Gene Editing and Healthcare Applications

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1.1 Introduction

The growing advances in the field of genetics and gene editing have led to fascinating applications of gene modification technology in the work of healthcare research. Genetic mutations and genetic diseases can be impacted by the therapies and treatments that have progressed due to CRISPR-Cas 9. CRISPR-Cas9 is a major breakthrough in the field of gene editing. It consists of a guide RNA (gRNA) which contains a section of the target DN) and an endonuclease that cuts DNA. CRISPR-Cas9 is specific because of the gRNA, relatively cheap compared to previous gene editing technologies, and highly programmable (Zhu 2021). CRISPR-Cas9 is great at knocking out gene expression and especially useful for autosomal dominant disorders because if CRISPR-Cas9 disrupts the function of a dominant, disease-causing allele, only the recessive allele would be expressed (Kathleen et al. 2020). Nevertheless, with the field of gene editing, it is imperative to keep in mind gene therapy progression as well. Gene therapy is the oldest form of genetic medicine and is based on gene transfer technology, such as insulin bacteria. Insulin bacteria are the result of the human insulin gene being inserted into a bacterial plasmid, a bacterial DNA molecule. The bacteria will then start producing insulin based on the newly encoded instructions in its DNA and replicate with this modification (Sanger 2017). A primary component of gene therapy is the type of cell delivery technique used. DNA can be delivered by a virus shell by "infecting" a cell & inserting the appropriate replacement DNA (Petrich 2020). All forms of gene therapy in humans must undergo clinical trials in order for any treatment to be approved by the FDA. Clinical trials must also follow a series of criteria that all involved must adhere to: trials must have strict eligibility criteria, researchers must communicate about serious adverse events in other participants, and participants must show informed consent (Gupta 2013). Lastly, because tempering with genetic material can be such a risky method for healthcare professionals to resort to, ethical and safety considerations of using gene editing for human health and disease must be considered. Overall, how genetic mutations cause diseases such as cystic fibrosis and sickle cell anemia, how CRISPR tools can be used to combat it, and what the bioethical considerations are of using gene editing treatments can all be better understood through continued gene editing research.

1.2 CRISPR-Cas9 Structure and Function

CRISPR-Cas 9 was regarded as a bacterial defense system by many scientists for an extended period of time, up until its rediscovery as a tool for gene editing. Thus, with the discovery of the gene editing technology CRISPR-Cas 9 in 1987, a series of breakthroughs in the field of genetics due to this revolutionary tool followed (Sternberg 2017). CRISPR-Cas 9 gene editing harnesses an innate bacterial defense mechanism that can be programmed in order to carry out acute modifications, deletions, or edits in various DNA sequences. Being an endonuclease, CRISPR-Cas9 has the ability to cut through DNA causing a double-stranded break, which is helpful in disrupting, deleting, or correcting a specific sequence. The Cas9 enzyme instills said double-stranded breaks in genomic sequences in order to activate the usage of one of these two pathways in order for the body to naturally repair a target gene. In order to further improve accuracy, the CRISPR-Cas 9 complex navigates to the correct target site via a guide RNA (gRNA). A gRNA is custom engineered by a team of scientists to align with a specific DNA sequence and is implemented into the CRISPR-Cas 9 complex. CRISPR-Cas 9 relies on the body’s two natural DNA repair pathways: Non-Homologous End Joining (NHEJ) and Homology-Directed Repair (HDR). The NHEJ repair pathway is utilized by the body to

