Future of CRISPR: Technological Advancements and Ethics

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ABSTRACT

CRISPR is a revolutionary gene editing technology that has the ability to transform key parts of our lives, including agriculture, medicine, and scientific research. CRISPR is still a relatively novel technology about which we are still learning a great deal. Many technological advancements and discoveries of CRISPR are being tested, such as base, prime, and epigenomic editing and alternative forms of the Cas9 protein. These have the ability to limit the chance of off-target effects arising in patients being treated using CRISPR. However, ethical concerns about using CRISPR in humans need to be taken into consideration by finding ways to minimize side effects. In this review, I will discuss how advancements in CRISPR technology can alter the future of human health while reducing potential safety concerns.

INTRODUCTION

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) is a revolutionary gene editing technology. Although its use is relatively novel in research, CRISPR was first discovered in 1989. While working on halophilic archaea, Dr. Francisco Mojica identified unusual repeated DNA sequences in the genome of *Haloferax mediterranei* (Mojica & Rodriguez-Valera, 2016). These sequences were named CRISPR and initially thought to be sequencing artifacts. At the same time, researchers also identified unusual repetitive DNA sequences in the genomes of various bacteria, including *E. coli*. However, the function of these repeats was unknown.

In the early 2000s, scientists discovered the function of CRISPR. Its original function was to serve as an adaptive immune system in bacteria and archaea (Vigouroux & Bikard, 2020). CRISPR allows these microorganisms to recognize and defend against viral infections. Upon infection, bacteria store fragments of the viral DNA (called spacers) into their genomes, creating these CRISPR sequences, which then enable the bacteria to "remember" previous infections and respond more effectively to subsequent attacks by the same or similar viruses.

Function of CRISPR in Bacteria

The spacer is a segment of DNA that is complementary to the viral DNA, which allows for the recognition of past infections (Koonin & Makarova, 2019). Following spacer acquisition into the CRISPR locus, transcription occurs. Transcription turns the CRISPR DNA into a long RNA molecule that includes these newly acquired spacers scattered throughout repetitive nucleotide (repeat) sequences. The RNA strand is then processed into shorter CRISPR RNAs (crRNAs). Each of these crRNAs contain a single spacer sequence with repeat sequences. The crRNA binds to Cas proteins (e.g. Cas9), forming a complex and guiding the Cas protein to the target DNA that matches the spacer sequence (i.e., the viral DNA). When the CRISPR-Cas complex

encounters the viral DNA or any matching DNA sequence, Cas binds to it. The Cas protein then cuts the target DNA by creating a double or single stranded break, neutralizing the threat.

Use of CRISPR in Mammalian Cells

Because scientists understand the function of CRISPR in bacteria, they can manipulate it for use in mammalian cells. As in bacterial cells, the CRISPR system used in mammalian cells consists of two main components: the guide RNA (gRNA) and the Cas protein. The use of CRISPR in a laboratory begins with the creation of a guide RNA, which is specifically designed to be complementary to a sequence of interest in the target gene of the mammalian cells' genome (Mani et al., 2021). After gRNA synthesis, the Cas protein binds to the gRNA, which directs the Cas protein to the region of interest in the DNA. Once brought to the DNA, the Cas protein recognizes the protospacer adjacent motif (PAM). PAM is a set of nucleotides in the mammalian cell's DNA sequence that is adjacent to the target DNA. The PAM sequence is essential for the Cas protein to bind to DNA and create the double stranded break. Each Cas protein has its own PAM recognition site. For example, NGG is the recognition site for the Cas9 protein. Once the Cas protein binds to the target gene, it is able to create a double stranded break in the DNA.

In order to repair the double stranded break, cells activate one of two main repair mechanisms, the predominant one being non-homologous end joining (NHEJ) (Miyaoka et al., 2016). This repair mechanism is fast but error-prone. Typically, nucleotides are inserted or deleted to rapidly repair the break. This can lead to disruption of the genetic code, which can subsequently cause the gene to be inactivated. The advantage of this method is that scientists can easily use CRISPR to inactivate harmful genes and potentially treat genetic disorders by knocking out mutated genes.

