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Crispr-Cas9 Gene Editing: Ethical Considerations and Future Applications

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Abstract— *CRISPR-Cas9 gene editing has emerged as a revolutionary technology with the potential to transform genetic research, medical treatments, and agriculture. Its unparalleled precision in modifying DNA sequences offers exciting opportunities for addressing genetic diseases and advancing scientific knowledge. However, this transformative power brings forth profound ethical considerations that resonate through medical, societal, and environmental realms. This abstract encapsulates an exploration of the ethical dimensions of CRISPR-Cas9 gene editing and its future applications. The advent of CRISPR-Cas9 gene editing has ushered in a new era of genetic manipulation, promising to reshape the boundaries of medicine, agriculture, and scientific understanding. This technology enables precise modifications to DNA sequences, offering a revolutionary means to correct genetic defects, enhance traits, and potentially revolutionize various fields. However, its immense potential is accompanied by a cascade of ethical concerns that require in-depth examination. CRISPR-Cas9 gene editing poses fundamental ethical dilemmas that intersect with human values, societal norms, and our understanding of nature. The ability to directly alter genetic material prompts questions about playing with the very essence of life. Concerns about unintended consequences, potential genetic enhancement, and the moral implications of altering the human germline weigh heavily on the ethical scales.*

Keywords— ***Ethical, Human germline, environmental, transform, Genetic.***

I. INTRODUCTION

The realm of genetics and biotechnology has been revolutionized by the advent of CRISPR-Cas9, a groundbreaking gene editing technology. This powerful tool enables scientists to modify DNA sequences with unprecedented precision, offering remarkable potential for addressing genetic diseases and advancing medical research. However, the immense capabilities of CRISPR-Cas9 also give rise to profound ethical questions and implications. This introduction delves into the revolutionary nature of CRISPR-Cas9 gene editing, the ethical dilemmas it poses, and the promising future applications it holds.

CRISPR-Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats and CRISPR-associated protein 9) is a genome editing technology that has opened new avenues for altering DNA sequences within living organisms. It allows scientists to target specific genes and make precise modifications, ranging from correcting mutations that cause genetic diseases to enhancing desirable traits.

The extraordinary precision and potential of CRISPR-Cas9 also raise ethical concerns. The ability to edit the human genome poses ethical questions about the boundaries of genetic intervention, the potential for unintended consequences, and the long-term impact on future generations. Balancing the benefits of medical advancements with the ethical implications of altering the human genetic code is a complex challenge.

The distinction between somatic and germline editing adds another layer of ethical complexity. Somatic editing modifies genes within an individual's body and is not hereditary, while germline editing alters genes in reproductive cells, potentially affecting future generations. The ethical implications of germline editing include concerns about unintended genetic changes and "designer babies."

As CRISPR-Cas9 technology advances, ethical considerations have prompted calls for strict regulations and responsible research practices. International and national regulatory bodies are working to establish guidelines that ensure the technology is used safely and ethically, promoting transparency, informed consent, and oversight.

Despite the ethical dilemmas, the future applications of CRISPR-Cas9 hold immense promise. In medicine, it offers potential treatments for genetic disorders like sickle cell anemia and cystic fibrosis. In agriculture, it could enhance crop yield and quality, addressing global food security challenges. Additionally, CRISPR-Cas9 has implications for environmental conservation, bioremediation, and more.

CRISPR-Cas9 gene editing stands at the intersection of scientific innovation and ethical introspection. While it holds remarkable potential to reshape the landscape of healthcare, agriculture, and beyond, its ethical implications demand careful consideration. As we explore the ethical dimensions of altering the genetic blueprint of life, society faces the challenge of ensuring that scientific progress is aligned with responsible and conscientious choices, ultimately steering CRISPR-Cas9 towards a future that benefits humanity without compromising our ethical principles.

II. LITERATURE REVIEW

Fani Memi (2018) More than 10,000 human monogenic disorders may be treatable with gene therapy, and an even larger number of complicated polygenic ailments may be helped by gene therapy as well. Researchers now have at their disposal a game-changing tool for gene therapy thanks to the adaptation of CRISPR/Cas9, a long-forgotten bacterial immune defense mechanism, into a gene-editing technique. There are still technological hurdles and ethical considerations to be made before clinical uses of this technology become routine, despite the growing scope of research and clinical applications of this technology. We provide an overview of CRISPR/Cas9 technology, its advantages and disadvantages in the lab and in the clinic, and the ethical concerns raised by CRISPR gene editing.

