New Targets for Human Gene Therapy

New work suggests that the many genetic diseases that originate in the liver may eventually be candidates for gene therapy

IN HUMAN GENE THERAPY, translating vision into reality has proved trickier than most people imagined. The major genetic diseases, like cystic fibrosis, sickle cell anemia, and thalassemia, remain unassailable—the genetics are too complex, or too poorly understood, to even contemplate therapeutic strategies at this time. Instead, investigations have focused on a handful of diseases that are simple enough to tackle now. By and large these are very rare disorders, like severe immunodeficiency, that affect only a few people worldwide.

Just getting cells in and out the body so that a new gene can be inserted has proved formidable. For that reason, the first experimental forays have been made on bone marrow cells, which can be removed from the body and reinserted with relative ease. The problem is that many diseases can't be approached by treating bone marrow cells.

Now, for the first time, a research team at the Whitehead Institute has inserted a foreign gene into liver cells and corrected a defect that causes a life-threatening human disease. Although this work is preliminary—it was done on liver cells taken from rabbits, not humans—it is the first step toward gene therapy for this disease, familial hypercholesterolemia.

Perhaps more important, this and similar work by several other labs paves the way for the later application of gene therapy to diseases of the liver, of which there are many.

Until a couple of years ago few people even tried to work with liver cells, or hepatocytes, in this way. They were notoriously hard to culture, and gene transfer itself looked problematic, as liver cells seemed to resist the standard gene transfer technique using retroviruses. In addition, no one had a clue as to how to reintroduce them to the body and have them survive.

In 1986 Jayanta Roy Chowdhury, Achilles A. Demetrious, and their colleagues at Albert Einstein College of Medicine developed an ingenious technique for injecting liver cells, on the surface of microscopic beads, into living animals. Then last year, within a couple of months of each other, two groups—one led by Theodore Friedmann of the University of California at San Diego, the other by Savio Woo of the Baylor College of Medicine—demonstrated that retroviruses could, in fact, be used to carry foreign genes into liver cells.

It is these techniques that are now being brought to bear on familial hypercholesterolemia, better known as FH. Victims of this disease are missing a receptor on the surface of their liver cells that is necessary for cholesterol metabolism. This leads to severely elevated cholesterol levels and premature heart disease. The underlying defect is in the gene that would normally make the receptor for low-density lipoprotein, or LDL cholesterol.

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Those with the severe form of the disease, who have two copies of the defective gene, often die of a heart attack in their teens or twenties, and sometimes as young as age one. Those with the mild form of the disease, who carry only one copy of the defective gene, often have a first heart attack in their twenties, thirties, or forties. Half have heart disease by age 50. Although the severe form of the disease is exceedingly rare, roughly 1 in 500 people in the United States has the mild form of the disease.

The disease also occurs in a particular type of rabbit, the Watanabe rabbit, which has proved immensely handy for researchers studying cholesterol metabolism and, now, gene therapy.

What Richard C. Mulligan, James M. Wilson, and their colleagues at the Whitehead Institute did was to use a retrovirus to insert a normal copy of the receptor gene into rabbit liver cells in culture. They actually tried four different virus vectors, all of which worked, to see which was the most efficient at infecting cells.

Once inside the cell, the newly inserted gene began pumping out the LDL receptor protein, in varying amounts, depending on which vector was used. "It makes the receptor in pretty decent amounts," says Mulligan. And, he adds, the receptor is working—it is taking up LDL cholesterol.

"In vitro, the defect is 'cured'," says Mulligan. Now the next and more difficult step is to get those altered cells back into the rabbit to see if they will work there as well, which is by no means a sure bet. As work with bone marrow cells has shown, introduced genes often work beautifully in culture but stop working once introduced into living animals.

Mulligan's group is now collaborating with Chowdhury on reintroducing these engineered cells into living rabbits. That step is the stumbling block, agrees Friedmann at the University of California. In work to be published soon in the *Proceedings of the National Academy of Sciences*, his group has also corrected the FH genetic defect in vitro, this time working with skin cells instead of liver cells.

Meanwhile at the National Heart, Lung, and Blood Institute, Kathryn Anderson, W. French Anderson, and their colleagues report that they have mastered the next step, at least in rats: they have reintroduced an engineered liver cell into living animals.

The heart institute researchers are using a slightly different approach than Chowdhury's. Instead of using microscopic beads, they attach liver cells to a polymer construct that looks like a cotton ball, which is then implanted into the peritoneal cavity. When those cells are removed a week or two later, the foreign gene, in this case a marker gene, is still functioning, they say in an article just submitted for publication.

These experiments, while promising, are just the first steps in what is certain to be, for both scientific and regulatory reasons, a long trek toward gene therapy for FH or other liver diseases.

If this approach does prove feasible, the early application will probably be for the small number of people with the severe form of FH, for which no alternative therapy exists except liver transplantation. Later, investigators speculate, it might be used to boost the supply of LDL receptors and thus help ward off heart attacks in the larger group of people who have the mild form of the disease. And in the distant future, Anderson envisions using these techniques as a preventive measure in healthy people who are somewhat deficient in LDL receptors.

At this stage it is not clear what other liver diseases might be candidates for gene therapy. "FH we know how to fix. Other diseases might not be so simple," says Mulligan. "There may not be a whole set of diseases we can easily fix." ■ LESLIE ROBERTS