VCHEI SCIENCE CLUB

Vigilance of Contemporary Health and Environmental Issues

END OF YEAR 2024

Review Articles

TOPIC: **GENE EDITING**

April 2025

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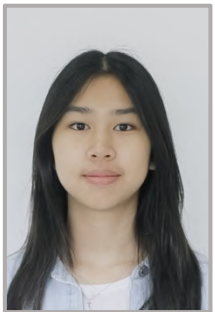

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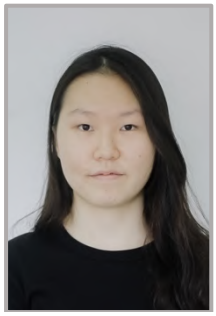

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Preface

Throughout history, the development of science and technology has always faced many challenges. Along with their development, many technologies inevitably receive backlash that require it to be halt and evaluated. Nevertheless, there is no doubt that the development of science and technology has also allowed us to improve humanity in many different ways, through the medical field, agriculture, environment, even in our daily life.

Gene editing is powerful yet controversial. Theoretically, it is a really promising tool that could be used for many advantageous benefit in a vast variety of field. It can be used to secure food resources, enhance biofuel production, and treat challenging diseases. At the same time, the boundaries of what it can and cannot do remains unknown and the unintentional effect of using it remains unpredictable.

In the last semester, Mountainview VCHEI Science Club members has come together to study contemporary issues surrounding Gene Editing technology. Each individual/pairs has study the benefits and uses of Gene Editing as well as the moral issues, concerns, and drawbacks behind the development of Gene Editing. We hope to show the current development, condition and status of gene editing and its advancement in the community. This combined information hopefully is enough to evaluate whether gene editing with all of its implications, the positive as well as the negative, is good or bad for humanity.

In a bigger scope this could also give us insight on how we should pursue and use science, any kind of it. Should we halt scientific development for the fact that it's impossible to predict all the dangerous risk and effect of a research procedure? Or should we overlook risks, in the pursuit of persume benefits? What are the moral boundaries and principles in conducting a science research? Should we pursue science just to see how far it may go, or should we pursue science only if it has possibilities to improve humanity? In the end, this issue will remain debatable, but engaging this through discussion, debates and reviews allow us to build perspectives upon science issues and help the science community to utilize science in the best way that could improve humanity.

April 2025, Editor

What Is the History of Gene Editing and What Has It Been Used For?

Debora W. Nuhamara

Introduction

All living things possess genetic information, which provides instructions for their growth, how they function and how they are able to reproduce [Matsumoto & Nomura, 2023; National Human Genome Research Institute (NHGRI), 2020]. The genetic information is stored in a form of a well-known molecule called DNA, which is the acronym of: deoxyribonucleic acid (Matsumoto & Nomura, 2023; NHGRI, 2020). Our DNA contains sequences of nucleotides called a gene that are essential to make protein which performs a specific task in our cell/body (Minchin & Lodge, 2019; NIH, 2020).

Nucleotide is the single unit of molecule that makes up DNA. It's made up of a (deoxy-)ribose sugar, a nitrogen base and phosphate group (Minchin & Lodge, 2019; NIH, n.d.). The chain of nucleotides is what makes one strand of the DNA, which bonds to another strand through base pair bindings, making DNA a double stranded molecule of nucleotides, as shown in Figure 1 (NHGRI, 2020).

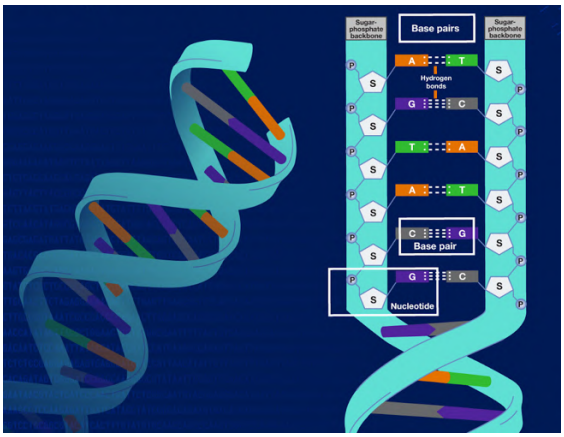

Figure 1. DNA Molecular Structure (NHGRI, 2020)

Since the discovery of DNA structure by Watson and Crick in 1953, the research about what we can do with this information has grown rapidly (Synthego, n.d.). The research has developed from understanding how the DNA functions in an organism's cell, imitating DNA processes in the cell, collection and creation of gene databases, until developing techniques to engineer DNA and alter their function in the cell (Illman, 2017). These discoveries eventually led to the invention of vaccines, gene therapies, and tools to engineer genetic materials in living organisms including plants, animals, microbes, even humans (Synthego, n.d.).

The revolutionary discovery of gene editing had paved ways to improve humanity. GMOs, for example, give a way to improve crop production and quality and improve the production of biofuel and other bioactive compounds (Sadeghi, 2023). In conservation, gene editing could also be used to promote species adaptation to harsh environment changes and exert extinction (Sadeghi, 2023). Gene editing in human's DNA also gives us means to potentially cure disorders/diseases that are caused by gene errors/abnormalities (Sadeghi, 2023). This includes some of the challenging diseases to cure and prevent like cancer, sickle cell anaemia, and AIDS (Sadeghi, 2023). However, the risks of using this technology in humans are still being evaluated and addressed (Datta, 2023).

As the development of gene editing continues, many concerns rise on the use and development of this technology. These concerns cover the aspects of humanity, social equality, human's consent, and the consequences of modifying nature processes. It leads to many controversies,

leaving many to question whether or not we should continue to allow genetic editing (Datta, 2023).

Just like any other technology, the discovery of gene editing has become a double edge sword. This raised a question, should we continue the development of gene editing or not? To answer this question, let's start by looking at the history of gene editing and evaluate how the discovery of this advanced but controversial technology has been utilized. This article will cover some of the milestones in the history of gene editing and then see how those discoveries have been used to impact our lives and the environment.

History and development of gene editing

Discoveries that leads to gene engineering technology. The concept of heredity has always been around in the science community as far as Charles Darwin suggested about natural selection in 1859. Gregor Mendel's experiments in 1865 on pea plant colours suggested that heredity is transmitted as units. In 1869, Frederic Miescher became the first who was able to isolate a DNA from a cell. Many discoveries later, the Mendelian unit of heredity is named as gene, and it is discovered that genes are made of DNA (genome.gov).

The discovery of DNA structure by Watson and Crick in 1953 continues to unfold the way heredity works. In 1958, Arthur Kornberg was able to make the first DNA in a test tube, by synthesizing all 5 nucleotides. Marshall Nirenberg was the one who cracked the genetic code in 1966 by figuring out the composition of nucleotides that encodes for 20 amino acids in protein. This allows scientists to understand how DNA works to encode proteins (Synthego, n.d.).

The discovery of DNA ligases in 1967 and restrictions enzymes in 1968 plays a significant role in engineering the DNA because these are the genes that are used in DNA repair and replication. The restriction enzyme was then successfully purified by Hamilton Smith the first time in 1970. The understanding of restriction enzyme function to "cut" DNA and how DNA protects itself become the basis of developing genetic engineering (Synthego, n.d.).

DNA Recombinant – 1971. With the "cut and splice" method, Paul Berg, in 1971, became the first scientist to create a recombinant DNA from more than one species. He first cut the DNA of two circular viruses, and modified the ends with enzymes to make them attach to each other. He then mixes the two cut and modified DNA together and incubates them so they will rejoin into a single circular DNA (Science History Institute, n.d.).

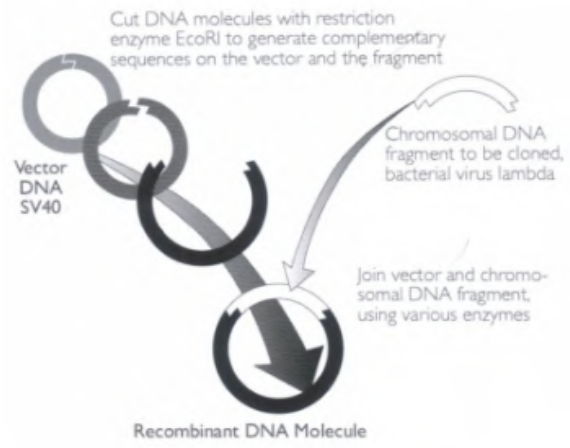

Figure 2. Paul Berg Cut and Slice method in DNA Recombinant
(Science History Institute, n.d.)

With the success of creating a recombinant DNA that can replicate naturally by Cohen and Boyer in 1972, DNA recombinant technology has been

used and developed for a lot of purposes. Some of them include recombinant insulin, vaccines (eg. HPV, Hepatitis, and pneumococcal virus), and antibiotics. It also leads to more advanced methods to create hybrid cells like monoclonal antibodies for genetic diagnostics and treatments purposes. In 1988, the first recombinant corn known as the Bt corn was introduced to the field, which contained genes from *Bacillus thuringiensis* (Bt) bacteria to adapt from pests (Synthego, n.d.).

Gene editing methods and application

Zinc Finger Nuclease (ZFN) – 1985. The discovery of ZFN has allowed "targeting genes" or directed genetic changes in a variety of organisms (Carroll, 2011). ZFN artificial proteins made with DNA recombinant technology that combines a non-specific restriction enzyme domain and 3 zinc fingers domain that each binds to a specific nucleotide, allowing scientists to make homologous cuts in a specific area of the DNA to repair/change them (Satheesh, Zhang, Wang, & Lei, 2019). Figure 3 shows how ZFN pairs are used to target a specific area of the DNA to insert/delete a gene in that area (Carroll, 2011).

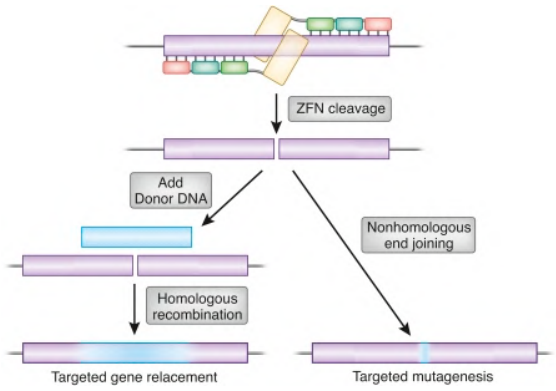

Figure 3. ZFN method
(Carroll, 2011)

The first example of the powerful use of ZFN is to block the expression of human oncogenes that was tested in a mouse cell in 1994 (Klug 2010). Successful attempts have been made in animals like fruit fly, silkworm, and mouse; plants like maize and tobacco; and in humans for economic and therapeutic importance (Carroll, 2011). The deletion of CCR5 gene by ZFN may allow the resistance to AIDS progression in HIV infection (Carroll, 2011).

TALE and TALEN – 2009. Transcription activator-like effectors (TALEs) are proteins from *Xanthomonas* bacteria that target promoters site in the DNA and induce gene expression (Becker & Boch, 2021). TALE-nuclease (TALEN) replaces 152 amino acids from TALE with a restriction nuclease and has been used more ever since (Becker & Boch, 2021). Like ZFNs, TALENs induce targeted DSBs that activate DNA damage response pathways and enable custom alterations (Gaj, Gersbach & Barbas, 2013). The enzymes were first sequenced and TALEN has the advantage of an increased target specificity, reduced toxicity, and a higher nuclease activity (Becker & Boch, 2021).

TALEN applications were wide in crops and livestock but also in other model and non-model organisms. It brought the first genome-edited crop to the market in 2019 which are resistant to pathogens. Because geneting editing of TALE sites are so precise and impact a small genomic mutation, TALE edited plants were classified as non-GMO in 2020. However, livestock such as micro pig and hornless dairy cows couldn't achieve this effect, raising anti-GMO concerns and did not achieve commercialization permit (Becker & Boch, 2021).

TALEN was the first genome editing tool that had saved life by curing cancer in 2015. Cellectis was the company that used TALEN to inactivate genes triggering non-self immune reactions from immune cells. The company used TALEN-edited CAR-T to kill tumor cells, curing two children (11 and 16 months) from leukemia (Becker & Boch, 2021).

CRISPR-Cas9 – 2013. The functions of CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) and CRISPR-associated (Cas) genes to eliminate invading genetic material as an adaptive immunity was first discovered in 2007 by Barrangau and colleagues (Reis, Hornblower, Robb, and Tzertzinis, 2014). But the identification of these genes was initially discovered in 1980 in *E. coli* (Reis, Hornblower, Robb, and Tzertzinis, 2014). Only in 2012, when Jennifer Doudna, Emmanuelle Charpentier, and their teams figured out the biochemical mechanism of CRISPR that it was able to be used as another technology for gene editing (Synthego, n.d).

Table 1: Gene editing technology throughout history and their application

Discovery	Year	Discovered/ developed by
DNA Recombinant	1971	Paul Berg
ZFN	1985	Klug et al.
TALEN	2009	Moscou et al., and Boch et al.
CRISPR-Cas9	2012	Jennifer Doudna, Charpentier

Discussion

The ZFN, TALEN and CRISPR-Cas9 method are the most prominent method used for genome editing. As ZFN method was discovered, it was the first method to allow "targeting genes" or directed genetic changes in a variety of organisms, allowing the future development of TALEN and CRISPR-Cas9. Figure 4. shows how all enzymes complexes that are used in each of the method are able to cut the DNA in a specific area using different site binding molecule, which develops to become more and more specific/precise as the technology develops. ZFN uses zinc molecules, to identify the nucleotides of the binding area, TALEN uses transcription activator like effectors, while CRISPR-Cas9 uses a chain of nucleotide that is complimentary to the nucleotide of the DNA in the binding site.

