The Development of Gene Editing Technology and Controversial Issues: A Discussion

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Abstract. The fields of genetics and biomedicine stand to benefit greatly from the innovative technique known as gene editing technology, especially with the potential uses of CRISPR-Cas9.the creation of crops resistant to disease, the treatment of genetic illnesses, and the advancement of biomedical research in fields like sickle-cell anemia and rice gene editing are all made possible by CRISPR-Cas9's capacity to precisely pinpoint and edit specific genes. Despite being beneficial to humanity, the quick development of human gene editing technology has also brought up ethical, legal, societal, and technical issues. Its management, informed consent, privacy, and social equality are among the issues that require considerable attention.In an effort to give the scientific community something to think about, this article examines the state of gene editing today, its potential for the future, and the debates surrounding its application. It concentrates on the way the CRISPR/Cas system works, how CRISPER/Cas9 gene editing is used, and the range of problems it has brought up.

Keywords: CRISPR/Cas; effects; ethics, laws.

1. Introduction

After Watson and Crick's 1953 discovering of the double helix structure of DNA, gene research has increased every year. Related studies have also advanced quickly, with gene editing technology emerging from the early homologous recombination to nuclease gene editing technologies, Zinc Finger Nucleases (ZFN), and Transcription Activator-Like Effector Nucleases (TALEN). Due to its quick progress, the third wave of editing gene technology, or clustered regularly interspaced short palindromic repeats(CRISPR), has surfaced in recent years. Because of its many benefits—high editing efficiency, simplicity of usage, and operational clarity—CRISPR is widely employed in the fields of pharmacological research, gene breeding, and medical treatment. CRISPR technology has been applied extensively in various domains such as gene breeding, medication research, and illness therapy. Because CRISPR technology has so many uses, more varieties of CRISPR/Cas systems have been gradually discovered and improved.

In order to further the development of gene editing technology, this paper describes the genesis and evolution of the CRISPR/Cas system, focuses on the features of this technology, and addresses a number of issues that have come up during the process. The primary event that is examined is the gene edited babies incident.

2. Organization of the Text

In 1987, Ishino et al from Osaka University, Japan, first discovered that the CRISPR/Cas system is characterised by short repeat sequences (SSRs). And in 2002, Jansen et al. named this sequence CRISPR. The four genes, from Cas1 to Cas4, that may be functionally related to it as Cas genes, which has been used since then. In subsequent studies, in 2012, Jinek et al found that a system using RNA to guide Cas9 protein can lead to DNA double-strand breaks in target genes. It is theoretically possible to edit genes through modifications to the RNA sequence, showing that the CRISPR/Cas system is a highly prospective gene tool. With the successful editing of mammalian cell genes in 2013, Cong et al. formally started the CRISPR/Cas system's practical application in the biological sciences [1].

The core of CRISPR / Cas system consists of two components: Clustered Regularly Interspaced Short Palindromic Repeats(CRISPR) and the CAS (CRISPR associate system), which is derived from phage DNA fragments that can infect prokaryotes. It consists of AT-containing leader sequences followed by special spacer sequences separated by short repeats. These short repeat sequences generally consist of 28-37 base pairs, with a total range of generally 23-55 base pairs, some of which exhibit dyad symmetry. While spacers generally consist of 32-38 base pairs. CAS is a nucleic acid endonuclease that recognises and cuts the specific DNA strands complementary to the spacers in the CRISPR sequence using the RNA guide corresponding to the spacer sequence.

3. Working Principle

3.1. CRISPR systems

CRISPR systems fall into two primary categories. A number of effector proteins are necessary for RNA-guided targeted cleavage in Class I. For Class II to cleave the DNA sequence, one RNA-guided endonuclease is needed. The specific CRISPR classification is shown in Table 1.

