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RESEARCH ARTICLE

CRISPR-Cas9, the genetic scissor

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Abstract

In the modern society, gene editing technology is a part of our lives. Genome editing refers to a series of technologies that allow doctors to manipulate an organism's DNA. These technologies allow for the addition, removal, or modification of genetic material at specific parts in the genome.Therefore, it is crucial to understand the process and the influence of it. In this study, our main focus will be on the gene editing technology named "CRISPR-Cas9", which is short for " Clustered Regularly Interspaced Short Palindromic Repeats". The CRISPR-Cas9 system is connected with the protein 9. The medical society is thrilled about this technology because it is more time efficient, more precise, and more inexpensive than other methods. In this paper, we will start with general information about the CRISPR-Cas9 system then move on to a more complex concept. For instance, what will happen if CRISPR modifies non-targeted genes, how CRISPR can assist in curing disease, and the relationship of CRISPR and Covid-19 vaccine. Moreover, we will discuss how CRISPR is associated with the world in the topic of positive and negative effects as well as enhancement in the future.

Introduction:-

CRISPR is a cutting-edge technology that allows genetic engineers to edit genes. They can add, delete, or modify parts of the genetic sequence. It is the simplest, most adaptable, most exact approach to genetic manipulation now available (Naeem, 2020), and it is making a change in the scientific community.

The CRISPR-Cas9 system was redesigned from a genome editing system which took place in bacteria. Bacteria usually catch fragments of DNA from invading viruses, we call them CRISPR arrays. Bacteria can recognize viruses thanks to CRISPR arrays. If the viruses resurface, the bacteria synthesize RNA segments from the CRISPR arrays to target the viruses' DNA. The bacteria then utilize Cas9 or an alike enzyme to rip the virus's DNA apart, which makes it out of action. Scientists are employing this defense mechanism of bacteria into editing the genome of humans.

Genetic engineering is of interest in order to create treatment of human diseases. The medicine community is still researching to measure whether this approach is secure and practical. It's being studied for a number of diseases, even the complex one.

When genome editing techniques like CRISPR-Cas9 are used to alter human genomes, ethical issues arise (Bowers, n.d.). Some are concerned that this technology will add to the regular human traits, this includes appearances or intelligence. Other concerns might include the safeness of human and undesirable effects of this technology.

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CRISPR-Cas9 methodology:
The CRISPR-Cas9 system includes two molecules that enable it to alter DNA. The first component is an enzyme called " Cas-9". This enzyme functions like scissors, allowing portions of DNA to be inserted or removed by cutting the two strands of DNA at a precise point in the genome. Another molecule that makes up the CRISPR-Cas9 system is an RNA called "guide RNA". The guide RNA is a particular RNA sequence that recognizes the desired target DNA region and leads the Cas9 enzyme to that location for editing. (Naeem et al., 2020)

The guide RNA's job is to find and bind to a certain DNA sequence. The guide RNA contains complementary RNA bases to those found in the genome's target DNA sequence. This indicates that the guide RNA will only bind to the target sequence and not to other parts of the genome. The Cas9 enzyme follows the guide RNA to the same spot in the DNA sequence. The Cas-9 enzyme cuts through both strands of DNA. As of this moment, the cell will notice that there is damage within it and will attempt to restore itself. Once the cut is restored, it introduces a change or mutation to one or more genes within the selected genome. Occasionally, this might not be the case since the guide RNA might locate at the wrong sight. ("What are genome editing and CRISPR-Cas9?, 2020)

Editing non-target gene:
In most cases, the guide RNA is made up of a 20-base sequence. These are complementary to the editable gene's target sequence. The guide RNA does not need to match all 20 bases to bind, this is where the concern arises. To illustrate, a sequence containing almost 20 complementary nucleotides could occur in a different part of the genome. This indicates that the guide RNA may bind there instead of or in addition to the target sequence. Modifications at the incorrect position will introduce a mutation in the wrong place.("What is CRISPR-Cas9?", n.d.)

Cutting at the wrong sight might not harm the individual in any way. Yet, it may affect a critical gene or another key component of the genome. Therefore, scientists are eager to figure out how to guarantee that CRISPR-Cas9 operates correctly. This can be accomplished in such an interesting way, The employment of a Cas9 enzyme that cuts only one strand of target DNA rather than the entire double strand. For the changes to be made, two Cas9 enzymes and two guide RNAs must be present at the same time. This decreases the chances of the cut going in the wrong direction. Another mathematical equation can be used to minimize off-target mutations too.

The mathematical model provides a better understanding of why CRISPR-Cas9 cuts at the wrong sight:
CRISPR-Cas9 was originally considered to only split a segment of DNA if it exactly matched the RNA it carries. Unfortunately, that premise has recently been revealed to be misconceived. The protein will occasionally cut DNA sequences that seem similar to the material it's seeking yet contain a variety of different letters. Martin Depken states that cutting such slightly different sequences is highly rational from an evolutionary standpoint. Viruses evolve all the time, and their genomic make-up may differ from what Cas9 is looking for, he explains. By breaking DNA sequences that are slightly different, the Crispr-Cas9 system may follow the evolution of a virus and better protect bacteria against its enemies. This bacteria's defense mechanism is bad for humans. If we wish to utilize Cas9 to remove diseases from our DNA, we need to make sure that no other genes are cut in the process. Cas9 can have disastrous repercussions if it damages other genomic material.

