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The Utilization of CRISPR/Cas9 in Monogenic Disorders

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Abstract

This paper is a literature review of various scientific research papers, exploring the recent scientific advancement in the field of genetic engineering. The research presented is a foundational tool, building awareness on the implications of CRISPR/ Cas9 technology. CRISPR/ Cas9 was first discovered through the study of bacterial immune systems, fighting against viral infections. Manipulation of the Cas9 protein would eventually lead to target specific, gene-altering medicines for human organisms. CRISPR/ Cas 9 technology has begun to show promise as an effective treatment for certain monogenic disorders. Despite this, time is required before its efficacy as a proven genetic treatment plan. Certain drawbacks like chromosomal rearrangements and the formation of cancerous cells, reveal the potential negative consequences of CRISPR/Cas9 treatment. Careful calculations will be required to ensure the safety and specificity of this technology throughout future clinical trials.

Keywords: CRISPR/ Cas 9, gene editing, genetic engineering, DNA mutation, non homologous end joining, homologous directed repair, Intellia, CRISPR therapeutics, monogenic disorders

Evolution of Gene Therapy to Gene Editing

Gene therapy has been a growing field for nearly 30 years. It has unbelievable potential to cure genetic diseases, making it a hot topic of discussion. Gene therapy generally refers to "gene transfer therapy" a process of transferring a functioning copy of a gene, a transgene, to the body. The transgene adds a functioning version of the gene to make a healthy protein (Kabadi, 2020). Genetic Therapy has proven to be successful in various therapies; for example, haematopoietic stem cell transfection has been used to treat Cerebral Adrenoleukodystrophy (ALD) (Ridler, 2017). ALD is caused by a mutation which initiates fatty acid breakdown, cerebral inflammation, and rapid demyelination. Fortunately, the gene therapy performed on 17 male ALD patients was "safe and effective" (Ridler, 2017). While it introduces astounding possibilities, the pursuit of gene therapy has included tragic setbacks. An 18-year-old boy, Jesse Gelsinger, enrolled in a gene therapy clinical trial for an ornithine transcarbamylase (OTC) deficiency, causing ammonia to accumulate in the blood. Gelsinger was "infused with a corrective OTC gene, in a dose of attenuated cold virus, a recombinant adenoviral vector" that progressively caused organ failure, leading to his death (Sibbald, 2001). After this fatal event, gene therapy was fairly overlooked until the discovery of zinc-finger nucleases (ZNF) and transcriptor activator-like effector proteins (TALEN). The utilization of proteins discovered from bacterial defense mechanisms evolved gene therapy into gene-editing. Gene-editing is different from gene therapy in that editing works by correcting the faulty gene through disruption, replacement, or correction. Both ZNF and TALENs are made up of two parts: a binding domain and a Fok1 endonuclease which cuts the double-stranded DNA (Nemudryi, 2014). Unfortunately, ZFNs and TALENS are time-

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consuming, bioengineers have to specifically program individual proteins to recognize single DNA nucleotides, whereas the CRISPR/ Cas9 system "eliminates the need for elaborate protein engineering" through the utilization of gRNA (Hong, 2018). Crispr's utilization of gRNA to locate specific DNA, and mechanisms of the CRISPR protein to cut DNA, may have long lasting implications on the bodies defense response to genetic mutations.

What is Gene-Editing?

CRISPR gene-editing was first uncovered in 2012, by studying microbial defense systems, how bacterial immune systems fight against viral infections through the use of a Cas protein (Wu, 2020). From this discovery, a CRISPR/ Cas9 complex was developed and has been meticulously studied for the usage of human genome editing (Zhang 2021). CRISPR/ Cas complexes have been organized into three major Cas systems followed by a wide variety of subtypes inside of those systems (Makarova, 2011). CRISPR/Cas 9 is considered to be a type II CRISPR system that "requires only one protein (Cas9) for the recognition and cleavage of a DNA site" (Makarova, 2011). The Cas9 protein contains an endonuclease that cleaves or cuts at a specific site. A spacer made of about 2-4 base nucleotides, called protospacer adjacent motif (PAM), is used to hold a specific place on the DNA (Barrangou & Doudna, 2016). The Cas9 protein binds to a guide RNA (gRNA), which directs the Cas9 protein to the PAM recognition sequence. Once the gRNA determines a match to the DNA nucleotides, a cleavage, or a cut is induced on the double-stranded DNA (Wu, 2020). The Cas9 protein in combination with a gRNA and PAM complex essentially forms a pair of molecular scissors. This is revolutionary because CRISPR Cas 9 nuclease technology can be used to either disrupt, replace, or correct mutated DNA (Zhang, 2021).

