CRISPR screening identifies potential HIV treatment targets

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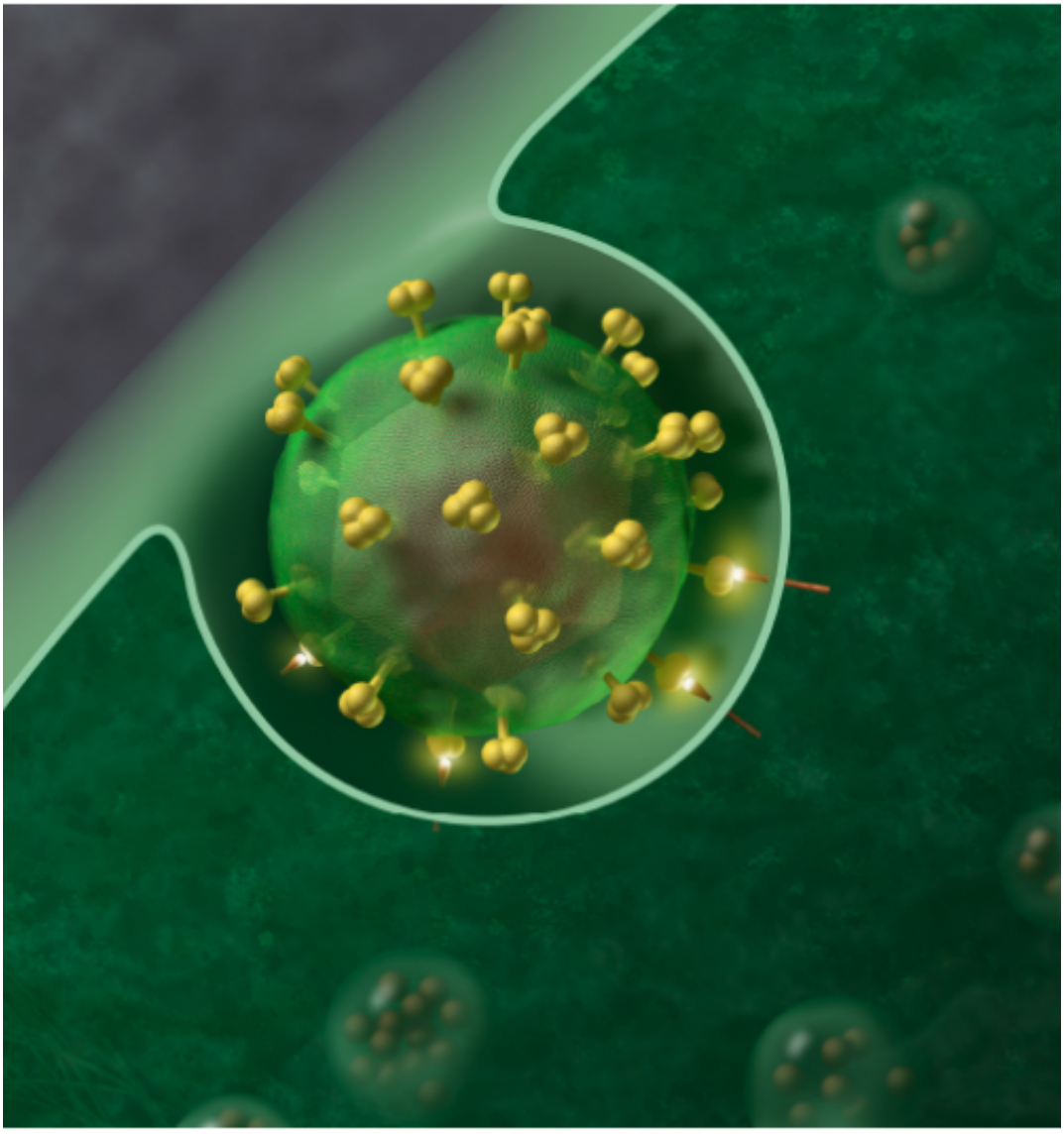

HIV-1 Virus. Credit: J Roberto Trujillo/Wikipedia

Investigators from Whitehead Institute, the Ragon Institute of MGH, MIT and Harvard and the Broad Institute of MIT and Harvard have used CRISPR-Cas9 gene-editing technology to identify three promising new targets for treatment of HIV infection. In their report receiving advance online publication in *Nature Genetics*, the research team describes how screening with CRISPR for human genes that are essential for HIV infection but not for cellular survival identified five genes—three of which had not been identified in earlier studies using RNA interference. Their method can also be used to identify therapeutic targets for other viral pathogens.

