Perspectives on the Current and Future Use of CRISPR Technology

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Since the discovery in 1993, CRISPR system has become one of the most significant technologies associated with gene-editing and has been rapidly evolving in recent years. CRISPR has been proposed for use in diagnostics, research and addressing serious hereditary diseases. Despite the call for a moratorium on human gene-editing through CRISPR, it is important to highlight the importance of gene-editing applications of CRISPR technologies; whether by developing antibiotic alternatives, using the system as a research tool, or even as a rapid detection system for pathogens. CRISPR technology can open doors to many solutions for modern-day medical issues, such as antibiotic resistance, targeted drug therapy, and many others. The most well-known CRISPR associated system is the Cas9 system, capable of Non-Homologous End Joining (NHEJ) and Homology Directed Repair (HDR). With the development of CRISPR technology, multiple variants have been discovered. Most variants are capable of causing specific DNA breaks, but other variants have been identified and characterized for their RNA cleaving capabilities such as the Cas13a (formerly C2C2) and Csm6 proteins. Here, we will explore the bioethical concerns surrounding CRISPR technologies. Additionally, we will highlight a multitude of gene-editing independent CRISPR applications and the most appropriate CRISPR systems for various applications. By addressing these topics, we hope to unveil the crucial effect of CRISPR upon our world and we will come to know where regulations and public discourse falls short.

Keywords: CRISPR, Cas13a, gene editing, disease eradication

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Introduction

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR), is a powerful gene editing tool that is currently streamlining gene-editing processes. CRISPR is a protein with endonuclease activity and RNA with guide properties. There are three main forms that CRISPR-Cas (CRISPR-associated) systems are found in, such as some bacterial genomes, and almost all archaeal genomes. There are also various forms of Cas proteins with varying target molecules such as Cas9, Cas13a, and Cas10 (Makarova and Koonin 2015). All these proteins differ from each other, but the difference between the two common Cas proteins, Cas9 and Cas13a, is that Cas9 is used to edit DNA, whereas Cas13a focuses on editing RNA. As previously mentioned, one of CRISPR's main properties is its ability to be a quick and efficient solution to edit genes, making previous tools such as TALEN not as desirable (Yeadon 2019). Although CRISPR allows genes to be edited easily, it has lead to controversial issues and viewpoints on the ethics of gene-editing in this highly novel field.

This paper will focus on discussing the controversy behind CRISPR and the reasoning behind the controversy. CRISPR is a very useful technique which not only edit genes, but has prospects for disease eradication, antimicrobial and drug therapy, as well as in various environmental aspects. Being informed on all its characteristics is important as it creates an informed viewpoint on the tool. As the future approaches, the novel techniques such as CRISPR will be increasingly used due its simple and effective properties. Therefore it is important to discuss various aspects of CRISPR, which will allow all the positive and negative aspects of this technology to be recognized.

Gene Editing

Gene editing is a fairly new field and with the emergence of CRISPR, gene editing has become easier and faster to accomplish (Figure 1). In 1993, the structure of CRISPR was discovered by Francisco Mojica in the archeal bacteria *Haloferax mediterranei*. *H. mediterranei* has an extreme salt tolerance and it was found that the salt concentration impacted how the genome was cut by the restriction enzyme. When the DNA fragment was observed, a sequence of repeats never seen before was discovered. Additionally, this similar structure was observed in a study in 1987 in *Escherichia coli*. Yet, many of CRISPR's properties would remain a mystery until the mid-2000s (Makarova and Koonin 2015) . Currently, the use of CRISPR systems to edit genes is a controversial field as there are many issues that could occur if the system does not work properly.

One of the main drawbacks that lie behind CRISPR technologies is that gene editing has become much easier to design and implement, allowing this technology to become commonly used by untrained or dishonest individuals. Although the ease of use seems to be a positive characteristic of CRISPR, the potential biosecurity issues make gene editing in humans a debatable subject. Recently, we have seen the negative response to Dr. He Jiankui and his announcement that he used CRISPR editing techniques on human embryos. His story brings to the surface many questions regarding CRISPR such as the ethics behind gene editing, as well as if the technology works as intended. There is still no definitive evidence that Jiankui actually succeeded in modifying the intended genes (CCR5) and he has since come under investigation by Chinese authorities (Zhang et al., 2015).

However, there are still positive impacts, gene editing by CRISPR can create as it is able to help in the eradication of diseases as well as successfully edit plants to produce stronger crops. These characteristics will be discussed further in the paper. Additionally, contrary to many people's beliefs, CRISPR contains many other proteins that do not edit genes such as Cas13a. The Lethbridge High School iGEM team is even creating a system that will administer and detect pathogenic bacteria using a Cas13a system (Aborawi 2019). The team will be utilizing Cas13a to target RNA and cleave the target bacteria for their project.

