Gene therapy for metabolic disorders

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Most diseases caused by genetic deficiencies could, in theory, be treated by the introduction and expression of a normal gene into an appropriate target tissue. It seems likely that gene therapy strategies for most metabolic disorders will not require strict gene regulation, as a fraction of the normal levels of gene activity could result in amelioration or significant improvement in the clinical outcome. Gene therapy is making rapid progress towards the goal of treating various disorders: here, we summarize the state of gene therapy for metabolic disorders.

Numerous inherited human metabolic disorders are known (Table 1), most of which are recessive. Many have devastating effects that may include a combination of several clinical features, such as severe mental retardation, impairment of the peripheral nervous system, blindness, hearing deficiency, metabolic instability, organomegaly and gross disfiguration. Fortunately, most of these disorders are rare; however, the majority cannot be treated with drugs, and some are fatal. A few can be alleviated by adhering to a strict diet supplemented by specific nutrients. Although the enzyme deficiency that underlies these disorders may either affect many tissues or be restricted to a particular cell type, the deficient cell type is not always the one most affected: for example, deficiency of adenosine deaminase, a ubiquitous enzyme, results in the accumulation of toxic intermediates in purine metabolism and selectively kills T cells.

Approaches to gene therapy for metabolic disorders: some considerations

When designing approaches to gene therapy for metabolic disorders, several issues must be considered. Gene therapy for most metabolic disorders, unlike therapies for diseases such as the hemoglobinopathies, will probably not require strict gene regulation. For most enzymes deficiencies, clinical disease results only when enzyme activity is severely reduced: 5–25% of normal enzymatic activity will often protect from clinical disease. An example is hemophilia B, which results from a deficiency of the serine protease plasma clotting factor IX. Individuals who have <1% of normal factor IX clotting activity are at severe risk of spontaneous hemorrhage, those with 1–10% are at low to moderate risk, while those with 10–20% may have no apparent clinical disease. Exceptions to this general rule include some of the porphyrias, which are transmitted in an autosomal dominant manner and result in clinical disease with only a 50% reduction in enzymatic activity.

Treatment of certain metabolic disorders may be more complicated. One of the more common of the hepatic deficiencies is phenylketonuria (PKU). PKU most often results from a phenylalanine hydroxylase

TABLE 1. Examples of inherited metabolic disorders

Type of disorder	Example	Underlying deficiency
Glycogen storage	Glycogen storage deficiency type 1A	Glucose-6-phosphatase
Gluconeogenesis	Pepck deficiency	Phosphoenolpyruvate-carboxykinase
Galactose metabolism	Galactosemia	Galactose-1-phosphate uridyl transferase
Aminoacidopathies	Phenylketonuria	Phenylalanine hydroxylase
	Maple syrup urine disease	Branched chain α-ketoacid dehydrogenase
	Tyrosinemia type 1	Fumarylacetoacetate hydrolase
Organic acidemias	Methylmalonic acidemia	Methyl malonyl-CoA mutase
Fatty acid metabolism	MCAD	Medium chain acyl CoA dehydrogenase
Urea cycle	OTC	Ornithine transcarbamylase deficiency
	Citrullinemia	Argininosuccinic acid synthetase
Lipoprotein metabolism	Familial hypercholesterolemia	LDL receptor
Bilirubin metabolism	Crigler–Najjar	UDP-glucouronosyltransferase
Purine and pyrumidine metabolism	Severe combined immunodeficiency	Adenosine deaminase
	Gout, Lesch–Nyan syndrome	Hypoxanthine guanine phosphoribosyl transferase
Vitamin metabolism	Biotinidase deficiency	Biotinidase
Lysosomal storage	Gaucher disease	β-Glucocerebrosidase
	Sly syndrome	β-Glucuronidase
Peroxisomal disorders	Zellweger syndrome	Peroxisome membrane protein 70 kDa
Heme biosynthesis	Acute intermittent porphyria	Porphobilinogen deaminase

