Gene Therapy

Inborn Errors of Metabolism

Inborn errors of metabolism (IEM) are characterized by inherited defects in a primary metabolic pathway. Lack of a given enzyme causes a block in the normal metabolism of nutrients, producing toxicity either by accumulation of toxic precursors "upstream" of the block or loss of critical "downstream" metabolic products. Over 100 different IEM have been described, and although most are rare, as a class they affect approximately 1 in 5000 live births.

For many IEM, current treatment is limited to supportive care for acute episodes and dietary restriction to avoid the defective metabolic pathway. The long-term success of these strategies varies greatly between diseases, and for many IEM, no effective therapy is available.

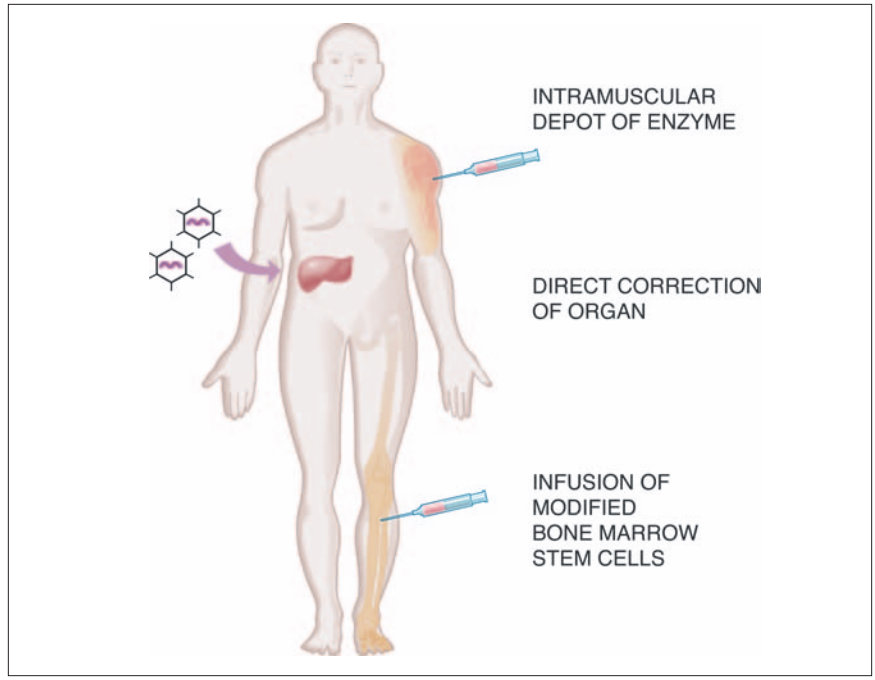
IEM have many features that make them attractive candidates for gene therapy. Most disorders are caused by a single gene defect that has been described on a molecular level, and replacement of this gene product will often correct the metabolic disorder. Second, subnormal amounts of enzyme can be sufficient to eliminate disease. Heterozygous carriers of a mutant allele (who have 50% normal activity) are usually unaffected, and for some IEM as little as 5% of normal activity is therapeutic in animal models. Finally, maternal clearance of toxic metabolites often delays symptoms in the fetus until after birth, and so prenatal detection followed by postnatal therapy may be a viable option for selected IEM.

The best treatment for each IEM will largely be dictated by the pathophysiology of the disease. IEMs can be broadly classified into diseases caused by localized toxicity, diseases caused by circulating toxins, or diseases with components of each. Each class of IEM requires a different gene transfer strategy.

In localized IEM, symptoms are largely due to direct toxic effects in tissues lacking the enzyme. For example, in the lysosomal storage disease mucopolysaccharidosis type VII (MPS VII), absence of the lysosomal enzyme β-glucuronidase results in an inability to break down certain long-chain glycosaminoglycans. This results in accumulation of undegraded storage material and progressive lysosomal distention in multiple tissues, leading to disruption of cellular functions. Effective treatment of diseases characterized by localized toxicity requires gene transfer methods to deliver the therapeutic enzyme directly to the affected tissues.

In toxin-mediated IEM, a metabolic block leads to accumulation of upstream metabolites or activation of alternative metabolic pathways. Potentially toxic molecules build up in the circulation and can cause widespread symptoms. This pattern is commonly seen in amino acid disorders, such as phenylketonuria (PKU).

In PKU, lack of hepatic phenylalanine hydroxylase (PAH) activity results in an inability to metabolize phenylalanine, leading to high circulating levels of phenylalanine and phenylketones. These metabolites are toxic to the developing



Gene therapy strategies for IEM. Although direct correction of single organs may be appropriate in some cases, not all diseases can be treated this way. Peripheral expression of enzyme in nonnative tissues could serve as a less-invasive option for diseases caused by circulating toxins. Finally, ex vivo infection of bone marrow would limit the patient's exposure to the viral vector.

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GENE THERAPY PROTOCOLS IN INBORN ERRORS OF METABOLISM

Disorder	No. of Protocols	Gene Defect	Vector	Method of Delivery
Canavan disease	2	Aspartoacylase	Plasmid DNA/lipofection	Intracranial
Canavan disease	1	Aspartoacylase	Adeno-associated virus	Intracranial
Familial hypercholesterolemia	1	LDL receptor	Plasmid DNA	Intraportal infusion to liver
Gaucher disease	3	Glucocerebrosidase	Retrovirus	Ex vivo infection of PBMC, followed by BMT
Hunter syndrome	1	Iduronate sulfatase	Retrovirus	Ex vivo infection of lymphocytes
OTC deficiency	1	Ornithine decarbamylate (OTC)	Adenovirus	Intraportal infusion to liver

By early 2003, only 9 of the 558 human gene transfer protocols reviewed by the NIH Recombinant DNA Advisory Committee had addressed gene therapy for IEM. However, recent vector advances have led to an increase in the number of protocols for other monogenic diseases (e.g., hemophilia, cystic fibrosis). The gene transfer methods developed in such protocols will likely be applicable to other diseases, including the IEM. (LDL, low density lipoprotein; PBMC, peripheral blood mononuclear cells; BMT, bone marrow transplant.)

brain and result in developmental delay, the primary symptom of PKU. For such toxin-mediated IEM, any intervention that lowers levels of circulating toxins can prevent disease, even if it does not directly reconstitute enzymatic activity in all tissues.