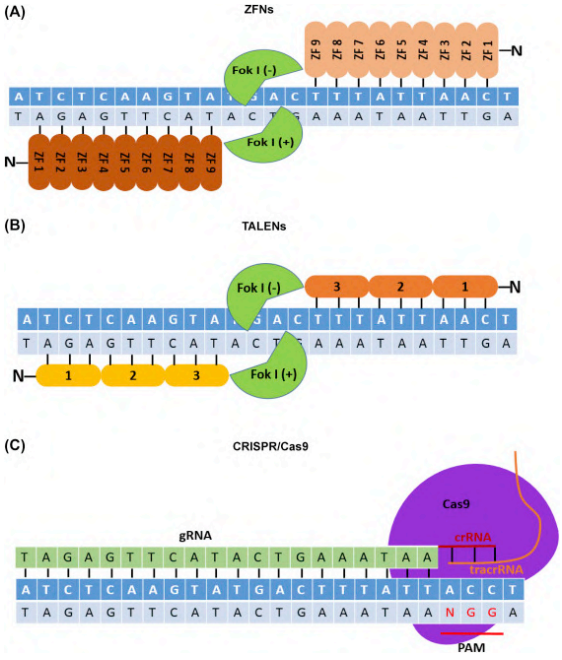

Figure 4. Comparison of ZFN, TALE/TALENs, and CRISPR-Cas9 method in gene editing
(Bao and Palecek, 2016)

Gene editing, especially in human are considered to be generally premature, having high risk and uncertainties (Cheng,

et al., 2022). Through the development of the 3 methods alone, we can see how genome editing methods are always developed to be better by time to time. But along with its rapid development, there are many unresolved issues and challenges surrounding gene editing. Some of the clinical products that are available today, could affect health negatively, even leading to death. The long term effect of using gene editing technologies in human has also not been able to be evaluated yet (Cheng, et al., 2022). Meanwhile, although not as concerning as in human, some issues that arise in the field of farm and agriculture are transparency (Cheng, et al., 2022).

While safety guidelines needs to be build and evaluated to make gene therapy safer, there are also effort being made to resolve the negative effects of gene therapy on health. For example, Retron Library Recombineering (RLR) was introduced in 2020 to eliminate the toxicity associated with CRISPR, and enables scientist to investigate mutation in genome level (Cheng, et al., 2022). However, just like the previous technologies, these does not automatically solve the issues and concerns in using gene editing as therapy in human and other organism. As biology is variable and complex, it is hard to 100% correctly predict the outcome when using these technology (Cheng, et al., 2022).

However, it is also clear that gene editing has enable us to treat what was previously a challenging disease, like cancer, sickle cell anaemia, and even deadly infections like HIV and hepatitis. Using gene editing in agriculture are also shown to more likely secure food and energy in a growing population and with climate change. Table 2 shows the application of each of the most prominent gene editing methods throughout the years.

The advancement of gene editing could still be viewed as a viable way for humanity to progress. This is why developing the technology is important. Many has feared that gene editing could also be used for designing babies for non-medical uses, to achieve traits that is viewed superior in babies. Although editing the embryo itself is still debated, this is a concern that is also being brought up in the scientific community. That is why it is important that the development of the technology is ethically bind with the necessary goals to improve humanity but also values what it means to be human.

Table 2: Gene editing technology and its application

Discovery	Application
DNA Recombinant	Recombinant insulin, vaccines (HPV, Hepatitis), antibiotics, Bt Corn.
ZFN	Fruit fly, silkworm, tobacco, maize, deletion of CCR5 gener to halt AIDS progression
TALEN	Resistant to pest corps in markets, TALEN edited CAR-T cells to kill leukaemia cells.
CRISPR-Cas9	Mostly in gene therapy for cancer, tumours, and genetic related diseases but also in agriculture, fisheries, and conservation.

Conclusion

The historical concept of gene comes back as far as Charles Darwin suggestion of natural selection in 1859. With the development of science, we were able to understand how heredity works and functions, and eventually even discovered ways to manipulate it. ZFN, TALE/ TALENs, and CRISPR-Cas9 has been the most prominent methods used to edit genomes in the past 4 decades. Even though there are drawbacks, what has been done so far in manipulating the genes are used to mainly improve humanity, through treating challenging diseases, securing food and energy, and conserving nature. This shows that developing gene editing is important for humanity. As improvements needs to be made to make the technology more reliable, guidelines should also be used to develop and use the technology more ethically.

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Advantages of CRISPR-CAS9

Jansen Thoe

Introduction

CRISPR-Cas9 is a gene-editing tool that edits genes by removing, adding, or altering sections of the DNA sequence. It was accidentally founded by Jennifer Doudna and microbiologist Emmanuelle Charpentier who were studying Streptococcus pyogenes, an aerotolerant bacteria in the genus Streptococcus. CRISPR-Cas9 is the most widely used genome editor due to multiple factors (Synthego, n.d.). This paper will show some of the advantages of CRISPR-Cas9 for gene editing and why it is widely used compared to other genome editing methods like TALEN or ZFN.

Comparison between CRISPR-Cas9, TALEN, and ZFN gene editing tool

Precision. The first advantage of the CRISPR technology is its precision. This comes from its two key components: a Cas nuclease enzyme and a guide RNA (gRNA). The Cas nuclease binds to and cuts DNA, while the gRNA directs the enzyme to the specific DNA sequence that needs to be edited. This level of accuracy allows scientists to target particular genes, making it a powerful tool for genetic editing (Synthego, n.d.).

Compared to TALEN, CRISPR-Cas9 is less precise, for it binds to a longer recognition sequence (32 nucleotides) than CRISPR (20-24 nucleotides), which means fewer off-target effects (Palmgren, 2024). CRISPR-Cas9 is more precise than ZFN because of CRISPR's single guide RNA, which makes it more precise when targeting the DNA strandeasier to design targets, compared to ZFN which uses protein as guide (Szczesna, 2023). The use of RNA as a guide in CRISPR also allows for an easy design to target the genome, reduce the possibility of off-targeting, and simultaneously edit the genome in multiple sites.

Efficiency. In addition to its precision, CRISPR is highly efficient and relatively fast. Scientists can easily design the guide RNA, which speeds up the editing process significantly compared to older techniques (Broad Institute, n.d.)

Cost. The cost of producing CRISPR components, like the gRNA and Cas9 enzyme, is also lower, making this technology more affordable and accessible to a wider range of researchers. Its simplicity makes it even more appealing because it's easier to program than other gene-editing tools. CRISPR-Cas9 is a flexible option for genetic research and medical uses, unlike TALEN and ZFN, which might take more time and money to operate.

Conclusion

In conclusion, CRISPR-Cas9 has multiple advantages that make it more popular than other gene-editing methods like TALEN and ZFN, mainly because of its efficiency and overall precision. However, much research and developments to improve CRISPR-Cas9 performance could still be done. Some of them include reducing the effect of off-targeting, Even with CRISPR-Cas9's stellar performance in gene-editing, it can still be improved. One way to improve it may be to optimize the sgRNA and anti-CRISPR proteins to reduce the off-target effects of CRISPR-Cas9. As

CRISPR main development and use is for therapy, this would improve therapeutic effects and benefits of CRISPR. (Mengstie, et al. 2024)

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CRISPR in Conservation: Innovation or Ecological Gamble?

Aurelia S. Tanuwijaya and So Yeon Choi

Introduction

Gene editing has been used to genetically modify organisms for years, but recently scientists have questioned whether they would be able to use gene editing to conserve species of endangered animals and environments. As modern science continues to advance, scientists and researchers alike began developing new approaches to genetic editing, such as CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats), cross breeding, cloning, and various alternative methods.

These aforementioned methods have proven effective, as demonstrated by the successful attempts at conservation of the American Chestnut, the Black-Footed Ferrets, and coral reefs. Despite their success, these efforts have raised numerous ethical concerns, especially in regards to their impact on the ecosystem. This article aims to explore the necessity of gene editing by analyzing its applications and notable successes. Ultimately, this article supports that gene editing is unnecessary for wildlife conservation due to its inherent unpredictability and potential to disrupt ecosystems, creating imbalances in natural environments.

Gene Editing in Conservation

Traditionally, conservation efforts generally focus on reducing specific threats to a species (Withers and Jenkins, 2023). On the other hand, a newer approach for conservation prioritizes the improvement of species resilience, characterized by a species' resistance and adaptability to threats (Withers and Jenkins, 2023). A combined strategy—mitigating threats while fostering resilience—can strengthen conservation outcomes, especially amid climate change (Withers and Jenkins, 2023).

Genetic diversity is a key component of resilience, as it increases the likelihood that some individuals within a species will possess traits that resist shocks like disease or extreme temperatures (Withers and Jenkins, 2023). Studies indicate that forests with higher genetic diversity or targeted traits, such as pest resistance, exhibit greater resilience to environmental threats (Withers and Jenkins, 2023). Through gene editing, specific adaptive traits can be introduced to species, helping them cope with evolving environmental challenges and improving conservation effectiveness (Withers and Jenkins, 2023).

Thus far, scientists and conservationists use gene editing to accelerate the natural selection process by adding resilient traits to species, enhancing their adaptability to environmental threats (Withers and Jenkins). Using CRISPR technology, they can introduce favorable traits via gene drives, which spread these traits rapidly throughout a population by making them dominant (Withers and Jenkins). Additionally, gene drives can also be self-limiting, ensuring the new traits only persist for a designated period (Withers and Jenkins, 2023). By increasing genetic diversity and resilience through selected traits, gene editing can bolster a species' survival chances (Withers and Jenkins).

How ethical the process of genetic editing is and how big of an impact it could cause on a species' environment, biodiversity, social structure, and food chain has been a major setback to genetic editing in conservation (Bosshert, 2024). Genetic modification imposes human control over the animal's behavior and characteristics, these can affect an animal's survival instincts and natural behavior; regardless of the intention to save them, animals have no choice over this raising the ethical concerns (Bosshert, 2024). Furthermore, releasing genetically modified animals can carry large unforeseen impacts on the environment (Bosshert, 2024). The ecosystem is very complex, every living organism is interconnected, a change in one organism may spark a chain reaction throughout the entire ecosystem (Bosshert, 2024). These consequences are far too great of a risk, leaving scientists unable to release their creations.

Gene Editing Applications in Conservation

As previously discussed, there have been three notable instances of gene editing applied toward species conservation. The first case involves the American Chestnut, an iconic tree species in the United States during the early 20th century (Withers and Jenkins, 2023). Its chestnuts served as a crucial food source and habitat for various animal species, and its wood, regarded as quality, contributed significantly to the economy (Withers and Jenkins, 2023). However, a devastating fungal disease known as chestnut blight emerged, nearly eradicating the population (Withers and Jenkins, 2023). To combat this disease, scientists cross-bred the American Chestnut with a blight-resistant species, rendering it less vulnerable to the fungus (Withers and Jenkins, 2023). Regrettably, while the disease no longer posed a threat, it also led to the loss of many defining characteristics of the American Chestnut, including its large growth potential and superior wood quality (Withers and Jenkins, 2023). In this particular case, gene editing becomes redundant as it leads to the loss of many distinct characteristics of the tree.

Coral reefs, essential to global biodiversity, are now threatened by rising ocean temperatures (Withers and Jenkins, 2023). Scientists believe gene-editing techniques could enhance coral resilience to heat stress (Withers and Jenkins, 2023). Predictive models have shown that introducing a broader range of resilient traits, rather than just heat resistance, could result in larger, more sustainable coral ecosystems with minimal human intervention (Withers and Jenkins, 2023). This approach, focusing on a wide range of adaptive traits, has shown to be more effective than selective breeding for heat tolerance alone, as genetic diversity generally strengthens species' defenses against multiple threats, including disease, proving gene editing to be beneficial for the environment.

The black-footed ferret, a species nearly driven to extinction by disease in the early 20th century, now has an estimated population of only 300 individuals, all descended from a mere seven ferrets, which has resulted in critically low genetic diversity (Withers and Jenkins, 2023). In 2021, scientists achieved a breakthrough by cloning a black-footed ferret using frozen cells from a long-deceased individual (Withers and Jenkins, 2023). The cloned ferret, named Elizabeth Ann, was

introduced to enhance genetic variability within the population, potentially improving the species' resilience to environmental changes (Withers and Jenkins, 2023). If deemed safe, this milestone could substantially enhance the black-footed ferret's capacity to endure future environmental challenges (Withers and Jenkins, 2023). Although such a feat is highly impressive, there remains much uncertainty surrounding her reproductive viability and the potential effects on the ecosystem. Her creation leaves too many unanswered questions that, at this point, are difficult to answer.

Discussion

While gene editing offers promising possibilities for advancing wildlife conservation, its application is not without significant uncertainties and risks. Techniques like CRISPR have demonstrated potential for enhancing species' adaptability and genetic diversity, as seen in efforts to protect coral reefs and boost the genetic viability of the black-footed ferret. However, these successes also highlight critical questions that remain unanswered about the long-term implications of gene editing on ecosystems. For instance, how will introducing genetically modified traits affect ecological interactions? Will these traits spread uncontrollably, leading to unforeseen consequences for other species and the environment? Even with gene drives designed to be self-limiting, the potential for mutations or unintended persistence of traits poses a serious concern.

Another unresolved issue is the difficulty in predicting how edited species will interact with their natural habitats. For example, if a modified species becomes more resilient to threats, could it outcompete other species and disrupt the delicate balance of the ecosystem? This is especially concerning in cases like the black-footed ferret, where cloning efforts aim to increase genetic diversity but raise questions about reproductive viability and ecological compatibility. What if introducing genetically modified individuals leads to unforeseen diseases or genetic vulnerabilities that were not apparent in controlled laboratory settings?

