LCR and the beta globin genes are turned off competitively. On the other hand, LCR postnatally interacts with beta globin genes resulting silencing the gamma globin genes; also, discovery of the LCR had a major impact on the investigation of mechanisms of switching and KLF1 and BCL11A axis.^{13,14}

KLF1 MUTATION and HbF

Krupple like factor 1 plays a key role in gamma globin switch from gamma to beta. It preferentially binds and activates the beta globin chain. KLF1 also regulates the expression of repressors of globin gene

transcription factors such BCL11A and MYB.

GATA1 regulates hemoglobin synthesis, It directly induce KLF1 expression, it is involved in alfa and beta globin loci. Gata 1 is a critical determinant of Hb synthesis, it directly regulates expression of genes encoding Hb subunits and heme biosynthetic enzymes. Gata 1 regulates hundreds of genes.¹⁵

HPFH is a condition characterized by elevated levels of hemoglobin F in adult individuals. This disease is related to deletions or mutations of negative DNA regulatory elements that repress gamma globin genes. BCL11A is a major regulatory repressor of gamma globin and mutation in BCL11A regulatory DNA elements were associated with HPFH. Heterogenous mutations in KLF1 cause HPFH. Deletional HPFH is associated with deletion of large regions of DNA between gamma and beta globin locus¹⁶. Point mutations in the proximal fetal gamma globin gene promoters are the cause of the nondeletional HPFH. This disease is good model for gene silencing phenomenon.

Discussion

Sickle cell disease and Beta-thalassemia are unique among genetic disorders with high mortality and morbidity with no curative treatment except allogenic hematopoietic stem cell transplantation but due to the absence of appropriate donor and transplantation related mortality, it is not an ideal final treatment option. So the nature learned that with fetal hemoglobin induction, human can control these disorders. For the last fifty years ago, it was known

that increased Hb F abolishes the anemic effect of beta thalassemia major. The opening of the field of recovering gene silencing in Hb F system is interesting regarding future planning of hemoglobin diseases such as thalassemia, and sickle cell disease, the two entities with higher mortality and morbidity. The major players of the Hb F gene silencing in future are targeting BCL11A, GATA1, CMYB. Studies of hemoglobin switching have provided major insights on the control of gene loci by remote regulatory elements. During the fetal stage development, there is interaction between the gamma genes and LCR the LCR interacts preferentially with the beta gene during adult stage of development, resulting the gamma globin silencing, this competition by epsilon and gamma genes silences the beta gene in embryonic erythropoiesis. Gene competition and autonomous silencing are two mechanisms of turning off the gamma genes during development. The targeting of the transcription factors such as BCL11A, KLF1, MYB, by siRNA shRNA delivery via genetic approach control hemoglobin disease and theoretically can be a treatment model in future. Thalidomide and related drugs also can induce gamma globin gene expression and the CRISPR will be the modern therapeutic plan in thalassemia and other hemoglobin diseases. CRISPR/cas9 can break BCL11A and clinicians collect hematopoietic stem cells, then with the method which can induce a mutation in the molecule.^{18,19,20} With CRISPR-CAS9 editing and destructing the remaining bone marrow of the patient with chemotherapy, then the CRISPR editing cells are injected

into the patient to create HbF cells providing higher oxygen in the body to compensate the effect of hypoxia due to underlying hemoglobinopathy.

Conclusion

The Future is bright for thalassemic and sickle cell diseases regarding the intervention in fetal hemoglobin gene switching by uncovering the genetic regulation of fetal hemoglobin. So, targeting BCL11A, MYB, KLF1 will be the therapeutic plan in the coming years.

Abbreviations

Hb=hemoglobin

Hb F=fetal hemoglobin

LCR=locus control region

MYB= myeloblastosis- a type of transcription factor

BCL11A=B cell lymphoma, leukemia 11A, regulator of globin gene expression

KLF1=Krueppel-like factor 1, encoded by the KLF1 gene, is a transcription factor that is necessary for the proper maturation of erythroid cells.

GATA1= Erythroid Transcription Factor, it is absent in other cell lineages types

HPFH=hereditary persistent fetal hemoglobin

DNA=deoxyribonucleic acid

SOX6= A Transcription factor, is a protein coded by SOX6 gene

SHRNA=Short Hairpin RNA

SIRNA=small interfering RNA

RNA=ribonucleic acid

CRISPR=Clustered Regularly Interspaced Short Palindromic Repeat, CRISPR-associated 9, Cas9

FDA=federal drug administration

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