directly rejoin broken ends of DNA quickly, but in doing so could cause insertion or deletion mutations that could affect the functionality of a protein encoded by the repaired gene (Uddin 202). However, the errors often caused by the NHEJ repair pathway are effectively utilized by researchers to eliminate certain genes by causing a disruption in their sequences. Comparatively, the HDR pathway has an increased level of accuracy as it bases its repairs off of a template DNA strand that holds the corrected sequence needed for the repair, however this process can usually take longer, so it is used less frequently by the body (Patrick 2023) . Both of these methods require a double-strand break and repair process in order to fully correct the mutated or broken gene. In the case of the HDR pathway, with the presence of a template DNA strand and a double-stranded break introduced by the Cas9 protein, the mechanism can grant precise gene insertions or corrections. Once the gRNA has helped the CRISPR-Cas 9 complex to correctly identify the target site, a double stranded break in a single location will cause the NHEJ repair pathway to take over. The DNA strand end joining provided by this pathway can cause small insertion or deletions that can inactivate the gene, knocking it out. If researchers insert two gRNAs and assign them to different locations, the CRISPR complex will issue a double- stranded break in both sections, allowing a larger section of DNA to be completely omitted from a genome. The NHEJ pathway will then connect the two strands, seamlessly deleting the portion of target sequences. Utilizing both the NHEJ and HDR repair pathways, the CRISPR-Cas 9 complex can serve as a valuable tool scientists can harness to issue acute modifications, deletions, or edits in genomic sequences.

1.3 The Genomics of Cystic Fibrosis

Cystic Fibrosis (CF) occurs when excessive or abnormal mucus builds up in the lungs, pancreas, or digestive system, and can lead to pulmonary or respiratory problems. CF is a genetically inherited disease that can be passed on through generations. The affected gene in an individual with CF is the cystic fibrosis transmembrane conductance regulator (CFTR) gene that is responsible for the production of the CFTR protein. This protein is responsible for the osmoregulation for cells in the respiratory and digestive systems. It serves as a chlorine ion channel and helps to maintain a thin and slippery mucus consistency (Hanssens 2021). Mutations in the CFTR gene vary in range of severity from Class I to Class V Mutations. Class I Mutations completely omit the production of the CFTR protein and are the most severe mutation. Due to the absence of this protein production, osmoregulation does not occur and mucus does not retain a proper slipper consistency, for healthy organ lining. Class II Mutations facilitate improper protein production, leading to a misfolded CFTR protein. Ion channels that may be formed are not effectively transported to the cell surface and cause thick mucus build-up. Class III Mutations, on the other hand, do allow this protein to reach the cell surface, but there is a defect in the chlorine ion channels. Ion concentrations become imbalanced in cells, leading to sticky mucus. Lastly, Class IV and Class V may exhibit a properly structured CFTR protein, but the functionality of the protein is diminished (Veit et al. 2016). This allows for the transportation of chlorine ions, but the rate at which is needed to yield proper mucus is not yet met. Mutations to this protein can lead to the accumulation of dense mucus and cause inflammation and chronic lung infections. In some cases, this mucus blocks digestive enzymes from being transported to the small intestine through pancreatic ducts, which could lead to malnourishment. For the improvement of those diagnosed with CF, it is important to understand the effects on functionality that can result from a mutation to the CFTR gene. In terms of gene editing, CRISPR- Cas 9 would be engineered to find the site of the CFTR mutation and repair that specific target site. Currently, gene editing relies on the cell's natural DNA repair pathways

and cell division, although lung cells affected by CF do not usually divide, which poses a challenge. Fortunately, precise stem cells methods can be utilized as these cells undergo cell division more frequently (Cystic Fibrosis Foundation n.d.).

1.4 Gene editing and Gene Therapy

Both gene editing and gene therapy are methods for altering gene expression. The main aspect of gene therapy that sets it apart from gene editing is the element of introducing a new gene into a specific target cell. If scientists pinpoint a faulty protein due to genetic abnormalities, they can introduce a new functioning gene copy to compensate for the non-functioning disease gene. Scientists can improve protein functionality without altering the mutated gene by simply injecting a different gene that encodes protein instructions for normal function. Central to gene therapy is the choice of cell delivery technique. One approach involves utilizing a virus shell to "infect" the target cells and introduce the desired DNA. Various viruses, like adenoviruses and AAVs, are evaluated based on transduction efficiency, immunogenicity, and packing capacity (Naso 2017). In-vivo gene therapy entails packaging the gene into a virus and directly injecting it into the target cells. On the contrary, ex vivo involves extracting cells from the body and delivering therapeutic genes through viral transduction in the laboratory. Gene editing works with pre-existing genes and precisely edits and modifies the genetic sequence of target cells. Gene editing, because it is more recent than gene therapy, can often be regarded as a type of gene therapy with similar goals. Gene editing also relies on the body's two main natural DNA repair pathways, NHEJ and HDR to make the desired changes. Some common types of gene editing technologies being used currently include TALENs, Zinc Finger Nucleases, and CRISPR-Cas 9. Both methods mentioned above have been used for several disease applications and can be used to improve genetic conditions.