Furthermore, there are ethical dilemmas regarding the role of humans in shaping natural evolution. Should we intervene to this extent in nature, and how do we determine which species or traits are prioritized for genetic enhancement? These questions are compounded by the unpredictability of climate change, which adds another layer of complexity to forecasting the success or risks of gene editing over time.

Conclusion

At present, the scientific community lacks the tools and data to comprehensively assess the long-term ecological impacts of gene editing. Without a thorough understanding of these risks, the use of gene editing in conservation must be approached with extreme caution. While it holds the potential to save endangered species and bolster ecosystems, it also risks causing imbalances that may be irreversible. Moving forward, a more balanced conservation strategy should focus on mitigating threats, preserving habitats, and fostering resilience through natural means, with gene editing serving as a last resort rather than a primary solution.

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Gene Editing for the Advancement of Biofuels

Mahardhika C. Ananta

Introduction

Biofuels play an important role in society because they can replace fossil fuels due to reduced emissions, resource depletion, and dependence on foreign suppliers (U.S. Environmental Protection Agency, 2022). However, they might use many resources like land, water, etc. Biofuels also positively impact the environment, such as increasing demand for biofuels, which could improve farm incomes by creating new markets for agricultural products (U.S. Environmental Protection Agency, 2022).

Microbial biotechnology has a crucial role in bioprocess, where microorganisms and their enzymes are used for the conversion of carbohydrates, lignins, and glycerols into various renewable resources like bioenergy production (Ramamurthy et al., 2021). The buildup of agronomic and industrial wastes causes significant environmental problems (Ramamurthy et al., 2021). To reduce this problem, microorganisms plays an important role in various biotechnological progressions such as in the microorganism fermentative methods (Ramamurthy et al., 2021). Furthermore, microbes are multifaceted moieties that are helpful in the utilization and bioconversion of biomass as they are essential sources of enzymes with biotechnological capability (Ramamurthy et al., 2021).

Microbial-produced biofuels are a sustainable way to make energy (Ali and Wilder, 2022). Different microbes can turn biomass into bioethanol and biodiesel (Ali and Wilder, 2022) For example, Saccharomyces cerevisiae can make bioethanol by fermenting non-food material like rice husks, wheat straw, and corn stover (Ali and Wilder, 2022). These materials are cheap and help reduce waste (Ali and Wilder, 2022). Microalgae's fast growth is also helpful for biofuel production (Ali and Wilder, 2022).

Fermentation is simple and efficient, so microorganisms are recognized as great sources for making biofuels through various processes, such as glucose catabolism, lipids oxidation, and using the isoprenoid pathway. However, the fermentation of microorganisms can also produce by-products that interfere with the desired biofuels. This problem is common in large-scale manufacturing systems, which in turn has called for a drastic change in their current strategy of efficient and cost-effective biofuel production. Fortunately, with the continuous advance of genetic and genome engineering, it is now possible to implement various microbial strains to address by-products issue. CRISPR-Cas system can help make specific genetic changes in industrial microorganisms to improve biofuel production (Garg et al., 2023). This article will talk about gene editing applications in microbial biofuel production to show the significance of this technology in the biofuel industry.

Genetic Engineering in Fatty Acids Pathway

Fatty acid metabolism in microbes requires multiple enzymes as shown in Figure 1. Genetic engineering could improve Fatty Acid production by improving its metabolic pathway in

microbes. There are some ways to achieve this goal including overexpression of fatty acid metabolic enzymes, blocking the lipid degradation pathway, and modifying the fatty acid profiles (Lin et al., 2012).

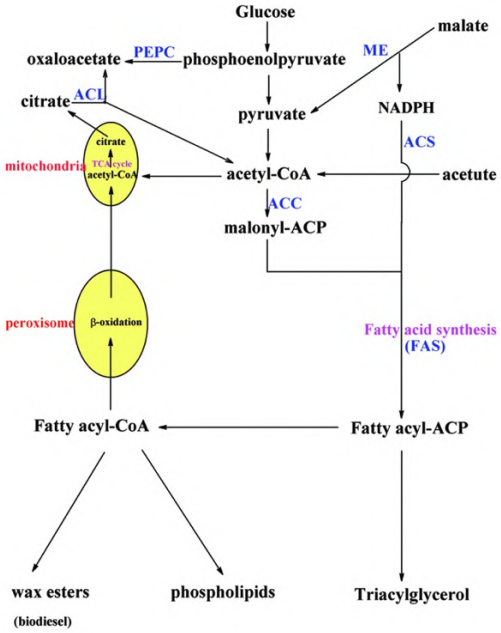

Figure 1. Fatty Acid Pathways in Microbes (Lin et al., 2012)

Overexpression of Fatty acid metabolic enzymes. The ACC enzyme is an important enzyme that is involved in the early stage of fatty acid biosynthesis in cells. When ACC is overexpressed in bacteria like E.coli, it can increase the levels of malonyl-CoA, leading to a 6x fatty acid production. Similarly, overexpressing ACC from Arabidopsis in potatoes resulted in a 30% increase in fatty acid content. Another example is the enzyme Acyl-CoA synthetase helps speed up the process of thiosterification of fatty acids, which is an important step in fatty acid metabolism. However, the process of overexpressing with ACC enzyme is not enough to optimize this process and still has to be supported with other gene editing (Lin et al., 2012).

Blocking the lipid degradation pathway. In Cells, lipids are broken down into energy, but blocking the breakdown pathway of the lipids can lead to an increase in fatty acid levels. One major pathway for lipid breakdown is β-oxidation, which can be inhibited by deleting the fadD gene in E.coli or blocking the acetyl-CoA transport system in Candida tropicalis. Additionally, lowering the activity of PEPC, an enzyme that converts a lipid component into oxaloacetate, can also increase fatty acid accumulation, as seen in Brassica napus. These highlight how preventing lip degradation can result in higher fatty acid production (Lin et al., 2012).

Modifying Fatty Acid Profiles. Modifying fatty acid profiles is key to improving biofuel production. Acyl-ACP thioesterases, which release fatty acid chains from acid synthase (FAS), determine the length of fatty acid chains. Overexpressing specific acyl-ACP thiosterases that favor shorter chains can increase the production of short-chain fatty acids, in microbes such as E.coli. Enzymatic transesterification using lipases is a common method for producing biodiesel, where fatty acids and alcohols are converted into biodiesel. The founding of an intracellular transesterification system will be helpful to the production of biodiesel to be more effective (Lin et al., 2012).

Current Application of Genetic Engineering in Microbial Biofuel

Genetic engineering is key to improving microbial biofuel production, especially by increasing fatty acid production and transesterification. Other methods include engineering microbes to secrete lipophilic compounds and use substances more efficiently E.coli is commonly used for these genetic changes,

but current strains are not yet good enough for large-scale biodiesel production. To compete with fossil fuels, microbes need to be more efficient and capable of cheaper substrates. Overexpressing enzymes may not have a big enough impact, so systems metabolic engineering, which looks a multiple pathways, could be more effective. It's also important to consider how well these engineered microbes will perform on a large scale in industry (Lin et al., 2012).

Conclusion

In conclusion, genetic engineering is important for improving the production of biofuels production through microbes. By making changes to how microbes produce fatty acids, such as increasing enzymes or blocking certain pathways, we can increase biofuel production. Scientists keep coming up with new research and the application of technologies like CRISPR-Cas addresses the challenges like large-scale use. As a result, microbial biofuels became more affordable and sustainable material as a substitute for fossil fuels.

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Application of Recombinant DNA Technology in Marine Microbes Research

Jasmine P. Laksono

Introduction

The vast biodiversity and abundance of marine microbes highlight their immense potential as a promising resource, offering significant opportunities for marine biotechnologists to discover economically valuable natural products. According to Dahuri (2003), Indonesia's marine biodiversity includes at least 782 species of algae, 12 species of seagrass, 38 species of mangroves, 850 species of sponges, 210 species of soft corals, and hundreds of thousands of other macro-micro marine species, showcasing the nation's rich oceanic wealth. Beyond these species, many types of marine microbes remain under-explored and underutilized. These organisms hold potential as sources of food, medicines, and various high-value products.

The discovery of restriction endonuclease enzymes in 1971, capable of catalyzing the cutting of DNA molecules at specific sites, marked the advent of a revolutionary technology in molecular biology known as recombinant DNA technology or genetic engineering (Murdiyatmo, 1992). Recombinant DNA technology involves combining DNA from different species to create hybrid organisms with desired traits (Hala, 1999).

In the field of marine and fisheries biotechnology, this technique can be applied to explore the potential biodiversity of marine organisms. Marine organisms, particularly marine microbes, which were once merely considered a potential natural resource without added value, can now be transformed into highly promising products through recombinant DNA technology. For example, functional genes from these microorganisms can be isolated, encoding one or more enzymes with significant applications in the medical field. Key enzymes regarded as essential in medicine include proteases, lysozymes, lipases, invertases, hemicelluloses, collagenases, glucosamines, dextranases, and catalases (Suhartono, 1989).

Advancements in molecular technology have enabled gene transfer between entirely unrelated organisms—for instance, transferring human genes into bacteria or pigs (Muladno, 2002). This capability to transfer genes from one organism to another holds immense potential, as genetic engineering can reduce costs and enhance the production of materials widely used in medicine, pharmaceuticals (Murdiyatmi, 1992), agriculture, industry (Prentis, 1990), and even marine science. Marine microorganisms are a fascinating reservoir of genetic and functional diversity, and their products could be utilized in many different biotechnological sectors. For instance, they can produce bioactive natural compounds, enzymes useful for industrial applications, or pharmaceutical agents with anticancer, antimicrobial, or anti-inflammatory properties (Zeaiter et al., 2018).

The application of genetic tools such as appropriate plasmids, and transformation methods are well understood for model organisms like *E. coli*, which has led to the development of modified strains that improved the

efficiency of transformation and maintenance of plasmids (Thoma and Schobert, 2009, Grant et al., 1990). Recent advances in sequencing technology, and -omic analyses have contributed to an increased understanding of the genetic potential of marine bacteria. This articles purpose is to explain DNA recombinant technology and show its many application in marine microbes.

Recombinant DNA Technology

The success of recombinant DNA techniques heavily relies on how microorganisms or phages are handled and developed, as well as the selection and cloning of bacterial strains used in the system (Artama, 1991). According to Brown (1991), plasmid and phage DNA molecules possess fundamental properties required as cloning vectors. However, these properties are only effective with experimental techniques for manipulating DNA molecules in the laboratory.

The basic steps of gene/DNA cloning require several specific skills:

1. Preparing pure DNA samples.
2. Cutting DNA molecules using restriction enzymes.
3. Analyzing DNA fragment sizes.
4. Joining DNA molecules with ligase enzymes.
5. Introducing recombinant DNA molecules into host cells.
6. Identifying cells that contain cloned recombinant DNA molecules.

(Artama, 1991)

Components of Cloning Experiments

In conducting a cloning experiment, there are five essential components that must be fulfilled for the experiment to proceed successfully. These components include the donor DNA, restriction endonuclease, vector, DNA ligase, and host cell. The function of each of these components is outlined in the table below.

Table 1: Components and Function of DNA Recombinant Components

Cloning Component	Function
DNA Donor	One of the sources of the DNA or genes being cloned from marine microbes.
Restriction Endonuclease	Enzymes are used to cut both the donor DNA and vector at specific sites, allowing the donor DNA to be ligated into the vector.
Vector	Plasmids or bacteriophages are used to introduce the gene to be cloned into a suitable host cell.
DNA Ligase	The enzymes used to ligate the splice ends of the vector and donor DNA, thereby forming a recombinant vector.
Host Cell	Usually a bacteria or yeast where the recombinant vector is introduced.

Restriction Endonuclease. The restriction endonuclease are bacterial enzymes that recognize specific nucleotide sequences within a double-stranded DNA molecule and cleave the DNA at those nucleotides sites. These enzymes cut the DNA into fragments of various sizes, depending on the frequency of the enzyme's recognition sites within the molecule. Restriction endonucleases are typically isolated from prokaryotic microorganisms (Artama, 1991).

According to Brown (1991), there are three types of restriction endonucleases that are recognized, each distinguished by

its slightly different mode of action. Types I and III endonucleases are relatively complex and have very limited roles in genetic engineering. On the other hand, type II restriction endonucleases are crucial enzymes in genetic engineering, particularly in gene cloning activities. Type II endonucleases used in cloning experiments recognize base pair sequences ranging from 4 to 8 nucleotides in length. These enzymes are named based on the bacterial species from which they were isolated. For example, the restriction enzyme EcoRI is named after the first restriction enzyme isolated from *Escherichia coli* strain R, and the enzyme Hindlll is named after the third restriction enzyme isolated from *Haemophilus influenzae* strain D (Stanfield *et al.*, 1997).

Since the early 1970s, approximately 300 types of restriction enzymes have been discovered (Prentis, 1990). These enzymes possess two key properties that make them highly useful. The first property is their specificity; each enzyme recognizes and cuts only a particular nucleotide sequence in DNA. The second property is their ability to generate sticky ends when they cleave double-stranded DNA, producing overhanging or "sticky" ends (Marx, 1991).

Most restriction endonucleases function effectively at a pH of 7.4 and in ionic strength, typically with NaCl. Additionally, all type II restriction endonucleases require Mg^{2+} to function properly (Brown, 1991; Artama, 1991). Furthermore, the activity of type II endonucleases is often enhanced by reducing agents such as 2-mercaptoethanol or dithiothreitol (Suhartono, 1989).

Creating the proper conditions for enzyme activity is crucial, such as the correct concentrations of NaCl or Mg^{2+}. If these conditions are not optimal, not only can enzyme activity decrease, but the enzyme's specificity may also be altered, causing DNA cleavage at non-canonical recognition sites.