Fatma Betül AYANOĞLU (2020) The field of genetics has seen radical shifts as a result of genome editing technology. CRISPR-Cas9 technology stands out among them because to its cheap price, excellent precision, and ease of use. It has quickly become a topic of discussion in areas that affect people, animals, and the environment, but it has also raised some challenging questions, applications, concerns, and bioethical difficulties. Germline genome editing, in which human sperm and eggs are altered genetically, is the primary source of worry about CRISPR-Cas9. There is a chain reaction of bioethical concerns that stem from germline genome editing, including the possibility of unintended modifications to the genome, who gives permission, and how it is given. The potential environmental, agricultural, and animal welfare concerns posed by CRISPR-Cas9 technology should not be overlooked. For CRISPR-Cas9 to be used responsibly across disciplines and to address any potential problems that may arise, international legislation should be drafted with input from life scientists, social scientists, policymakers, and other sector stakeholders. However, scientific independence must not be compromised by these regulations. Ethical concerns about employing CRISPR-Cas9 for genome editing are addressed, and many medical and agricultural uses of the technique are detailed. There is discussion of social and bioethical issues as they pertain to people, other living things, and the natural world.'

Adam P. Cribbs (2017) The scientific use of CRISPR-Cas9, a method of editing genomes that emerged in 2012, has spread fast over the world in a short amount of time. By binding to complementary DNA sequences, RNA guide molecules engage the endonuclease Cas9 to create double-stranded breaks in the target DNA. Repairing the ensuing double-stranded split makes it possible to add, subtract, or otherwise alter individual DNA bases. The procedure has become popular in the lab since it is simple, fast, and inexpensive. To create even more sophisticated animal model systems, it is also being used in vivo. Genome editing has been shown to be successful and may one day be used as a treatment for both inherited and acquired disorders. Genome editing is in its infancy, but it has the potential to be a game-changing medicine for patients. As the technology advances to more difficult concerns, like as manipulation of the germline, the legal and ethical frameworks have yet to be completely debated and will become an increasingly critical subject. We discuss some scientific and ethical concerns that might affect future research and clinical use of this technology.

Biology and function of CRISPR-Cas9 technology

DNA breaks are a common byproduct of genome editing technology. According to the literature (Kim and Kim, 2014; Roh et al., 2018), nucleases are the only building blocks of ZFNs, TALENs, and CRISPR-Cas9. The power of these methods lies in the fact that the researcher may choose where in a target sequence they want to generate cracks. This paves the way for practical genome editing everywhere (Memi et al., 2018). Genome modifications are largely dependent on the cells' ability to repair damaged DNA (Lau et al., 2018). DNA double-strand breaks may be repaired in two distinct ways, both of which are present in all cells. Homologous-dependent repair (HDR) is one, while nonhomologous-end-joining (NHEJ) is another. While

HDR relies on sequence homology to repair a damaged DNA site, NHEJ does not need it and instead uses a rapid, direct connection between the ends of the fractures. Homology is achieved when DNA damage is repaired using the undamaged sister chromatid as a template (Urnov, 2018).

As previously discussed (Barrangau et al., 2007; Marraffini and Sontheimer, 2008), CRISPR-Cas9 is a naturally existing defensive mechanism in bacterial species that confers resistance to plasmids and bacteriophages. CRISPRCas9 permits the incorporation of small viral DNA molecules into the CRISPR site when the virus or plasmid enters a bacterial cell. Clustered regularly interspaced short palindromic repeats (CRISPRs) are short DNA repeats of viral or plasmid origin that are often found in bacterial genomes. CRISPR-associated (Cas) genes code for proteins with the ability to cleave or dissolve DNA and are linked to CRISPR repeat sequences (Jansen et al., 2002). Originally discovered in Streptococcus pyogenes, Cas9 is an endonuclease with the ability to cleave DNA at either end of the molecule (Doudna and Charpentier, 2014; Rodriguez, 2016). The Cas protein is activated by the CRISPR-Cas system, which detects viral or bacterial DNA and sends it packing (Otieno, 2015). Genome editing was made possible with the discovery that the CRISPR-Cas system can be instructed to locate and snip off particular areas of DNA (Jinek et al., 2012; Hsu et al., 2013). The discovery that CRISPR-Cas9 may be used to alter the human genome ushered in a new era in genetic engineering and opened up the possibility of using genome editing for therapeutic reasons (Lau et al., 2018; Roh et al., 2018).

