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Researchers apply new technique to manipulate virus, make it a possible cancer treatment

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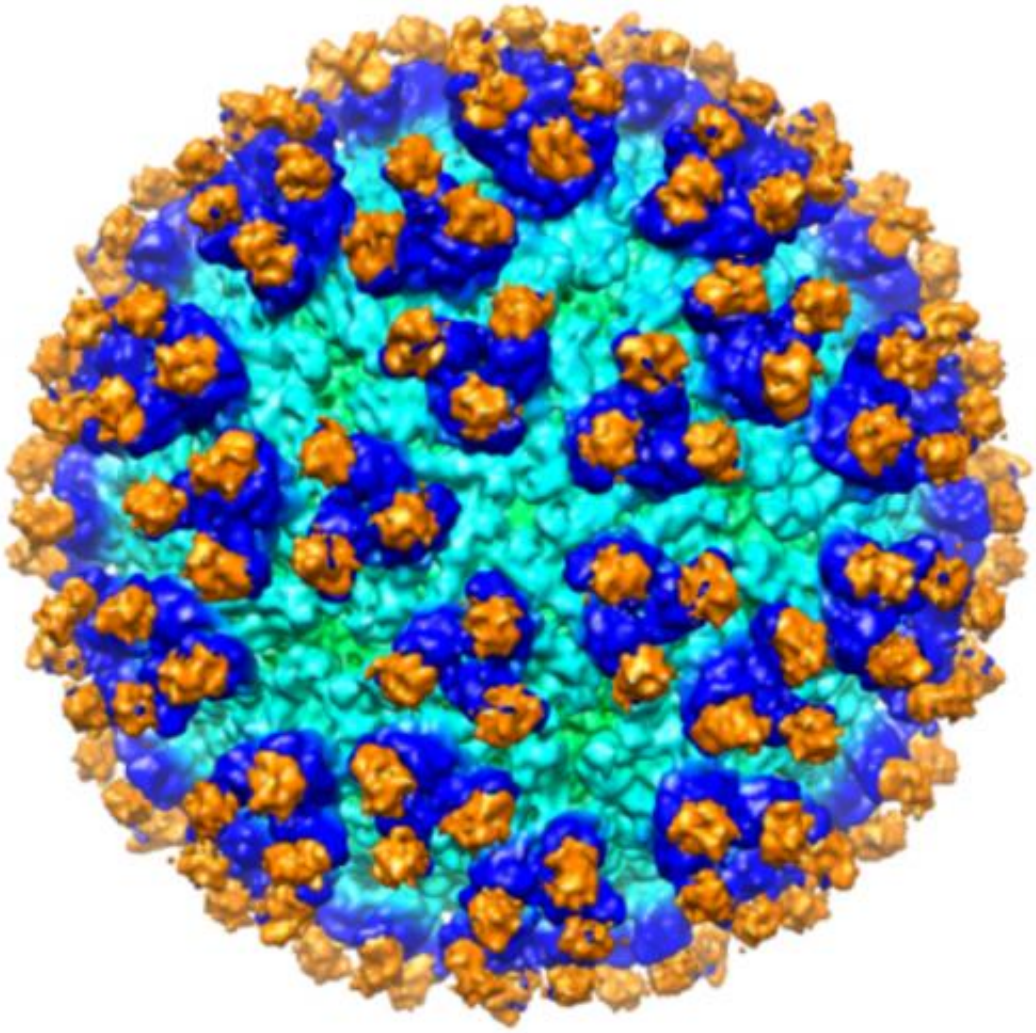

A cryoelectron microscopy reconstruction of ESV1 is shown. A team of Purdue researchers successfully engineered the virus to target and kill cancer cells and

eliminated its native infection preferences. Credit: Kuhn research group

(Medical Xpress)—Purdue University researchers successfully eliminated the native infection preferences of a Sindbis virus engineered to target and kill cancer cells, a milestone in the manipulation of this promising viral vector.

"This virus had been known to be a good vector for delivering therapeutic cargo, however it naturally infected all kinds of cells, and these diversions would compete with what we were instructing it to target," said Richard Kuhn, the Gerald and Edna Mann Director of Purdue's Bindley Biosciences Center. "We have now overcome a major challenge by not only inserting a targeting molecule of our choice, but also successfully stripping the virus of its native entry preferences. This was a big step in unlocking the potential of developing this virus into a platform for both targeted drug delivery, where it would sneak drugs inside cancer cells, and oncolytic virotherapy, where the virus itself destroys cancer cells."