1.5 Current research on applications of gene editing for Sickle Cell Anemia

Sickle cell anemia is a genetically inherited disease in which the production of the hemoglobin protein results in a “sickle” or crescent shape that can block proper blood flow to various parts of the body. Because hemoglobin is a red blood cell that transports oxygen, its absence in regions of the body due to blockades can cause pain episodes, known as pain crises (Sickle cell disease 2019). Failure to prevent common blockades of the blood vessels could lead to organ damage, chronic complications, stroke, and anemia. Due to the nature of sickle cell disease, and the fact that it affects 3.2 million individuals globally, novel treatments have been given high priority and critical demands are gradually increasing yearly (Sundd 2019). The currently known treatment for the disease surrounds stem cell transplants, in which allogeneic hematopoietic stem cells, or donor stem cells responsible for the production of various blood cells, replace dysfunctional hematopoietic cells in a patient's bone marrow. However, more research being done in experimental gene editing clinical trials shows promising treatment options and potential cures for sickle cell anemia patients. This type of research explores the two types of Hemoglobin: Hemoglobin A (HbA) and Hemoglobin F (HbF), both found in the human body, but can compensate for abnormalities in the protein’s production. Hemoglobin A (HbA) is the predominant type of protein found in adult blood streams, whereas Hemoglobin F (HbF) is the fetal hemoglobin majorly found in fetal or newborn blood streams. The transformation of the production of HbF to HbA is facilitated by the BCL11A gene, which can effectively silence the expression of genes that produce fetal hemoglobin in erythroid cells (Sankaran 2013). A single point mutation to the HBB gene, which codes the beta globin chain of HbA, a protein subunit, leads to the production of Hemoglobin S, ultimately causing red blood cells to become rigid and sickle shaped (Arishi 2021). Eliciting the production of a normal HbF

instead of a mutated HbA, requires the BCL11A gene, which prevents the production of HbF, to be disrupted. The use of CRISPR-Cas 9 gene editing tools can effectively target the Erythroid enhancer region of the BCL11A gene and prevents it from silencing HbF production. Removing the Erythroid enhancer region can disrupt the gene expression and transcription factor of the BCL11A gene in red blood cell development for HbA. Various studies show that after the disruption of the BCL11A gene, levels of fetal hemoglobin increased rapidly from their initial baseline. Early adverse events show that patients undergoing this treatment are at risk for developing neutropenia or hepatic veno-occlusive disease, a non-obstructive coronary artery disease. Currently, more data is being gathered on overall efficacy and preclinical on-target and off-target analyses for regulatory approval and further follow-up studies (Psatha et al. 2018). With the applications of gene editing technology expanding, finding an effective therapeutic intervention for sickle cell anemia may not be as out-of-scope as previously believed.