Table 1. CRISPR Classification [1]

category	type	subtype	Cas protein
The first category	typeI	I-A, I-B, I-C, I-D, I-E, I-F1, I-F2, I-F3	Cas1, Cas2, Cas3, Cas4, Cas5, Cas6, Cas7, Cas8
	typeIII	III-A, III-B, III-G, III-D, III-E, III-F	Cas1, Cas2, Cas5, Cas6, Cas7, Cas10, Cas11
	typeIV	IV-A, IV-B, IV-C	Cas1, Cas2, Cas5, Cas6, Cas7
The second category	typeII	II-A, II-B, II-C1, II-C2	Cas1, Cas2, Cas4, Cas9
	typeV	V-A, V-B, V-B2. V-C, V-D, V-E, V-F1, V-F1(V-U3), V-F2, V-F3, V-G, V-U1, V-U2, V-U4, V-K(V-U5)	Cas1, Cas2, Cas4, Cas12
	typeVI	VI-A, VI-B1, VI-B2, VI-C, VI-D	Cas1, Cas2, Cas13

Through three stages, the CRISPR/Cas system protects against viruses and foreign genetic material. The proto-spacer region acts as a spacer between CRISPR-derived RNA (crRNA) repeats in the host CRISPR site in the early stage of the procedure. The Cas protein's expression comes next. The spacer is transcribed into pre-crRNA using it. Furthermore, pre-crRNA is cleaved by the Cas protein to produce mature, functional crRNA. Third-stage genomic cleavage is triggered by the crRNA helping the Cas protein identify the target. Many CRISPR systems depend on Protospacer Adjacent Motif (PAM), which is located adjacent to the target genome's crRNA-specific region. Owing to CRISPR/Cas system’s distinct characteristics, researchers think that it could be the most effective gene editing technique now available. Among them, the most widely used system is CRISPR/Cas9.

3.2. CRISPR/Cas9 System

TracrRNA, crRNA, and the Cas9 protein are the three parts of the natural CRISPR-Cas9 system. Through local base pairing, crRNA and tracrRNA combine to generate guide RNA (gRNA), which binds to the Cas9 protein and directs it to identify and cleave the target DNA sequence. With its short cycle time, high efficiency, ease of usage, and simplicity in synthesis, CRISPR/Cas9 is the most widely utilized gene editing technique to date. the system is distinct from conventional gene editing methods such as TALEN and ZFN in that it can be used to reprogramme the sequence specificity of Cas9 with just a modest guide RNA (sgRNA) sequence alteration. In addition, Cas9 proteins restrict transcription by inactivating nuclease structural domains, which translates into RNA-guided homing-devices (dCas9). The utilization of effector fusions (sgRNAs or Cas9 proteins) to alter the transcriptional state of specific genomic loci or rearrange the genome can significantly broaden the

scope of applications for CRISPR/Cas9 system modifications in genome engineering [2]. Thus, the CRISPR/Cas9 system has been widely embraced and deployed by researchers across a wide range of domains, including microorganisms, plants, mammals, insects, and human cell lines.

4. Advantage

4.1. Gene Therapy

By inserting foreign genes into target cells, gene therapy is a therapeutic strategy to treat or compensate for diseases brought on by genetic anomalies or deletions.Because CRISPR/Cas9 technology can target numerous genes at once and is incredibly efficient at editing them, it has been utilized in gene therapy to treat diseases like AIDS, neurological diseases, tumors, and hematopoietic disorders.

Gene abnormalities affecting the beta protein cause beta-hemoglobinopathy, which includes illnesses including sickle cell anemia and beta-thalassemia. Fetal hemoglobin (HbF) can be used as a substitute for insufficient adult hemoglobin (HbA) in some cases. Yet, HbF expression is normally inhibited at approximately one year of life because of repressor binding locations in the HBG1 promoter. The HBG1 gene in CD34+ hematopoietic stem and progenitor cells was altered by Humbert et al. using CRISPR/Cas9 technology. After the modified cells were reinjected into a model of non-human primates, it was shown that up to 18% of the erythrocytes in the peripheral blood were capable of expressing HbF and the gene-edited cells imposed at a rate of up to 30% for more than a year. This implies that the modified cells effectively and reliably restored HbF expression, which may alleviate sickle cell anemia and beta-thalassaemia symptoms [3].

