THREE BLIND MICE

HOW CRISPR/CAS9 TREATING VISUAL IMPAIRMENT IN ANIMALS IS USHERING IN A NEW ERA OF GENE THERAPY.

OR SHOULD I SAY, THREE BLIND RATS?

Retinitis pigmentosa is a genetic disorder that causes vision loss and can lead to blindness. Scientists have been exploring various methods to develop effective treatments for this condition. One promising approach is the use of CRISPR/Cas9, a cutting-edge gene-editing technology. In a research paper, scientists successfully used CRISPR/Cas9 to treat retinitis pigmentosa in a rat model. This breakthrough has opened up new possibilities for targeted gene therapies and provides hope for individuals suffering from this debilitating eye disease.

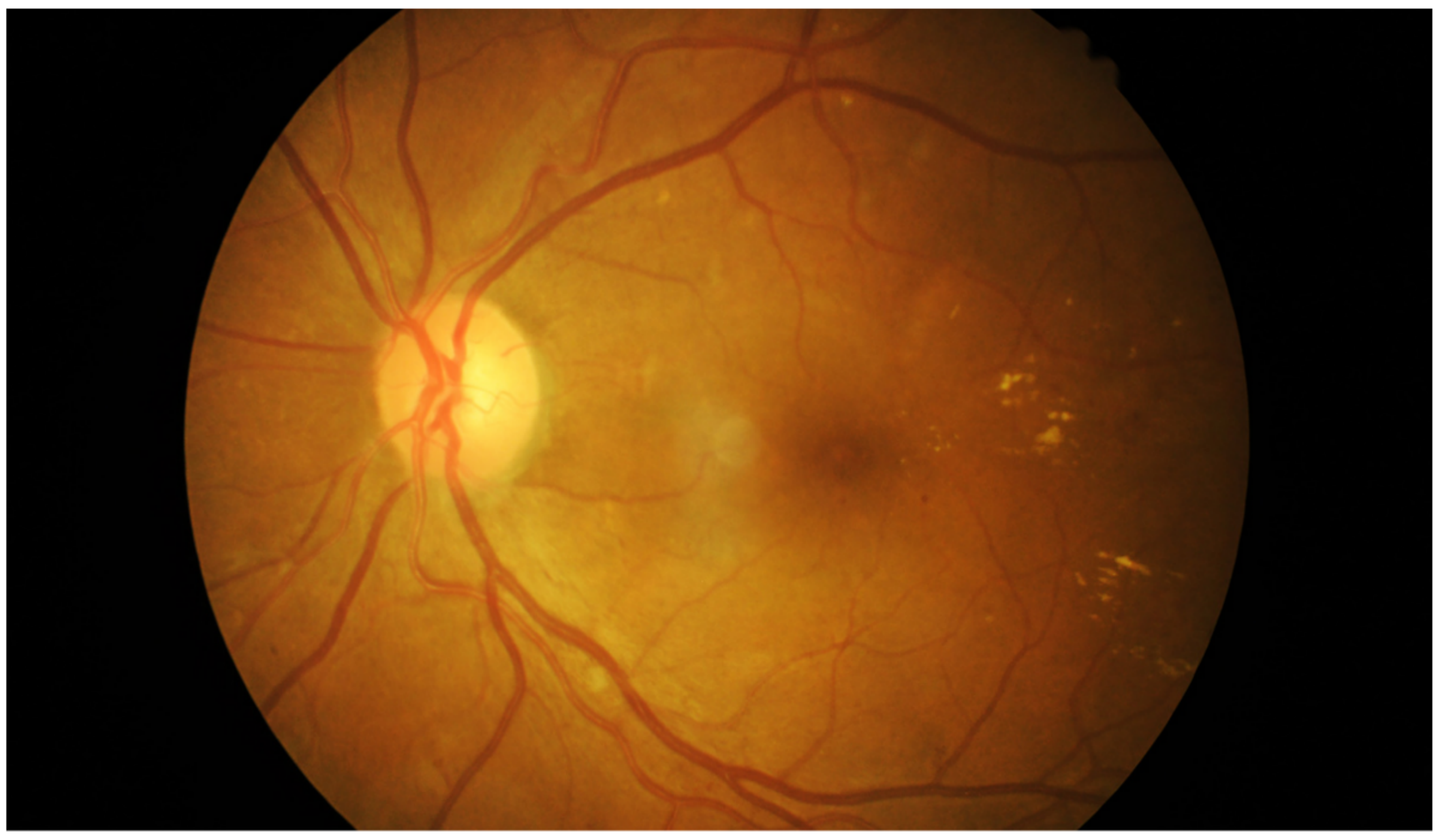
WHAT EXACTLY IS RETINITIS PIGMENTOSA?