deficiency, causing an inability to convert phenylalanine to tyrosine. PKU can result in mental retardation, but this can be prevented by a protein-restricted diet. All infants are therefore screened for PKU at birth so that those affected can be identified and started on dietary management before the onset of mental retardation. Unlike factor IX deficiency, which could in theory be treated by delivery of the normal gene into ectopic tissues[1,2], it is unlikely that deficiencies such as phenylketonuria can be treated in this way, because of the requirement for hepatic-specific cofactors necessary for phenylalanine hydroxylase activity. Recently, a mouse model for PKU has been developed and will be used for gene therapy studies[3]. Similar strategies will be necessary for treating some disorders of glycogen storage and the urea cycle in which normal function depends on the presence of additional hepatic enzymes. Rare disorders of bilirubin metabolism, such as Crigler-Najjar type 1, result in severe liver damage and, unless treated by liver transplantation, in death. The availability of the Gunn rat[4], a model for the human disease, has been important in trials for therapies of Crigler-Najjar type 1. An animal model is now also available for tyrosinemia type 1, in which absence of the hepatic enzyme fumarylacetoacetate hydrolase (FAH) leads to an accumulation of toxic metabolites that destroys hepatocytes[5].

Thus the underlying defect involved, the need for cofactors and the affected and target tissues are all important considerations in developing protocols for gene therapy for a particular disorder. Below, we discuss approaches to gene therapy for various metabolic disorders that have been undertaken in animal models and humans.

Vectors for gene therapy

One of the biggest obstacles to gene transfer has been the efficient transfer of genes to target tissues. A number of vectors, both viral and non-viral, have been developed for transferring therapeutic genes into primary cells.

Viral vectors

Retroviral vectors. To date, the vector that has received most attention is a recombinant retrovirus derived from the mouse Moloney leukemia virus (MMLV), which is replication-defective[6,7] and allows cloning of promoter and cDNA sequences for expression. The cloned DNA is packaged into virions in cell lines that express the essential viral genes required, and virions recovered from the cell supernatant at concentrations of 10^5–10^7 p.f.u. ml^{-1}. Because these particles lack the genes necessary for replication and virion production, no additional virus can be produced by the cells they infect. When recombinant virus enters a cell, the RNA genome is reverse transcribed and the DNA product becomes integrated into the host chromosomal DNA. Efficient integration of the virus into the host cell requires cell replication, limiting the use of this vector to transferring genes into proliferating tissues. To date, MMLV-based vectors have been the most widely used in clinical gene therapy trials.

Adenoviral vectors. Adenoviral vectors have recently featured in gene therapy strategies[8,9]. The linear double-stranded DNA adenovirus has a natural tropism for respiratory epithelium, but can also infect most other cell types, and preparations of the wild-type virus have been given orally as vaccines[10]. The E1A region of the viral genome responsible for viral gene expression and replication can be deleted and replaced with therapeutic genes, and the replication-defective virus propagated in the human kidney cell line 293, which supplies the E1A products *in trans*. Virus is recovered after cell lysis, purified using cesium chloride gradients, can be concentrated to very high titers (10^{11}–10^{12} p.f.u. ml^{-1}), and is efficient at transferring genes into both non-dividing and dividing cells. However, the adenoviral genome does not integrate into host cell chromosomes and is slowly lost from infected cells. Because of their tropism for respiratory epithelium, these vectors are currently being tested for the treatment of cystic fibrosis in humans[11].

Other viral vectors. A number of other viral vectors are being developed for gene transfer. The adeno-associated virus[12] is non-pathogenic and infects the respiratory epithelium. In certain cell types, the virus DNA appears to integrate at a specific location on human chromosome 19; however, it is less clear whether the recombinant vectors can integrate efficiently and whether their integration is site-specific. Two additional problems with these vectors are that the entire viral genome is only 5 kb, so the amount of transgenic DNA that can be delivered is limited, and that it has so far been difficult to produce recombinant vectors at high titers. *Herpes simplex* virus type 1 (HSV1)[13] is a 152 kb neuronotropic DNA virus. Although HSV1 DNA does not integrate into the host genome, in the latent phase it exists as an episome in infected neurons. Expression of latent virally encoded genes can be reactivated in infected human neurons. Additionally, some viral genes are cytopathic in infected cells. Although HSV1-based vectors hold promise for the future, more basic molecular biological and genetic studies are needed.

