Prospects for developing a molecular cure for thalassemia

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The delineation of the thalassemia syndromes started in the late 1950s with the distinction of alpha and beta thalassemias and the initial studies that pointed out to the considerable genetic heterogeneity of these disorders. Molecular investigations, however, had to wait for the development of recombinant DNA and molecular cloning techniques in the 1970s. The thalassemias were the first human disorders to be delineated at the DNA level and indeed, by the mid 1980s their molecular pathology had been determined. Investigators in this field always hoped that the molecular understanding of thalassemias would provide clues for the development of molecular therapies and perhaps cures. This hypothesis, however, was proven wrong; no clues for therapies have come from the molecular analysis of the large number of thalassemia mutations that have so far been characterized. Hopes for a molecular cure are now resting on the development of gene therapy. Special for beta thalassemia and the other beta chain hemoglobinopathies is a form of therapy that has been revealed to us by nature, i.e. the alleviation of the beta chain deficiency by the production of fetal hemoglobin in the patient’s red cells.

Prospects for the development of gene therapy for beta thalassemia syndromes. Gene therapy for thalassemia has been an early goal of investigators working in this field. Indeed, we were thinking about gene therapy even before the molecular understanding of the control of globin genes had reached a stage that could make this goal realistic.

The ultimate goal of gene therapy of thalassemia is to correct the mutant globin gene in the patient’s hemopoietic cells. Gene correction, however, requires technologies which are still at a very early stage of development. The current goal of gene therapy for thalassemia is to add in the stem cells of the patients a normal β globin gene or a fetal globin gene (gene addition). In order to be successful, gene additions require that the therapeutic beta or gamma globin gene (1) is transferred to a large number the patient’s pluripotent repopulating stem cells; (2) it is regulated like a normal globin gene; (3) it is expressed at the very high level characteristic of the beta globin genes of normal individuals.

During the last 20 years a major effort has been directed towards the development of vectors that fulfill these requirements. For about 15 years retroviral vectors were used because of the very good understanding of the retroviral biology, and because they integrate into the cell’s genome. Stable integration of therapeutic genes into the patient’s stem cells is an absolute requirement for gene therapy of thalassemia. However, retroviral vectors (oncoretroviral vectors) require cell division for integration and this is a disadvantage because the vast majority of hemopoietic stem cells are at rest.

Systematic studies of globin gene transfer using retroviral vectors started in the mid 1980s but the results were disappointing because globin gene expression was inconsistent and very low. The discovery of the major regulatory element of the β locus, the locus control region (LCR) brought new impetus in the field and a large effort was made in several laboratories to produce new globin gene vectors containing the beta or gamma globin genes and components of the LCR that hopefully would have stimulated high globin gene expression. The initial excitement, however, was followed by new disappointment because these vectors were incapable of achieving therapeutic levels of globin gene expression; they were also unstable. The field was resurrected with the introduction of lentiviral vectors. These vectors are superior to oncoretroviral vectors because (1) they do not require cell division to enter the cell’s nucleus and to integrate into the cell’s genome. (2) They allow the incorporation in the vector of larger regulatory sequences from the locus control region;
this in turn guarantees high levels of globin gene expression; (3) they are stable.

Lentiviral globin gene vectors have now been used by six groups for preclinical studies in murine beta thalassemia and sickle cell anemia models and have cured thalassemia and sickle cell anemia in these models. Lentiviral globin vectors completely correct the thalassemia hematological phenotype in cultures of erythroid cells from patients with Cooley’s anemia. Although several improvements are expected to be implemented in the future, it is safe to state that the basic molecular tools for achieving curative levels of globin gene expression using gene therapy vectors are now available.

In order to translate these advances to gene therapy of our patients, two major challenges need to be met. The first is safety. Patients with XSCID were cured after receiving gene therapy with retroviral vectors containing the \( \gamma^c \) gene (which is abnormal in this disease) but 3 of 10 patients developed leukemia. Subsequent studies of these patients and several other investigations established that integration of viral vectors is not random and can have unwarranted effects because of activation of protooncogenes. A goal of the gene therapy field is to develop approaches that decrease the probability of unwarranted interactions between the integrating viral vectors and the genes of the host. The second challenge stems from the requirement for genetic modification of a large number of pluripotent stem cells; this is necessary in order to achieve therapeutic levels of globin gene expression in the patient’s blood. It has been estimated that gene transfer to about 20 to 30% of the patients pluripotent hematopoietic stem cells is required to cure Cooley’s anemia. Achieving this goal will necessitate bone marrow conditioning. It is expected that a moderate degree of conditioning will create enough spaces to allow homing by the genetically modified hematopoietic stem cells.