After the slicing and dicing process has been completed, cells have the ability to detect broken DNA, initiating a cell's repair response. The two major methods for repair in the CRISPR/ Cas 9 complex are non-homologous end joining (NHEJ) and Homologous Directed Recombination Repair (HDR) (Humbert, 2021). Non-homologous end joining (NHEJ) describes the body's natural repair process, which simply reattaches the broken ends of DNA. If correctly repaired, this pathway can be harnessed to "knockout" or silence an undesirable gene mutation (Uddin, 2020). The drawback of this repair pathway is that certain errors can occur from unintended insertions or deletions called INDELS (Nemudryi, 2014). INDELS essentially cause a frameshift which influences the way a three codon DNA sequence is read. If the frame is shifted by one nucleotide, it will cause an incorrect amino acid sequence, resulting in abnormal proteins (Education, n.d.). Homologous Directed Recombination Repair (HDR), is a method that has significantly intrigued the scientific community because it requires the insertion or "knock-in" of a new genetically engineered template at the cleaved coding site. HDR is a process which has never been achievable historically, it eliminates the body's natural DNA repair process to insert desirable genetic coding.

What is CRISPR/Cas 9 being used for?

By harnessing the power of the Cas9 protein, leading scientists Jennifer Doudna along with collaborator Emmanuelle Charpentier along with a group of various scientists, discovered they could utilize CRISPR/Cas9 protein complex to potentially cure genetic diseases. Since their initial breakthrough discovery of CRISPR technology in 2012, they have since founded their own companies Intellia and CRISPR Therapeutics. It should be noted, there are a multitude of venture pharmaceutical companies that have been formed surrounding CRISPR. This technology will be tested on various genetic diseases, yet for the purpose of simplicity this review will focus on monogenic blood disorders. By following the industry application of this scientific breakthrough, we can begin to track the implication of this technology and its effects on monogenic disorders in humans.

Intellia: Partnered with Regeneron and Novartis: June 27, 2021, the first-ever clinical data from human trials have been recorded on the effects of gene editing. Specifically, Intellia's NTLA-2001 intravenous infusion is designed to prevent the unfolding of transthyretin protein to treat amyloidosis, a protein buildup that can interfere with the body's organ functions (Leonard, 2021). ATTR is a monogenic disorder, meaning only one gene has been affected, making it an ideal candidate for CRISPR/ Cas9 "knockout" repair method (Gillmore et al., 2021). Intellia, currently in the first phases of their clinical

trial, reports that a single high dose of NTLA-2001 has a 96% reduction in ATTR and a low dose has a 52% reduction in ATTR (Gillmore et al., 2021). Further tests are being done to determine the effects of increased doses, so follow-up research will be necessary as NTLA-2001 moves further into clinical trials. Overall, from this research, we see a positive effect from the usage of CRISPR/Cas9 "knockout" method for the treatment of ATTR Amyloidosis.

Intellia's additional gene-editing therapy for hemoglobinopathy is currently in the first phases of clinical trials. The therapies will be administered ex vivo, meaning they will edit cells outside of the body, to be delivered back in. Intellia plans to use CRISPR/Cas9 editing to induce fetal hemoglobin (HbF) expression, to treat Sickle Cell Disease (Hong, 2018). Sickle Cell Disease is a monogenic disorder that causes misshapen red blood cells, interfering with the delivery of oxygen throughout the body. The sickle cell life span typically survives 10-20 days as opposed to a healthy red blood cell's 90-120 days (Mayoclinic, 2021). Typically, HbF is found only in infants, but Intellia predicts they can edit this gene to activate in fully grown adults, minimizing the negative effects of genetic blood disorders. Further details on these trials will be eagerly anticipated as the usage of CRISPR/Cas9 continues to develop in human clinical trials.