1.6 Bioethical Concerns

The field of genome editing comes with substantial challenges, one of the most pressing being bioethical concerns. In order for a gene treatment to be considered “ethical” it should adhere to basic bioethical principles proposed initially by Beauchamp and Childress in 1979: Autonomy, Justice, Beneficence, and Non-Maleficence. Decision making for the patient should be free of coercion and fully informed, burdens and benefits of new treatments should be equally distributed among all societal groups, intentions must be good, and treatments should not harm the patient or others in society. Specifically in the diagnosis of prenatal sickle cell anemia (SCA), researchers and other healthcare workers raise some bioethical concerns. Diagnosis methods typically involve obtaining tissue sampling from the mother such as chorionic villus sampling (CVS) or amniocentesis. Both of these methods are considered invasive and could disclose potential harm such as risk of infection, bleeding, injury to the fetus or surrounding tissues, and in some cases, a risk of miscarriage (Fadare 2009). A major question to consider is whether the need for accurate diagnosis precedes any mentioned risks to the fetus. Gene editing also gives parents the option to choose from what poses to be a very difficult question: Whether or not to continue the pregnancy when an identified fetus is affected by sickle cell anemia. Disclosing information about a SCA diagnosis could call into question the rights of autonomy and reproductive choice/consent. This raises the discussion of the rights of the fetus and protection of a potential life as well as whether or not a meaningful life should be determined by a health condition. Another option that may be pursued more heavily in the future is genetic selection in which families can choose an embryo that is free of the genetic mutation causing sickle cell anemia. SCA affects many people on a global scale, not just nationally; almost 1 in 4 Nigerians have the sickle cell trait and Nigeria itself is considered the epicenter zone of the disease (Nwabuko et al. 2022). However, the Principle of Justice does not align with resource-limited locations such as Nigeria when considering the availability and accessibility of prenatal diagnosis of sickle cell disease within all of its societal groups. The benefits of new and novel treatments are not reaching the right demographic of people because of socio-economic limitations in Nigeria, whereas it is easily accessed in more Western parts of the globe. It is imperative to note that this issue is not only limited to Nigeria but other countries as well. In fact, some 40 percent of people in specific African countries have the sickle cell trait, and not all of these countries have services that are equitable and available to all the segments of its society (Serjeant 2013). Of course, this issue can have many individual solutions that may work for different families based on factors such as cultural beliefs, religious values, social standing, and economic factors. These are often sensitive matters and should be thoroughly discussed before

moving forward with any forms of treatment from both healthcare workers, researchers, and patients. Knowing the different perspectives that individuals may be concerned about is vital for giving personalized care and understanding how a treatment can impact a patient and society.

Other ethical concerns include “Designer Babies,” as they have often been called, which raise bioethical questions about tampering with the genetic pool and genetic makeup of future generations. Designer Babies cross the line of necessary medical intervention and enhancement of personal traits such as intelligence, physical appearance, or even personality. Eradicating hereditary illness has spurred some debate, although comparatively, there is massive controversy over using gene editing for non-medical enhancement purposes (Rothschild 2020). One cause for concern is the ability to access modification to new physical and intellectual capabilities creating a social divide. If access is based on wealth, the economic gap between the rich and the poor would increase not only monetarily, but in an entirely different quality of genetic “superiority” (Funk 2020). Additionally, “playing God” could have unintended consequences to the overall gene pool of humans and could impact the diversity of the human species. Not to mention, the ethical query of consent is ever present when genetically modifying embryos as it's impossible to obtain informed consent from an unborn baby. Any decision made on the parents behalf could have severe impacts on the baby’s autonomy and self-identity as well as the culture that surrounds those of the future generation. Inherited human qualities would be perceived as inferior to genetically enhanced qualities and could foster distorted societal standards of beauty, intelligence, and worth (Almeida et al. 2022). There is unfortunately no right method or answer of which side to the ever-growing debate is correct. Ultimately, Ethicists and Researchers should work concurrently to prevent any damaging courses of action from surfacing.

1.7 Conclusion

The precision and flexibility of CRISPR- Cas 9 has amazing implications in healthcare and on diseases such as Sickle Cell Anemia and Cystic Fibrosis. However, as the scientific community ventures into the field of genetic editing a fine and delicate balance must be found between scientific progress and ethical considerations, a major one being equitable access to treatments. Looking ahead, gene editing can open the field of medicine to more exciting, more personalized treatments. By matching treatments to individuals' genomes, gene editing could be a step in the right direction for preventative care for entire family lines. The future of gene editing is promising, but caution must be kept in mind as responsibility to future generations and impacts on overall society is a significant danger of misuse of genetic editing. How gene editing impacts the needs of the people must be the primary consideration researchers answer before going through or funding any major studies, trials, or treatments.

Resources

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