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Researchers develop improved gene therapy agent

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Replacing one amino acid on the surface of a virus that shepherds corrective genes into cells could be the breakthrough scientists have needed to make gene therapy a more viable option for treating genetic diseases such as hemophilia, University of Florida researchers say.

Reporting in the journal *Proceedings of the National Academy of Sciences* today (May 19), UF geneticists say they have developed a new version of the adeno-associated virus used in gene therapy that works about 30 times more efficiently in mice than vectors scientists currently rely on.

The discovery could be the solution to a problem that has plagued researchers and doctors using AAV as a gene therapy vector — how to administer enough of the gene-toting virus to yield a therapeutic benefit without triggering an attack from the body's immune system, says Arun Srivastava, Ph.D., the George H. Kitzman professor of genetics and the chief of cellular and molecular therapy in the UF College of Medicine department of pediatrics.

AAV is considered ideal for gene therapy because it possesses the viral ability to infect cells yet does not lead to disease. But scientists discovered they had to administer trillions of AAV particles for the corrective gene to take root in a cell's nucleus and begin working.

"Based on our studies and those of others, it's become clear that the reason you need so much is because about half the AAV particles get

stuck in the cytoplasm," said Srivastava, the senior author of the study and a member of the UF Genetics Institute. "It doesn't get to the nucleus very efficiently. The reason for that is obvious. AAV is seen by the body as an invading protein and it tries to block it."

The body mistakenly tags many AAV particles as junk proteins and sends them into cellular trash cans called proteasomes, where they are destroyed, Srivastava said. And a particular amino acid, tyrosine, is to blame.

Tyrosine has as part of its makeup a group of molecules called a hydroxyl group, which attracts phosphates in the cell. When a phosphate binds to the hydroxyl group, it sends a signal to the proteasome — the cellular equivalent of taking the trash out to the curb.

So Srivastava and his UF College of Medicine colleagues decided to test what would happen if they took the phosphate out of the equation.

To do that, the researchers replaced tyrosine with another amino acid, phenylalanine. The two amino acids are identical except for one thing — phenylalanine lacks the part that attracts phosphate.

"We didn't change anything except the amino acid that does not allow phosphorylation to occur," he said. "It was very simple. You can buy a kit from a company and can mutate any amino acid you want."

Tyrosine is found at seven spots on the surface of AAV, Srivastava said. The scientists created seven new vectors, replacing a different tyrosine in each one and inserting in them the gene that triggers production of the blood-clotting protein Factor IX. Patients with hemophilia B, a common form of the disease, do not naturally produce this protein.

In tissue samples and in mice, all the new vectors worked better than a

commonly used version of AAV. One of the versions in particular worked 11 times better in tissue samples after 48 hours. In mice, the results were staggering. Two weeks after the mice were injected with the corrective gene, one of the new AAV-gene combos was working 29 times better than the standard vector was at incorporating the new gene into cells, at a 10-fold lower dose.

“We were very surprised,” Srivastava said. “It’s amazing to think that changing one amino acid could produce these results.

“Now the virus actually completely avoids being phosphorylated, so it doesn’t become degraded and it goes into the nucleus, and we get therapeutic levels of proteins. We can generate therapeutic levels of Factor IX.”

The researchers are creating additional new vectors based on this concept, with the goal of creating what Srivastava calls “a perfect vector” that lacks all seven phosphate-attracting tyrosines. They are also teaming with University of North Carolina researchers to test the vectors in dogs with hemophilia. If these studies are successful, the vector could be used in human gene therapy trials.

In addition to being more efficient, the new version of AAV could also prove to be more economical, Srivastava said. Current gene therapy trials are expensive because scientists must administer so much of the vector containing the therapeutic gene to see results. Using the new vector, scientists could potentially scale back to using as little as 100 billion particles instead of 10 trillion, Srivastava said.

“I think this is a very promising step forward,” said John Engelhardt, Ph.D., the director of the University of Iowa Center for Gene Therapy, who was not involved with the study but also plans to use the UF-developed vector in upcoming research. “From a basic biological

standpoint, this clarifies our understanding of how the virus acts in the cell. The more we understand, the better we are going to be at engineering viruses for use in humans."

Source: University of Florida