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Leading article

Devising a cure for β-thalassaemia by targeting α-globin

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Key words: β-thalassaemia, targeting α-globin

Abstract

β-Thalassaemia is a disorder of haemoglobin synthesis which does not have an effective cure for a majority of patients affected. Most patients have poor quality of life and die prematurely. The basic pathophysiology of β-thalassaemia is haemolysis and ineffective erythropoiesis due to the imbalance of β-globin chains in red blood cells. Studies done on the molecular pathology and naturally occurring mutations among patients have conclusively shown that decreasing the synthesis of α-globin chains ameliorates the severity of anaemia in β-thalassaemia. A series of recent in vitro and animal studies described in this paper shows that therapeutic inhibition of α-globin synthesis is feasible through genome editing of its major enhancer and pharmacological disruption of epigenetic enzymes. These novel pathways would invariably pave the way for an effective cure for β-thalassaemia which will be available for all patients in the future.

Introduction

β-Thalassaemia is one of the most common genetic diseases in the world[1]. Approximately, 70,000 new births are reported every year[2]. In Sri Lanka, 50 to 60 babies are born with severe forms of β-thalassaemia annually[3]. There are nearly 1800 patients receiving treatment from 26 different centres all over Sri Lanka at present[4]. Out of these patients, 68% has homozygous or compound heterozygous β-thalassaemia major while 20% have severe haemoglobin E β-thalassaemia. Remainder consists of rare forms of thalassaemia that include sickle β-thalassaemia.

The medical management of β-thalassaemia has improved considerably over the past few years with the availability of safe blood products and effective oral iron chelators[5]. However, these treatment modalities only provide supportive care which should be continued throughout life[6,7]. Therefore, patients with β-thalassaemia experience a poor quality of life and die prematurely[8]. Consequently, the average life expectancy of a patient in Sri Lanka with β-thalassaemia is still 30 to 40 years[9]. The only mode of cure for β-thalassaemia at present is haematopoietic stem cell transplantation (HSCT) which is not available to a majority of patients due to lack of suitable donors and morbidities associated with the procedure[10]. Therefore, multiple research studies are ongoing to discover a permanent cure that is suitable to all patients with β-thalassaemia[11].

Pathophysiology of β-thalassaemia

The molecular pathology of thalassaemia is due to the defective synthesis of globin chains responsible for the production of adult haemoglobin (Haemoglobin A, HbA) in human red blood cells (RBCs). In β-thalassaemia, over 250 genetic mutations in the β-globin gene lead to defective synthesis of β-globin peptides resulting in either absent (β^0-thalassaemia mutations) or reduced (β^+ or β^{++}-thalassaemia mutations) production of β-globin chains in RBCs[12]. However, the synthesis of α-globin continues normally and this unopposed production leads to the accumulation of α-globin chains within RBCs of patients with thalassaemia. These insoluble α-globin chains precipitate in mature RBCs and their precursors to

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result in haemolysis and ineffective erythropoiesis[13]. RBC destruction by these mechanisms is the primary factor leading to anaemia in patients with β-thalassaemia.

α-Globin as a genetic modifier of β-thalassaemia

Although β-thalassaemia is a prototype autosomal recessive genetic disease, it has a remarkable clinical heterogeneity. While most patients with homozygous β-thalassaemia are transfusion dependent, a significant proportion have variable phenotypes with inconsistent requirements for blood, and are transfusion independent (β-thalassaemia intermedia or non-transfusion dependent β-thalassaemia)[14]. This clinical heterogeneity can at least be partly explained by the pathophysiology which revolves around the destructive effects of the unbalanced synthesis of α- and β-like globin chains. A number of clinical studies done in the past identified two main naturally occurring mechanisms that modify the disease severity of β-thalassaemia[15,16]. Firstly, decreasing the synthesis of α-globin as seen in patients who co-inherit α-thalassaemia ameliorates the disease severity of β-thalassaemia by favourably modulating the α- to β-like globin chain imbalance. Secondly, increasing the production of γ-globin as seen in individuals with hereditary persistence of fetal haemoglobin, increases the β-like globin proportion in RBC and improves the clinical phenotype (17). Therefore, we hypothesised that if we can control the production of α-globins in patients with β-thalassaemia, it should reduce the excess of α-globins thereby the destruction of RBCs which should lower the severity of β-thalassaemia.