As a general rule, vectors used for gene therapy of IEM must reconstitute enzymatic activity in vivo, must provide lifelong expression of the relevant enzyme, and must be nontoxic and nonimmunogenic. However, these vectors will vary among IEM depending on the relative contributions of localized and systemic toxicity in a particular syndrome.

The most obvious use of gene therapy in IEM is to directly transfer a functional copy of a gene to tissue that normally expresses the enzyme. In such native tissue, the therapeutic enzyme will be in the appropriate metabolic environment, allowing it to interact with any cofactors or associated proteins required for function. Because many IEMs involve defects in hepatic enzymes, gene transfer to the liver has been widely studied. To date, the greatest preclinical successes in hepatic gene transfer for IEM have been achieved using replication-deficient adenoviral vectors.

In the PKU mouse model, a single intraportal injection of adenovirus coding for PAH resulted in the rapid reduction of phenylalanine levels with 48 hours. However, PAH expression was transient, and hyperphenylalaninemia returned within 2 weeks.

Transient expression is a common characteristic of adenoviral vectors and is largely due to an immune reaction against adenoviral proteins. This short-term expression, coupled with concerns about the toxicity of these vectors, has limited their application for treatment of IEM in humans.

Adeno-associated virus (AAV) vectors are based on a non-pathogenic human parvovirus, which can efficiently infect a number of tissues in vivo, including the liver. Long-term expression of transgenes for more than a year has been achieved in mouse models using AAV vectors, with minimal immune response.

In neonatal MPS VII mice, a single injection at birth of AAV coding for β-glucuronidase produced widespread gene expression in liver, brain, heart, lung, and kidney, which persisted for greater than 1 year. The levels of correction achieved in this mouse were sufficient to prevent development of many of the disease symptoms. AAV vectors are currently being used in human trials for the treatment of hemophilia B, and results from those trials will help determine their safety for human use.

Although liver-directed gene therapy is one approach for treating IEM, relatively invasive methods such as portal vein injections have been required to efficiently infect the liver in animal models. For the subset of IEM in which disease symptoms are primarily due to circulating toxins, less-invasive peripheral gene transfer techniques may be a viable alternative. In one example of a peripheral approach, skeletal muscle is infected with a gene transfer vector by intramuscular injection. This intramuscular "depot" of enzyme then clears toxic metabolites from the circulation, reducing symptoms.

A proof-of-principle experiment in the PKU mouse model demonstrates both the benefits and limitations of this strategy. A transgenic animal was produced that expressed PAH in skeletal and cardiac muscle. These animals were then bred with PKU mice to produce offspring that expressed PAH in muscle, but not in liver. These hybrid mice were initially hyperphenylalaninemic, because the intramuscular PAH lacked appropriate cofactors for functional activity. However, after treatment with the PAH cofactor tetrahydro-

biopterin, circulating phenylalanine levels decreased.

As seen in this example, a lack of appropriate cofactors for function is one potential disadvantage of gene expression in nonnative tissues. However, this approach may still be useful for enzymes that do not require cofactors or if oral supplementation of cofactors can be provided.

A related approach is the use of peripheral tissues, such as muscle, to produce and secrete a therapeutic product. This product can then act in the circulation or be taken up by distant organs to exert a beneficial effect. Such approaches have been used experimentally for diseases such as α_1-antitrypsin deficiency, MPS VII, and hemophilia B and provide another alternative to reconstitution of enzyme in a native tissue.

The described approaches are appropriate for disorders in which only a single organ is affected or in which removal of circulating toxins is the primary goal. For many IEM, however, direct reconstitution of enzyme in multiple tissues is required to prevent localized disease. Direct gene transfer to multiple organs at sufficient levels to correct disease is not currently possible in humans.

An alternate approach for this class of IEM may be bone marrow transplantation. In patients with IEM, bone marrow transplantation can lead to the dissemination of enzyme-producing hematopoietic progeny throughout the body, which can locally secrete enzyme to correct disease. However, the widespread application of this technique is limited by a lack of matched donors for many patients and by the morbidity associated with allogeneic transplantation.

Gene therapy could be used to allow autologous bone marrow transplantation following genetic correction of the patient's own bone marrow. In this approach, stem cells mobilized from the patient are infected ex vivo with a vector carrying the therapeutic gene and then expanded. This corrected bone marrow is then reinfused, providing the patient with an autologous bone marrow transplant of normal marrow.

Successful application of this technique has been demonstrated in the MPS VII mouse, where bone marrow transplantation of retrovirally transduced bone marrow achieved lifelong correction of disease in multiple tissues. The primary limitation of this technique has been the difficulty in isolating and efficiently transducing early hematopoietic stem cells in humans.

Gene therapy approaches have the potential to greatly expand the treatment options for patients with IEM. Because of the large number and variety of IEM, treatment will require knowledge of the underlying pathophysiology of each disease to determine what gene therapy approaches are likely to succeed. Because most IEM are rare in humans, extensive preclinical testing in animal models will be required to determine the safety and efficacy of various therapeutic approaches.

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