CRISPR Therapeutics partnered with Vertex Therapeutics: Similarly, CRISPR Therapeutics has begun clinical trials to treat hemoglobinopathies, genetic blood disorders such as B-thalassemia, and Sickle Cell Anemia. Traditional treatment for blood disorders are transfusions, a procedure in which donated blood is delivered into the body to replace old blood cells (Mayoclinic, 2020). CRISPR Therapeutics shares the same goal as Intellia, to treat blood disorders by increasing fetal hemoglobin (HbF) levels. HbF is oxygencarrying hemoglobin that is present in a fetus, followed by rapid decline shortly after birth. If CRISPR Therapeutics is successful, the activation of HbF can alleviate pain symptoms and eliminate blood transfusions. According to the New England Journal of Medicine, early reportings of CTX001 show that fetal Hemoglobin levels effectively rose in both a 19-year-old female patient with TDT and a 33 yr old female with SCD, yet each patient also experienced adverse health events (Frangoul, 2021). Specifically, the 19-year-old experienced 32 adverse events, the most severe being pneumonia and the 33-year-old experienced 114 adverse events, the most severe being sepsis (Frangoul, 2021). All conditions were monitored and resolved with treatment, yet these early results confess a need for further experimentation for CRISPR/Cas9.

Victoria Gray is known as the first patient to recieve CTX001 gene editing treatment for sickle cell disease. Gray was treated in 2019 at the Sarah Cannon Research Institute in Nashville, Tenessee. In an exclusive interview with NPR news, Gray admitted that sickle cell disease has put her through tremendous pain, weakened her immune system, and damaged her heart (Stein, 2021). Desperate to heal, she volunteered for CRISPR/CAS9 DNA editing treatment to reactivate fetal hemoglobin. In the most recent interview on December 15, 2020, Victoria Gray has claimed that "It's amazing! It's better than I could have imagined. I feel like I can do what I want now." Since her treatment in 2019, she has not experienced severe bouts of pain or emergency hospital visits (Stein, 2021). Further observation of her condition will continue for years to determine the effectiveness of CRISPR treatment in Sickle Cell Disorder.

Complications in Gene Editing

The major ongoing concern of CRISPR systems, appears to be a question of specificity. CRISPR/Cas 9 increases specificity, compared to ZNF or TALENs. However, gRNA nucleases may induce cuts on base pairs, at different locations other than the intended sites. A knockout CRISPR/Cas9 study was conducted on HBB gene, responsible for B-hemoglobin production, and CCR5 gene, an attachment point for HIV. Substantial off-target cleavage, resulting from edits on HBB and CCR5 genes, show large mismatches between gRNA and complementary target sequences (Cradick, 2013). "A high rate of off-target cleavage may result in large indels, causing significant potential of mutagenesis and chromosomal rearrangement" (Cradick, 2013). Meaning, off-target edits may spawn undesirable mutations in the human genome, an immense concern.. Furthermore, larger than expected deletions may have pathogenic consequences (Kosicki, 2018). In an attempt to prevent off-target edits, variants of the Cas9

enzyme have been engineered, and continue to be engineered, to detect and prevent unintended edits.

Supplemental complications with CRISPR/ Cas9 is the activation of p53 protein, which initiates the cell's repair systems to fix damaged DNA, causing gene edits to be more challenging (Hong, 2018). Cells without the p53 protein are more ideal candidates for gene editing, yet p53 protein is necessary as a tumor fighting cell response (Hong, 2018). It presents a dilemma from which there is no escape, because of mutually conflicting conditions. "Absence of p53 in cells makes them more likely to become tumorous, as DNA damage can no longer be corrected" (Hong, 2018). Increased cell populations, without the p53 pathway, will decrease the cell's tumor-fighting capability. The body's response to edits may result in oncogenesis, the formation of cancerous cells. The greatest shortcomings of gene editing is the body's defense response to alterations, as well as, off-target edits resulting in mutations. Meticulous steps are required to ensure the safety of participants in clinical trials.

Conclusion

The purpose of this paper is to explore the CRISPR/Cas9 complex and the potential applications for single-point mutations. Additionally, offer insight into the pharmaceutical industry's usage of gene editing therapy in clinical trials. The CRISPR/Cas9 is one of many variations of the CRISPR protein. Modifications to the CRISPR protein have already begun to take place in order to enhance specificity. Further research is required to understand the full implications of this technology as well as its effects on patients. While the potential for this technology can be life altering, negative consequences may take place in the form of mutations or cancerous cells. A great deal of observation of testing will be required to ensure the safety of this groundbreaking technology.

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