4.2. Cultivation of Plants

In plant breeding, CRISPR/Cas9 gene editing technology is frequently employed. It offers several advantages over conventional breeding methods, including high precision, high efficiency, low cost, and short cycle. This system is essentially similar to radiation or chemical mutagenesis breeding, but at the same time can achieve molecular breeding of directional design.

The eIF4E gene's three copies on the A, B, and D subgenomes of hexaploid wheat were successfully deleted by the Institute of Crop Science, Chinese Academy of Agricultural Sciences, using CRISPR/Cas9 technology. The findings of this research offer new target genes for the biological breeding of wheat yellow mosaic disease and show that wheat and barley share a similar recessive viral resistance mechanism. This study provides an important scientific basis and technical means for the improvement of disease resistance in wheat [4].

In plant agriculture, particular genes can be precisely targeted with CRISPR/Cas9 system to enhance critical features including disease resistance, adaptation, and yield. This offers a fresh method for breeding plants and should hasten the process of producing better crops.

5. Disadvantage

5.1. Off-target Effect

Although the target DNA specificity of CRISPR-Cas9 nuclease is determined by the gRNA, base sequences in other locations of DNA may have random similarities with targeted genes. So the editing process may accidentally alter the DNA sequence in non-target regions of the genome. Their use in clinical applications therefore raises safety concerns and there is an urgent need for systematic identification of these off-target events.

5.1.1 Biased off-target.

The CRISPR/Cas9 system may occasionally incorrectly recognize and alter the DNA sequence of non-target genes. The target gene's resemblance to other genes or a feature of the CRISPR/Cas9 system could be the root of this off-target deviation.

5.1.2 Random off-target

Even in the absence of obvious similar sequences, the CRISPR/Cas9 system may still edit in unrelated gene regions. This random off-targeting may be due to non-specific binding of the Cas9 protein or other unknown factors.

To address the problem of off-target effects, scientists are continuously improving and optimising the CRISPR/Cas system. Some approaches include the use of more precise Cas proteins, by altering or optimizing the design of gRNAs, and detecting and reducing off-target effects through the use of ancillary technologies such as genomics analysis. While genome-wide approaches for identifying and characterizing CRISPR-Cas nuclease off-target sites exist, no tool is currently available to reliably anticipate all off-target editing events, particularly the off-target events that occur at low rate [5]. Thus, it's possible that there isn't a better way to deal with off-target consequences yet.

5.2. Gene Mutation

A group of scientists from the University of Maryland, Sanford Burnham Prebys Medical Research Institute, and the National Cancer Institute discovered that close observation of mutations linked to cancer is necessary when employing gene therapy with CRISPR-Cas9.

CRISPR-Cas9 gene editing technology works by cutting the genome of organisms by targeting sites with an endonuclease enzyme, causing DNA double-strand breaks.

As a negative regulator, p53 is essential for controlling the cell growth cycle. The p53 gene recognizes when the DNA double strand is damaged and stops cell division. The team investigated p53's response to DNA double-strand breaks in almost 1,000 human cell lines. Researchers discovered that following the CRISPR-Cas9 system's removal, nearly all cell types showed decreased growth in cells with the normal p53 gene, whereas cells with p53 gene alterations grew as quickly as normal cells—and in some cases, even faster than normal cells [6]. It's crucial to keep in mind that p53 gene alterations usually cause cancerous cells. Furthermore, the researchers discovered that CRISPR gene editing might raise the likelihood of cells growing that have mutations in other genes linked to cancer, such as the KRAS oncogene.

Another research has demonstrated that the use of CRISPR-Cas9 technology to alter genes in human pluripotent stem cells led to notable genetic changes, involving the removal of numerous gene fragments and more intricate genome recombination [7]. Therefore, diseases could arise from the cellular genome damage seen during CRISPR-Cas9 gene editing.