Vector. A variety of vectors from prokaryotic, eukaryotic, and synthetic sources have been widely used in DNA cloning systems. Generally, there are four vectors commonly used in gene cloning processes (as cloning vectors): plasmids, phages, yeast artificial chromosomes (YACs) (Suharsono, 2000), and cosmids (Artama, 1991). According to Suharsono (2000), the choice of vector depends on the cloning objective. For constructing cDNA libraries, plasmids are typically used as vectors, whereas for constructing genomic libraries containing large DNA fragments, phages, cosmids, or YACs are more commonly employed.

A cloning vector must have restriction on endonuclease recognition sites where genetic information can be inserted. Some cloning vectors are engineered to include a multiple cloning site (MCS), which contains several distinct restriction sites. The key characteristics required for an effective cloning vector include:

1. Stability in the host cell (Noviendri 2007).
2. Containment of specific antibiotic resistance genes to facilitate the selection of recombinant genes (Artama, 1991).
3. Possession of an origin of replication (Ori) to enable self-replication or multiplication (Marx, 1991). This allows the plasmid to replicate autonomously and control replication.

4. The presence of restriction sites or MCS that can be cleaved by specific restriction endonucleases (Artama, 1991).
5. Small size is ideal for ease of handling (Noviendri, 2007).
6. Cleavability at a single site by restriction of endonucleases (Noviendri, 2007).
7. Non-conjugative properties, ensuring it is not transferred by conjugation (Noviendri 2007).
8. Distinct detectable traits (Stanfield et al., 1997).
9. Ease of isolation from host cells (Stanfield et al., 1997)

Based on their natural replication abilities, plasmids can be categorized into two types:

1. Stringent plasmids: These plasmids have strict replication control, resulting in only one copy of the plasmid per bacterial genome.
2. Relaxed plasmids: These plasmids have less stringent replication control, allowing 1 to 20 copies per cell (Artama, 1991).

The copy number of a plasmid refers to the number of plasmid molecules found in single bacterial cells. Plasmids with relaxed control can replicate independently and produce between 10 to 200 copies per cell. This high copy number makes them valuable for recombinant DNA research, as they provide abundant DNA for experiments. These plasmids replicate independently of the host cell's chromosomal DNA (Nicholl, 1994). In contrast, plasmids with stringent control at a rate similar to the host cell's chromosomal DNA, resulting in only one or a few copies per cell. This low copy number reflects their tight replication regulation (Brown 1991).

Antibiotic Resistance Markers. To study various bacterial activities in their habitat—whether genetically engineered or not—molecular markers are essential. These markers involve using genes that are easy to assay in terms of their expression and activity (Noviendri, 2007). This enables monitoring of bacterial activity in their habitat, allowing for informed decisions about subsequent steps or interventions (Wahyudi, 1997).

In labs, antibiotic resistance markers are commonly used as selectable markers to confirm that bacteria in culture contains a specific antibiotic-resistant gene (Brown, 1991). These markers are the most frequently used and serve as the foundation for developing their marker techniques. The advantage of this method lies in its ability to selectively distinguish live bacterial populations from marked bacteria. This selection can be achieved through simple plating techniques using the appropriate antibiotic, making the process efficient and straightforward (Noviendri, 2007).

Transformation plasmid. Naturally, plasmids can be transferred to a new host cell through processes such as conjugation or other mechanisms. However, in the lab, they can be transferred artificially through a process called transformation. During transformation, plasmids are introduced into bacteria that have been made competent, meaning the cell walls temporarily become permeable, allowing small DNA molecules to enter (Artama, 1991). To facilitate plasmid entry, the host cells must first be made competent. The cells used in the transformation process are referred to as competent cells (Muladno, 2002).

In the transformation process, competent cells mixed with recombinant

DNA molecules face three possible outcomes:

1. The competent cells fail to take up any DNA (Noviendri, 2007).
2. The competent cells take up vector DNA without the target gene (Noviendri, 2007).
3. The competent cells take up recombinant vector DNA carrying the target gene (Muladno, 2002).

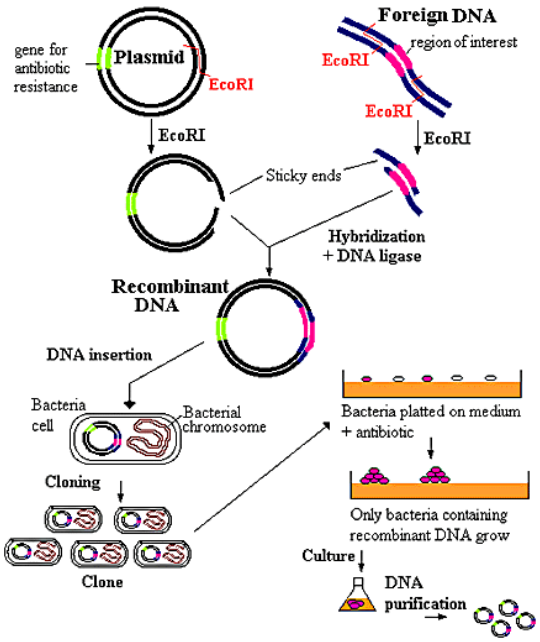

Figure 1. Steps in DNA Cloning (Singh et al., 2012)

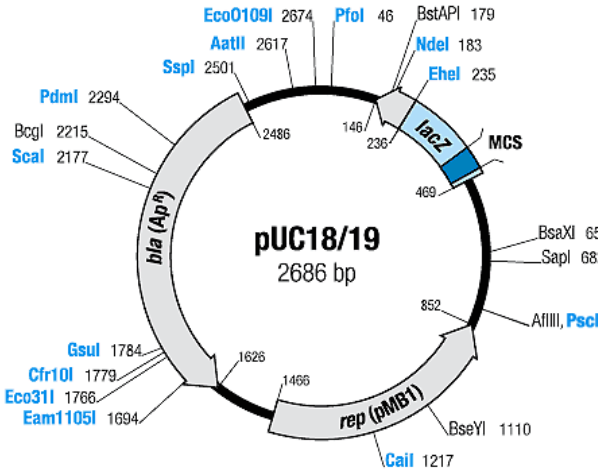

Figure 2. Diagram of a plasmid – pUC18 (El-Gendy et al., 2006)

Recombinant DNA Technology Application

The use of recombinant DNA technology for exploring microbes, particularly marine microbes, has been widely applied. The objectives of employing this technology in marine microbiology are diverse. Consequently, as this method is adapted to various purposes, modifications to the procedures are often made. This approach does not adhere to fixed sequence steps.

The choice of method depends on the gene to be transferred and the type of organism receiving the new genetic information. These decisions are also influenced by the preferences and expertise of the individual scientist involved (Prentis, 1990). However, the fundamental principles underlying recombinant DNA technology remain consistent (Noviendri, 2007).

Recombinant DNA Technology Application in Marine Microorganism

Recombinant DNA technology has been extensively applied to marine microbes for various purposes, significantly enhancing their value beyond being mere components of marine biodiversity. These applications include construction gene libraries (Libl, 2006), engineering gene clusters for polyketide synthase I (PKS-I) (Grozdanov et al., 2006), discovering antitumor metabolites (Calle, 2006), cytotoxic compounds (Webb et al., 2006), lycopene β-cyclase inhibitors (antioxidants) (Tofighi et al., 2006), and novel antimicrobial enzymes (Eck et al., 2006). Additionally, new exopolysaccharides (EPS) (Delbarre-Ladrat et al., 2006) have been identified, as well as genetic diversity explored for comparative studies (Suwanto, 2004). The technology has also supported

bioremediation efforts, such as developing microbes capable of degrading oil spills, and enabled the production of biodegradable plastic precursors like poly-β-hydroxyalkanoates (PHA) (Noviendri, 2004). These advancements demonstrate the transformative potential of recombinant DNA technology in unlocking the vast industrial and environmental benefits of marine microbes.

Conclusion

In the development of marine and fisheries biotechnology, recombinant DNA technology can be utilized to explore the potential and biodiversity of marine organisms. Marine organisms, particularly microbes, which were previously considered merely as part of the ocean's natural wealth with untapped potential, can now have their value significantly enhanced through recombinant DNA technology, enabling the production of highly promising and valuable products.

Recombinant DNA technology has revolutionized our ability to manipulate genetic material, providing invaluable tools for research and industrial applications. The success of this technology hinges on precise experimental techniques, including the preparation and manipulation of DNA, selection of suitable cloning vectors, and creation of competent host cells for transformation. Essential components such as restriction enzymes, ligase, and specific vectors facilitate the integration and expression of target genes, while markers like antibiotic resistance genes ensure the successful identification of recombinant constructs. These foundational elements highlight the systematic and methodical nature of recombinant DNA processes.

The application of recombinant DNA technology extends significantly into marine biotechnology, where it has unlocked the potential of marine microbes. These organisms, previously seen as untapped biodiversity, are now sources of high-value products such as antitumor agents, biodegradable plastics, antioxidants, and enzymes with antimicrobial properties. From bioremediation efforts to genetic diversity studies, the versatile application of this technology demonstrates its transformative impact. As methodologies continue to evolve and diversify, recombinant DNA technology will remain central to advancing marine biotechnology and addressing global environmental and industrial challenges.

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Genome Editing for Genetic Disorder

Midem Kim

Introduction

Germline mutations are a primary cause of genetic disorders. It occurs when mutation takes place in the parent's reproductive cells and the mutated gene is passed down to the next generation (Cleveland Clinic, n.d). This is the cause of disability from birth.

Genetic disorders can bring great limitations to a person. Such an example would be physical and mental limitations to a person with Down syndrome. People with Down syndrome have developmental disorders like autism, higher risks of mental illnesses like depression and anxiety as well as experiencing physical limitations having to carry celiac disease that causes digestive problems and hypotonia that causes their poor muscle tone (National Institute of Health, 2023). These are what makes genetic disorders a limitation to the human race.

As technology advances, humanity has created ways to treat and approach patients as new treatments enter into the medical field (Rubeis and Steger, 2018). Such an example is the Germline gene editing (GGE) which is a technology that modifies the DNA of embryos to prevent inherited genetic disorders. GGE alters the genome in a way that affects not only the individual but also their descendants as it edits the genetic inheritance (Rubeis and Steger, 2018). While GGE focuses on preventing disabilities before birth, alternative approaches include somatic gene editing.

Somatic gene editing is a type of gene editing that aims to treat disorders or diseases by correcting genetic mutations in specific tissues. However, it does not contribute to the body's germline, meaning that the changes made in the somatic cells would not be inherited by the future generations. These advancements in the medical field will enhance the quality of life for those with disabilities (Saha, et al., 2021).

Both approaches hold promising possibilities for treating down syndrome (Kolanu, 2024). However, such practices, especially germline editing raise ethical questions related to safety, long-term consequences, potential risks, and the potential for eugenic (inheritance) applications (Kolanu, 2024). In this article, we will compare the role of germline and somatic gene editing in treating genetic disease and discuss why one method is more reliable than the other.

How does Germline Gene Editing (GGE) and Somatic Gene Editing work in Down syndrome?

Germline Gene Editing (GGE) is a cutting-edge biotechnology aimed at altering the DNA of an embryo to correct or eliminate genetic disorders before birth. It primarily uses tools like CRISPR-Cas9, which act as molecular scissors to precisely target specific DNA sequences. This process involves identifying the faulty gene responsible for a condition, cutting the DNA at that site, and either repairing the error or replacing the defective sequence with a healthy version. Changes made through GGE affect every cell of the individual, including reproductive cells, making these edits heritable across generations. Extensive preclinical studies

are vital to refine its accuracy and minimize risks such as off-target effects or unintended changes to other parts of the genome (Rubeis and Steger, 2018; Schleidgen et al., 2020).

Germline gene editing offers innovative strategies to address Down syndrome by targeting the genetic root cause: the presence of an extra copy of chromosome 21. One approach involves using CRISPR-Cas9 to introduce double-strand breaks at specific sites along chromosome 21, allowing its removal through cellular repair mechanisms. This method has shown success in patient-derived stem cells but faces challenges in embryos due to the potential lethality of chromosomal deletions. Alternatively, instead of deleting the extra chromosome 21, it can be inactivated by the insertion of the X-inactive specific transcript (XIST) gene, which epigenetically silences the extra chromosome without physically removing it. Another emerging method focuses on removing the Down syndrome-critical regions of chromosome 21, which contain genes responsible for neurodevelopmental impairments. While these strategies hold promise, significant hurdles remain, including ensuring precise targeting to avoid off-target effects, and addressing ethical concerns related to heritable genetic modifications.

Methods to achieve somatic gene editing using CRISPR in children or adults can be divided into two categories: ex vivo and in vivo gene editing. Of the CRISPR strategies discussed here, the scientific and medical evidence suggest that ex vivo therapies are the most likely to become a meaningful option for intended parents in the United States in the near future. Ex vivo gene editing involves isolating a patient's cells, editing the cells in culture, and then transfusing corrected cells back into the patient. Pre-clinical ex vivo studies are nearing clinical application for various heritable blood disorders, such as sickle cell disease and b-thalassemia. To achieve in vivo gene editing, CRISPR components must be delivered either systemically to a patient or injected directly into easily targeted tissues like the eye or skeletal muscle. In vivo gene editing is limited by the ability to deliver CRISPR to target organs. Viral delivery plasmids and lipid nanoparticles are the two most commonly used delivery platforms for CRISPR-based

Table 1: Comparison of GGE and Somatic Gene Editing in treating genetic disorders.

Gene editing approach	Affected cell	Advantages	Disadvantages
Germline	Embryonic cell	• Able to be inherited • Possibility of permanently curing the disorder	• Lethal to the embryonic cell • Unethical • Off-target effects
Somatic	Body cell	• Reduces the symptoms of Down Syndrome • Effect of the gene correcting is permanent	• Not hereditary • Does not completely cure the disorder • Off-target effects

therapeutics, each with their own advantages and limitations. The correction of inherited blindness, muscular dystrophy, and liver disorders, all of which impact easy to target tissues, have shown promising results in animal models and are likely to be the first in vivo CRISPR therapeutics to enter clinical trials (Kofler & Kraschel, 2018).