Non-viral vectors. Non-viral DNA-based vectors have been developed by a number of laboratories. Complexes of protein and DNA can be used to transfer genes into specific cell types by receptor-mediated endocytosis; this principle has been demonstrated *in vivo* and *in vitro* by using desialolated orosomucoid, which is normally taken up by the asialoglycoprotein receptor on hepatocytes[14,15]. Transferrin has also been used as a ligand for DNA transfer; however, unlike the asialoglycoprotein receptor, the transferrin receptor is present on many cell types[16]. The DNA–protein complexes are internalized but gene expression is low and transient. The DNA is trapped in endosomes: Curiel *et al.*[16] demonstrated that treatment of transfected cells with an endosomal lysis agent such as an inactivated adenovirus increases gene expression from these vectors 2000-fold *in vitro*. Moreover, when the adenovirus is chemically linked to the DNA–protein complexes, gene expression is enhanced another tenfold[17,18]. These strategies have been combined to produce high levels of transgene expression in cultured hepatocytes, but have not yet been shown to work efficiently *in vivo*[19].

Ex vivo versus *in vivo* gene therapy

Recombinant vectors have been used to deliver genes to various cells and tissues (Table 2). Here, we

discuss examples drawn mainly from hematopoietically derived cells and hepatocytes. Two general strategies are used to deliver genes to specific tissues. The first, *ex vivo* transfer, involves removing cells from a patient, introducing appropriate genes, and transplanting the genetically reconstituted cells into the individual. The second, *in vivo* transfer, involves introducing the gene directly into the affected tissue, and requires that the vector be targeted specifically and at sufficiently high frequencies to the desired cell types. Both approaches are currently being investigated in animals and in clinical trials; both have been used to transfer genes to hepatocytes, whereas introduction of genes into cells derived from the bone marrow has only been achieved using *ex vivo* transfer (Table 2).

TABLE 2. Targets for gene therapy

Cell type	Feasible approach		Approaches being tested in clinical trials
	Ex vivo	*In vivo*	
Hematopoietic progenitor cells	+	−	*Ex vivo*
Peripheral lymphocytes	+	−	*Ex vivo*
Hepatocytes	+	+	*Ex vivo*
Fibroblasts	+	+	
Neuronal cells	+	+	
Keratinocytes	+	+	
Endothelial cells	+	+	
Skeletal muscle	+	+	
Pulmonary epithelium	−	+	*In vivo*
Chondrocytes	+	+	
Cancer cells	+	+	*Ex vivo* and *in vivo*

Introduction of genes into hematopoietic cells

Ex vivo gene transfer by recombinant retroviral vectors has been used to reconstitute most types of hematopoietic stem cells in mice[20,21]. However, much lower levels of gene transfer have been achieved in hematopoietic stem cells in dogs and non-human primates[20,21]. Gene transfer into stem cells is important for therapy of genetic disorders, as a single successful transfer could result in permanent reconstitution. Transduction of stem cells in non-rodent models has been inefficient; however, a therapy has been developed for one form of severe combined immunodeficiency that results from adenosine deaminase deficiency. Although individuals with only 2–5% of normal enzyme activity have normal immune function[22], those more severely affected develop life-threatening opportunistic bacterial infections at an early age. Reconstitution of enzymatic activity in peripheral T lymphocytes by retrovirus-mediated gene transfer improves immune function in treated individuals. Because mature T lymphocytes are constantly being replaced, repeat treatments are needed. These findings, while encouraging, are not completely conclusive, since it is not yet certain that they are the result of gene therapy alone. Protocols are being designed for transducing hematopoietic stem cells in such a way that even at a low frequency of transduction, early progenitor lymphoid cells derived from transfected stem cells may have a selective advantage and repopulate the blood. However, since for most disorders, an endogenously based selection secondary to the disease process will not be available, methods for introducing genes into the stem cells must be improved. The recent development of strategies for isolating and characterizing bone marrow stem cells in mice and humans should be instrumental in this[23].

Several metabolic disorders are caused by the absence of specific lysosomal enzymes that degrade specific compounds, whose accumulation can cause organ dysfunction; some affect primarily visceral organs, others the central nervous system. Depending on the underlying defect, lysosomal deficiencies that cause dysfunction of the central nervous system may require gene transfer into cells of the viscera, such as hematopoietic cells or hepatocytes, or into the central nervous system.

