CRISPR and Sickle Cell Disease: A Breakthrough in Medicine

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CRISPR Background

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) are repetitive DNA sequences separated by "spacer" sequences that match sequences in viral DNA. They were discovered in archaea cells but are known for being used as a bacterial defense mechanism. [1]

Why does CRISPR exist?

When a virus infects bacterial cells, the bacteria takes the virus' genetic information (DNA) and incorporates it between the CRISPR sequences located in the bacteria's genetic code. These viral DNA sequences are used as a kind of genetic memory. If the same virus invades again, those viral DNA sequences are turned into a guide RNA that attaches to **Cas9**, an enzyme that cuts through DNA or RNA. The guide RNA leads the enzyme to the invader with the matching viral sequence, and Cas9 cleaves through the viral DNA. This kills the virus. [2]

How is it used in modern science?

CRISPR can be used by scientists to edit genes. When a scientist wants to edit a gene, they insert two things into the cell containing the gene- the CRISPR complex, and a piece of template DNA that will take the place of the original gene. The complex finds the gene and cleaves through the DNA. When the cell's repair mechanisms try to fix the break, they pick up the template DNA and use it to replace the broken gene, effectively "editing" the DNA.

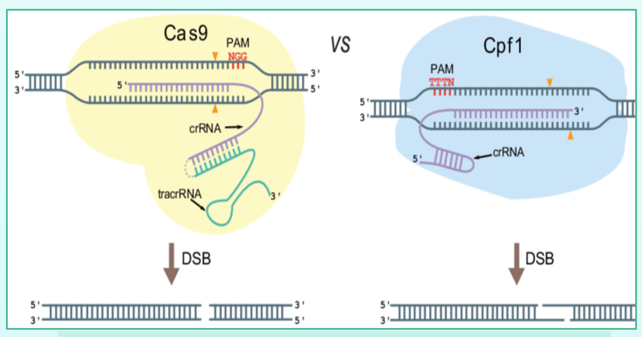
There are two complexes researchers can use to genetically engineer DNA. The first is **CRISPR-Cas9**, as used in bacterial cells. The second is **CRISPR-Cpf1**. Although they have similar functions, they produce different types of cuts in DNA- this difference leads to CRISPR-Cpf1 producing more precise insertions. [1]

Sickle Cell Disease

Sickle cell disease (SCD), also known as sickle cell anemia, is a genetic condition that affects red blood cells, making them crescent shaped instead of round and affecting their function.

People with this condition often feel fatigued and pained, as their red blood cells cannot properly carry oxygen- at any given time, the blood in their veins can clot and lead to intense episodes of pain (called pain crises) that lead to hospitalization. These patients tend to die earlier due to their condition.

These symptoms can be traced back to one single mutation in DNA- within 3 billion nucleotides, one T base is deleted and replaced with an A. This distinct base leads to the production of a mutated amino acid sequence, which in turn leads to a dysfunctional hemoglobin protein. Found in red blood cells, hemoglobin helps bring oxygen to other body cells as well as giving the blood cell its distinctive red color and its round shape. Sickle cell disease patients have dysfunctional hemoglobin that clumps together, giving their red blood cells their characteristic sickle shape. [10]

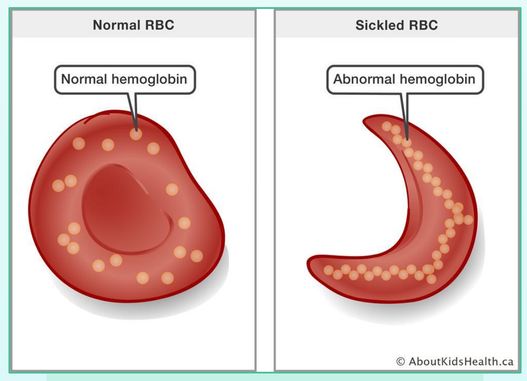


A diagram comparing the way Cas9 and Cpf1 cut DNA [b]

How is CRISPR used to treat sickle cell disease?