DEVELOPMENT OF THE CRISPR-Cas9 SYSTEM: A HISTORICAL PERSPECTIVE

Since the development of CRISPR-Cas9 technology in 2012, so-called gene editing in live cells has dominated the biological research community. Genome editing is nothing new, however; scientists have been using transgenic mice in experiments since the 1970s with great success. Because of this, transgenesis has become a very useful method for studying the complex biological systems that contribute to illness. Despite its widespread use, this method of introducing a genetic component (transgene) into a cell proved unsuccessful in carrying out a targeted insertion into the genome. Research in the 1980s showed that embryonic stem (ES) cells, which kept their pluripotency to give birth to numerous different cell types, could be modified to attain directionality. It was technically difficult and was still a rather ineffective approach (with a success rate of less than 10%).

Because of these restrictions, researchers have been working hard to create new gene targeting methods. Zinc finger nucleases (ZFNs) were the first systems that could be built to detect particular sequences of DNA. Transcription activator-like effector nucleases (TALENs) followed in 2010. To avoid cell death, the cell might repair the double-stranded DNA breaks produced by these enzymes by recognizing their unique target sequences. There are two possible mechanisms for this fix (see Figure 1). The cell's repair mechanisms begin by re-joining the DNA break ends in a process called non-homologous end-joining (NHEJ). The inaccuracy of this repair process, however, may lead to the introduction of DNA insertions or deletions (Indels), which can interfere with the proper translation of the targeted gene. Second, a homology-directed repair (HDR) may take place to specifically mend the double-stranded break by using a complementary matching sequence of the DNA. This method is less effective than NHEJ, but it may be used to insert or delete particular DNA sequences at the location of the double-stranded break.

APPLICATIONS OF THE CRISPR-Cas9 SYSTEM IN HUMAN HEALTH

The CRISPR-Cas9 system's capacity to break the genome at a precise targeted site has opened up a wide range of possible applications in biological research. Genome editing has been used in the creation of functionally stable cell lines for the purpose of creating experimental models of illness. Numerous species, from bacteria to fish to birds to fruit flies to humans, have shown that CRISPR-Cas9 can successfully edit their DNA. In 2012, a unique variant of CRISPR-Cas9 engineered by this technique was successfully deployed in human cells. CRISPR has now been used as a revolutionary approach to study a wide range of biological processes and diseases. For instance, it has been tweaked such that certain transcription factors may be instructed to seek for and either activate or suppress a given set of genes. Cas9 was modified further, allowing researchers to examine the effects of methyl group modification on gene expression at precise DNA locations. Recently, scientists have utilized CRISPR-Cas9 to create genetic switches to regulate cell destiny and program conditional behaviors, transforming cells into programmable computers. These examples show how flexible the CRISPR-Cas9 system may be when it comes to producing in vitro research tools for fundamental science.

CRISPR-Cas9 is useful not only for in vitro applications, but also for the creation of in vivo animal models for the enhanced investigation of illnesses. By using the system to introduce loss-of-function mutations in tumor suppressors or gain-of-function mutations in oncogenes, for instance, animal models have been established to assess the harmful impact of mutations in cancer. Germline gene editing in mice has allowed for the creation of whole-organism and

conditional models of human illness. The use of human embryos in the study of the development of illness is an exciting use of germline editing. However, until recently, research committees were hesitant to approve such studies. Recently, a team of Chinese researchers has conducted a comprehensive study of gene functions in CRISPR-Cas9-modified human embryos to determine the tool's potential for future study.

CRISPR-Cas9 genome editing has great promise for improving healthcare and eliminating inequalities. CRISPR-Cas9 has shown promise in controlling and even curing diseases by targeting the genomes of pathogens like Hepatitis B and HIV. In the case of HIV, for instance, CRISPR-Cas9 has shown that introducing Indels is fatal to the virus, while other alterations to the virus have demonstrated greater virulence. An increasingly appealing therapeutic use of genome editing is training the immune system to target HIV. Treatments for leukemia and other blood malignancies have mirrored these approaches. Cell-based treatments, in which cells are extracted, altered, enlarged, and reintroduced into the patient to increase the therapeutic impact, have demonstrated considerable benefits. However, given the current level of the technology, CRISPR-Cas9 is not anticipated to be beneficial for numerous other illnesses, such as solid tumor malignancies or those that damage tissues or organs. Despite the obstacles, researchers are still exploring ways to use CRISPR-Cas9 to alter genes for diseases including Cystic Fibrosis (CFTR) and Duchenne muscular dystrophy (dystrophin). Despite these advances, such research is still in its early stages, and the technology still faces hurdles relating to delivery systems, similar to those experienced by gene transfer technologies in the 1990s.