The most common lysosomal storage disorder is Gaucher disease, a deficiency in β-glucocerebrosidase that causes accumulation of glucosylceramide in reticuloendothelial cells. Individuals with Gaucher disease develop hepatosplenomegaly and bone problems, and a rare subset who have a neuronopathic form develop neurological sequelae. Enzyme replacement therapy has had some success in treating the visceral manifestations of this disorder[24].

Gene therapy stategies also seem promising. Retroviral vectors that produce human β-glucocerebrosidase mRNA have been used to transduce mouse hematopoietic cells *in vivo*[25,26]. Recently, a mouse model of the disease has been created by gene targeting[27]. Mice that are completely deficient in the enzyme die soon after birth, and may parallel a rare neonatal or perinatal lethal form of the human disease. Although this particular model may not be useful in preclinical studies for the most common form of the disorder[28], mice carrying less-severe mutations are being generated and may prove more appropriate. Clinical trials in which retroviral vectors are used to transfer β-glucocerbrosidase cDNA into bone marrow progenitors or stem cells are under way[29,30].

Sly syndrome, or mucopolysaccharidosis VII, is a lysosomal storage disorder in which β-glucuronidase deficiency results in the accumulation of sulphated glycosaminoglycans in the lysosomes of most cells, causing bone and joint abnormalities, hepatosplenomegaly and mental retardation. A mouse strain homozygous for the mutant allele *Gus^{mps}* has a similar disease, and has been used in preclinical gene therapy trials. Wolfe *et al.*[31] transferred the β-glucuronidase cDNA by retrovirally mediated gene transfer into enzyme-deficient bone marrow progenitors, which were transplanted into affected mice. The low level of enzyme activity that resulted in the liver and spleen reduced the lysosomal storage lesions in these animals. A similar finding was obtained when autologous fibroblasts were transfected using appropriate retroviral vectors and transplanted into these mice[32]. The enzyme is secreted by the genetically modified fibroblasts and transported into tissues via the mannose-6-phosphate receptor. It is not yet clear whether similar approaches can also alleviate the effects of these disorders on other organ systems, including the central nervous system.

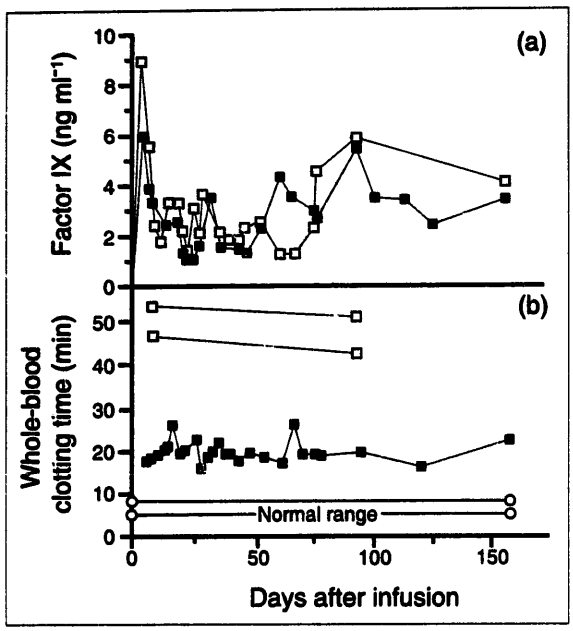

FIGURE 1. Graphs showing partial correction of the clotting defect in haemophilic dogs after hepatic transduction with a retroviral vector that encoded factor IX. (a) Plasma concentrations of factor IX were measured immunologically (results plotted as filled squares) and by bioassay (results plotted as open squares). (b) Whole-blood clotting times in a treated haemophiliac dog (filled squares), two haemophiliac littermates (open squares) and the normal range seen in unaffected dogs (open circles). Measurements taken at day 0 represent those before treatment measurements were then taken at varying times after the first treatment by retroviral infusion.