Somatic gene editing provides a promising strategy for treating Down syndrome by alleviating its symptoms by targeting specific body cells that cause it. Unlike germline editing, somatic editing modifies the specific tissues and body cells of the already existing individuals without making heritable changes. The process begins with identifying the target cells, such as neural cells, where the extra chromosome disrupts cognitive function. In Somatic Gene Editing, Gene-editing tools like CRISPR-Cas9 are designed to target and either remove or inactivate the extra chromosome. Viral vectors or nanoparticles transport these tools to the target cells. Once delivered, the tools modify the DNA, restoring normal gene expression levels. The outcomes are evaluated to ensure corrected cellular function, and the edited cells are either reintroduced into the patient or edited directly in vivo. This approach focuses on alleviating Down syndrome symptoms while avoiding the ethical concerns of altering the germline, making it a viable therapeutic option.

Implication of Germline Gene Editing and Somatic Gene Editing to Treat Genetic Disorders

When GGE is reliable and safe, it offers transformative benefits. It can prevent serious hereditary conditions like cystic fibrosis or sickle cell anaemia from being passed down, sparing individuals and their families from significant health and emotional burdens. Beyond individual health, GGE could reduce healthcare costs by minimizing lifelong treatments for genetic disorders. The technology also has societal implications, potentially eradicating certain diseases from the gene pool over generations. Compared to traditional treatments or somatic therapies, GGE addresses conditions at their root cause, offering a one-time, permanent solution rather than ongoing symptom management. However, as promising as it sounds there are also potential ethical and safety concerns regarding Germline Gene Editing (GGE). It raises the question of its heritability as it may cause potential unintended consequences in descendants by the inherited gene that has been modified. The risk of off-target effects as well as ethical issues of ‘playing God’ and increasing social inequality. The lack of clinical trials also questions the long-term safety and the efficacy of GGE.

Somatic gene editing offers significant potential for treating genetic disorders by enabling precise modifications to an individual's non-reproductive cells, thereby avoiding the transmission of changes to future generations [World Health Organization (WHO), 2021]. This approach allows for the correction of disease-causing mutations in specific tissues, providing targeted therapies for conditions such as sickle-cell anaemia and cystic fibrosis. Also, it has already been used in treatments for some genetic diseases unlike Germline Gene Editing that has never been used before (WHO, 2021) However, challenges include ensuring efficient and accurate delivery of gene-editing tools to the appropriate cells,

minimizing off-target effects that could lead to unintended genetic alterations, and addressing ethical considerations related to the modification of human cells. Additionally, the long-term effects of somatic gene editing are not yet fully understood, necessitating comprehensive clinical trials and regulatory oversight to ensure safety and efficacy.

Based on the information gathered from the Table 1 and the previous discussion, we can compare the reliability of the Somatic Gene Editing and the Germline Gene Editing. We can conclude that the Germline Gene Editing is more unreliable than the Somatic Gene Editing. If the procedure in the individual goes wrong it could permanently affect the individual and even become lethal, causing death. It is also in terms of ethical issues, harder to pursue this technique for it is illegal in many countries that consider editing trials on human embryos unethical. While on the other hand, Somatic Gene Editing has been approved by many countries and has several clinical trials using it to treat many other types of genetic diseases and disorders. Some of these treatments have been already used, making it more reliable than the Germline Gene Editing that has not been tested enough and have never been used legally.

Conclusion

Overall looking at the discussion in this article the somatic gene editing is more reliable to be used in the medical field compared to the germline gene editing when looking at terms of safety and ethics to treat genetic disorders. Somatic gene editing has already been used in treatments for genetic diseases and has lower concerns about gene mutations in descendants since it is not heritable. However, the germline gene editing has not yet been used outside of clinical trials as it is illegal to be used outside of research due to its unethical process and lacks enough study to ensure the safety and efficiency of the technique. This concludes that somatic gene editing has more potential to be used in the medical field.

Through the somatic gene editing, the race of humanity would advance. People with genetic diseases or genetic disorders will be able to seek out the full potentiality of life, being able to get treatment to reduce its symptoms and acquire the ability to function normally. Through further usage of somatic gene editing, it would be revolutionary to both the scientific field and the medical field. It will bring out more solutions to other genetic diseases and disorders as well as help advance genetic tools, creating new ways to treat and give potential cure. However, it is still in need of more clinical trials in order to minimize off-target effects and be able to study the long term effects of the technique in order to fully achieve the goal of treating genetic disorders.

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How Can CRISPR-edited iPSCs Treat Genetically-Mutated Diseases?

Cherish Andreea

Introduction

Genome editing, which is the modification of DNA at a specific target site, and its applications have been highly studied by scientists on a wide range of cell types and organisms, including humans (Xu and Li, 2020). With genome editing, faulty genes can be altered, eliminating the potential negative health effects it can impose on an organism (National Academies of Sciences, Engineering, and Medicine, 2017). This is done through modifying a target site of DNA in the form of either an insertion, deletion, or replacement of a new DNA (Xu and Li, 2020). These modifications can result in the inactivation of the target gene, the adoption of a new genetic trait, or the correction of a gene mutation from pathogens such as bacteria, fungi, and viruses (Xu and Li, 2020). As a result of this discovery, scientists have developed various techniques and tools to modify genes in specific sites (National Academies of Sciences, Engineering, and Medicine, 2017). Some of these methods have been shown to be effective in the clinical applications practiced (National Academies of Sciences, Engineering, and Medicine, 2017).

One of the most renowned tools used in genome editing is CRISPR/Cas9 (National Academies of Sciences, Engineering, and Medicine, 2017). CRISPR, clustered regularly interspaced short palindromic repeats, refers to sequences in a genome (Redman et al., 2016). This was first discovered in the DNA of the bacteria *Eschericia coli* (Gostimskaya, 2020). The CRISPR-Cas system is an adaptive immune system in bacteria and archaea that helps prevent them from getting infected by invasive pathogens (Xu and Li, 2020). As scientists studied about this further, the gene-editing tool CRISPR/Cas9 (CRISPR-associated protein 9) was developed (Redman et al., 2016). The CRISPR/Cas9 is made up of 2 main components: a guide RNA that matches a desired target gene, and a protein called Cas9 that has the ability to break double-stranded DNA (Redman et al., 2016). In order for a cut by the Cas9 protein to occur, a synthetic guide RNA (sgRNA) must be used to guide the Cas9 to the specific target site (Redman et al., 2016). With the sgRNA binding onto the genomic DNA, a cut can be made (Redman et al., 2016). After this occurrence, during the reparation of the cut, a DNA can be inserted, deleted, or replaced with a desired genome sequence (Redman et al., 2016). This ability of CRISPR/Cas9 serves the potential of viral infections, hereditary diseases, and cancers in humans to be treated (Liu et al., 2021).

iPSCs, or induced pluripotent stem cells, are artificial stem cells produced from somatic cells that are “induced” or “reprogrammed” in a culture (Moradi et al., 2019). Transforming somatic cells into iPSCs requires the somatic cells to undergo a process called ectopic co-expression (Moradi et al., 2019). iPSCs, similar to embryonic stem cells, can reproduce rapidly and are pluripotent: they can develop into any type of cell (Moradi et al., 2019). These artificial cells can be used to treat diseases by replacing diseased or

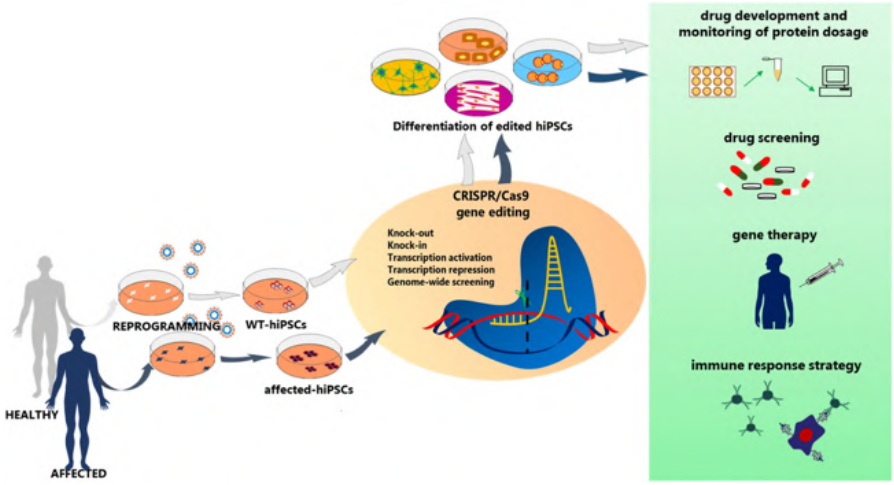

Figure 1: The workflow of research of CRISPR-edited human iPSCs for the development of novel gene therapy
(De Masi et al., 2020)

damaged tissues (Moradi et al., 2019; Sen and Thummer, 2022).

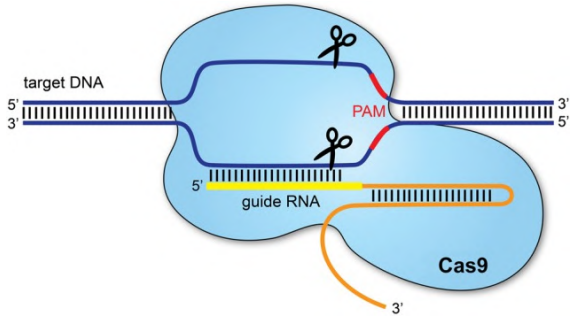

Figure 2: CRISPR-Cas9 system
(Redman et al.)

The development of iPSCs and CRISPR/Cas9 were huge breakthroughs in the medical field (Sen and Thummer, 2022). Scientists have done numerous studies to combine CRISPR/Cas9 and iPSCs together, creating CRISPR-edited iPSCs (Sen and Thummer, 2022). Research has shown that CRISPR-edited iPSCs can eliminate the limits each technology has in treating diseases, especially in diseases that are rooted in pathogenic mutations (Sen and Thummer). This literature review will explore the applications of CRISPR-edited iPSCs in two different areas: cardiovascular diseases and HIV (human immunodeficiency virus).

CRISPR-edited iPSCs to Model and Correct Cardiovascular Diseases with Disease-Causing Mutations

iPSCs are abundant sources of human cardiomyocytes helpful for the cardiac field to study human *in vitro* models (Motta et al., 2017). Though the phenotype of iPSC-derived cardiomyocytes (iPSC-CMs) are still far from that of human adults, scientists have made protocols to optimize their *in vitro* maturation for better cardiac pathology models (Motta et al., 2017). iPSC-CMs have been used to study several cardiac diseases, such as inherited arrhythmogenic disorders, catecholaminergic polymorphic ventricular tachycardia (CPVT), dilated cardiomyopathy (CDM), and hypertrophic cardiomyopathy (HCM) (Motta et al.). In fact, in the study of iPSC-CMs in DCM patients carrying a mutation in the gene that encodes for cardiac troponin t – a sarcomeric protein – has shown to successfully represent both morphological and functional phenotypes of real affected hearts, one being the decrease in contractility of the heart (Motta et al., 2017). Similarly iPSC-CMs from an HCM

patient with a missense mutation in the MYH7 gene has shown the realistic symptoms of a heart suffering from HCM, such as its electrophysiological irregularities (Motta et al., 2017).

With the many investigations being done on the pathophysiological mechanisms of inherited cardiac diseases in human cell models, CRISPR/Cas9 has been used as a tool to effectively create gene knockouts and knockins in iPSCs, including in cardiac disease models, allowing for the correction of genetic mutations (Han and Entcheva, 2023; Motta et al., 2017). Especially, the use of patient-derived human iPSCs (hiPSCS) provide scientists with the opportunity to investigate both common and rare diseases of varied genetic backgrounds, and create more subjective complex models that represent those genetic disorders (Han and Entcheva, 2023).

Table 1: Application of CRISPR/Cas9 in hiPSCs in cardiac diseases (Han and Entcheva. 2023)

	Disease Type	Gene Target	Disease
Cardiac applications	Cardiomyopathy	*PRKAG2 KI*	Familial Wolf-Parkinson-White Syndrome
		MYH7 variants *KO*	Hypertrophic cardiomyopathy model
		TNNT2 KO	Ventricular Arrhythmogenesis model
	LQTS	*KCNH2 KI*	LQT2 model
		CACNA1C KO	LQT8 model
		SCN5A KO	LQT3 model
	DMD	DMD *KO*	Duchenne muscular dystrophy correction
	Disease-Modelling	Multi-gene *KO*	
iPSCs	Screens	Multi-gene *KO*	

One example of the successful combinations of CRISPR/Cas9 and iPSC is the deriving of iPSCs from patients with Barth syndrome – an X-linked genetic heart disease – and the introduction of a mutation in the tafazzin (*TAZ)* gene to the iPSCs from healthy donors using CRISPR/Cas9 (Motta et al., 2017). This revealed that the *TAZ* gene affected the mitochondrial phenotype (Motta et al., 2017). Knowing that the Barth syndrome is associated with mitochondrial CM abnormalities, scientists administered an antioxidant for the mitochondria called mitoTEMPO to the Barth syndrome-derived iPSC-CMs to suppress the excess of mitochondrial ROS (reactive oxygen species) production (Motta et al.). Doing so normalized contractility in the heart (Motta et al., 2017). In addition to this, the iPSCs derived from a patient with Long QT syndrome (LGTS), who was a carrier of the heterozygous *CALM2* mutation, had shown positive results when edited using CRISPR/Cas9 (Motta et al., 2017). When the mutant allele was edited using CRISPR/Cas9, the abnormal electrophysiological properties of the

LGTS affecting the patient were reduced (Motta et al., 2017).