"We were surprised to find that there are so few host factors required for HIV infection given some of the previous literature," observes David M. Sabatini, Whitehead Institute Faculty Member and co-corresponding author of the *Nature Genetics* paper. "The beauty of the CRISPR-based genetic screens is the clear and robust results they yield," notes Sabatini, who is also member of the Broad Institute and Professor of Biology at Massachusetts Institute of Technology.

"Current anti-HIV medications overwhelmingly target viral proteins," says Ryan J. Park of the Ragon Institute and the Broad Institute, co-lead author of the report. "Because HIV mutates so rapidly, drug-resistant strains frequently emerge, particularly when patients miss doses of their medication. Developing new drugs to target human genes required for HIV infection is a promising approach to HIV therapy, with potentially fewer opportunities for the development of resistance."

Bruce Walker, director of the Ragon Institute and co-corresponding author of the Nature Genetics paper, explains, "Viruses are very small and have very few genes - HIV has only 9, while humans have more than 19,000 - so viruses commandeer human genes to make essential building blocks for their replication. Our goal was to identify human genes, also called host genes, that are essential for HIV to replicate but could be

eliminated without harming a human patient."

Tim Wang, a doctoral student conducting research at Whitehead Institute and the Broad Institute, and co-lead author of the report, explains, "CRISPR makes it possible to completely knock out genes at the DNA level; and our genome-wide, CRISPR-Cas9-based approach targets more than 18,500 genes, the vast majority of human protein-coding genes. Our study demonstrates how CRISPR-based screens can be applied to identify host factors critical to the survival of other viral pathogens but dispensable for host cell viability. Broad application of this method should pinpoint a novel class of potential therapeutic targets that have previously been underexplored for the treatment of infectious disease."

Co-corresponding author Nir Hacohen, an institute member at the Broad Institute and director of Cancer Immunology at Massachusetts General Hospital (MGH), adds, "An important aspect of our study was to focus on human T cells, the primary targets of HIV, and to identify host genes with the most dramatic role in viral infection of T cells."

Previous research has identified several host dependency factors, including two proteins required for HIV to enter CD4 T cells, the primary target of the virus: the CD4 molecule itself, to which the virus binds, and CCR5, which facilitates the binding of common HIV strains. Individuals with a particular CCR5 mutation are immune to those viral strains - indeed the only individual considered cured of HIV infection received a bone marrow transplant from a donor with that CCR5 mutation - but while therapeutic CCR5 inhibitors have been developed and are in clinical use, they can cause serious side effects.

Three 2008 studies that used RNA interference (RNAi) to identify potential host dependency factors identified more than 800 possible targets; but the little overlap among the results of the studies suggested a high rate of false positive results. In addition, none of those studies was

performed using the immune cells targeted by HIV, which also reduces the likelihood that the identified genes actually participate in HIV's infection of CD4 T cells.

Whitehead Institute's Tim Wang explains that, "RNAi suppresses but does not completely block gene expression - which could allow a targeted gene to produce enough protein to permit HIV infection - and it also can suppress expression of additional genes besides the intended target, leading to a false positive result."

Using CRISPR to screen a cell line derived from HIV-susceptible CD4 T cells identified five genes that, when inactivated, protected cells from HIV infection without affecting cellular survival. In addition to CD4 and CCR5, the screen identified genes for two enzymes—TPST2 and SLC35B2—that modify the CCR5 molecule in a way that is required for the binding of HIV. An additional gene identified through the screen was ALCAM, which is involved in cell-to-cell adhesion. When CD4 T cells are exposed to low amounts of virus, as might be seen in natural transmission, loss of ALCAM was associated with striking protection from HIV infection.

Park explains, "ALCAM is necessary for cell-to-cell adhesion in our cell line, allowing more efficient viral transfer from one cell to the next. In fact, we found that artificially inducing the aggregation of cells lacking ALCAM restored the cell-to-cell transmission of HIV. Further studies are needed to investigate whether targeting these genes would be toxic to humans. However, even if systemic inhibition has toxic effects, gene therapy approaches that selectively target these genes only in CD4 T cells or their precursors may avoid these toxicities, although it's important to note that gene therapy remains a challenging and potentially costly therapeutic approach."

More information: *Nature Genetics*,

nature.com/articles/doi:10.1038/ng.3741