Introduction of genes into hepatocytes

Both *ex vivo* and *in vivo* methods have been developed for transferring genes into hepatocytes using vectors based on retroviruses[33–38], adenovirus[39–44] and *Herpes simplex* virus[45], and DNA-protein complexes[46–48]. To date, long-term constitutive expression of trangenes has only been demonstrated using recombinant retroviral vectors. Direct *in vivo* gene transfer into hepatocyes has been used to partially correct the coagulation defect in dogs deficient for factor IX (Ref. 38). Because retroviruses require cell division for gene transduction, a two-thirds partial hepatectomy was performed before gene transfer. Transduction of hepatocytes with a retroviral vector that expresses canine factor IX resulted in low but constitutive production of biologically active factor IX (Fig. 1a). At plasma concentrations as low as 0.1% of endogenous factor IX, there was a greater than 50% reduction in the whole-blood clotting time of the animals after gene therapy treatment (Fig. 1b). However, it is not known whether these levels are sufficient to protect the dogs from spontaneous hemorrhaging. *Ex vivo* gene transfer into hepatocytes has been used in dogs to transfer a recombinant retroviral vector carrying the gene for human α-1-antitrypsin[33]. A similar approach has been used to transfer the gene for LDL receptor, partially correcting the hypercholesterolemia seen in Watanabe rabbits[35]. More recently, this technique has been successfully used in humans with familial hypercholesterolemia[47]. *Ex vivo* gene transfer involves partial hepatectomy followed by cell culture, gene transfer and cell transplantation, making this an extremely labor intensive process. Unfortunately, both *in vivo* and *ex vivo* approaches result in transduction of only a small proportion of hepatocytes, and relatively low levels of transgene expression. A different approach, *in vivo* delivery of a DNA–protein complex, has been used in rats to transiently express the gene for human factor IX in the liver[48], and to produce albumin in albuminemic rats[49].

Early studies using recombinant adenoviral vectors showed that in mice deficient for the urea cycle enzyme ornithine transcarbamylase, some of the phenotypic features of the disorder could be corrected[40]. Since then, efficient *in vivo* transfer of genes to the liver by recombinant adenoviral vectors has been demonstrated, without the need for partial hepatectomy; however, only transient expression of such transfected genes has been achieved so far[39,43,44]. Recombinant adenoviral vectors have also been used to introduce the gene for factor IX into dogs with hemophilia B, transiently ameliorating their clotting abnormalities[44], and to transiently reverse the hypercholesterolemic effects of LDL receptor deficiency in mice[50].

Concluding remarks

Numerous monogenic metabolic disorders of humans are known. Although each individual disorder affects only a very small proportion of the population, many have devastating effects, resulting in death, multiple organ dysfunction or mental retardation. A number of therapies have been developed over the years in an attempt to treat patients with these disorders; most are designed to ameliorate the symptoms, rather than effect a cure. The possibility of therapy by gene transfer into somatic cells opens a new area of therapeutics and hope for individuals afflicted with these genetic disorders. Clearly, several technical hurdles must be overcome before successful and complete cures are possible for many of these diseases, and technologies must continually be improved upon if the many disorders are to be treated. Like all medical therapies, certain gene therapies will ameliorate some, but not all, symptoms of a particular disorder, and might improve the quality of life of affected individuals. Partial replacement of enzyme activity in a specific target tissue or a subset of affected tissues or cells may slow the accumulation of toxins in lysosomes, but not completely prevent it. Intervention may inhibit the progress of a chronic illness, but offer little in the way of reversing a pre-existing degeneration. For example, if a specific defect in the urea cycle has caused significant neurological impairment before gene therapy is begun, the achievement of metabolic homeostasis will not reverse the neurological sequelae. The same may be true for the treatment of muscular dystrophies: introduction of the appropriate normal gene may inhibit further muscle degeneration, but is less likely to reverse long-term muscle atrophy.

The ultimate goal of gene therapy for metabolic disease is to deliver a vector by non-invasive means such that the normal gene product will be produced in sufficient quantities in an appropriate cell type to alleviate all clinical manifestations of the disorder. It seems likely

that no single vector system will be appropriate for treating all such disorders, or will cure all metabolic disease. The next several years will see the development of improved gene delivery systems that will satisfy stringent criteria. We can then look forward to improved treatments for patients affected by these hereditary metabolic disorders.

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