CRISPR-edited iPSCs in HIV Research

Knowing the mechanics of the CRISPR/Cas9 system in protecting archaea and bacteria from viruses, scientists have applied the introduction of CRISPR/Cas9 to HIV as a therapeutic strategy (De Masi et al., 2020). To remove the virus from the body and help the body fight against this particular infection, hiPSCS have been used to mimic the antiviral defensive system of CRISPR/Cas9 (De Masi et al., 2020). This was done by engineering the hiPSCs against the reverse-transcribed products of the viral RNA genome of HIV (De Masi et al., 2020). The applications of CRISPR/Cas9 and hiPSCS have been shown to be positive in antiviral response (De Masi et al., 2020).

CRISPR/Cas9 was used on the homozygous *CCR5Δ32* mutation in iPSCs in HIV research (Xiao, Guo, and Chen, 2019). The CCR5-modified iPSCs were discovered to have the ability to develop into monocytes and macrophages that were resistant to the HIV-1 infection (Xiao, Guo, and Chen, 2019). Thus, CRISPR/Cas9 was combined with sgRNAs to target the fourth exon of CCR5, disrupting its expression (Xiao, Guo, and Chen, 2019). With the use of two specific sgRNAs, the efficiency of TZM-BL cells – cells permissive to a vast variety of HIV-1 infection – is cut by over 60% (Xiao, Guo, and Chen, 2019). CRISPR/Cas9 was able to silence the expression of the CCR5 expression, protecting cells from the infection of HIV-1 (Xiao, Guo, and Chen, 2019).

Conclusion

The use of CRISPR/Cas9 in genome editing has given scientists positive results in eliminating genetically-inherited diseases by cutting a specific target site and either inserting, deleting, or replacing the cut with a new DNA (National Academies of Sciences, Engineering, and Medicine, 2017; Xu and Li, 2020). The protein Cas9 and sgRNA have been developed into a significant technology used to treat genomic mutations (Liu et al., 2021; Redman et al., 2016). Additionally, iPSCs – with their ability to develop into any cell type – have been used to treat damaged tissues, and its combination with CRISPR/Cas9 have advanced their use in treating more complex diseases (Moradi et al., 2019; Sen and Thummer, 2022). CRISPR-edited iPSCs have been studied in various diseases, including cardiovascular diseases and HIV. In cardiovascular diseases caused by genetic mutations, iPSC-CMs edited with CRISPR/Cas9 have shown to alleviate the negative symptoms experienced by the heart (Motta et al., 2017). Similarly, in HIV, CRISPR-edited iPSCs have also been proven to silent the expression of a gene mutation, protecting cells from getting infected by the virus (Xiao, Guo, and Chen, 2019).

With the ongoing and growing research scientists are conducting today, we will be able to understand better how CRISPR-edited iPSCs work. This can benefit the medical field as more accurate complex models of varied genetically-mutated diseases can be developed using iPSCs. On top of that, CRISPR-edited iPSCs will lead to the creation of more specific treatments of genetically-mutated diseases, beyond cardiovascular diseases and HIV.

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Gene Therapy for Alzheimer's Disease

Abigail A. Santoso

Introduction

Alzheimer's Disease or related dementia is a devastating neurodegenerative disorder primarily affecting older individuals. It is characterized by progressive memory loss and cognitive decline, ultimately leading to severe disability. Alzheimer's is a progressive and incurable neurological disorder which causes memory loss along with other cognitive impairments. (Prabhune, 2021).

There are several risk factors that influence Alzheimer's Disease (Prabhune, 2021). This includes age, family history genetics, individuals with down syndrome, air pollution, head trauma (someone who has experienced a traumatic brain injury above the age of 50), excessive alcohol consumption, unhealthy lifestyle, etc. Even though there are multiple ways to reduce the risk factors of the development of AD, such as exercising regularly, eating a diet of fresh produce and quitting smoking, Alzheimer's disease is unpreventable.

One of the ways to treat and possibly cure Alzheimer's is with the help of genetic engineering, specifically, with the help of clustered regularly interspaced short palindromic repeats, or also known as CRISPR. CRISPR is a technology of modifying certain genes in a living organism. They are able to guide the RNA to the specific gene and are able to cause a double-stranded DNA break. This technology was adapted from the naturally occurring genome editing found in bacteria (Redman et al., 2016).

Currently, gene therapy is used to reduce symptoms, slow the progression, and delay onset in many diseases (Ruwa, 2023). Unfortunately, in the case of AD, gene therapy is only able to reduce the amount of risk genes with hopes that it might reduce the chance of getting Alzheimer's disease. How can we use CRISPR to prevent and possibly eliminate Alzheimer's disease in the future? This article will further explain the genes that play a role in the development of AD and how gene editing helps provide or eliminate these genes.

CRISPR-Cas9 gene editing tool for Alzheimer Disease

In the meantime, CRISPR-Cas9 is the most promising gene editing tool that can be used to cure and prevent AD due to its capability to edit certain genes and correct its mutations. It has the capability to target certain genes in the cell tissues and in animal models to make modifications to those genes (Barman et al., 2020; Thapar et al., 2024). It is adapted from the bacteria mechanism of defence when infected. When infected, bacteria are able to recognize and neutralize the conquering bacteriophages (virus) and other unwanted nucleic acids incorporation. Using CRISPR, the bacteria is able to target a specific area of RNA from the bacteriophages, so the cas9 enzyme could cut that area and deactivate the virus. This is how the CRISPR-Cas9 combination is used to modify our DNA. (Barman et al., 2020).

In Alzheimer Disease, CRISPR-Cas9 is primarily used to reduce the production of beta amyloid, a toxic protein that boosts the development of AD as shown in a study in mice (Redman et al., 2016). In this study, using CRISPR-Cas9 to introduce or eliminate genes that involved in beta amyloid production could help in preventing the development of AD (Redman et al., 2016).

Editing genes that are involved in beta amyloid production

A673T gene mutation. Even though there are around 50 genes that may increase the possibility of Alzheimer's, there are

several genes known to cause less beta amyloid production such as A673T. The A673T is reported to be five times more common in dementia-free elderly individuals than people with AD. Additionally, it is found to be able to protect against age-associated cognitive decline. It was further shown that a woman who carried the A637T variant had dementia at the age of 104, however it was due to hippocampal sclerosis instead of AD, providing additional evidence to show the protective effect of A637T (Alzforum, n.d.). CRISPR editing to introduce this gene may have a huge opportunity to completely prevent Alzheimer's disease.

In an effort to prevent Alzheimer's, researchers from Laval University in Canada have edited genes in brain cells using CRISPR. The Jacques Tremblay-led research discovered a genetic mutation known as A673T that can lower beta-amyloid, a biomarker for Alzheimer's disease, and has been shown to decrease the risk of Alzheimer's by a factor of four. About 40% of the lab-grown brain cells had the A673T variation activated by the researchers using CRISPR in petri dish experiments (Prabhune, 2021).

While this may appear to be exciting news, but it has only worked on roughly 40% of lab-grown brain cells, and scientists will need to duplicate this in animal trials before testing it on humans. Nonetheless, this suggests that gene therapy to introduce this gene may be able to prevent Alzheimer's disease in the future. (Prabhune, 2021).

PSEN1 and PSEN2 genes. In general, the genes PSEN1 and PSEN2 typically cause early-onset dominant familial AD. Even though the majority of causes that cause AD are still unknown, over 300 mutations have been discovered in the PSEN1 gene, whereas the PSEN1 is still a rare cause of early-onset AD. PSEN1 and PSEN2 are both a subunit of y-secretase which causes a higher production of β-amyloid peptide. CRISPR-Cas9 can potentially correct these harmful mutations and prevent AD. In the past, CRISPR-cas9 has already been used to correct PSEN2 dominant mutation genes (Barman et al., 2020).

APOE-e4 gene. APOE-e4 is one of the three major isoforms of the human APOE. APOE-e2 is the rarest out of those three as it has shown to have reduced the risk of developing AD by 40%. APOE3 does not provide any risks nor rewards. However, APOE4 is present in 10%-15% of the population and increases the development of AD. With just one copy of E4, it increases the risk by 2-3 times and with two of them present, it immediately increases the risk by 10-15 times. CRISPR-Cas9 can be used to convert APOE4 into APOE2 or APOE3 showing signs that prevention is possible for APOE4 carriers (Barman et al., 2020)

Challenges and Risks of CRISPR-Cas9

Several issues need to be addressed to find a safe and effective delivery mechanism in order to turn the technology CRISPR-Cas9 fully into practical use. First, The usage of viral vectors in delivering CRISPR-Cas9 may lead to serious problems in which the subject may lead to accidental mutations which could be life threatening. CRISPR-Cas9 application still has a low percentage of success rate in fully preventing AD as there are still many unidentified causes of AD. Researchers found out that late-onset AD has progressed for a very long time before symptoms appear, thus creating an uncertain window for intervention (Barman et al., 2020).

Second, one of the fundamental priorities of CRISPR-Cas9, is specificity and accuracy. Gene transport to the targeted areas may be ineffective as off-target mutations are one of the main obstacles that may affect the functionality of altered cells. This could also intensify germline mutations, providing great danger (Barman et al., 2020).

Conclusion

In conclusion, CRISPR-Cas9 has brought hope for treating target diseases efficiently and correcting gene mutations by correcting and editing multiple numbers of autosomal-dominant mutations that may cause early-onset AD or any genetics with a high risk of developing late-onset AD. This proves that genetic editing may be a reliable solution in treating AD in the near future. However, scientists need to focus their research on providing a more specific RNA guide to prevent the "off-target" behaviour of CRISPR-Cas9. Even though gene therapy shows great potential and scientists have utilized CRISPR to cure Alzheimer's, it is still very challenging as the disease is caused by many different factors and may lead to many other possible issues including death.

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How Gene Editing Helps Our Body Defends Against Cancer Cell

Soo Han Kim

Introduction

Cancerous cells are body cells that begin to reproduce uncontrollably due to a mutation in parts of the DNA. This mutation is the reason why body cells reproduce uncontrollably and spread to different areas in our body. Cancerous cells take away nutrients and oxygen from cells in our body and keep dividing to form a tumor. The immune system does not notice the developing cancer cells because they are still body cells that were mutated resulting in the immune system ignoring the cancer (NCI, 2021). A reason why cancer is so feared is because cancer can impair organ functions, and can be extremely hard to cure (NCI, 2021). Cancer is a life-threatening disease that can spread through our body and deprive our working body cells of oxygen and nutrients without being noticed by our immune system (NCI, 2021).

Gene editing is a promising way for doctors in the future to cure cancer patients in an easy and fast manner by only editing the portion of the DNA that has been mutated. Gene editing technique, for example CRISPR, is a way to modify or delete parts of DNA that are causing other areas in the body to be cancerous (Chehelgerdi, 2024). Gene editing may shape the future of how we cure cancer in time ahead because of its fast and efficient treatments of cancer.

Unlike traditional cancer treatments like chemotherapy where the treatment kills all fast growing cells (both healthy and unhealthy cells), gene editing targets a specific gene or DNA to stop the tumor from growing, becoming a much more efficient way to cure cancer (Mayo Clinic, 2024). This allows for a more localized effect of cancer treatment compared to the systemic effect of chemotherapy. However scientists are still perfecting the way CRISPR works on our body without harm. The CRISPR gene editing tool has the potential to become the future of the cure for cancer as it easily can stop tumors by swiftly editing the gene that causes the growth of cancer cells.

Gene mutations in cancer

The oncogenes (OCGs), or a modified version of a proto-oncogene, begins the formation of cancer cells as they carry out a mutation while going through the process of cell division (Sinkala, 2023). The proto-OCG is a class of genes that follow a normal routine of cell division, however when there is a mutation of producing excessive amounts of the cells copies, the proto-OCG turns into an oncogene increasing its ability to divide cells (Sinkala, 2023). Through these mutations spreading around the body, the immune system loses its ability to regulate growth control as the cancer alters the cells abilities to regulate it (Sinkala, 2023). This is how the cancer cell grows and gets introduced to the body and other organs and tissues in the body without any limiting factors (Sinkala, 2023).

Tumor suppressor genes can also be a factor of cancer development, when these genes get mutated, they lose the function to regulate cell division, hence, being called a loss-of-function activity (Sinkala, 2023). In general, DNA mutations may change the order of nucleotide in the DNA, altering

proteins which can change the cells and other functions of the body. A function that may subject to cancer development is uncontrollable production of cells.

CRISPR-Cas9 to edit gene mutation in cancer

Gene editing or the CRISPR editing tool helps our body prevent cancer from spreading in our body and making it less lethal and easier to cure [National Cancer Institute (NCI) Staff, 2020]. A potential application of using this gene editing tool to prevent cancerous cells from growing is by inactivating the gene that caused the tumors to grow (NCI, 2020). Inactivating the gene can slow down or completely halt the progress of the tumor to grow, this does not yet cure the disease but is very helpful at stopping the disease from growing to become more dangerous (NCI, 2020). The process of inactivating a gene consists of the CRISPR gene-editing tool pairing with the Cas9 enzyme to bind and cut the DNA that has been mutated (NCI, 2020). Then, the Cas9 enzyme replaces the DNA with new genetic material (Figure 1.).