Function and Origin of the Crispr/Cas9 System

The CRISPR/Cas9 system occurs naturally as an immune system in prokaryotes that helps them combat plasmids and bacterial viruses. The "clustered regularly-interspaced short palindromic repeats" (CRISPR) that arise when a bacterium is exposed to a virus or plasmid are responsible for this adaptive response. These repeats consist of short lengths of DNA sequence followed by shorter segments of spacer DNA. The DNA-cutting and DNA-unwinding enzymes encoded by the cas (CRISPR-associated) genes are CRISPR repeat-associated nucleases and helicases, respectively. The CRISPR system works by encoding RNA sequences to remember the DNA sequences of invading viruses or plasmids, and then directing a cas nuclease to cut the DNA of the same virus if it invades again. Cas9 is a nuclease that can cleave DNA at two distinct locations on each strand of the double helix. It was first discovered in the bacteria Streptococcus pyogenes. Transcribing spacers and palindromic DNA into a lengthy RNA molecule, the bacteria react to invading phages by employing trans-activating RNA (tracrRNA) and protein Cas9 to break the RNA molecule into pieces (called crRNAs). Later, it was found that Cas9 could be programmed to find and cut specific target DNA segments using a guideRNA composed of both tracrRNA and spacer RNA; crRNA contains a segment able to bind tracrRNA forming a hairpin loop; cas9 is able to modify DNA utilizing crRNA as guide; and crRNA contains a segment able to bind tracrRNA forming a hairpin loop. In either Non-Homologous End Joining (NHEJ) or Homology Directed Repair (HDR), a DNA repair template is utilized to guide the insertion of a new segment of DNA with the desired function. Transfecting target cells using CRIPSPR/cas9 often requires the use of a plasmid or virus as vector. The goal of designing crRNA is to direct Cas9's binding to the target DNA sequences in a cell. The DNA insertion sequence is encoded by a template that is intended to overlap with sequences on either side of the break.

Ethical Issues

Balance of risks and EHQH: The advantages of a study must outweigh its hazards if it is to be considered ethically sound. Risks need more consideration since they may cause harm to people and the environment. There is a chance that unwanted target alterations will be created as a result of using the CRISPR/Cas9 technology. Human cells have a high frequency of target effects, whereas mice and zebrafish have a low frequency. The issue of many DNA sequences in big genomes being identical or extremely similar to the desired target DNA sequence is a common challenge. These undesired sequences may also be cleaved by CRISPR/Cas9, leading to alterations that might lead to cell death or transformation. (Efforts have been made to decrease off-target mutations, but there's still room for advancement, particularly with regards to the pinpoint accuracy of mutations required for therapeutic interventions. CRISPR-Cas9 transport into cells and tissues that are difficult to transfect or infect efficiently is another pressing issue.

Ecological disequilibrium: It is important to consider off-target effects while doing studies using RNA-guided gene drives based on the CRISPR/Cas9 technology. The potential for off-target mutations persists and, potentially, increases with each successive generation of artificially made creatures as gene drive continues to function. The same goes for transmitting changed sequences, which might potentially impart the undesirable feature to related creatures outside national boundaries, if there is a possibility of transferring genes to other species. It may be challenging to regulate the spread of gene driving traits. Furthermore, the loss of an entire population via gene drive

may have severe repercussions for the stability of the ecosystem as a whole. For instance, new forms of disease might emerge. Some researchers have expressed concern about the potential consequences of unintentional environmental discharge of gene-driven experimental organisms. Each possible use and the need of regulatory standards must be carefully evaluated. Precautions must be taken to prevent the spread of organisms that might harm the environment or people.

Regulations for consumers: The efficiency of the CRISPR/Cas9 approach to achieve precise genetic alterations makes it harder to both identify a genetically modified creature after it has left the lab and to control these species in the market. It is unclear how regulatory agencies, like as the Food and Drug Administration in the United States, would deal with the potential of an enlarged market employing CRISPR/Cas9. The patenting system needs better oversight, for example. There are a lot of financial vested interests at stake. The huge expansion of biotechnology may be traced back to the practice of patenting transgenic organisms with industrial applications and human gene sequences with medical applications. However, patenting is a common activity that might spark legal disputes. Controversy and tensions have arisen among biotech firms about patenting CRISPR/Cas9 for human medicinal usage.

III. CONCLUSION

Numerous people have called for a public discussion to address the moral, ethical, and legal concerns raised by genome-editing technology's potential use in the human germline. Many other social and ethical issues, including the non-maleficence principle in risk assessment, safety concerns to prevent ecological impairment, and the prospect of CRISPR/cas9 being used for genetic enhancement, are brought back to the forefront by the technology's widespread application.

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