SCD is a genetic disease. Since CRISPR edits genetic material, it can be used to edit the genetic information in the stem cells in one's bone marrow. Stem cells in the bone marrow turn into red blood cells, so if those stem cells are edited by CRISPR, they will start producing normal red blood cells, rather than sickled ones. Because of CRISPR, a near-complete cure to sickle cell anemia is possible.

There's also germline editing- if the genes that caused sickle cell anemia were fixed in a just-fertilized egg, this would not only prevent that unborn baby from developing SCD, but also its entire future family line- if every fertilized egg that contained the SCD mutation was edited, in a few generations, sickle cell would be eradicated. Although this is unrealistic, germline editing could still reduce rates of Sickle Cell Disease occurrence if implemented correctly.



Normal vs mutated sickle cell hemoglobin [a]

Cause of sickle cell determined by a doctor in England. 1957 [7]

Blood transfusions are used to treat sickle cell anemia patients 1960 [7]

Stem cell transplants are used for the first time to treat sickle cell anemia. 1983 [7]

Discovery of CRISPR in E. coli bacteria by researchers at Osaka University, 1987 [4]

Francisco Mojica hypothesizes that CRISPR is an "adaptive immune system" 2005 [3]

Alexander Bolotin discovers Cas9. 2005 [3]

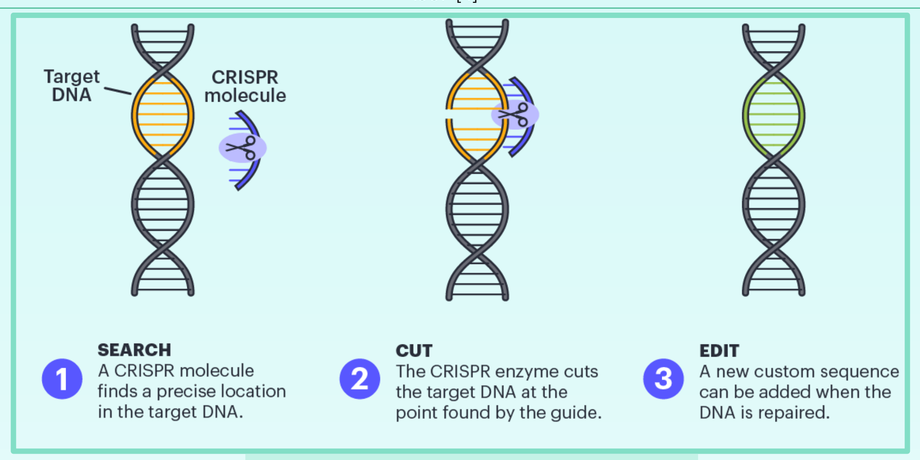
Mojica's hypothesis is proven correct by an experiment at the food company Danisco. 2007 [3]

Feng Zhang adapts CRISPR for use in eukaryotic cells and discovers that CRISPR can be used to edit multiple genomic locations. 2013 [3]

He Jiankui edits genes of twin embryos, making them resistant to HIV and the first genetically engineered humans in the world. 2018 [5]

Victoria Gray becomes the first sickle cell anemia patient to be treated with CRISPR. 2019 [8]

Casgevy, a treatment for sickle cell anemia, becomes the first ever FDA approved drug using CRISPR-Cas9. 2023 [6]



A diagram explaining how CRISPR edits genes [c]

Ethical, Legal and Social Issues

CRISPR sometimes edits the wrong genes, potentially causing life threatening side effects. [2] There is also a slippery slope when it comes to germline editing, which could quite literally change the path of human evolution. We have yet to fully understand how different genes affect each other, and editing one gene could cause irrevocable changes in the human gene pool overall. These dangers lead some to believe that it would be best to simply ban gene editing. But another ethical issue arises if scientists were to stop using CRISPR. With Casgevy turning out to be a success, the world knows that there is a potential cure for genetic diseases. If we decided not to even try to treat the conditions, wouldn't that be unethical too?

