Hemoglobin Gene Therapy for β-Thalassemia

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• Clinical trials

Human globin gene therapy is an exciting approach to curing homozygous β-thalassemia (β-thalassemia, Cooley anemia) as well as sickle cell anemia. These diseases are particularly suitable for this approach because the specific genetic defects that cause them are known: sickle cell disease is caused by a point mutation in the human β-globin gene; most β-thalassemia mutations are also caused by single nucleotide changes, all of which lead to either decreased or absent normal β-globin protein. Human β-globin gene therapy with autologous modified stem cells has been envisioned for many years by patients, physicians, and scientists as a logical and ideal way to cure the disease. However, it is only recently that some limited success has been achieved.

The only cure for β-thalassemia (Cooley anemia) is allogeneic stem cell transplantation (ASCT), using stem cells from adult peripheral blood, bone marrow, or umbilical cord blood sources. ASCT is discussed in detail by Gaziev and Lucarelli elsewhere in this issue , as well as by Kanathezhath and Walters. ASCT is limited by immunologic differences between patients and potential donors; less than 30% of patients have suitable donors. A curative result occurs in the most eligible patients who fit the criteria for transplantation, most of whom are children. The potential development of graft-versus-host disease, a potentially life-threatening complication caused by immune reactions, has tempered the use of ASCT, especially when a completely compatible donor is not available.

There are 2 general approaches to providing normal β-globin function by gene therapy in these disorders: correction of the DNA defect in the β-globin gene by homologous recombination, or addition of a normal β-globin gene to the genome. Gene correction has the great advantage of maintaining the β-globin gene in its native

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chromosomal environment, and thus is the preferred gene therapy approach. However, homologous recombination occurs at too low a frequency at present to be useful for human globin gene therapy.

Gene addition has been used successfully in human gene therapy clinical trials, with viral vectors transferring and expressing corrective genes in human hematopoietic cells. So-called γ-retroviral vectors containing Moloney viral components have been used to cure patients with severe immune disorders such as subacute combined immunodeficiency (SCID) and adenosine deaminase deficiency.[1–3] In these conditions, the gene-corrected lymphocytes are naturally selected for survival and expansion in preference to the patient's own defective cells, and even low-level transduction (infection) and expression of the corrective gene results in immune reconstitution of the affected T lymphocyte compartment, and cure.

No such selection currently exists for gene-corrected hematopoietic stem cells (HSC) containing and expressing the human β-globin gene in sickle cell disease or β-thalassemia. Thus, high levels of normal β-globin transfer and expression are required to cure these diseases.

GLOBIN GENE THERAPY

The current approach to human gene therapy for thalassemia is theoretically simple, using autotransplantation. HSC are taken from the patient, a normal hemoglobin (Hb) gene is added to the cells outside the body, and the human β-globin gene–corrected cells are returned to the patient intravenously. They automatically home and engraft in the marrow.

Gene therapy for β-thalassemia has been believed to be feasible since 1972, when β-globin complementary DNA, (cDNA), a copy of globin messenger RNA, was described.[4,5] Then, it was believed that the globin cDNA itself could be used as the source of the normal human β-globin gene sequences that could cure the disease. However, in the 1980s, it became clear that, in addition to the coding sequences present in globin cDNA, other important regulatory elements are required for successful and high-level human β-globin gene expression. These sequences include the intervening sequences within the gene, and regulatory sequences upstream and downstream of the human β-globin gene. In the late 1980s, Grosveld and colleagues[6] described important regulatory sequences far from the β-globin gene itself, called the β locus control region (β LCR) that are necessary to provide high level of expression of the human β-globin gene. The β LCR provides position-independent high-level enhancement of globin expression, and its discovery was seminal in moving β-globin gene therapy forward.[6]

Viruses as Vectors

Naked DNA can theoretically be used as the vector (or carrier) to transfer and express genes in human gene therapy, including those for human β-globin. However, viruses are much more efficient. Viruses are pieces of RNA or DNA wrapped in specialized viral proteins: after infecting cells, viruses use the host cell's molecular machinery to encode specific viral proteins, and express and assemble the proteins into viruses. Specific viral proteins on the surface of the viruses allow them to enter cells. After infection and integration, the viral DNA directs the synthesis of more of viral proteins; more viral particles assemble and are eventually extruded to infect more cells.

After infection, certain classes of viruses, adenoviruses, and, to some extent, adeno-associated viruses, remain in the cytoplasm of cells; they do not enter the nucleus and do not integrate into chromosomal DNA. These viruses are not useful