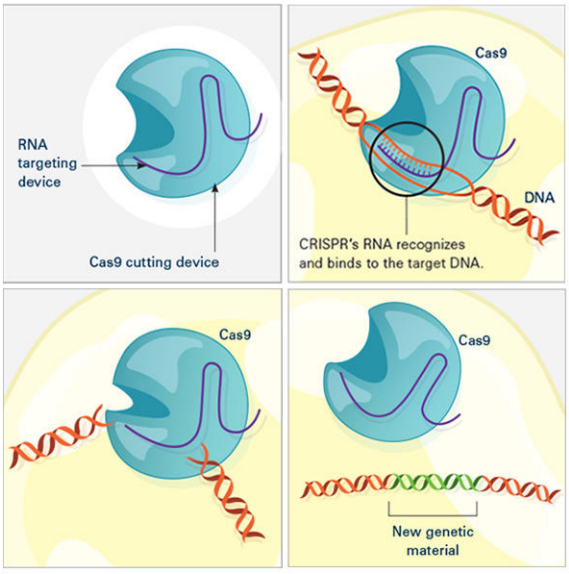

Figure 1. CRISPR-Cas9 Gene Editing method (NCI, 2020)

According to Chehelgerdi et al. (2024), there are some ways/ideas of how gene editing can be incorporated when treating cancer cells.

1. One approach of treating or halting the development of cancer is **by immobilizing genes that allow or create tumor growth**. This can be done by using the CRISPR tool to inactivate the oncogene MYC, the gene which has been proved to be overactive in the developments of many cancer cells.
2. Another approach of decreasing cancer's danger is to **enhance immune response to cancer** by decreasing the amount of PD-1 protein produced. These proteins latch on to T-cells which inhibits them from killing cancer cells and other cells that may develop into cancer.
3. Another solution can be to **repair genetic mutations that cause cancer**. This can be done by correcting mutations such as BRCA1 and BRCA2 using the CRISPR-Cas 9 tool.
4. Additionally, another approach is using **immunotherapeutic strategies**. In this approach, the T-cells can be engineered to fight off cancer cells more efficiently, such as expressing the receptors to target tumor cells more effectively, enhancing the body's ability to fend off cancer cells.

Possible Risks of Using the CRISPR Tool

The CRISPR tool may have the potential to cure cancer in the future, however as of right now, there are still some technological errors in how the CRISPR tool works. Sometimes the tool edits the wrong genome, or also known as "off-target editing". This may change how a cell works

and cause harm in the body instead of giving cure (Chehelgerdi, 2024). Scientists are still not fully sure how this gene editing tool may affect our immune systems when applied to our treatments. Nevertheless, this tool will completely alter the way we treat diseases like cancer in the future once research is being made to discover how to make it safer and more effective (NCI staff, 2020).

Conclusion

Gene editing gives us a glimpse of how treating cancer in the future may look like, whether this invention is successful or not, it would inspire the medical world to create better tools to battle cancer. The CRISPR tool already shows us multiple possible ways of halting the spreading of cancer in our body by inactivating genes that promote tumor growth, enhancing the efficiency of T-cells, repairing genetic mutations such as the BRCA 1 and 2 mutations. However even though there are many solutions, there still can be further improvements, such as lowering the rate of "off-targeting", which is editing the wrong DNA and the possibility of getting autoimmune diseases. Overall, gene editing will be a great advancement in the future of cancer treatment because of its efficient way of stopping cancer growth.

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CAR-T Cells for Cancer

Ji Hyun Kim

Introduction

Chimeric Antigen Receptor T (CAR-T) cells have emerged as an effective approach to fighting cancer in immunotherapy, particularly for hematologic malignancies such as leukemia and lymphoma. CAR-T cells are a type of genetically modified T-cell which plays a crucial role in a person's immune system by helping the body identify and respond to infected or abnormal cells such as cancerous or virally-infected cells. T-cells are typically produced by the bone-marrow and develop within the thymus (Alberts, 2015). However, CAR-T cells are genetically engineered to express a receptor specific to tumor-associated antigens, enabling them to recognize and kill cancer cells more effectively than traditional treatments (Brentjens, 2013).

The first CAR-T therapies were developed in the early 2000s and after promising preclinical results, were successfully translated into clinical applications. Notably, the approval of Kymriah and Yescarta for treating certain types of leukemia and lymphoma in the 2010s marked a significant accomplishment in the field (Maude et al., 2014; Neelapu, 2017).

CAR-T cells in cancer

CAR-T cells are modified immune system cells that are responsible for antigen recognition via the T cell receptors bound to the cell's surface (Davila & Sadelain, 2016). CAR-T cells can be specifically designed to target B cell malignancies, which are generally hard to treat due to its therapeutic resistance and repeated likelihood of relapse. This specialization of a patient's immune system cells allows for B cell malignancies to continue to be targeted despite mutations or a build-up of drug-resistance to various known cancer treatments (Lu, et al., 2021). CAR-T cell production involves genetic engineering that results in adding costimulatory molecules, either CD28 or 4-1BB, creating costimulatory domains which help normal T-cells better target specific antigens particular to the malignant cancer cells doctors are aiming to reduce (Lu, et al., 2021)

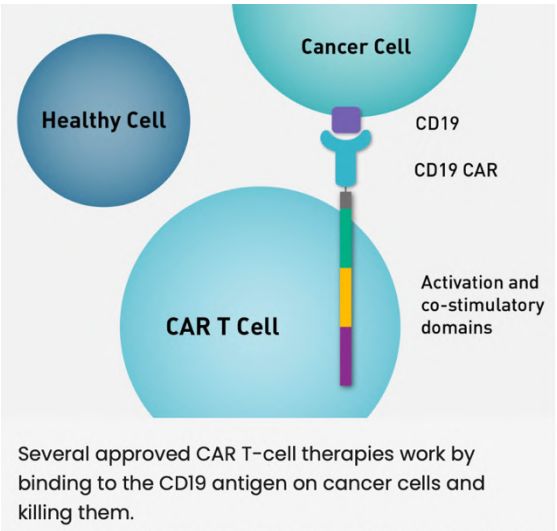

Figure 1. CAR-T Cell
(NCI Staff, 2022)

Negative side-effect of CAR-T cells

While CAR-T cell therapy has demonstrated remarkable efficacy, especially in patients with refractory or relapsed cancers, challenges remain, including managing side effects like cytokine release syndrome (CRS) and neurotoxicity, as well as improving responses in solid tumors (June et al., 2018). Side effects like neurotoxicity are

currently serious health complications that can arise from the use of CAR-T cells to fight cancer, and it involves the damaging of the nervous system due to exposure to toxic substances (in this case the CAR-T cells) which can lead to seizures, severe neurological impairments, and speaking difficulties (Lee et al, 2014).

To address the issue of CAR-T cell treatment's potential to induce neurotoxicity and CRS, strategies have been formulated to reduce the toxicity while strengthening the effectiveness of the treatment. In order to reduce toxicity, researchers have started creating multiple different structures of CAR-T cells such as tandem CARs, dual CARs, and tri-specific CARs which help CAR-T cells to respond to a variety of different antigens and reduce the likelihood for antigen-negative tumor recurrence (Wang, et. al., 2023).

Furthermore, researchers have implemented a "safety switch" within the CAR-T cells which allow medical personnel to control and monitor the activities of the CAR-T cells and effectively terminate these modified immune cells if a patient starts developing CRS (Wang, et. al., 2023). CRS is the over-production of cytokines, with symptoms of fever, hypotension, and hypoxia typically developing early after CAR-T cells are administered within patients that experience side effects (Blud et al., 2024). These preventative strategies have proven to be making progress in reducing the risk of employing CAR-T cell treatments, and further indicates promising results as more research is invested in this method.

Conclusion

Despite these potential side effects, CAR-T cell research is still ongoing and further improvements are not impossible to achieve. Overall, CAR-T cells bring ground-breaking advancements in the treatment of cancer, and further research has been focusing on enhancing the persistence and specificity of CAR-T cells, reducing toxicity, and expanding the therapy's applicability to a broader range of cancers in order to improve upon this method (Pule et al, 2012; Morgan et al., 2006).

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The Disadvantages of Genetic Engineering

Sung Yun Kim

Introduction

Over the years genetic editing was debated in the science community whether it is moral or immoral to perform genetic editing on a human being. According to WHO (n.d.), genetic editing, which has a big risk but also a big reward, is a method to make specific changes in the DNA of a cell or organism.

The goal of genetic editing is to cure diseases that are impossible to avoid, but trying to cure these diseases can cause severe consequences both to the individual and community. For example, in one case doctors may try to genetically edit the cancer cells to disappear, but this has the risk to potentially mutate cells and become more dangerous. In other words, trying to fix a cell can actually make the disease worse than it already was.

Genome editing may be detrimental because of the potential pernicious effects it could have on the patient. The striving to cure the disease can lead to DNA changes and precede the destruction of the body. DNA is the genetic instruction to the body that is built based on that instruction. If this instruction is alternated negatively, then the organs pursuing the instruction could malfunction. The malfunction can cause a generational effect that can reverse the effects of natural selection on humans.

Why would such a promising goal not be pursued with rapid pace with the new technology called the CRISPR? A recent research emphasized the potential dangers of genome editing; the dangers could be the permanent damage of the DNA that can lead to more severe destruction to the body than the actual disease. While efforts are being made to achieve the original goal of genome editing, scientists are reluctant due to the many deficiencies of this technology. This paper will show some major drawbacks of gene editing and why some countries banned the further development of genome editing.

Cases of Genome Editing:

Case #1: Jess Gelsinger (18 y.o.) – The first death from genome editing. An 18 year old boy named Jesse Gelsinger suffered from a rare disease called ornithine transcarbamylase (OTC) deficiency which is the deficiency of an enzyme that causes accumulation of ammonia in the blood. Since in Jess Gelsinger's case, he only had partical OTC deficiency, he became a perfect candidate for gene therapy trial. disease led Jess to have the desire to cure this disease. The corrective gene for OTC was delivered using a virus that has been weakened, which was injected through the blood vessel. It turns out that the virus carrying used in this therapy caused Gelsinger to have a severe immune reaction that eventually leads to his death 4 days after the injection (Sibbald, 2021).

Case #2: 20 children in The Necker–Enfants Malades Hospital. At the same time, the Necker-Enfants Malades Hospital in France did the first trial for 20 children who had who had severe combined immunodeficiency (SCID) due to adenosine deaminase (ADA) deficiency. In this trial, 5 children developed cancer and passed away due to this genome therapy. When the children were inserted with correcting genes using viral vectors to their T-cells, it activated cancer genes in the T-cells that led the children to have leukemia.

This gradually led to the abandonment of genome editing until 2018 even causing the banning of this technology in countries (Sadeghi, 2023).

Case #3: He Jiankui. In 2018, a Chinese man named He Jiankui conducted an experiment to prevent HIV in twin babies by genetically editing the embryo. Jiankui's experiment is highly debated among the science community (Sadeghi, 2023). While He claims to edit the CCR5 gene to prevent babies from having HIV, critics suggest that He's experiment was unnecessary as it posed serious risk of gene editing to the babies while other successful methods of prevention was already established. The science quality of He's experiment is also very poor and superficial, with unreliable result (Wang & Yang, 2019).

Chinese researchers in the field of gene editing was completely shocked when the news came out, especially because it was done despite the concern of the scientific community and societies on prohibition of gene editing in human embryos. The experiment was done in secret and no noteworthy scientific papers has been published upon the experiment (Sadeghi, 2023; Wang & Yang, 2019). What also infuriated the science community is the misinformation he must have fed to the victim's parents judging by the poor rationale of his experiment and unreliable result that he presented.

Countries that banned genome editing

As discussed before, deaths in therapy has become an important issue in the use of gene editing. This affects legal frameworks of using gene editing in countries. At least 19 countries announced the banning of genome editing in human embryo including Canada, Sweden, Belarus, and Australia (Sadeghi, 2023). Australia has strongly stated the banning of gene editing; the illegal performance of gene editing can lead anyone up to 15 years in jail (Global Gene Editing Regulation Tracker, n.d.). Similar to Australia, countries should make appropriate penalties for those who illegally perform gene editing to cease the potential dangerous altercation of gene editing.

Conclusion

As shown in from the discussed cases, there are infinite unpredictable possibilities that can be caused by the CRISPR. Similar to our question, countries are afraid and are banning CRISPR technology due to the possibility of mutations. Possible future research should be conducted if and only if the CRISPR technology can perform genetic engineering in a more precise way. Our community needs to be careful with this new technology that can both solve problems and cause problems with the risk it holds.

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Benefit, Risk, and Concerns of Genetic Engineering in Human

Aditi Nayak

Introduction

Gene editing (also known as genome editing) is a group of technologies that enable scientists to change an organism's DNA to helping treat genetic diseases, improving plants and animals, or even to possibly changing human traits [U.S. Food and Drugs Administration (FDA), 2022]. The latest tool utilized in genome editing is known as CRISPR-Cas9, which was developed in 2012 (Mah & Roberts, 2022). CRISPR-Cas9 is a molecular scissor that is designed to cut a specific DNA sequence in living organisms to either delete a damaged gene or add a new gene (Yourgenome.com, n.d.).

Currently, there are two types of gene editing, 'somatic gene editing' and 'germline gene editing' (Bergman, 2019). While the former involves making DNA edits in bodily cells of a human to treat diseases like cystic fibrosis and sickle cell anemia, the latter involves making edits to DNA in germ cells which are: egg, sperm, or embryo cells and, therefore, is passed down to future generations (Bergman, 2019). This is where the ethical concerns start to get serious because it affects not just one person, but their children and even grandchildren.

In the field of medicine, gene editing could be advantageous as it offers new treatments and even cures diseases that have no cure right now like cystic fibrosis and sickle cell anemia (Bergman, 2019). However, there are concerns about fairness, safety, and also whether it is appropriate to customize someone's DNA without their consent. The choices we make today about genetic editing could shape the future of society, and, therefore, we have to think carefully about how we want to advance in using this technology.

Historical context of Gene Editing

Gene editing has come a long way from its beginnings. In the 1970s, scientists discovered Restriction Enzymes (Mah and Roberts, 2022), where they can identify and then cut a particular nucleotide sequence to replace it with a new DNA material. However, this method can only recognise a few nucleotide patterns, and thus is rarely used for gene editing.