Retinitis pigmentosa is a complex genetic disorder that primarily affects the retina, a vital component of our visual system. The retina is a thin layer of tissue located at the back of the eye, responsible for capturing light and converting it into electrical signals that are sent to the brain for visual processing.

This condition arises due to mutations in specific genes, which disrupt the normal functioning of the retina. The genetic mutations can be inherited from parents who carry the faulty genes or can occur spontaneously in an individual's DNA. These mutations can interfere with the production of proteins necessary for the proper functioning of the retinal cells. (Campochiaro & Mir, 2018)

The most affected cells in retinitis pigmentosa are the photoreceptor cells. These specialized cells come in two types: rods and cones. Rods are responsible for vision in dim or low-light conditions, while cones are responsible for colour vision and visual acuity in bright light. As retinitis pigmentosa progresses, both types of photoreceptor cells may be affected.

As the disease advances, the photoreceptor cells in the retina begin to degenerate, leading to a gradual loss of vision. Initially, individuals may experience difficulty seeing in low-light conditions, known as night blindness. They may have trouble adjusting to darkness and may struggle with activities like driving at night or navigating in dimly lit environments. Over time, the loss of photoreceptor cells continues, and individuals may start to experience a narrowing of their visual field, a condition known as tunnel vision. Peripheral vision is gradually lost, making it challenging to see objects or people in the outer edges of the visual field.



In severe cases, retinitis pigmentosa can lead to legal blindness or even complete blindness. It's important to note that the progression and severity of the disease can vary from person to person, even within the same family with the same genetic mutation.

While there is currently no cure for retinitis pigmentosa, researchers and medical professionals are continually exploring innovative approaches, such as gene therapies like CRISPR/Cas9, to slow down or halt the progression of the disease and potentially restore vision. The advancements in genetic research and gene-editing technologies offer hope for individuals affected by retinitis pigmentosa and bring us closer to finding effective treatments in the future. (Suzuki et al., 2016)

THE PROMISE OF CRISPR/CAS9:

CRISPR/Cas9 is an incredibly powerful and versatile gene-editing tool that has revolutionized the field of genetic research and holds tremendous promise for medical applications. It offers scientists the ability to make precise modifications to the DNA of living organisms, including humans, with unprecedented accuracy and efficiency.

At the core of the CRISPR/Cas9 system is a molecule called RNA, specifically guide RNA (gRNA), which acts as a molecular GPS, directing the Cas9 enzyme to specific locations within the genome. The gRNA is designed to recognize and bind to a specific DNA sequence that corresponds to the target site for genetic modification. Once the gRNA-Cas9 complex reaches its intended target, the Cas9 enzyme acts as a pair of molecular scissors, capable of making precise cuts in the DNA strands.

The power of CRISPR/Cas9 lies in its ability to harness the natural DNA repair mechanisms of cells. After the Cas9 enzyme makes its precise cut, the cell's own repair machinery comes into action. There are two primary DNA repair pathways: non-homologous end joining (NHEJ) and homology-directed repair (HDR). The NHEJ pathway, which is more error-prone, often leads to small insertions or deletions (Indels) in the DNA sequence at the site of the cut, generally leading to disruption of gene function. The HDR pathway, on the other hand, can be harnessed to introduce specific genetic changes by providing a DNA template that serves as a repair blueprint. NHEJ, however, is generally more efficient in cells than HDR.

By utilizing the CRISPR/Cas9 system, scientists can exploit these DNA repair pathways to correct disease-causing mutations.

THE STUDY

The study presented a novel approach called homology-independent targeted integration (HITI) using CRISPR/Cas9 technology for efficient gene insertion in both dividing and non-dividing cells. The hope was to take advantage of the efficiency of NHEJ while redirecting that repair pathway to be used for integrating desirable changes. Previous methods were limited in their effectiveness, especially for non-dividing cells, which posed challenges for understanding biological principles and developing treatments for genetic disorders.