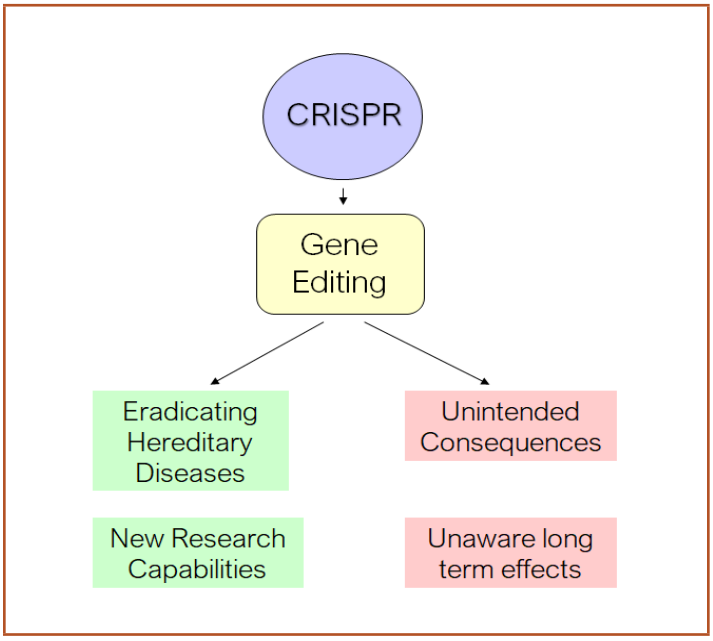

Figure 1. *Using CRISPR method for gene editing*

Disease Eradication

Diseases and illnesses are prevalent human health concerns among the world as they decrease quality of life drastically in addition to contribution towards mortality. Genetic diseases, many of which have no known cure have great impact on all socioeconomic classes. Therefore, it is a major goal to develop cures and prevention methods of these non-curable diseases. One possible, yet controversial, method of disease eradication is using CRISPR (Figure 2). CRISPR is an innovative piece of technology that has opened many doors in the world of disease eradication. Clinical trials are being run in China to observe the effectiveness of CRISPR on patients with cancer of the esophagus. So far, there have been at least 86 people with different forms of cancer treated (Suga and Hyakumachi 2004). Many other common and life-threatening diseases may potentially be treated using CRISPR including beta-thalassemia, genetic blindness, HIV, cystic fibrosis, muscular dystrophy, and Huntington's disease (Fernández 2019).

Despite these potential life-saving cures, many researchers still oppose CRISPR technology. CRISPR is incredibly innovative tool and new research to enhance its applicability are being conducted at a rapid pace. But currently, we are not yet aware of any long term effects of this treatment. Furthermore, there are concerns regarding non-targeted cell attacks. In order to ensure the safety of the patient, the CRISPR technology being used must be incredibly accurate and have minimal to no non-targeted cell attacks. In addition to these concerns, the effectiveness of this treatment is also under discussion, though few patients have been successfully treated. Application of CRISPR technology in disease treatment will not be available until further research and successful experimental modeling. While experimenting with CRISPR, many unintended consequences may arise and if so, they could be disastrous. For instance, scientists were working on examining the uses of CRISPR and in a study by Lander they examined two systems where CRISPR was targeted at blood-producing cells in mice and retinal cells in humans. Here 21% of the targeted cells had DNA deletions up to 6000 bases long (Lander 2016). This is a problem as it can result in genomic instability as well as alter the function of the gene (Begley 2018). CRISPR is an incredibly powerful tool that must be handled with extreme caution.

The ability of CRISPR to target any specific sequence means it shows great promise in antimicrobial therapy. It allows for CRISPR to target either very specific species of bacteria, or a wider range including even a whole genus (Barrangou and Doudna 2016). This specific targeting could allow for the survival of commensal and beneficial bacteria, thus avoiding a major issue that arises from the usage of broad-spectrum antibiotics. However, one obstacle in this usage of CRISPR is that delivery methods tend to be narrower than CRISPR itself, thus limiting the system's ability to target widerranges i.e., a whole genus. This is because vector phages tend to have a relatively small host range. Work to circumvent this problem includes attempts to engineer phages having wider host ranges and exploring other delivery methods altogether.

Another concern with the usage of CRISPR for disease eradication is the potential for the evolution of CRISPR resistance or anti-CRISPR genes (acr). CRISPR resistance tends to occur in the form of point-mutations in the targeted sequence (Pursey et al. 2018). Anti-CRISPR genes have the ability to deactivate important parts of the CRISPR system. There are many acr genes with somewhat different target ranges (Harrington et al. 2017). It has been said that, over a long period and on a large scale, it seems inevitable that certain methods of resistance arise. However, an attempt to fight point-mutation resistance involves multiplexing, or the targeting of multiple sequences at a time (Bikard and Barrangou 2017). Upon similar premise, to combat the rise of acr genes, one could attempt to use multiple CRISPR-cas variants at once.

