GENE THERAPY

Step Taken Toward Improved Vectors for Gene Transfer

Only a few years ago, the idea of introducing genes into the body to treat disease seemed futuristic, at best. But in the past 5 years, gene therapy has gone from pipe dream to experimental treatment. Early versions of the method are now in clinical trials for conditions ranging from cystic fibrosis to cancer. But exciting as they are, the first-generation therapies are far from ideal.

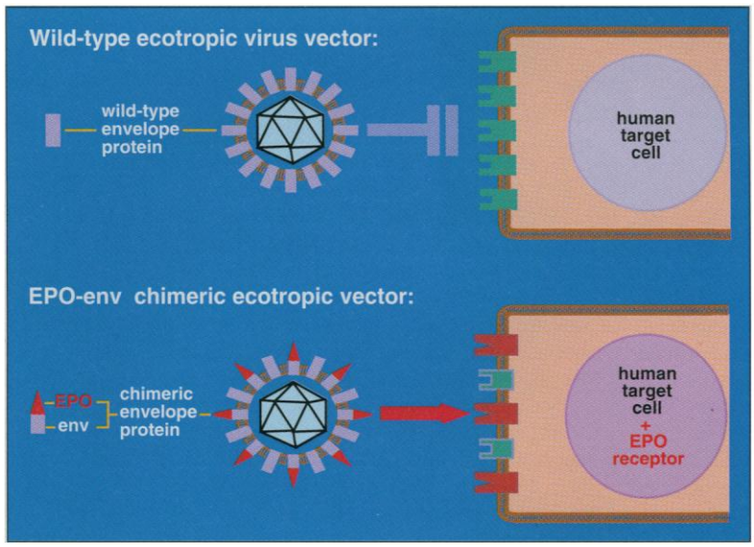
One aspect of gene therapy crying out for improvement is its delivery system: the viral "vectors" that carry foreign genes into the body. Most of them infect all kinds of cells, but in many cases researchers would much rather use a virus that would infect only one cell type. "Those [early approaches] are all just interim steps," says gene therapy researcher W. French Anderson of the University of Southern California (USC). "The goal is to be able to target."

On page 1373 of this issue, Yuet Wai Kan and his colleagues Noriyuki Kasahara and Andrée Dozy of the Howard Hughes Medical Institute at the University of California, San Francisco (UCSF), report that they've taken a major step toward such targeting. Although their work is preliminary, many in the field expect it to lead to clinically useful vectors.

The team started with a retrovirus, a virus that converts its RNA genome to DNA and inserts it permanently into the chromosomes of the cells it infects. They then altered the retrovirus so that it specifically infects cultured human cells that carry the receptor for the blood protein erythropoietin (EPO), which in the body appears on the cells that give rise to red blood cells. "It's a great paper," says retrovirologist and gene therapy researcher Dusty Miller of the Fred Hutchinson Cancer Research Center in Seattle. "People have been trying to redirect retroviruses to specific receptors for some time now. ... This is the first attempt I know of that really worked well."

Retroviruses infect cells by attaching themselves to the cell surfaces by one of their own surface proteins, known as the envelope protein. Many gene therapy experiments have used a type of mouse leukemia virus known as an amphotropic virus, which can also infect human cells because its envelope protein docks with a phosphate transport protein that is very similar in the two species.

But because the protein is ubiquitous, the amphotropic retrovirus can attach itself to a wide range of human cells. That promiscuity is not a problem in most of the first-generation gene therapy approaches, which involve injecting virus into a local site, such as a tumor, or even removing cells from a patient, infecting them with the virus vector, and then replacing them. But it would be a concern if the virus were injected into the bloodstream and had access to a wider variety of cells that are not the intended targets. Before gene delivery by intravenous injection can become a reality, researchers must develop more specific viral vectors.



Unlocking the door. The normal envelope protein cannot dock the ecotropic virus to human cells, but the modified protein will bind to cells carrying the EPO receptor.

Working toward that end, the UCSF team used a different strain of mouse leukemia virus which, unlike its amphotropic cousin, infects only mouse cells. Its envelope protein binds to an amino acid transporting protein on mouse cells, but not the comparable protein on human cells. The team engineered the virus so that its envelope protein would instead recognize the EPO receptor. "We replaced about 150 amino acids of the envelope protein with approximately the same number of amino acids from EPO," says Kan. Their experiment worked: The virus infected cultured human cells that have the EPO receptor on their surface, but retained its aversion to other types of human cells.

The UCSF group's finding "suggests that hybrid envelopes are really a feasible way to achieve specificity," says Doug Jolly, vice president for research at Viagene, a gene therapy company in San Diego. "No one was sure of that until now," he says, because when other groups have tried to use that approach, "the efficiency [of gene introduction] has always been very low."

Why did the Kan group succeed where others failed? USC's Anderson, who is among those trying to make targeted retroviruses, credits the UCSF workers' success to a novel twist they used. The problem with other modified viruses may have been that they carried only an abnormal hybrid envelope protein, Anderson says, and that may have interfered with its ability to gain entry into the cell.

The Kan group, in contrast, produced a virus that carries both the normal and hybrid proteins, possibly facilitating its entry into the human cells. Kan says he is not sure that explanation is correct, but whatever the reason, Anderson says the Kan group's success sets a new starting point for efforts to make even more efficient vectors.

Kan points out that the clinical utility of the new vector is limited by the fact that the red blood cell precursors that carry the EPO receptor turn over rapidly in the body, and so any gene therapy targeted at those cells would not be long-lived. But his group is already using the same approach to make more clinically relevant vectors, targeted at proteins expressed on cancer cells and hematopoietic stem cells in the bone marrow. The stem cells, which replenish all the cell types of the blood and immune system, are a major target for gene therapists because therapy directed at them would be a long-lasting way of getting corrective genes into the blood and immune tissues.

Even if these targeting schemes succeed, gene therapists have to solve other problems before they realize their dream of genes delivered by intravenous injection. There is the need to prove that genes can be delivered to high enough numbers of target cells to be effective when administered systemically to a human being. And researchers also must show that targeted vectors really are specific and won't infect other cells, where they might cause harm. "The technology at present is a long way from a clinical application," says Anderson. "Nonetheless, this is a very important step forward."

–Marcia Barinaga