A decade later, the Zinc finger nucleases [ZFNs] were discovered to solve the problem of accuracy. Although this tool had the advantage of recognizing longer DNA sequences and was slightly more specific, it was still not perfect. A better tool emerged in 2011 which was called Transcription Activator-Like Effector Nucleases [TALENs] which was quite similar in design to ZFN but differed in size and procedure. (Mah and Roberts, 2019) This tool however, was also not widely used because it was expensive and time-consuming.

Then around 2012, a huge breakthrough happened with the discovery of CRISPR-Cas9. This tool changed many scientists' perspectives about gene editing by making it much easier and more efficient. CRISPR-Cas9 allows geneticists and medical researchers to modify the genome by deleting, inserting, or changing specific DNA sequences (Yourgenome.com, n.d.). To be more specific, CRISPR technology uses the 'Cas9' enzyme to cut DNA at precise

locations, guided by a 'guide RNA'. When the cell attempts to repair this damage, scientists make edits to the gene (Yourgenome.com, n.d.).

Benefits of Gene Editing

Gene editing actually has a lot of benefits but here are some big and famous benefits of gene editing. Gene editing is a tool that can change people's lives by fixing gene problems that cause diseases (FDA, 2022). Scientists can cure diseases like Alzheimer's and Cancer by using tools like CRISPR to replace or repair faulty genes (Mayo Clinic, 2024). By repairing faulty genes, it's possible to stop these diseases before they even start [National Heart, Lung, and Blood Institute (NHLBI), 2022]. This also means that gene editing can help individuals with their suffering so that they can have good mental health as well. This means that it could help ageing populations because individuals will be able to live longer by not having diseases.

Gene editing can also help people with genetic disorders like people who can't have children. Sometimes, couples cannot have children because of a problem in their DNA. To fix this problem, scientists found a new way called in vitro gametogenesis (IVG), which helps make custom human eggs and sperm in the laboratory that originated from any cell of a person's body (Rao, 2023). With this tool, scientists can help people have healthy children.

Gene editing can also help make custom medicines to treat diseases like cancer, and genetic problems, according to the individual's DNA (World Economic Forum, 2024). It helps doctors create better treatments because they are designed specifically for the patient and have fewer side effects. This makes it easier to target the exact cause of the disease, improving results and reducing side effects.

Risks of Gene Editing

While gene editing may have a lot of benefits it may have some cons. Another big concern of Gene editing is unintended consequences. Unintended Consequences in gene editing happen when unexpected consequences happen while editing human DNA. Since human genes are so complex, even scientists and doctors make mistakes. These mistakes whether big or small can affect other genes too because everything in our DNA is connected (Schleidgen et al., 2020). This is a significant concern. Mistakes in gene editing could lead to health risks, genetic disorders, or other harmful effects. For instance, a study by Alanis-Lobato et al., 2021 revealed that while most mutation by CRISPR-Cas9 were small changes in the DNA, there were large unintended mutations in about 16% of samples that would not be found by conventional methods. This shows how hard it is to find all risks even while using standard methods.

Another big concern is germline editing. When there are changes made in embryos, they get passed onto future generations (Bergman, 2019). While this could help individuals with diseases, it also means any mistakes are permanent, with long-term effects we don't even know yet.

Concerns

Gene editing has a lot of important concerns, which we need to think about carefully before using it. Here are some of the main ones:

Designer Babies

Designer babies are babies whose embryos are either selected from a preimplantation genetic diagnosis or having their genes edited to influence the traits of the resulting babies (Pang & Ho, 2016). This process is done through IVF (in vitro fertilization), which allows doctors or scientists to select embryo's with desired genes or make changes to the embryo's DNA (Pang & Ho, 2016). In this section, you will learn about how fascinating it is to imagine how designer babies could shape our world, especially in culturally diverse places like yours.

However, the cost of these procedures can be excessively high, creating a financial barrier for many families. For example, in 2018, a single cycle of IVF alone accounted for approximately 57% of the average annual income of an American household (Bill of Health, n.d.). The high cost of these procedures raises important ethical questions about wealth inequality. This shows that only rich families can afford these technologies.

There are also other issues, such as selecting specific traits that could change how we value people, raising questions like, is 'ugliness' or having a below-average IQ considered as a disability that needs to be corrected by genetic engineering? (Thompson, 2019). Parents might choose traits based on what society thinks is "perfect" instead of letting nature decide. This could make future generations of individuals feel like they have to fit into the "perfect" standards, and people who are different might feel left out. As we think about the future generations it's important to remember how these changes can affect our society.

Playing God

The idea of "playing God" is a big concern when it comes to changing human DNA. The phrase often refers to humans taking control over things beyond their power. Changing someone's DNA raises big questions about whether we should even have the right to decide things like how intelligent, good-looking, athletic, or even disease-resistant a person should be.

Some philosophers and religious leaders think that by changing people's DNA, we might be going too far and crossing moral boundaries. They argue that it goes against the natural way of life, which many people believe is guided by forces like nature or a higher power (Locke, 2020). Trying to make humans "better" based on what we believe is desirable, could negatively impact the natural balance of life.

In religions like Christianity, Islam, and Judaism, the idea of "playing God" is even more important. They believe that life is sacred and created by God, so changing DNA might be seen as disrespecting God's original idea (Locke, 2020). Some people believe that we should leave life the way it was naturally made instead of trying to change it because it could lead to unexpected consequences.

As technology is growing, we need to make sure to be as careful as possible about how we use gene editing. We should think about whether changing DNA fits the moral values of existing beliefs that are out there

Informed Consent

Germline editing makes permanent changes to the DNA of embryos or reproductive cells, affecting not only the individual but also future generations (Rubeis and Steger, 2018). The main issue

is that the individuals who are born with these changes aren't able to give their consent. This challenges the principle of autonomy, which says that people should have the right to control their own bodies and make decisions about their lives (Sander and White, 2023).

Since these genetic changes are passed onto future generations, any mistakes or unforeseen effects could have long-term consequences (Rubeis and Steger, 2018). According to Rubeis and Steger (2018), since modification in embryos is passed on, errors in modification may have negative effects in future generations. Since genes may also interact with each other, it is hard to predict the exact effect or trait that will be manifest in the future individual (Rubeis and Steger, 2018). This shows that germline editing can be very unpredictable and risky.

For example, genetic modifications might unintentionally cause health problems or other issues that the individual didn't sign up for. Parents might also select traits based on their personal preferences, which may not align with what the child or future generations would prefer. This lack of choice means future individuals are forced to live with permanent decisions they had no say in, which raises questions about fairness and respect for their rights.

As genetic editing continues to improve, we should be cautious and should not overlook the risks of it just by focusing on the benefits. Changing the DNA of future generations without their consent could go against their rights, leading to unexpected issues. It's important to make sure that scientific research also respects the rights of people who will be affected later on.

Conclusion

Gene editing technology is being developed to answer many health problems, even in unborn babies. Although really promising, the long-term effects of gene editing on the human body are still unknown. People also view gene editing as unnatural or contrary to their personal religious beliefs where every individual is intendedly created by God for a purpose, raising religious concerns. Furthermore, there are concerns about unequal access to these things, benefiting only those who can afford them and expanding the gap between rich people and poor people, which could negatively affect society. Ethical concerns also arise as there is no consent in editing the genes of an unborn individual.

In the future, research should focus on making gene editing safer, minimizing the risk and elevating the success rate. Gene editing development should also be accompanied by the development of rules or guidelines for how to use it while thinking about how it could affect society. As technology gets better, it's important to make rules that balance its benefits with ethical concerns., Everyone should be able to access the technology, not just the rich people, otherwise it might be used for unnecessary purposes that can affect society. Future research should also look at how gene editing might change ideas about things like identity and fairness. We need clear guidelines to make sure that gene editing is used in ways that align with our values and protect everyone's rights. Furthermore, scientists should work on creating safety rules and plans that consider ethical concerns, so we can use gene editing without any fear while making sure its benefits are shared fairly.

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Gene Editing and Global Food Security

Roah Kim

Introduction

With the global population projected to reach 9.7 billion by 2050, the challenge of ensuring food security has become increasingly urgent (FAO, 2023). Climate change, soil degradation, pests, and diminishing arable land intensify this challenge. Gene editing, particularly CRISPR-Cas9 technology, presents promising solutions for enhancing crop yield, improving resistance to environmental stressors, and increasing nutritional value. This review examines the role of gene editing in bolstering global food security, assessing the technological advancements, and the potential for long-term sustainability. The review seeks to evaluate how gene editing can help alleviate global food insecurity and its implications for future agricultural development.

Gene Editing as a Pillar for Global Food Security

Gene editing technologies, particularly CRISPR-Cas9, have revolutionized plant science by enabling precise modifications to DNA sequences (Idris et al., 2023). CRISPR-Cas9 operates by employing a guide RNA to target a specific sequence in the DNA, where the Cas9 enzyme then induces a double-strand break. This break can be repaired by the cell's natural repair mechanisms, allowing scientists to disable genes or insert new genetic material at the targeted site. This precise mechanism renders it highly effective for modifying crop genomes to enhance desirable traits. These techniques facilitate the development of crop varieties with improved resistance to drought, pests, and diseases, which directly supports global food production and availability. For example, researchers have successfully edited the genomes of rice and wheat to boost drought resistance and reduce dependency on chemical pesticides (Amoah et al., 2024).

In Africa, gene-edited maize varieties have shown promise in resisting the Fall Armyworm, a major pest affecting corn yields (Prasanna et al., 2018). According to a study by the FAO, gene editing is regarded as a vital tool for sustainable and climate-resilient agriculture worldwide (FAO, 2023) These scientific advancements suggest that gene editing could be a viable tool in addressing food insecurity in vulnerable regions by enhancing crop resilience, ensuring consistent production, and mitigating agricultural losses.

Enhancing Nutrition to Combat Malnutrition

Beyond yield resistance, gene editing supports food security by addressing nutritional deficiencies in populations that rely heavily on staple crops. Biofortification, or the process of increasing the nutrient content of crops, has been successfully achieved using gene editing. For instance, scientists have developed gene-edited rice that contains higher levels of provitamin A, addressing vitamin A deficiency in regions where rice is a primary food source (Senguttuvel et al., 2023). Similarly, CRISPR has been used to increase the iron and zinc content in wheat and cassava, which are essential micronutrients for human health (Senguttuvel et al., 2023). A recent methodological advance using the CRISPR-Cas system has further improved the precision and efficiency of such modifications in a cost-effective way (Ceasar and Kavas, 2024).

Table 1: Nutrient concentration improvement in gene-edited crops

Crop	Nutrient Enhanced	% Increased
Rice	Provitamin A	+30%
Wheat	Iron	+25%
Cassava	Zinc	+22%

These improvements could significantly reduce malnutrition and related health issues in developing countries, contributing to food security by ensuring access to nutritionally adequate food.

Environmental Sustainability and Agricultural Resilience

Gene editing can also promote environmental sustainability by reducing the need for chemical fertilizers and pesticides. Modified crops that are pest-resistant or drought-tolerant reduce the frequency of chemical treatments, which often contaminate soil and water sources. In one study, gene-edited soybeans demonstrated a 30% reduction in pesticide use without compromising yield (Shen et al., 2024)

This sustainable approach not only supports ecological balance but also reduces input costs for farmers, making agriculture more economically pratical and viable. Moreover, the environmental benefits of gene editing technologies align with global sustainability goals and climate change adaptation strategies, which are essential for long-term food security (FAO, 2023).

Ethical Considerations and Public Acceptance

Despite its potential, gene editing faces ethical and regulatory challenges. For instance, the United Kingdom's Genetic Technology (Precision Breeding) Act 2023 facilitates the development of gene-edited crops, yet ongoing negotiations with the European Union may delay its implementation due to regulatory alignment concerns (Idris et al., 2023). In contrast, China has approved several gene-edited crop varieties, including soybeans and rice, to enhance yields and reduce import dependence, reflecting a more permissive regulatory stance (Idris et al., 2023). These examples illustrate the diverse regulatory landscapes that influence the adoption and advancement of gene editing technologies globally. Public concerns regarding genetically modified organisms (GMOs) extend to gene-edited crops, even though gene editing does not always involve transgenic modifications. The lack of clear global regulations and varying national policies complicates the adoption of gene-edited crops (Idris et al., 2023). Ethical issues include potential biodiversity loss, corporate monopolies over seed patents, and the socio-economic divide between nations that can afford advanced technologies and those that cannot (Amoah et al., 2024). As emphasized by Niu et al. (2022), public engagement and inclusive policymaking are crucial to ensure equitable and transparent use of gene editing in agriculture. To gain public trust, transparent labeling, education, and equitable access must be prioritized to enable broader adoption that truly enhances food security.

Conclusion

Gene editing presents a transformative opportunity to tackle global food insecurity by improving crop yield, resilience, and nutrition while reducing environmental impact. The connection between gene editing and food security is evident in its ability to stabilize food systems under climate pressure, reduce nutritional deficiencies, and create more sustainable farming practices. However, the success of gene editing in agriculture depends on robust regulatory frameworks, ethical considerations, and public engagement.

Current research shows promising results, but gaps remain in long-term ecological impact studies and equitable technology distribution. Future research should focus on assessing these long-term effects and developing frameworks that ensure the safe and equitable application of gene editing. Interdisciplinary collaborations among agricultural research institutes, international organizations such as the FAO, and universities with strong biotechnology programs could lead these efforts. Additionally, partnerships with policymakers and local farming communities will be essential to ensure that research outcomes are both scientifically sound and

socially relevant. If implemented responsibly, gene editing could be a key component in achieving global food security in the 21st century.

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