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CRISPR/CAS9 Genome Editing System and its use in Infectious Disease

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Abstract

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) is an exciting new genetic engineering technology that was found in the chromosomes of certain bacteria and archaea. Bacteriophage are viruses that insert their DNA and hijack the host cell's machinery to make new phage that can go on to infect new cells. Some microorganisms use CRISPR as a defense mechanism to disrupt the bacteriophage DNA after it is inserted into the cell. CRISPR/Cas9 uses genome editing as a means to alter very specific sections of a foreign genome. CRISPR works with a RNA-guided DNA endonuclease called Cas9. This enzyme can locate and cut viral DNA and inactivate it so that it cannot replicate. Cas9 elicits the help of two forms of RNA called tracrRNA and gRNA (guide). These RNAs work with Cas9 to make modifications within the viral DNA. The CRISPR/Cas9 system is a genetic discovery that will aid researchers in the ability to target viral genomes and edit them. This technique may be able to clear latently infected cells as well as prevent new infections.

- **Infectious diseases are a leading cause of death worldwide. Novel therapeutics are urgently required to treat multidrug-resistant organisms such as *Mycobacterium tuberculosis* and to mitigate morbidity and mortality caused by acute infections such as malaria and dengue fever virus as well as chronic infections such as human immunodeficiency virus-1 and hepatitis B virus. (1)**
- **The recent development of the clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9) system as a genome-editing technique has significantly facilitated gene modifications in both pathogen and host cells and enables profound analysis of the molecular mechanisms that are involved in pathogenesis of infection. (2)**

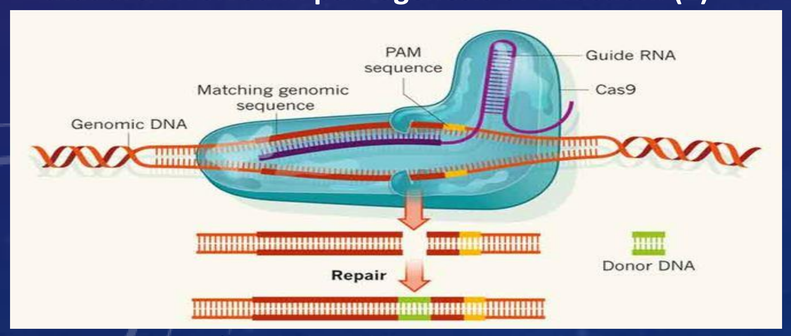

Figure 1. How CRISPR/Cas9 finds and locates target DNA for cleavage and repair

Applications for CRISPR/Cas9

- **CRISPR is being used to target latent HIV-1 provirus.**
- **When LTR-targeting CRISPR/Cas9 components were transfected into HIV-1 LTR expression-dormant and -inducible T cells, a significant loss of LTR-driven expression was observed after stimulation. Sequence analysis confirmed that this CRISPR/Cas9 system efficiently cleaved and mutated LTR target sites. More importantly, this system was also able to remove internal viral genes from the host cell chromosome. Results suggest that the CRISPR/Cas9 system may be a useful tool for curing HIV-1 infection. (3)**

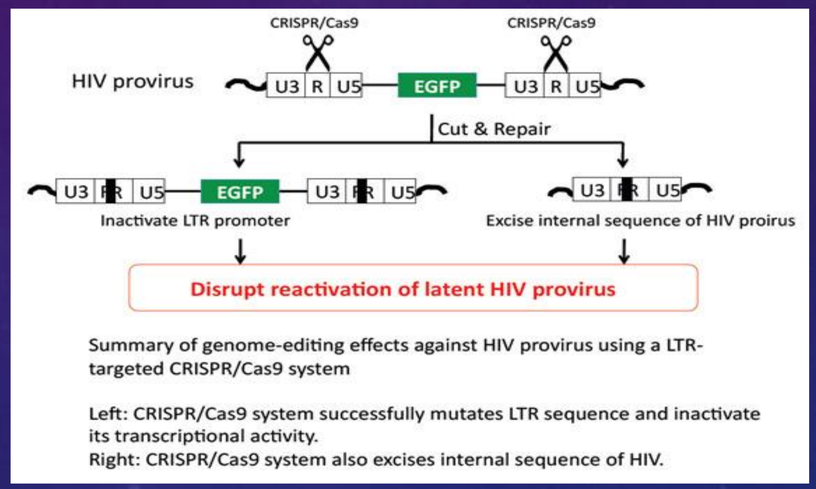

Figure 2. HIV-1 genome editing effects using LTR-targeted CRISPR/Cas9 System

- **Researchers tested different HBV-specific guide RNAs and demonstrated that they could inhibit HBV infections up to eightfold. Inhibition was due to mutations and deletions in cccDNA similar to those observed with chromosomal DNA cleaved by Cas9 and repaired by non-homologous end joining (NHEJ). Interferon alpha (IFN-α) did not have a measurable effect on the antiviral activity of the CRISPR/Cas9 system, suggesting that Cas9 and NHEJ activities are not affected by induction of an innate immune response with the cytokine.(4)**