**Prospects for a molecular cure through activation of fetal globin genes**

The possibility that Cooley’s anemia can be cured by induction of synthesis of fetal hemoglobin was realized when the pathophysiology of the thalassemia syndromes started to be delineated in the late 1950s. It was then realized that the patients with Cooley’s anemia survive beyond the period of the fetal to adult hemoglobin switch because synthesis of fetal hemoglobin continues in the patient’s erythroid cells. The level of fetal hemoglobin in the patient’s cells is, however, inadequate; hence the severe anemia with all its pathophysiological consequences.

The molecular control of globin gene switching was up to recently unknown, but this did not prevent us from attempting, in the 1970s, to investigate the control of fetal hemoglobin synthesis in adult individuals. A significant advance was the discovery that fetal hemoglobin can be readily induced in cultures of adult erythroid progenitors. Such studies in culture led to the concept that the adult erythroid cells have an inherent potential to produce fetal hemoglobin but that potential is lost during downstream differentiation. Another clue came from the discovery that fetal hemoglobin can be induced in primates and in humans under conditions of rapid erythroid regeneration. These observations led us test whether cytotoxic drugs which secondarily produce rapid erythroid regeneration can also activate fetal hemoglobin production. The outcome of these investigations was the introduction of hydroxyurea as an inducer of fetal hemoglobin in patients with beta chain hemoglobinopathies. Results of hydroxyurea treatment have been very encouraging in sickle cell disease and in sickle cell beta thalassemia but they have been rather poor in Cooley’s anemia.

The understanding of the molecular control of globin gene switching started in the late 1980s when it was realized that globin gene expression reflects an interaction between globin genes and the locus control region. This interaction between fetal globin genes and the LCR activates the fetal globin genes. Inhibition of this interaction silences the fetal globin genes. A large research effort has been focused on understanding the specifics of these interactions. We now know that specific components of the locus control region and specific sequences of the fetal globin gene promoter are involved in the fetal globin gene activation. We also have started identifying the determinants of the fetal globin gene promoters which are involved in silencing. Both gene activation and gene silencing are mediated through transcriptional factors which interact with the globin genes and the locus control region. We hope that eventually specific molecular therapeutics for induction of fetal hemoglobin will be developed by modulating the action of these factors involved in fetal gene activation or silencing. However, in spite of considerable effort, the specific transcriptional factors participating in \( \gamma \) gene activation or silencing remain unknown.

It is however possible to affect fetal globin gene expression indirectly by manipulating the chromatin of the beta globin locus. Extensive evidence obtained from studies of loci of model organisms indicates that modifications of histones affect gene expression. Recently the histone code of the human fetal and adult erythroid cells has been defined in our laboratory and it is clear that globin gene switching is associated with substantial switches in acetylation of histones of the fetal globin genes; the gamma genes are highly acetylated in the erythroid cells of the fetus, where fetal globin gene expression is very high; but they are hypoacetylated in the adult erythropoiesis where fetal globin gene expression is very low. Since
fetal globin gene silencing is associated with histone deacetylation, it is expected that inhibition of histone deacetylation can lead to $\gamma$ gene activation.

Practically, induction of fetal hemoglobin through inhibition of the enzymes responsible for histone deacetylation has been achieved before information on the globin histone code became available. Butyrate, a histone deacetylase inhibitor, has been shown to induce the $\gamma$ genes in vitro, in animal models and in patients with sickle cell anemia and patients with thalassemia. Other short chain fatty acids and related compounds that are potent fetal globin gene inducers have been found. Certain fetal hemoglobin inducers also have erythropoietic effects and are active by oral administration. Clinical development of these compounds provide the best hope for production of a molecular therapeutic which will have an impact on the treatment of thalassemia patients all over the world.

References