Would CISPR-CAS9 and CRISPR-CAS13A system from bacteria be an effective treatment against viral infections (especially HIV)?

Luqi Wang

Introduction:

Viral infections is a problem that have troubled humans for decades. CDC estimates that flu has resulted in 9 million – 41 million illnesses, 140,000 – 710,000 hospitalizations and 12,000 – 52,000 deaths annually between 2010 and 2020. With countless other viral diseases such as HIV, more recently COVID-19 and much more affecting countless people. Vaccinations have been offered as a solution to these viral infections, and are the promising solutions to many viral diseases so far. However, there are a significant number of deadly viruses which no vaccine, or no effective vaccine and treatment has been developed yet. For example: HIV, respiratory syncytial virus, the cancer-causing Epstein-Barr virus and the deadly Ebola virus.

For all of these problems CRISPR-CAS9 and CRISPR-CAS13A could be the ultimate answer.

What is the CRISPR system:

The CRISPR system is found in bacteria. It is an effective defence mechanism against bacteriophages. It could be considered as a simplified version of an immune system against bacteriophages. It integrates short virus sequences in the cell's CRISPR locus, allowing the cell to remember, recognize and then initiate specific CAS proteins to perform target RNA degradation. Thus, preventing viral production inside the bacteria.

What is the CRISPR system used for right now:

The RNA - guided CRISPR-CAS9 nuclease system provides a powerful tool for genetic engineering as it can recognize and cut out parts of specific DNA strands. The basic method of the RNA – guided CRISPR – CAS9 is by the tethering endonuclease catalytic domains to modular DNA-binding proteins for inducing targeted DNA double-stranded breaks (DSBs) at specific genomic loci.

Despite the excitement brought up by the potentials of CRISPR in genetic engineering, a significant number of scientists are also investigating the potential of the CRISPR system becoming a future vaccine technology and treatment against viral infections. This news is extremely exciting as it has limitless potential. With changes and modifications to the CRISPR guide – RNA, it could theoretically provide a cure and immunity to every possible virus that is in existence on Earth.

Who first exploited this tool:

The 2020 Nobel Prize in Chemistry was awarded to Emmanuelle Charpentier of the Max Planck Unit for the Science of Pathogens and Jennifer Doudna of the University of California, Berkeley, for their discovery of the CRISPR/Cas9 genetic scissors that have revolutionized genome editing

How would this treatment work?

Once the viral infection is identified in early stage, a complementary strand of guide RNA is made artificially that would bind to a specific part and recognize a specific and unique part of the virus genome. Then this guide RNA would be inserted into the cells of the human body with the CRISPR-associated endonuclease. When the virus infects the cell with the guide RNA and the CRISPR-associated endonuclease, the guide RNA will bind onto the virus RNA and the CRISPR-associated endonuclease would be activated and trigger the degradation of the virus RNA therefore preventing the synthesis of viral particles, thus stopping viral replication. Ultimately curing the viral infection, or massively reducing the stress of the immune system, and will most likely prevent a cytokine storm that would heavily damage the body.

CRISPR-CAS induced immunity:

Using the CRISPR gene editing technology, we can achieve immunity against viral infections and also control spreads of viruses by using the CRISPR technology on viral vectors. Using the CRISPR system to edit the genes of immune cells to adapt them into fighting effectively against viruses and other pathogens which no vaccine or no effective vaccine has been developed yet. The main method is to genetically engineer B-lymphocytes so that it can produce an effective antibody and effectively identify the specific viral antigens or other viral components as soon as traces show up in the circulatory system. This method can prevent the situation in which B lymphocytes don’t rearrange their DNA segments in a way necessary to manufacture the needed antibodies to combat that specific virus.

How to deliver the CRISPR-CAS proteins and the guide RNA into the cells?

In order to safely deliver the CRISPR-CAS proteins and the guide RNA into the cells, nano cages are an option. A protein nanocage formed of protein assemblies will be able to offer biochemical engineering to the interior, the exterior and the inter-subunit to allow specific targeting, enhanced efficiency in the delivering process and reducing any toxic side effects when introduced in mass quantities.

Another option would be using a natural nanocage, which is an engineered virus that does not contain any genetic material inside the protein coating. Viruses are extremely efficient in delivering and are highly specific in the cells that they deliver to due to the spikes ability to recognize one or a limited amount of surface glycoproteins or glycolipids. However, the potential side effects of using viruses as nanocages are largely unknown and could trigger an immune response, most likely would lead to a cytokine storm that will severely damage the body.

Another possible way to deliver the CRISPR is by using exosomes. It is recently discovered that cells in the human body exchange RNA through carriers called exosomes. Though the mechanism of how this is done is not fully understood, once it is understood it will be a powerful tool used for targeted delivery in biomedicine.

How to reduce guide-RNA mismatching:

In order for the CRISPR-associated endonuclease to activate and to trigger degradation of the viral RNA, it is vital that the guide-RNA correctly matches onto the complementary part of the viral RNA. Therefore, the accuracy of guide-RNA matching is vital to the effectiveness of the treatment.

There are several ways to reduce mismatching. The first way is to increase the length of the guide RNA to at least 200 bases so that the probability of mismatching is greatly reduced.

The second way is to identify a specific area of the virus genome that is unique and special enough to be sufficiently different from our own genome, e.g. the RNA sequence for the synthesis of the virus spikes.

Summary:

Overall, I think there is great potential for this new piece of technology as it brings 2 distinct but related new ways of combating viral infections. This could be the new answer to treat HIV and other viral diseases in which no vaccine or no effective vaccine has been made.

Bibliography:

Nano cages:

<https://www.nature.com/articles/am2016128>

CRISPR/Cas technology as a promising weapon to combat viral infections:

<https://pubmed.ncbi.nlm.nih.gov/33569817/>

Exosomes:

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3738855/>

Engineering protein nanocages as carriers for biomedical applications:

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7091667/>

Exosomes: Biological Carriers and Promising Tools for Cancer Immunotherapy:

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7565712/>

Exosomes, a New Star for Targeted Delivery(review article):

<https://www.frontiersin.org/articles/10.3389/fcell.2021.751079/full>

Exosomes: biogenesis, biologic function and clinical potential:

<https://cellandbioscience.biomedcentral.com/articles/10.1186/s13578-019-0282-2>

The CRISPR-Cas immune system: Biology, mechanisms and applications:

<https://www.sciencedirect.com/science/article/pii/S0300908415001042>

Molecular Mechanisms of CRISPR-Cas Immunity in Bacteria:

<https://pubmed.ncbi.nlm.nih.gov/32857635/>

Systematic analysis of CRISPR–Cas9 mismatch tolerance reveals low levels of off-target activity:

<https://www.sciencedirect.com/science/article/pii/S0168165615300419>

Guide-target mismatch effects on dCas9–sgRNA binding activity in living bacterial cells:

<https://academic.oup.com/nar/article/49/3/1263/6121456>

Massively parallel Cas13 screens reveal principles for guide RNA design：

<https://www.nature.com/articles/s41587-020-0456-9>

The CRISPR-Cas immune system: Biology, mechanisms and applications：

<https://www.sciencedirect.com/science/article/pii/S0300908415001042>