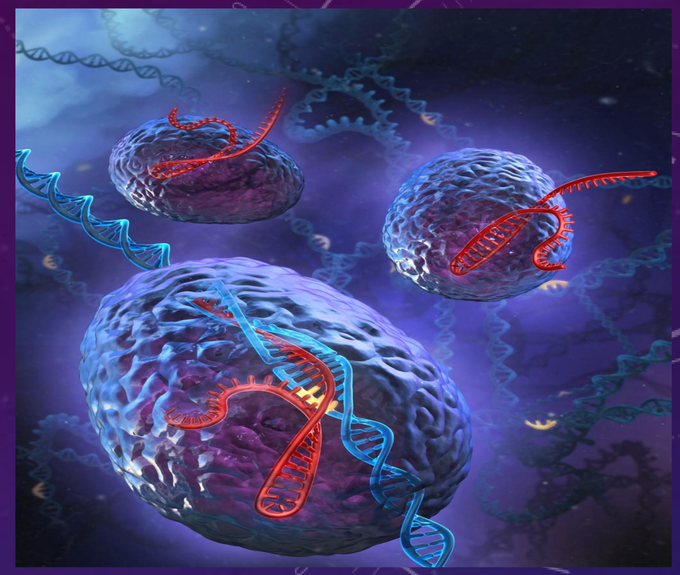

Figure 3. Computer animated artistic rendition of the CRISPR/Cas9 Gene editing system.

Discussion

- **The discovery of the CRIPSR/CAS9 system has simplified the approach for current genome editing techniques.**
- **CRISPR/Cas9 is revolutionizing the way that we are utilizing genetic engineering as a means to prevent and cure infectious disease.**
- **CRISPR/Cas9 is a promising genetic tool that may help facilitate a cure for chronic autoimmune disorders.**
- **CRISPR/Cas9 system has great potential in the development of novel antimicrobials and vaccines and therefore has the ability to open new avenues both for treatment and prevention of infectious diseases. (5)**

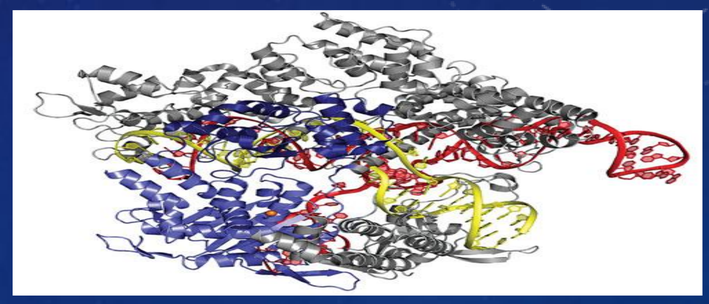

Figure 4. Ribbon Model of CRISPR/Cas9 Complex

References

(1) (2) (5) CRISPR/Cas9—The Ultimate weapon to battle infectious disease? (M. Doerflinger, W. Forsyth, G. Ebert, M. Pellegrini, M.j. Herold)
(3) Harnessing the CRISPR/CAS9 system to disrupt latent HIV-1 provirus (Hirotaka Ebina, Naoko Misawa, Yuka Kanemura, Yoshio Koyanagi)
(4) Targeting Hepatitis B Virus With CRISPR/Cas9 (Christoph Seeger, Ji A Sohn)

Pictures

Figure 1.
https://scienceofsingularity.com/tag/crisprcas9/
Figure 2.
Harnessing the CRISPR/CAS9 system to disrupt latent HIV-1 provirus (Hirotaka Ebina, Naoko Misawa, Yuka Kanemura, Yoshio Koyanagi)
Figure 3.
https://www.washingtonpost.com/national/health-science/scientists-are-growing-anxious-about-genome-editing-tools/2015/05/18/0a4db63c-ef4e-11e4-8abc-d6aa3bad79dd_story.html?utm_term=.f37c73979659
Figure 4. http://www.genengnews.com/Media/images/Article/MassGenl_RibbonDiagram_Rev3119927111.jpg