In summary, there are many uncertainties and possible sequelae associated with gene editing technology in the CRISPR/Cas9 system. For these problems, people need to continue in-depth research and find ways to optimize them.

5.3. Ethical Issue

On November 26, 2018, He Jiankui, an associate professor at the Southern University of Science and Technology, revealed that two gene-edited babies, named Lulu and Nana, were born in China in good health. It was reportedly that the twins' altered gene (CCR5) gave them a natural resistance to the HIV/AIDS virus. The news of the "gene-edited babies" incident was reported, and it quickly stirred up a furor that shocked the world. The first occurrence of the "gene-edited babies" case was publicly declared on January 21, 2019, December 30 in the Shenzhen Nanshan District People's Court. According to the law, He Jiankui, Zhang Renli, Qin Jinzhou, and the other three defendants were held criminally responsible for collectively engaging in the illegal practice of medicine, which included the gene editing of embryos of humans for reproductive purposes.

Regarding gene editing science and technology, ethics is one of the trickiest and most delicate topics. Human nature, existence, and dignity may be significantly impacted by the use of human gene editing techniques. Gene editing technology can be categorized into reproductive and non-reproductive modes, which are trans-generational in nature. In this context, if gene editing technology is not guided by proper ethical standards, it is highly likely to undermine the dignity and value of human beings.

5.3.1 Individuals

As stated above, CRISPR/Cas9 technology is still immature, with risks of off-target effects and induced mutations. The first human CRISPR gene editing cancer trial also shows that gene editing technology is not yet a perfect solution to the problem of human survival and has many technical problems[8]. Therefore, if gene editing technology is not regulated, malicious users may cross the ethical line, falsify the procedures required for clinical trials, and apply gene editing technology to human beings, which may lead to terrible consequences.

In the case of "gene-edited babies", the targets of manipulation are in vitro embryos and germ cells, which have the potential to develop into human beings. Thus, the exploitation of human beings as manufacturing and editing subjects is against human autonomy, and gene editing has the possibility to violate human dignity[9]. Gene editing of germ line embryos would not only have an impact on the embryos that will develop into human individuals in the future, but also on the genetic make-up of the offspring, which would be irresponsible for any individual.

5.3.2 Society

At the societal level, the alienation of gene editing technology is leading to the alienation of people's needs [10]. People can see that gene editing technology is widely used, and if it is not reasonably perceived, human desires will continue to expand, the ability to think will gradually be lost, and the pursuit of reasonable needs will disappear. Society at large is often prone to herd mentality, which may rationalize alienated needs, thus affecting the values and ethics of society. Although our understanding of gene editing technology is still limited, people's social psychology and social and ethical values are being impacted. The emergence of "designer babies" signals that the widespread spread of gene-editing technology will lead mankind down a dark path, reminding humankind again of the need to remain vigilant in the use of technology.

5.4. Capital Intervention

The application of gene-editing technologies to "designer babies" may have implications for the mechanisms of equality and social mobility in society. If left unchecked, genetic optimization could lead to the stratification of societies, exacerbating inequalities and becoming a long-term challenge for humanity. In families with higher economic incomes and better-educated parents, parents usually have more time and resources to spend with their children and invest in their children's education, and thus their offspring tend to be more advantaged in terms of socio-economic status. Nonetheless, education is seen as an important pathway to social mobility, and those individuals from less favourable family backgrounds have the same opportunity to change their social status through hard study and work. However, once gene editing technology intervenes, the situation may change. Families with higher socio-economic status have the economic base and capital to give their offspring an advantage in terms of appearance, intelligence and physical fitness from an early age. As a result, the basic principle of "all men are born equal and free" may be broken, and people may lose their right to free choice.