The other repair mechanism is homology-directed repair (HDR). Unlike NHEJ, HDR relies on a donor DNA template to repair the break. This allows for precise editing of the genome. While this repair pathway has less errors compared to NHEJ, the efficiency of gene editing is fairly low. HDR has an edge on NHEJ because it allows for the insertion of donor DNA in a gene, the correction of mutations, or specific modifications to the genome.

While CRISPR has the ability to precisely edit genes, there is a risk of off-target effects, which can disrupt beneficial or essential genes. In this review, I will discuss the technological advancements used to improve the limitations of CRISPR and the ethical considerations for its use in human gene editing.

TECHNOLOGICAL ADVANCEMENTS IN GENE EDITING

As scientists are using CRISPR in laboratories, they are making new discoveries which have led to advancements in the use of CRISPR. Four major advancements in CRISPR technology include (1) base editing, (2) prime editing, (3) epigenome editing and (4) the use of different Cas proteins.

Base Editing

Base editing is a method that allows for the change of a single nucleotide. The process of base editing relies on the action of a nickase version of Cas9 (nCas9) and deamination (Llado, 2023). nCas9 only cuts one strand of DNA at a time instead of the double stranded break that tradition Cas9 makes. Deamination is a process in which a deaminase enzyme, or a base editor, can remove an amino group from RNA which allows for the modification of one base.

Base editing accurately and efficiently converts specific base pairs, limiting off target effects because less nucleotides are affected by avoiding double strand breaks. Base editing is also much more specific (very high specificity) and does not rely on these repair processes (i.e. NHEJ and HDR) to introduce large-scale changes like CRISPR does such as insertions, deletions, etc. (Thomas, 2023). However, the high specificity of base editing limits it to specific types of base pair changes compared to conventional CRISPR. Base editing allows for two main changes: cytosine base editors change cytosine-guanine pairings to thymine-adenine pairing and adenine base editors change adenine-thymine pairings to guanine-cytosine pairings.

Prime Editing

Prime editing is a gene editing tool that substitutes, inserts, or deletes bases. This tool is referred to as a “search and replace” gene editing technology due to its ability to find a specific gene locus and precisely modify it (Anzalone et al., 2019). Unlike base editing, prime editing allows for a wide range of genetic modifications (substitutions, insertions, deletions, and large sequence replacements), allowing for increased flexibility. Prime editing relies on the use of reverse transcriptase, an enzyme that synthesizes a DNA strand based on an RNA template, which is used to write the desired DNA sequence into the target site after the nick is made using nCas9. Specifically, a prime editing guide RNA (pegRNA) guides the Cas9 nickase to the target DNA sequence, then it carries the desired edit in the form of a template sequence that is used by reverse transcriptase to insert the desired DNA change into the target site. This method allows for increased gene editing capabilities and higher purity compared to HDR.

Despite these advantages, since prime editing uses both a Cas9 nickase and reverse transcriptase, it can be less efficient at delivering the edit compared to the simpler CRISPR/Cas9 system that relies on a single enzyme (Cas9) to create double-strand breaks (Scholefield & Harrison, 2021). When it comes to large-scale genomic rearrangements or multi-locus edits, prime editing faces limitations compared to Cas9 mainly due to the fact that the template for the edit in the pegRNA is typically limited to relatively small DNA sequences.

Epigenome Editing

Epigenome editing involves using tools to specifically add, remove, or alter epigenetic marks at specific genomic loci to regulate gene expression, without changing the underlying genetic code (DNA sequence). Epigenetic marks are chemical changes to DNA that allow for cellular identity and the regulation of gene expression (Ueda et al., 2023). For example, a methyl mark on DNA typically represents gene repression and can be inherited through multiple cell divisions. Methyltransferases are the enzymes that add these methyl groups ($-CH_3$) to

specific molecules. These epigenetic modifiers (e.g. DNA methyltransferases) can be tethered to a catalytically inactive Cas9 (dCas9) protein, and when guided by a gRNA to the specific target loci, they either add or remove epigenetic marks.