Using HITI, the researchers achieved high knock-in efficiencies in both dividing and non-dividing cells compared to other methods like HDR and microhomology-mediated-end-joining (MMEJ). They observed that HITI resulted in successful gene insertion without inducing indels or genetic modifications. The researchers further optimized HITI by testing different nuclear localization signals (NLS) fused to the Cas9 enzyme, finding that the bipartite SV40NLS or BPNLS was more effective in enhancing Cas9 activity and genome editing. (Suzuki et al., 2016)

The HITI technique was not only successful in vitro but also demonstrated its potential in vivo. By delivering HITI constructs to the brains of live mice via in utero electroporation, the researchers achieved efficient gene knock-in in post-mitotic neurons. Moreover, HITI was effective in non-dividing cells in various tissues such as muscle and kidney. To enhance its in vivo applicability, the researchers sub-cloned HITI constructs into adeno-associated virus (AAV) vectors, which have shown efficacy and safety in human studies.

In a significant demonstration of the therapeutic potential of HITI, the researchers applied it to a rat model of retinitis pigmentosa, a retinal degeneration condition. By injecting HITI-AAV vectors into the subretinal space of rat eyes, they successfully inserted a copy of the Mertk gene, leading to increased Mertk mRNA expression, improved preservation of the outer nuclear layer (ONL) thickness, and the presence of MERTK protein. Functional assessments using electroretinography (ERG) revealed significantly improved visual responses in the treated rats. (Suzuki et al., 2016)

The versatility of HITI was further demonstrated through systemic delivery via intravenous injection in mice, resulting in successful gene knock-ins in multiple organs, including the liver, heart, and skeletal muscle. The researchers found that HITI exhibited higher knock-in efficiency compared to HDR in liver and heart tissues. Importantly, they observed minimal off-target effects at predicted genomic off-target sites.

The ability to achieve targeted transgene integration in post-mitotic neurons using HITI is unprecedented and holds immense potential for advancing neuroscience research. It enables precise manipulation of gene expression in specific cell types, such as inserting optogenetic activators for cell-type-specific control of neuronal activities. HITI also offers opportunities for generating knock-in reporters to trace cells in live animals, particularly valuable in animal models with limited transgenic tools. (Suzuki et al., 2016)

SO WHAT DOES THIS MEAN?

The study's results highlight the utility of HITI for targeted gene therapies and establish new avenues for basic research. With further improvements in efficiency, HITI holds promise for revolutionizing gene replacement therapy and advancing the understanding and treatment of genetic disorders.

The findings of this study have significant implications for the future of gene editing and targeted gene therapies. The development of the homology-independent targeted integration (HITI) technique using CRISPR/Cas9 technology opens up new possibilities for
precise and efficient gene insertion in both dividing and non-dividing cells. Here's what it means for the future:

- Improved gene therapies:
HITI provides a powerful tool for targeted gene therapies. It offers a more efficient and reliable method for correcting disease-causing mutations and inserting healthy genes into the genome. This advancement holds promise for treating a broad range of genetic disorders and will pave the way for more effective gene replacement therapies.

- Enhanced understanding of biological principles:
The ability to perform targeted gene insertions in non-dividing cells, including post-mitotic neurons, is a groundbreaking achievement. This capability will greatly advance basic research in neuroscience and other fields by enabling scientists to study gene function and manipulate gene expression with unprecedented precision.

- Expanded applications of optogenetics:
HITI opens up possibilities for using optogenetics to control neuronal activities with cell-type specificity. By inserting optogenetic activators into specific gene loci using HITI, researchers can gain precise control over neuronal circuits and further unravel the complexities of the brain's functioning. This has implications for understanding and potentially treating neurological disorders.

- Advancements in animal modelling:
HITI's ability to insert knock-in reporters into live animals provides valuable tools for studying cell tracing and dynamics. This is particularly significant in animal models where transgenic tools are limited, such as non-human primates. The availability of HITI will expand the possibilities for studying complex biological processes in vivo.

- Progress in gene replacement therapy:
The successful application of HITI in a rat model of retinitis pigmentosa demonstrates its potential for gene replacement therapy. By inserting a corrected gene into the retina, the researchers were able to improve visual function. While further refinements are needed, HITI shows promise for future treatments of inherited retinal degenerative conditions and other genetic disorders.

- Clinical translation:
The use of AAV vectors for delivering HITI constructs enhances its potential for clinical translation. AAVs have already shown efficacy and safety in human studies, making them an attractive delivery method for targeted gene therapies. The development of HITI-AAVs further improves in vivo efficiency and expands the possibilities for clinical applications.

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