Forcing viruses to evolve to help, not harm

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Viruses and humans have evolved together over millions of years in a game of one-upmanship that, often as not, left humans sick or worse. Now, a University of California, Berkeley, researcher has shown that viruses - in this case, a benign one - can be forced to evolve in ways to benefit humans.

The adeno-associated virus, or AAV, is a common, though innocuous, resident of the body that has received a lot of attention in recent years as a possible carrier for genes in gene therapy. Because as many as 90 percent of people already have the virus, however, their immune systems are primed with antibodies to quickly tackle and neutralize it, thwarting any attempt at gene therapy.

UC Berkeley's David Schaffer, associate professor of chemical engineering and a member of the Helen Wills Neuroscience Institute, with colleagues Narendra Maheshri, James T. Koerber and Brian Kaspar, decided to speed up the process of viral evolution and direct the change in a way that would allow the virus to slip past the body's immune defenses, making it a more viable vehicle for gene therapy. In essentially two generations of accelerated evolution, requiring about two months of lab work, they succeeded.

Schaffer and his team at UC Berkeley and at Ohio State University report their success in the current issue of Nature Biotechnology.

"Directed evolution has mainly been done to change the activity of an enzyme - to make it more effective toward a new substrate or better able

to catalyze a reaction, for example - or to make antibodies better binders against specific targets," said Schaffer, who also is an affiliate of the UC Berkeley wing of the California Institute for Quantitative Biomedical Research (QB3). "In the viral realm, this approach is essentially untapped."

This technique could be used to improve many other characteristics of AAV to make it a better delivery vector for genes.

"We think there are a huge variety of new problems we could address as well, such as targeting the virus to cells it is ordinarily not good at getting into, or speeding its transport through the body," he said.

Though Schaffer acknowledges that the technique could be used to help pathogenic viruses evade the human immune system, potentially making them more virulent, he said that other and easier techniques already allow this frightening possibility.

AAV consists of two genes enclosed within a ball, or capsid, of proteins. The capsid proteins are what antibodies recognize, and as a result were the target of directed evolution. To provide the raw material for evolution - the genetic variation from which nature selects the best-adapted organism - the researchers created mutant viruses by introducing small variations in the genes through an error-prone polymerase chain reaction (PCR) coupled with a test tube recombination technique. After reassembling the mutant viruses inside their capsids, they introduced them to blood serum pooled from rabbits immunized against AAV, and thus containing many types of antibodies to AAV. Only the mutant viruses good at evading antibodies to AAV survived the serum.

After passing the viruses three times through increasingly more potent serum, they isolated the survivors and subjected them to another round of PCR that introduced more mutations. After passing this second

generation through serum three times, they isolated viruses that could survive AAV antibodies much better than the original strain of AAV. One strain of virus was 96 times more effective than the wild AAV, and two evolved strains survived injection into mice with nearly 1,000 times the level of antibodies required to neutralize the wild virus.

By sequencing the survivor strains, the researchers discovered that the capsid proteins of the survivors differed from those of the original strain by only seven amino acid building blocks, two of which were responsible for most of the altered interaction with antibodies.

"Starting from scratch, just trying to rationally decide which two amino acid changes to make on the virus, there is no way you would have guessed those two," Schaffer said. "Using the same algorithm as nature came up with - evolution - to solve the problem, is the best way to do it."

Since each generation takes about a month, Schaffer predicted that many types of new and improved strains could be created in a few months' time, and certainly in less than a year. He is pursuing experiments now using pooled human blood serum.

"This virus is kind of a gift from nature, a very safe and efficient virus, but nature never evolved it to be a human therapeutic. So, in a sense, we have to re-evolve it for that purpose," he said.

Source: University of California - Berkeley