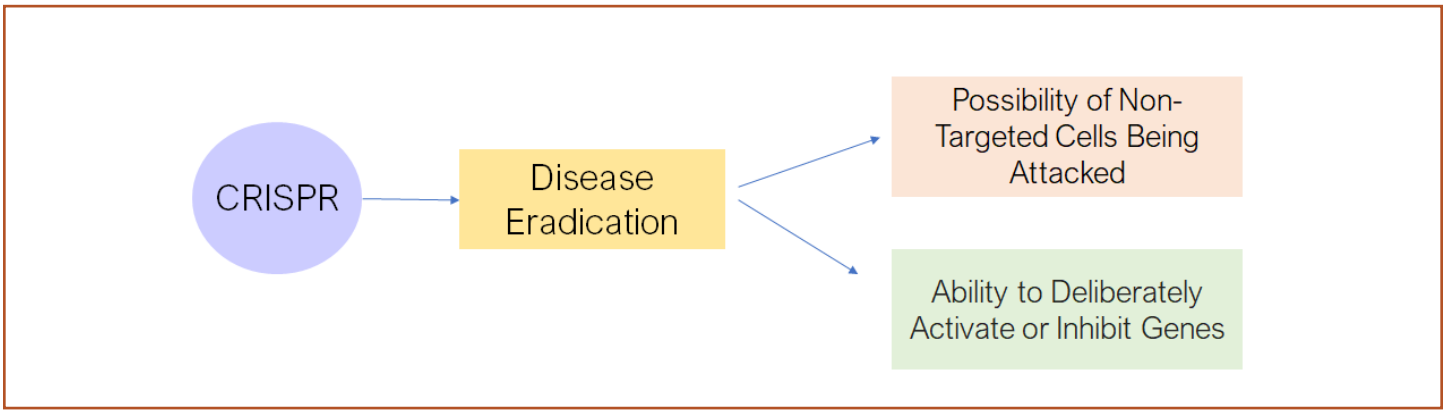

Figure 2. *Using CRISPR methods for disease eradication*

Targeted Drug Therapy

CRISPR-cas systems have been depicted as a powerful tool for cancer treatment. They can be used to target oncogenes necessary for the formation and growth of tumors.hus can reduce the proliferative abilities of certain cancer cells (Zhen et al. 2014). These immunotherapy methods have a number of advantages over other therapy methods such as chemotherapy, radiotherapy etc and even may be usable for the types of cancer that cannot be treated with conventional methods. However, these novel technologies are still in infancy stages. Stanford bioethicist Mildred Cho, Ph.D., expressed concern for CRISPR as a cancer treatment stating, "... the cancer protocol is so complex… it requires manipulating lots of different systems at the same time, especially the immune system, which is not fully predictable." Cho also worries that CRISPR treatments will be far more costly than available cancer treatments.

Environmental Aspects

Along with exponential increase in human population, there is a constant increase in human needs such as food and medicines. In order to sustain the population needs, scientists had to genetically modify many organisms to produce better yielding GMOs (Genetically Modified Organisms). Using CRISPR technologies, scientists will be able to make unique targeted deletions and edits to the genome of organisms. CRISPR would allow specific desired segments of DNA to be added into a plant or even to delete an undesirable gene segment (Cyranoski 2019). In addition to this, CRISPR opens up new research prospects in plant biology. Traditionally, biochemical research was done by overexpressing a protein within a genetically engineered plant. CRISPR allows for knockout studies in a quicker and more efficient way than any other current method. This innovation could completely revolutionize the agricultural sector and improve the survival of crops in disease filled areas. Recently, scientists are trying to produce a disease resistant variety of cocoa plants that will make the plant resistant to deadly virus that has destroyed many West African crops (Pilger 2017). Also, the scientists in Alberta, s are working on *Fusarium*, which is a current agricultural risk. *Fusarium* is a genus of soil fungi, that causes deadly diseases to plants and devastates crops. Using an innovative system such as CRISPR would allow the plant to be modified in a way which improves it defence mechanism against fungus and other pathogens. CRISPR has also been proposed to be used in fast-growing cannabis industry. With the help of this technology, instead of spending years or decades on cross-breeding plants, new strains with specific and predetermined characteristics could be produced within a few plant generations.The use of CRISPR technologies would therefore make current gene editing substantially more efficient. Research has also been done into the use of CRISPR to create biofuels.

Conclusion

It is concluded that in order to enhance applicability of CRISPR in future, its scope should be properly defined.. Also strict regulations should be imposed to minimize controversies involving this technology. Looking past the controversial actions of individuals, it is seen that there are many useful applications of CRISPR. Therefore, to ensure that CRISPR systems are being used for their intended purposes, careful regulation and detailed collection of research using CRISPR should be produced to keep researchers accountable. This would also aid other researchers as it could help keep things up to date as CRISPR evolves.

The future of CRISPR will likely remain complex due to the different opinions individuals have about the system. Therefore, maintaining an informed viewpoint is important, as it will also allow CRISPR to evolve and more properties of this novel tool to be discovered.

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