Epigenetic Editing: The Temporary Tattoo of the Genome

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THE PROBLEM

When lawyer Sonia Vallabh's mother died suddenly from a genetically-determined dementia, she entered a race against the clock to find a cure so she would not have to suffer the same fate.[1] It was discovered that the dementia stemmed from a malfunction in prions: a type of protein which is non-essential and harmless in a healthy state. However, for a variety of reasons, including genetic mutation, a prion may misfold, and once in this state, it will act as a template for proceeding proteins to follow suit. These abnormally folded proteins can form clumps in the brain, which result in neurodegeneration.[2] This discovery placed gene editing in the spotlight as a potential cure.

CAN CRISPR-CAS9 GENE EDITING HELP?

Clustered Regularly Interspaced Short Palindromic Repeats, or CRISPR, are regions in the bacterial genome that work alongside associated proteins such as Cas9 to provide adaptive immunity to viruses. When a bacteriophage (a virus specific to bacteria) attacks a cell, it injects its genetic material into the cytoplasm of the cell, where it can hijack the host machinery to replicate, producing enough viral particles to cause the cell to burst.[3] The CRISPR-Cas9 mechanism works by incorporating fragments of viral genetic material into the CRISPR locus, creating a "memory" of the infection. If the bacteria are attacked by the same bacteriophage again, the CRISPR locus is transcribed into short RNA transcripts, which guide the Cas9 protein to the complementary viral DNA region to cut and disable it, inhibiting the hijacking process.[4]

With the use of plasmids—-small loops of DNA that can be manufactured to carry various external genes—-this system has now been adapted as a tool to create precise changes in the genome. Specifically designed plasmids deliver Cas9 and a guide RNA—engineered to mimic the translated CRISPR gene—into the cell. This induces breaks at chosen points in the genome, and small regions of DNA will be lost on either side. Ordinarily, the cell will repair itself by stitching together the two ends, which can create small random insertions and deletions. Alternatively, if a template sequence is provided in the plasmid, the cell may use this to resynthesize the DNA, allowing for the introduction of foreign genetic information (Figure 1).[5]

CRISPR has transformed the world of genetics, with applications in gene therapy, cancer treatment, research, and drug development. However, due to the cascading nature of prion disease, CRISPR-Cas9 gene editing is not the most appropriate treatment for Dr. Vallabh's disease. The first prion protein to misfold in a cell may do so due to a genetic mutation, which, in theory, could be fixed by gene editing. However, if the mutation is not

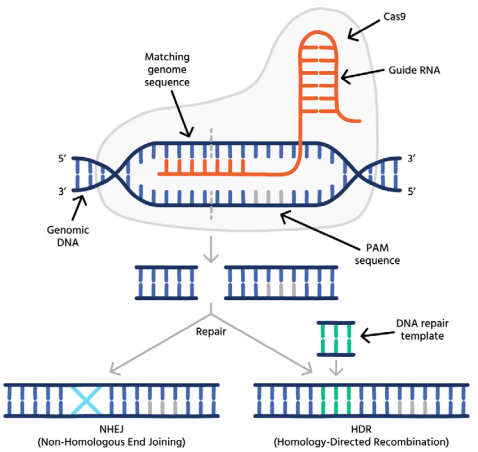

Figure 1: A Schematic of CRISPR-Cas9's Mechanism. The Cas9 protein and the guide RNA (orange) are shown inducing the double-stranded break. The two possible repair mechanisms are displayed, with the template sequence shown in green.

caught before the protein is synthesised, future proteins can still be affected, even if the DNA is unmutated. Because of this, CRISPR-based genome editing would be redundant. Instead, the expression levels of the prion protein must be targeted to combat the characteristic build-up of the diseased phenotype, or expressed trait.

WHAT IS EPIGENETICS?

In the field of genetics, the prefix 'epi'—meaning on or above in Greek—refers to the level of control of genetic activity that does not alter the genetic code itself. The epigenome consists of a series of heritable chemical modifications of DNA that create variation in how genes are expressed between cells and individuals. Modifications to the epigenome are common and can be acquired during conception, gestation, or as a result of behavior and environment throughout life. Studies have identified various lifestyle choices that play a part in epigenetic regulation, such as diet, physical activity, and psychological stress.[6]

Epigenetic change can take many forms, and often involves acetylation or methylation—two processes that add different small chemical groups to molecules. Compacted DNA, known as chromatin, is formed when DNA wraps around packing proteins known as histones. Histones, at precise locations in the genome, can be modified via acetylation to promote gene expression, or via location-dependent methylation to either promote or repress gene expression. Alternatively, gene expression can be silenced completely by the methylation of the DNA molecule itself. Cells also synthesize non-coding RNA, a molecule that can regulate gene expression through interaction with DNA, RNA, or proteins. Each of these small modifications to the structure of DNA can have substantial effects on the gene expression, and hence phenotype of an individual. For example, a study conducted with rat subjects suggested that epigenetic changes caused by maternal care can impact offspring behavior.[7]

A SOLUTION?