In the US, germline editing is illegal, but that doesn't mean the law is set in stone. Governments and scientific organizations around the world still debate when and how CRISPR should be used. This debate was intensified after **He Jiankui**, a Chinese scientist, edited two twin embryos (without permission of the Chinese government) to be resistant to HIV [5]. It may take years for world governments to come to a consensus on laws concerning germline editing for SCD, despite its numerous economic benefits.

Another facet of the ethical issue has to do with social inequality. Casgevy is incredibly expensive right now, and most people with SCD will not have access to it until prices come down. Although there are hundreds of Sickle Cell centers that can use CRISPR in the USA, sub-Saharan Africa, which has a much higher SCD rate (up to 40% in some regions), has only three for the entire continent. [13] These are examples of the overall disparity in access to CRISPR- if only the wealthy people in certain developed countries can edit their genes (giving them healthier and more productive lives), the already large wealth and health gap between upper- and lower-income families will widen further.

Biotech Innovators + Economic Impact

Innovators

Vertex Pharmaceuticals is a biotechnology company that specializes in finding small molecule drug treatments, mainly for cystic fibrosis. They partnered with **CRISPR Therapeutics** to develop a CRISPR-based sickle cell anemia treatment called **Casgevy.** [9] This therapy involves taking the patients stem cells and editing them so, when they turn into red blood cells, they produce fetal hemoglobin- this type of hemoglobin helps prevent cells from sickling. It is a single-dose, one time treatment. Because it edits the stem cells themselves, the treatment is virtually permanent. In 2023, Casgevy became the first CRISPR-based gene editing treatment approved by the FDA, a momentous occasion for the scientific community.

Another CRISPR based sickle cell treatment is **Lyfgenia,** which is like Casgevy, but it edits stem cells to produce HbA^{T87Q}, which is a type of hemoglobin typically found in adults. Lyfgenia was invented by scientists at **Bluebird Bio**. [6]

Economic Impact

Insurance companies pay 1.7 million dollars on average per person living with Sickle Cell, and those with SCD pay four times more for healthcare than those without. Patients with sickle cell have shortened and painful lives, and likely cost the economy millions of dollars in lost productivity. Studies estimate that the total cost of hospital care and visits for all adults with Sickle Cell was nearly $3 billion in 2015. For kids with SCD, that cost was nearly $60 billion. A cure for SCD could save the healthcare industry millions. [11] Casgevy currently costs $2.2 million dollars, which is significantly more costly than what the cost for non-curative SCD treatment is. Right now, many people will not be able to afford the treatment without financial aid. [12] However, this is the case for most new innovations, medical or not. CRISPR technology is continuously advancing, and treatment will likely become more affordable as time passes.

Scientists are currently working on new gene editing treatments that don't require a bone marrow cell transplant- when ready, they could potentially bring the cost of gene editing down from millions of dollars to just thousands. [13] If germline editing became legal and widespread for embryos with the SCD mutation, in a few decades, rates of Sickle Cell Disease would drop drastically, further reducing money spent on SCD hospital care and aid.

References

[1] Broad Institute. "Questions and Answers About CRISPR." *Broad Institute*, Broad Institute, n.d., https://www.broadinstitute.org/what-broad/areas-focus/project-spotlight/questions-and-answers-about-crispr.

[2] The Jackson Laboratory. "What Is CRISPR?" *Personalized Medicine & You*, The Jackson Laboratory, n.d., https://www.jax.org/personalized-medicine/precision-medicine-and-you/what-is-crispr.

[3] Broad Institute. "CRISPR Timeline." *Broad Institute*, Broad Institute, n.d., https://www.broadinstitute.org/what-broad/areas-focus/project-spotlight/crispr-timeline.

[4] Ishino, Y., Shinagawa, H., Makino, K., Amemura, M., & Nakata, A. "Nucleotide sequence of the iap gene, responsible for alkaline phosphatase isozyme conversion in Escherichia coli, and identification of the gene product." *Journal of Bacteriology*, vol. 169, no. 12, 1987, pp. 5429–5433, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9377665/#:~:text=CRISPR%20%E2%80%93%20clustered%20regularly%20interspaced%20short,1987%20by%20Ishino%20et%20al.

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