The achievement also demonstrates the ability to use methods of manipulation previously only applied to proteins. The team combined the methods of rational design, in which certain portions of the virus were strategically altered based on known information about their structure and function, and directed evolution, in which random mutations were introduced to millions of copies of the virus and the results are then screened for the desired traits, he said.

"These experiments demonstrate that these two methods can be combined and used to create complex molecular machines, like this viral vector with a tailored targeting receptor," said Kuhn, who also is head of the Department of Biological Sciences. "We've now reached the point

where we can easily change the virus to carry a variety of cargos and to seek out specific types of cells. We know where and how to add the characteristics we want and eliminate those that we do not."

A paper detailing the work was published in the October issue of the *Journal of Virology* and is currently available online. In addition to Kuhn, co-authors include Purdue postdoctoral researcher Zheng Liu, associate professor of biological sciences Wen Jiang and former postdoctoral researcher Hong-Sheng Dai. The National Institutes of Health funded this research.

Through standard rational design procedures the team inserted a human epidermal growth factor, or EGF, targeting sequence into the genome of the virus, and changed some of its amino acids to stabilize the addition, Kuhn said.

Cancer cells have large quantities of this growth factor receptor and it has been studied as a target for cancer therapies for nearly 30 years. When the ESV1 virus binds to the EGF receptor on a cancer cell it triggers a natural signal for the cell to internalize, or consume, the growth factor and the virus is carried in along with it, he said.

"This receptor is found in healthy human cells, however these cells won't be harmed by the ESV1 virus because they don't consume the growth factor in anywhere near the quantities cancer cells do," he said. "A healthy cell will easily be able to manage this virus, but cancer cells will gobble it up in overwhelming and deadly amounts."

The team developed a new directed evolution system, named tandem selection and enrichment, to shed the targeting to natural receptors and generate the desired characteristics within the virus. In the tandem selection and enrichment system the evolving viruses are transferred between two different host cells. One of the host cells was used as they

screened for viruses with the desired traits and the other was then used to enrich the most promising viruses from that group.

Random mutations were introduced into the virus through the use of an error-prone polymerase, an enzyme involved in the DNA replication process. The result was many dead or non-functional viruses, but the team screened those that survived to find viruses that both retained the EGF targeting and dropped the intrinsic targeting of Sindbis virus. The most promising candidates were then enriched to amplify certain characteristics, and once the desired virus was achieved, it was cloned to create multiple exact copies, Kuhn said.

The resulting virus, named Epidermal Growth Factor Receptor Specific Virus 1, or ESV1, doesn't replicate well and its effects will be more like a drug than a viral infection within a patient. It will likely only remain in the body for a short period of time and will not continue to infect the body. Patients will likely need more than one dose over the course of a cancer therapy, he said.

Because one's immune system will recognize and attack ESV1 after an initial exposure, the virus will have to be modified for each dose by switching out the envelope proteins, of which there is a panel available that could be easily swapped in and out, he said.

Kuhn and his research group studied the virus for years and built the foundation for this work by successfully assembling pieces of the virus in-vitro, placing different packages inside it and uncovering details of its structure and the behavior of its surface proteins.

The team next plans to further modify and refine ESV1 and prepare it for tests in animal models, he said.

More information: Directed Evolution of a Virus Exclusively

Utilizing Human Epidermal Growth Factor Receptor as the Entry Receptor, *Journal of Virology*, 2013.

Abstract
Rational design and directed evolution are powerful tools to generate and improve protein function; however, their uses are mostly limited in enzyme and antibody engineering. Here we describe a directed evolution strategy, named tandem selection and enrichment system (TSES), and its use in generating virus with exclusive specificity for particular cellular receptor. In TSES, evolving viruses are sequentially and iteratively transferred between two different host cells, one for selecting receptor specificity and the other for enriching the fittest virus. By combining rational design and TSES, we generate a human Epidermal Growth Factor Receptor (EGFR) Specific Virus 1 (ESV1). ESV1 has the backbone of Sindbis virus (SINV) and displays an EGF domain engrafted onto structural protein E@ after residue Pro192, together with eight amino acid changes stabilizing the E2-EGF chimera. ESV1 utilizes EGFR to initiate infection and has lost the capacity to interact with all known SINV receptors. A 12.2 Å cryoEM reconstruction of ESV1 reveals that the E2-EGF fusion adopts a fixed conformation with EGF sitting at the top of E2 spike; the EGFR binding interface faces outward and the EGF domain completely masks SINV receptor binding. The cryoEM structure of ESV1 explains the desirable properties of ESV1 and provides insights for its further modification. TSES expands the scope of directed evolution and can be easily extended to other targeting molecules and viral systems.

Provided by Purdue University