Epigenetics can be hijacked using a modified version of CRISPR, known as CRISPRoff. By using a nuclease dead version of Cas9, alongside a scaffolding protein and an enzyme, histone modification and DNA methylation can be induced at specific points in the sequence.[9] Unfortunately, this method is limited in its applications to neurodegeneration. Typically, adeno-associated virus (AAV) vectors are used to transport gene therapies to the brain; however, they have a limited capacity, and the tools needed to carry out CRISPRoff are too large.

Instead of being defeated by this setback, Sonia Vallabh decided not to hope for scientific innovation, but to make it happen herself.

Dr. Vallabh and her colleagues developed CHARM (Coupled Histone Tail for Autoinhibition Release of Methyltransferase), a mechanism that silences genes by inducing over-methylation of specific promoters. The system functions by recruiting DNA methyltransferase

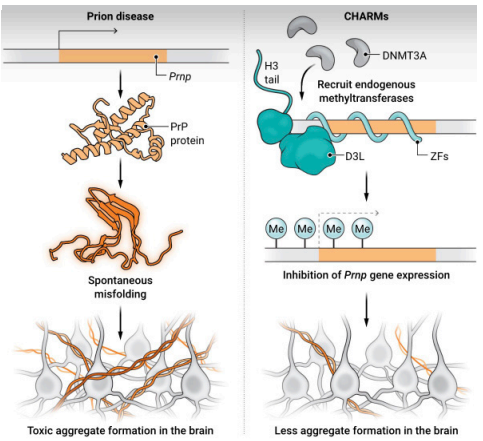

Figure 2

(DNMT3A), a cellular enzyme, to carry out methylation to silence gene expression (Figure 2).

This technology evades many issues found with both CRISPR and CRISPRoff. The use of an enzyme that is native to the human body avoids the toxicity that can come with using a foreign one, as in CRISPRoff. Additionally, CHARM surpasses CRISPR-based technologies in its adaptability by fitting into a single AAV vector, enabling efficient brain delivery. All forms of gene editing pose a risk of innaccuracy through off-target effects, causing unwanted and potentially harmful changes. However, CHARMs have the ability to self-silence their expression by targeting the AAV vector's promoter, minimizing the risks of prolonged expression, and therefore improving their accuracy.

Despite its strengths, epigenetic editing is still flawed. Without making edits to the DNA itself, altering epigenetic status will only have temporary effects. While this fortunately means that any off-target effects will be non-permanent and likely less damaging, even beneficial modifications will eventually be returned to their wild-type (original) state. As a result, a realistic model for the use of epigenetic editing in healthcare would require patients to receive regular treatment to maintain the changed status of the genome. This carries the risk of an increased financial burden and repeated exposure to the risks that come with gene editing. Having said this, there are also ethical concerns to be raised with permanent gene editing using CRISPR, which could be challenged by the temporary nature of epigenome editing.

THE FUTURE

Aside from the benefits and drawbacks that can be weighed for the development of epigenetic editing, the technology is still in its infancy. There are many years of research needed before we can see any real impact of the discoveries made by Dr. Vallabh and her team. Nevertheless, her work outlines a plethora of potential applications, helping to inspire continued progression in this field. What if we use DNMT3A to silence cancer-causing genes? Perhaps we could streamline the process of reprogramming cells or even explore the concept of slowing the process of aging— a topic linked to epigenetics in many studies.[8] There is a lot of promise with this discovery, but with each new application, we introduce a whole new scope of ethical concerns.

REFERENCES

1. Kasman, L. M. & Porter, L. D. Bacteriophages. Brenner's Encyclopedia of Genetics: Second Edition 280–283 (2022) doi:10.1016/B978-0-12-374984-0.00131-5.
2. Jinek, M. et al. A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. Science (1979) 337, 816–821 (2012).
3. Doudna, J. A. & Charpentier, E. The new frontier of genome engineering with CRISPR-Cas9. Science (1979) 346, (2014).
4. Epigenetic editors are a gentler form of gene editing. https://www.economist.com/technology-quarterly/2025/02/24/epigenetic-editors-are-a-gentler-form-of-gene-editing (2025).
5. Developing treatments for prion diseases | National Institutes of Health (NIH). https://www.nih.gov/news-events/nih-

research-matters/developing-treatments-prion-diseases.
6. https://pmc.ncbi.nlm.nih.gov/articles/PMC3752894/
7. https://pmc.ncbi.nlm.nih.gov/articles/PMC2682215/
8. https://www.nature.com/articles/s41392-022-01211-8
9. https://pmc.ncbi.nlm.nih.gov/articles/PMC11429707/

IMAGE REFERENCES

1. Banner image: Bertagnolli, M. M. (2024, July 25). Epigenetic Editor Silences Toxic Proteins in the Mouse Brain, Offering Promising Path to Treat Deadly Prion Diseases. NIH Director's Blog. https://directorsblog.nih.gov/2024/07/25/epigenetic-editor-silences-toxic-proteins-in-the-mouse-brain-offering-promising-path-to-treat-deadly-prion-diseases/
2. Figure 1: iotaSciences. (2024, November 17). CRISPR-Cas9 Gene Editing - Iota Sciences. Iota Sciences. https://iotasciences.com/applications/crispr-cas9/
3. Figure 2: Whittaker, M. N., & Musunuru, K. (2024). An epigenetic editor to silence genes. Science, 384(6703), 1407–1408. https://doi.org/10.1126/science.adq3334