New gene-therapy techniques show potential

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The promise of gene therapy—the replacement of dysfunctional genes with useful ones— has gone largely unfulfilled because the microbial delivery agents used to insert the desirable genes into needy cells haven't been up to the job.

Early in the research, scientists seized on viruses as ideal vectors to deliver genes to patients since these microbes insert their genome into a host cell. However, the agents have proved less than perfect. Viruses can be expensive to prepare and store. Moreover, one of the most promising viruses isn't big enough to tote the large genes required to overcome some troubling diseases.

More serious obstacles have also arisen. Even viruses that scientists have partially disabled sometimes replicate, and the microbes can attract unwanted attention from a patient's immune system.

A research team at Stanford University School of Medicine now reports success at circumventing the viral approach altogether, while other groups are testing ways to expand a virus's cargo capacity.

The Stanford work employs a transposon, or naturally mobile piece of DNA, as the gene-delivery truck. Geneticist Mark A. Kay and his colleagues reasoned that a selected gene delivery truck might be packaged into such DNA, which then could easily insert itself into a patient's chromosome.

They performed experiments on more than 50 mice, some with hemophilia, a disease in which the blood doesn't clot properly. The researchers sought to replace a defective version of the gene for a coagulation protein called factor IX. Using transposon DNA as a carrier for the functional gene, they implanted the whole package into liver cells in the mice.

The transposon, which in this study consists of DNA engineered from a fish gene, encodes an enzyme called transposase. Once produced, this enzyme attached the coagulation-factor gene to the host chromosome. The transfer was successful in 5 to 6 percent of liver cells sampled, Kay and his colleagues report in the May *Nature Genetics*.

Mice treated with the transposon gene therapy showed vastly improved blood coagulation. It didn't seem to matter precisely where on the chromosome the gene attached, Kay says.

The implanted genes have so far functioned correctly, directing the production of factor IX for at least 5 months—a long time in the typical 2- year life span of a mouse. Kay suggests that the gene might work indefinitely, which would make such treatment essentially a cure.

"Our experience is that anything that integrates into the liver of a mouse lasts as long as the mouse lives," he says. Now 8 months after the gene therapy, Kay has still detected no immune backlash in the mice.

Hemophilia provides a good test for gene therapy. The absence of a single factor can sabotage the body's ability to stanch bleeding. Correcting this genetic abnormality yields clear results, Kay says.

Because of the problems of using viruses, any advance in nonviral gene therapy is welcome, says virologist David T. Curiel of the University of Alabama at Birmingham. Using a transposon to carry a gene is a "very significant accomplishment," he says.

Molecular biologist Xiao Xiao of the University of Pittsburgh agrees that the experiments are "a nice piece of work" but adds that the high volume of fluid that the researchers pumped into the mouse veins may require that the method be modified for use in people.

Meanwhile, three other studies address a problem nagging current gene therapy: the inability of an otherwise ideal virus to carry large genes into a cell. All three studies use recombinant adeno-associated virus (rAAV), a genetically engineered virus incapable of replicating but able to deliver a selected gene. This virus is being used in some ongoing trials in people.

Two of the studies split a gene from its promoter region, the nearby DNA that switches on the gene. Two rAAV vectors then deliver the separate cargoes into mouse cells, where the gene and its promoter reunite. Kay and his Stanford colleagues in experiments described in the May *Nature Biotechnology* were able to deliver the gene for the enzyme betagalactosidase. In the May *Nature Medicine*, John F. Engelhardt and his team at the University of Iowa in Iowa City reported successful transfer of the erythropoietin gene.

Taking another tack, Xiao and his colleagues split a large gene in two and used rAAV to deliver the parts, one of which included the promoter. In mouse muscle, the two pieces produce a complete protein. The transplanted gene encodes factor VIII, another coagulation protein.

"These studies really expand the utility of rAAV," says Brian K. Kaspar, a neurobiologist at the Salk Institute for Biological Studies in La Jolla, Calif. Cystic fibrosis and a common form of muscular dystrophy—both of which stem from defects in large genes—may also make good targets for these new technologies, he says.

In gene therapy until now, "everybody was forced to work within certain gene size limitations," says Richard Jude Samulski, a molecular virologist at the University of North Carolina in Chapel Hill. "I think now they can approach [techniques using rAAV] without that reservation."

However, these virus-loading methods may introduce new problems. For example, splitting a promoter region from its gene and then trying to reunite the two pieces might leave the promoter free to switch on another gene, with unforeseen consequences, Samulski says.

All these methods will require animal testing "until they come up squeaky clean," he concludes.

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