Vaccinia virus MacGyvers a makeshift tool to repair its DNA, exposing a vulnerability that could be targeted

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Paula Traktman, Ph.D., dean of the College of Graduate Studies at the Medical University of South Carolina (MUSC) and Conor Templeton, Ph.D. in the Traktman laboratory. Traktman and Templeton are co-authors on the Journal of Virology paper. Credit: Medical University of South Carolina. Sarah Pack.

Instead of relying on the cell's repair mechanisms, the vaccinia virus MacGyvers a tool for DNA repair from one that it already uses to copy DNA, reports a team of researchers at the Medical University of South Carolina (MUSC) in the *Journal of Virology*. Blocking that tool—an enzyme known as polymerase—at once disrupts the virus's ability to copy and to repair DNA, exposing an Achilles' heel that could be targeted with a therapeutic.

"For vaccinia virus, polymerase is a Sawzall—a tool that you can use for everything" said Paula Traktman, Ph.D., senior author of the article and dean of the College of Graduate Studies at MUSC, who has studied the virus for decades. "Viruses have smaller chromosomes, and so they've evolved to be able to use their tools for different things."

"It's like the virus's Swiss Army knife," said Conor Templeton, Ph.D. lead author of the article, who was a predoctoral candidate in the Traktman laboratory during the study and has since completed his doctorate. "It's a protein that's involved in replicating or copying DNA, but it also seems to be involved in repair."

Such detailed basic science findings about the way viruses copy and repair their DNA have paved the way for breakthrough antiviral therapies in the past 20 years, said Traktman.

"HIV antiretroviral drugs were made by really painstaking analysis of which proteins in the virus are essential, leading to drugs that now have made it a chronic disease," she said. "A curative treatment for hepatitis C was made possible by painstaking analysis of which proteins are essential for the virus. The more we know about the enemies, the better the weapons we can develop against them."

Better therapies for pox viruses are certainly needed. The vaccinia virus is a close relative of the virus causing smallpox and was used in the

vaccine that successfully eradicated it in the late 20th century. Although smallpox no longer naturally occurs, the threat that it might be used as a bioweapon remains, and currently, there is only one approved antiviral agent against it. Other pox viruses, most notably monkeypox, continue to afflict humans and can be lethal.

Vaccinia is a large DNA virus made up of about 200 genes, and its approach to survival differs markedly from that of smaller, nimbler RNA viruses, such as that which causes COVID-19. The RNA viruses mutate quickly to outrun the body's immune system. However, they do so at the cost of corrupting their genome. Vaccinia virus prefers a slower, steadier approach and is less likely to make mistakes, helping to ensure genomic stability.

"Vaccinia has gone for 'I may not be a Ferrari, but I'm a jeep, and I'm going to come out undamaged, and I'm going to be stable, and I'm going to stick around,'" said Traktman.

Unlike other DNA viruses, vaccinia virus does not set up shop in the cell's nucleus but, instead, stays in the cytoplasm, where it begins reproducing itself using only the tools it brought with it.

"The nucleus is like the kitchen of the cell," said Traktman. "If you came into somebody's house to cook dinner, you would go to their kitchen because that's where all the necessary equipment is. You wouldn't decide to go downstairs to their basement because then you'd have to start from scratch. But that's what vaccinia does. It says 'I'm not going into the kitchen where you cook. I'm going to just set up shop in the basement, where there's lots of space. I'll build everything I need.'"

The MUSC team wanted to see how vaccinia virus would react to damage to its DNA caused by ultraviolet (UV) radiation. They chose UV radiation because it is already known to affect viral replication

negatively. They also wanted to know whether exposing the cell to UV radiation one hour before infection with vaccinia would affect the virus's ability to copy and repair DNA.

The MUSC team found that exposing cells to UV radiation either one hour before or four hours after infection with vaccinia virus reduced the number of mature viral units, or virions, vaccinia was able to produce.

The enzyme polymerase is known to be necessary for successful viral replication, and UV radiation can prevent it from doing its job.

"Polymerase is like a car running down the road," said Templeton. "It runs smoothly when the road is nice and flat. But UV radiation acts like a speed bump, stopping it in its tracks."

The UV radiation can cause damage that makes it impossible for DNA replication to continue.

The team found UV-caused damage in the viral DNA of cells irradiated four hours after infection but not in those irradiated an hour before infection. The cells irradiated at four hours after infection also produced 30 times fewer new viruses. This UV-caused damage could account for the reduced infectivity of these cells. Although viral DNA levels were able to recover slightly by 10 to 18 hours, suggesting some viral DNA repair, blocking polymerase resulted in a further tenfold to twentyfold reduction.

"Polymerase is a well-known character in DNA replication," said Traktman. "It's a well-known character in actually synthesizing the genome, but this is its debut in repair."

In essence, the virus's polymerase "multitasks," but in so doing makes the virus vulnerable. Because vaccinia relies on polymerase both for

DNA copying and repair, blocking it could be a particularly devastating weapon against the virus. The current blocking agent, however, is too broad, and a much more tailored one would be needed for the clinic.

Next, the MUSC team wants to understand better why blocking polymerase makes the DNA more vulnerable to damage and less able to repair itself.

"We want to establish the ensemble of culprits in that process and then try to understand why it is that when you inhibit polymerase function, you see this sensitivity," said Templeton.

More information: Conor W. Templeton et al, UV Irradiation of Vaccinia Virus-Infected Cells Impairs Cellular Functions, Introduces Lesions into the Viral Genome, and Uncovers Repair Capabilities for the Viral Replication Machinery, *Journal of Virology* (2022). DOI: 10.1128/jvi.02137-21

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