The "editing baby" incident, which was identified as a gene-editing activity in pursuit of fame and fortune, revealed that the inappropriate combination of high technology and capital may lead to greater inequality. In this instance, public concerns and financial resources drove the development and deployment of gene-editing technologies, pushing the bounds of ethical and legal bounds before producing "designer babies." This episode serves as a reminder that a small number of powerful corporations may control the world's gene pool and intellectual property rights related to gene patents

if capital is used to advance the development of gene editing technology. The affluent class's desire for "designer babies" may result in the illicit exploitation of gene editing technology. Such actions might make the issues of social inequality and class solidarity worse.

5.5. Law and Autonomy

Currently, the issue of the law and autonomy of gene-editing technologies faces a complex reality. There are differences in the legal frameworks and regulations governing gene-editing technologies in different countries and regions. Some countries have developed relevant laws and ethical guidelines to restrict and regulate the application of gene-editing technologies. For example, some countries prohibit gene editing on human embryos and only allow gene editing on individuals who have already been born. Germany is one such example, which through the German Basic Law and Embryo Protection Act clearly stipulates that embryos belong to the subjects of the right to life under its provisions and strictly protects human embryos [11]. However, some other countries take a more open stance and allow gene editing experiments and clinical trials under certain conditions. The UK is one such example, with laws allowing gene editing of human somatic cells in research and clinical settings and authorized licensed gene editing research on human embryos, but prohibiting the transfer of genetically altered embryos into the womb [11].

Meanwhile, the right to autonomy is also an important issue. Everyone has their own thinking about whether scientists should have autonomy in gene editing research [12]. Conflicts of interest, codes of ethics and ethical standards need to be discussed in depth.

6. Initiative

6.1. Recognize the Potential Risks of Gene Editing Technology

First of all, people need to recognize the potential risks of gene editing technology. Although the technology can treat certain genetic diseases, it may also bring about some unforeseen consequences. For example, gene editing may trigger undesirable side effects such as cancer or immune system problems. In addition, gene editing may lead to irreversible consequences, such as gene mutation or gene inactivation. Therefore, people need to conduct in-depth research and assessment of gene editing technology to ensure its safety and effectiveness.

6.2. Strengthen Regulation and Management

Both human society and people's understanding of the world are continuously changing. The progress of science and technology has led to an improvement in people's comprehension and investigation of life. The research and application of human gene editing involves the common interests of all mankind. If the technology is abused, it will threaten human dignity, social justice and security. At present, the development of the technology is still immature, and its risks may far outweigh the benefits. People should be cautious about its application. The research and application of human gene editing technology is a global issue that requires extensive discussion and attention on a global scale and cannot rely solely on the internal governance of a single country. Therefore, if conditions permit, a global regulatory mechanism can be established to ensure the legality and safety of gene editing technology. At the same time, people also need to strengthen international co-operation to jointly promote the development and application of gene editing technology, so as to achieve the common interests and well-being of mankind.

6.3. Collaborate with thePublic and Scientific Community

Along with the government, the scientific community is a major force behind the creation and advancement of gene editing technologies. Proficient practitioners ought to fortify their self-control and adhere to the professional code of ethics and ethical standards. Public education and participation are also very important for the popularization of gene editing technology and the development of

public opinion. Therefore, public dialogue and education should be carried out to enhance people's understanding and awareness of gene editing technology, and to promote social consensus and acceptance.

7. Conclusion

Advances in genome editing have been improved by the advent of CRISPR/Cas techniques. The discovery of the Cas protein has also altered the direction of natural science study at the same time. Gene editing technology, as a technology with revolutionary potential, brings great hopes and challenges to mankind. It has exciting prospects for treating hereditary diseases, increasing crop yields and so on. However, gene editing technology has also triggered widespread controversies and ethical issues.

Despite the promising development of gene editing technology, people must proceed with caution. Scientific and moral principles should be followed to ensure the safety, feasibility and ethical acceptability of the technology. Only by adhering to scientific rigour, respect for ethics, and social and international participation can gene editing technology benefit mankind and make a positive contribution to sustainable development in the future.

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