Decreasing α-globin as a treatment for β-thalassaemia

All human genes are controlled by three interrelated mechanisms namely, enhancers, transcription factors and epigenetic mechanisms. Enhancers are DNA sequences that facilitate the transcription of genes. The regulation of the expression of α-globin is controlled by four enhancers located upstream of the α-globin genes. Of these, 'MCS-R2' enhancer which is located 40kb from the α-globin genes is considered as the main enhancer that promotes transcription of β-globin genes[18]. Similarly, several epigenetic signatures including methylation and acetylation of histone proteins are important modulators of the regulation of β-globin gene expression. Therapeutically, it is feasible to target these enhancers and epigenetic enzymes to devise a cure for β-thalassaemia.

α-globin enhancer genome editing

Targeting of α-globin enhancer is feasible through a new genetic engineering tool – CRISPR-Cas9 (clustered, regularly interspaced, short palindromic repeat-CRISPR associated system) genome editing technology. The basic principle of CRISPR-Cas9 genome editing technique is to mutate a specific gene by cutting at a pre-determined target site of the human genome, commonly creating a deletion within the gene. In simple terms CRISPR-Cas9 reagents act as molecular scissors to cut DNA[19].

As described previously, the MCS-R2 is the most important enhancer of human α-globin gene and is crucial for normal α-globin production in RBCs. Using CRISPR-Cas9, we designed a strategy to mutate MCS-R2 enhancer to decrease the synthesis of α-globin and examined this approach *in vitro* in erythroid cells and *in vivo* in mice. Firstly, we demonstrated successful deletion of MCS-R2 enhancer *in vitro* in human erythroid cells differentiated from CD34+ haematopoietic stem and progenitor cells (HSPC) using CRISPR-Cas9. As expected, the deletion of MCS-R2 enhancer was associated with a decrease in the expression of the α-globin gene. Next, we performed the same experiment in erythroid cells generated from CD34+ cells of patients with β-thalassaemia. The deletion of MCS-R2 enhancer normalised the α/β globin imbalance in RBC of patients with β-thalassaemia. Finally, we validated this approach *in vivo* in mice. We performed stem cell transplantation (xenotransplantation) of genome edited CD34+ HSPC to immune compromised mice and showed that the edited cells are viable, retain their stem cell properties and are able differentiate *in vivo* similar to unedited cells[20]. These experiments provide proof of principle that genome editing of α-globin enhancer is a potential and a feasible pathway to devise a cure for β-thalassaemia.

The steps of any future clinical application of this approach would include isolation of CD34+ HSPC

from patients with β-thalassaemia, genome edit them *ex vivo* to delete MCS-R2 enhancer and autologous transplantation of edited HSPC back to the patient. This approach will alleviate the need for HLA-matched sibling donors for HSCT therefore, would be available to all patients with β-thalassaemia. Similarly, as the transplanted cells are autologous it will eliminate the need for immune-suppression and minimize serious adverse effects of transplantation[21].

Pharmacological modulation of epigenetic enzymes to down regulate α-globin

The other potential pathway of decreasing α-globin is through modulation of epigenetic factors that regulate α-globin gene expression. We performed a screen of several novel epigenetic compounds in a small-scale erythroid differentiation cell culture system to identify potential drugs or compounds that decrease the production of α-globin[22]. We identified and validated two compounds which showed favourable changes in globin gene expression. Firstly, we showed that 'IOX1', which is a newly synthesised chemical compound that inhibits histone demethylase enzyme down regulates the expression of α-globin without altering the expression of other globin genes or perturbing erythroid differentiation[23]. Secondly, we demonstrated that 'vorinostat', which is a FDA approved histone deacetylase inhibitor drug has the potential to down regulate the expression of α-globin while upregulating γ-globin thus demonstrating synergistic beneficial effects for β-thalassaemia[24]. Out of the two drugs, IOX1 requires further optimisation of its medicinal properties before it can be utilised in animals or humans. In contrast, being a FDA approved drug for other indications, vorinostat is a suitable drug to be tested directly in phase 2 clinical trials in patients with β-thalassaemia.

Conclusions

The clinical severity of β-thalassaemia primarily depends on the degree of imbalance between α- and β-globin chains in RBCs. Natural reduction of α-globin in individuals who co-inherit both α- and β-thalassaemia have conclusively shown to decrease the need for blood transfusion in patients with β-thalassaemia. Down regulation of the expression of α-globin through genome editing of its enhancers or altering epigenetic factors are promising new strategies to devise a cure for β-thalassaemia.

Authorship

Contribution: This is the sole work of the author.

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