Cas Proteins

Cas proteins in general provide adaptive immunity against the invasion of foreign nucleic acids in archaea and bacteria. The CRISPR-Cas system achieves immunity by capturing small DNA fragments or spacers from foreign nucleic acids and integrating them into the host CRISPR locus. Over the years, this system has been harnessed to perform various genomic engineering tasks.

There is not just one Cas protein; in fact, there are many alongside the most widely known one (Cas9), such as Cas12 and Cas13. Although Cas9, Cas12, and Cas13 are not the only Cas proteins, they are the most influential. The Cas12 protein induces sequence-specific double-strand breaks (DSBs) in DNA, which are then repaired by cellular repair pathways similar to how Cas9 works (Clovis, 2022). Unlike Cas9, Cas12 only requires a single crRNA for targeting, and it creates a staggered break in the DNA, known as sticky ends, rather than a blunt break. This system is smaller and potentially more efficient than Cas9 (Shigemori et al., 2023).

While Cas9 and Cas12 target DNA, Cas13 is an RNA-editing system. Cas13 can knock down gene transcripts with high efficiency, making Cas13 a powerful technique for manipulating RNA expression (Huang et al., 2022). Additionally Cas13 has been utilized for RNA-based diagnostics, which are techniques that detect and analyze RNA molecules to diagnose diseases or identify pathogens. One example is the use of SHERLOCK (Specific High-sensitivity Enzymatic Reporter Unlocking), which enables detection of viral and bacterial pathogens (Kellner et al., 2019). Guided by crRNA-targeted ssRNA, Cas9 cleaves RNA adjacent to the target region. Because of this, it provides a defense against RNA viruses that affect eukaryotic cells and also permits fluorescent-based viral detection. Its programmability and specificity make it a promising tool for therapeutic applications, including treatment of RNA viruses and genetic disorders caused by abnormal levels or patterns of RNA expression in a cell.

ETHICAL CONSIDERATIONS IN GENE EDITING AND EMBRYO EDITING

Ethical Issues in Embryo Editing

CRISPR is an extremely powerful technique. That being said, we should take into account the ethics of using it, particularly when using it in human embryos. One of the possible side effects of using CRISPR in embryos is mosaicism. Mosaicism is a condition where an individual has a mix of genetically distinct cells. If CRISPR is used and not all cells are successfully edited, especially during developmental stages, a mix of edited and unedited cells may arise, leading to inconsistent outcomes with unpredictable impacts on development (Mehravar et al., 2019). While mosaicism can lead to inconsistencies in development, it allows

for better evaluation of gene function in animal models. Additionally, direct comparisons between mutant and wildtype cells within a mosaic animal can be made.

Editing cells at early developmental stages raises ethical questions about the implications of such interventions, especially regarding consent and potential long-term effects on future generations. In regards to consent, parents usually hold the legal authority to make medical decisions on behalf of their minor children (including embryos). The decisions parents make for their child or future child should and would be made with the notion that it is in the child's "best interest" as they weigh the potential benefits and risks. Under this notion, parents would be able to edit their embryos with the intention of creating a healthier child. But where is the line drawn? While considering gene editing in your embryo, the main focus should be on treating genetic disorders in order to give a future child the healthiest life possible, instead of editing accessory genes such as those that control eye color, height, etc.

Risk Concerns

Understanding the differences between somatic and germline editing is important when evaluating possible risks involved with gene editing. With germline editing, something parents should consider, apart from the fact that their child could be free from genetic disorders, is how these edits will affect future generations. Any off-target effects can also be passed down because sex cells, not somatic cells, are edited in germline editing.

Somatic editing impacts only the individual, not future generations, because a single organ is being edited, so sex cells are not affected as opposed to germline editing. However, there are still risks. One of the issues with somatic editing is that not all the cells will end up being edited. This could result in mosaicism and decreased efficiency in removing mutations. There is also a possibility for an autoimmune response where the body will attack the CRISPR edited cells thinking that they are foreign substances.

