Sleeping Beauty hooks up with herpes to fight brain disease

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Neuroscientists have forged an unlikely molecular union as part of their fight against diseases of the brain and nervous system.

The team has brought together the herpes virus and a molecule known as Sleeping Beauty to improve a technology known as gene therapy, which aims to manipulate genes to correct for molecular flaws that cause disease.

The work, detailed in a paper published online in *Gene Therapy*, has allowed scientists at the University of Rochester Medical Center to reach a long-sought goal: Shuttling into brain cells a relatively large gene that can remain on for an extended period of time.

"We've broken what is in effect a size barrier - a limit to how much genetic material we can put into the nucleus of a cell and keep functioning for a long period of time," said neuroscientist William Bowers, Ph.D., a scientist in the Center for Neural Development and Disease and the leader of the team. "That opens up more diseases to possible treatment with gene therapy."

The first author of the paper is Biochemistry graduate student Suresh de Silva, who defends his doctoral thesis later this month.

The molecular rendezvous of Sleeping Beauty and herpes in human brain cells could spell good news in the search for treatments for horrific brain diseases known as pediatric leukodystrophies, or a group of diseases

known as lysosomal storage disorders. In many of these diseases, even though just a single gene or protein is defective, the effects are devastating - the diseases slowly rob children of their brain cells and are often fatal after years of severe symptoms.

The findings bolster the tools that researchers have when approaching certain diseases, said Bowers, including Usher syndrome, which results in deafness and vision loss; Niemann-Pick disease Type C, a fatal childhood lysosomal storage disorder; and von Willebrand disease, an inherited disease that causes extensive, chronic bleeding.

"The field of gene therapy is just beginning to yield some successes for patients. Improvements like this are crucial for increasing the number of patients who might benefit from such an approach," said Bowers, who is an associate professor of Neurology, Microbiology and Immunology, and of Pharmacology and Physiology.

The research is part of a decades-long endeavor by scientists trying to get the right genes into the right cells at the right time to improve human health.

In the new work, scientists dramatically increased the size of the "genetic payload" they can deliver to brain cells compared to some conventional techniques, nearly tripling the amount of genetic material by some measures. They hope to deliver even bigger genes in the future.

The team did this by bringing together in a new way two molecular players, herpes and Sleeping Beauty, which are commonly used in molecular technology.

For years Bowers' team has been using the herpes virus - HSV-1, the type that causes cold sores - to shuttle genes into cells. Viruses like herpes are adept at infecting human cells, and scientists like Bowers use

such viruses to carry into cells genes that would help people who are sick. Bowers and colleagues modify the viruses extensively, removing the portions that could make a person sick and using the portions that the virus uses to gain access to human cells.

Many scientists use other viruses, such as lentiviruses or a cold-related virus known as adeno-associated virus (AAV), to do a similar job. Each virus has its strengths and weaknesses when it comes to gene therapy. Herpes, for instance, readily infects cells, and it can carry a huge amount of genetic material, typically 15 to 30 times the amount of DNA that other viruses can carry into a cell.

But herpes as a genetic tool has a couple of big weaknesses. While the virus can deliver DNA into the nucleus of a cell, the genetic payload it carries does not become part of the package of genes that cells pass from one to another. Simply put, herpes cannot integrate the new DNA into the host genome. Instead, the DNA is adrift in the nucleus, where it's silenced within a few weeks. The short time span spells trouble when scientists are trying to treat a disease that requires the genes to be active for months or years.

That's where Sleeping Beauty comes in.

In molecular biology, Sleeping Beauty is a mobile genetic element that jumps into and out of longer segments of DNA. It's normally silent, but years ago a team of scientists was able to activate or "awaken" the snippet - hence, Sleeping Beauty. Since Sleeping Beauty actually integrates segments of DNA into mammalian genomes, it sidesteps the main difficulties that herpes encounters inside a cell: Genes integrated within the cell's chromosomes by Sleeping Beauty operate for much longer periods of time. The drawback: The molecule can insert only small snippets of DNA.

So the Rochester team brought herpes and Sleeping Beauty together in an attempt to get the best of both worlds: Delivery of the bigger genetic package made possible by herpes, and the integration of the DNA into the host genome made possible by Sleeping Beauty.

And that's exactly what happened. In the tag-team approach funded by the National Institute of Neurological Disorders and Stroke, herpes gets the genetic package into the right neighborhood, the cell's nucleus, and then Sleeping Beauty delivers the package precisely where it needs to go to be most effective - into the cellular genome.

In the current experiments, the herpes virus carried into cell nuclei the gene for green fluorescent protein, which allows scientists to track where the gene is active. The team also outfitted the herpes package with special molecular signals that Sleeping Beauty would recognize. Separately, the team introduced Sleeping Beauty into the cells. When the two met, Sleeping Beauty transferred the gene for GFP from the herpes package to the genome of the human cells, where the gene was stably expressed.

The team has previously shown that the Sleeping Beauty/herpes combination works efficiently in brain cells known as neural progenitor cells, which go on to form brain cells known as neurons. Modifying these cells - perhaps by adding a gene that creates a protein crucial for health - is one technique scientists are experimenting with to try to treat several brain diseases that are currently untreatable.

The gene segment used in the experiment described in Gene Therapy was about 12 kilobases long, which is larger than the limit of either AAV (4.5 kb) or lentiviruses (9 kb). Those few kilobases matter, a lot. The ability to transfer bigger genes gives scientists room to try to address more diseases with a gene therapy approach. The added space also makes it possible to include more regulatory elements - instructions that

help determine how and when genes are turned on or off. This allows scientists to package additional safety directives, in the form of more DNA, along with the gene designed to treat the disease.

Provided by University of Rochester Medical Center