Strategies to Mitigating Risk of Germline Editing

In addition to the technological advancements made to CRISPR technologies, additional approaches can allow for safer germline editing. One of these strategies includes dual-system approaches. These systems require two independent signals to induce gene editing. In doing so, the likelihood of unwanted gene edits and off-target effects are lower since both conditions must be met for editing to occur. One example of this is CRISPR/Cas9 with inducible systems that allow for temporal (time) control. An inducible system allows for control over when gene expression is turned on or off in response to specific signals or conditions, such as the use of a doxycycline promoter. Doxycycline must be added to the cells, activating the Cas9 gene and leading to the production of the Cas9 protein. This method creates the ability to regulate when a genetic modification takes place during an organism's development or at a specific point in time. Another example of temporal control is temperature-sensitive systems. These gene regulation systems are sensitive to temperature changes. By raising or lowering the temperature, researchers can activate or deactivate genes that are controlled by temperature-sensitive promoters or repressors.

Another dual-system approach involves spatial control. Spatial control works by using precise regulatory systems, including tissue-specific promoters, genetic targeting, and delivery vectors (like AAV), to ensure that editing only occurs in the desired location (Zhu et al., 2019). The goal is to ensure that certain biological processes or modifications occur only in specific locations within the body or organism, preventing unintended effects in other areas. An example of spatial control is the use of CRISPR/Cas9 with AAV (Adeno-associated Virus) to ensure delivery to specific tissues. AAVs are small, non-pathogenic viruses that integrate into the host cell without disrupting the genome, allowing efficient delivery of the CRISPR system into cells/tissues, thus lowering chances of off-target effects (Fuentes & Schaffer, 2018).

Socio-Economic and Social Justice Issues

The integration of genetic advancements, such as embryo editing, has the potential to deepen socio-economic disparities. Gene editing therapies currently cost millions of dollars (Subica, 2023). This would allow wealthier families to gain access to genetic enhancements that improve cognitive abilities, giving their children a significant advantage in education and employment, creating an unlevel playing field over families unable to pay for gene therapy. Moreover, due to access to gene editing, these high-income groups may be able to reduce the prevalence of genetic disease, which can cause there to be a higher rate of disorders amongst lower-income groups. This would further increase the social divide between upper and lower classes because wealthier families would have expanded access to better healthcare.

Even though wealthy individuals may have access to expensive gene editing, this could lead to a reduction in genetic diversity. Genetic mutations are key to biodiversity, and by editing these mutations, the genetic variation among the rich could diminish. This might result in the possibility of shared genetic disorders within affluent communities, particularly if they continue to interbreed with each other.

Socio-Economic Disparities

Due to all of these socio-economic disparities, it is important to ensure equality in the emerging field of genomic medicine. This requires a combination of regulatory oversight by medical officials, larger distribution in financial accessibility, and ethical considerations. Governments and international organizations can implement policies that financially support genetic treatments and screenings for lower-income populations, ensuring that life-saving advancements are not reserved for the wealthy (Halbert, 2022). Expanding public healthcare systems to cover genomic interventions, much like vaccines or essential medications, can also help bridge the gap. Key stakeholders could invest in public research institutions to prevent monopolization of these genomic tools by private companies, keeping costs down and promoting wider accessibility.

Conclusion

CRISPR technology has revolutionized gene editing, offering groundbreaking applications in medicine, agriculture, and research by enabling precise genetic modifications

through mechanisms like non-homologous end joining (NHEJ) and homology-directed repair (HDR). While traditional CRISPR-Cas9 editing has challenges such as off-target effects and precision limitations, advancements like base editing, prime editing, and epigenome editing have improved accuracy. Alternative Cas proteins, such as Cas12 and Cas13, further expand CRISPR's potential by only targeting a single-strand of DNA and RNA, respectively. However, ethical concerns still arise, particularly regarding embryo editing, as issues like mosaicism, consent, and unintended consequences pose risks to individuals and future generations. Somatic editing, though safer, still carries challenges such as autoimmune responses, leading to strategies like inducible systems and tissue-specific targeting for improved safety. Socio-economic factors also play a role in the accessibility of gene editing, with high costs potentially widening disparities and reducing genetic diversity among wealthier groups. Addressing these issues requires strong regulatory oversight, financial accessibility, and public investment to ensure equal benefits. As CRISPR continues to evolve, it is essential to balance its groundbreaking potential with ethical considerations, socio-economic disparities, and potential unintended consequences.

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