Scientists from Martin Depken's research group, led by Ph.D. student Misha Klein, have been researching the CRISPR-Cas9's preference of cutting. According to Depken, the explanation is straightforward: it all comes down to how much energy it takes to create base pairs that differ from the RNA template. Cas9 starts at one end of the strand when it examines if a DNA sequence is a match, Depken explains. Then it verifies each letter in the strand one by one. Cas9 is rewarded with energy for each match, but any mismatch costs energy. The more mistakes in a DNA sequence there are, and the closer they are to the beginning of the sequence, the less likely the protein is to cut. Instead, it will unbind from the DNA and continue looking for genetic material that is more similar to its RNA template. According to Depken, the basic mathematical model built by his team accurately predicts existing evidence on Cas9's cutting behavior. According to the model, Cas9 does not cut when it detects a mismatch at the start of a sequence or when two mismatches are close together. If an error occurs towards the end of the sequence, the protein will have accumulated enough energy to overcome it, increasing the likelihood of cutting. ("Mathematics explains why CRISPR-Cas9 sometimes cuts the wrong DNA", n.d.)

CRISPR-Cas9 helping diseases:
CRISPR has been a giant transformation in how scientists conduct their research, which allows a variety of applications. However, the technology could have a lot of promise as a cure for human diseases. CRISPR might

theoretically allow us to edit any genetic mutation at will, allowing us to treat every disease that has a genetic cause. On the other hand, it is still in the early phases of its applications in therapy. CRISPR-Cas9 technology is being used to heal diseases.

At the University of Pennsylvania, The first CRISPR-based cancer clinical study in the United States was introduced in 2019 and is still underway. Researchers are employing a patient's genetically engineered immune cells in that trial to test an immunotherapy treatment. T cells (immune cells that may be able to detect and kill cancer cells) are modified in the immunotherapy treatment. Three genes that may interfere with or hinder the cells' ability to destroy cancer were eliminated using CRISPR. The medication slowed tumor growth in one patient with multiple myeloma and another with a solid tumor initially, but subsequently, it started growing again Despite this, the research may offer some promise, particularly in the treatment of solid tumors according to Edward Stadmauer, MD, a professor at the University of Pennsylvania. The long-term effects of CRISPR-edited cell therapy must be evaluated, according to Stadmauer, who expects to follow the study participants for years, if not decades. ("Does the DNA editing tool CRISPR have a future in cancer treatment?, 2021)

CRISPR Therapeutics and Vertex Pharmaceuticals are in a partnership to expand CRISPR-Cas9 in tackling blood diseases such as beta-thalassemia and sickle cell disease. The treatment includes harvesting bone marrow stem cells from the patient to construct fetal hemoglobin(The human fetus's major oxygen carrier protein, it is far more effective than the adult's form.) using the CRISPR technology. The patient is then reinfused with the altered cells. Lead-in results revealed in December that all five patients with thalassemia haven't needed any blood transfusions since starting treatment. In addition, the two patients with sickle cell disease haven't had any of their disease's typical bleeding episodes.(Fernández, 2021)

There are high hopes that the CRISPR-Cas9 system could play a role in curing problematic diseases. Genetic diseases such as Cystic Fibrosis, Muscular dystrophy, and Huntington's disease might be conditions that CRISPR-Cas9 can heal. Scientists are exploring its capabilities and limitations as a device in medicine.

CRISPR-Cas9 and Covid-19 vaccine:

CRISPR-Cas9 has also supported the development of the Covid-19 vaccine. According to the Innovative Genomics Institute, a CRISPR-based DNA-vaccine enhancer for COVID-19 is being developed, which will substantially reduce the time it takes to develop vaccinations against current and future viral threats. The DNA vaccine approach appeals because, unlike traditional immunizations, it allows for accurate antigen design, is easily adaptable to new, emerging pathogens eliciting both humoral and cell-mediated protection, and can be scaled up and manufactured quickly. Despite the obvious benefits of DNA vaccine technology, it has yet to be harnessed for widespread clinical use since DNA vaccines have so far needed high doses, repeated dosing schedules, and have shown low overall efficiency. (Ullah, 2021)

Their goal is to create a DNA vaccine adjuvant that will work with current DNA vaccination technologies, such as electroporation devices. This will boost immune responses from a single dosage, allowing two to three times the number of people to be vaccinated with a given vaccine stock, considerably shortening the time it takes to develop protective herd immunity. This has a lot of potential for reducing the bad effects of the present epidemic, and it would completely change the schedule for vaccine development in the future.

Another excellent example of CRISPR-Cas9 assisting with Covid-19 vaccinations is a study by Muhammad Farhat Ullah and colleagues on treatment techniques as well as how CRISPR/Cas9 gene-editing technology contributes to the coronavirus disease. Because gene editing is faster, less expensive, and more precise than prior genome editing technologies, CRISPR/Cas has aroused the scientific community's interest, particularly for disease diagnosis and therapy. Researchers can use data from gene mutations in specific patients gathered using CRISPR/Cas to work out the best treatment technique for each patient, as well as other research areas for example coronavirus replication in cell culture, including SARS-CoV2.

These findings demonstrate how CRISPR-Cas9 can aid in the improvement of the global pandemic by developing vaccines to assist people all around the world in overcoming and remaining safe from the virus. ("CRISPR-based enhancers of DNA vaccines for covid-19", 2020)

Risks:
CRISPR-Cas9 is a phenomenal technology that holds such promises. In theory, it can add, delete, or modify parts of the genetic sequence. This indicates that it can cure a wide range of diseases. However, we are in the early phase of its development. As tremendous as CRISPR-Cas9 technology sounds, a lot of concerns have arisen because of it.

It is very crucial to consider ethical issues that arise because of the CRISPR-Cas9 system. There has been a lot of interest in editing germ cells. However, in some countries, this is considered a crime. Genome editing can be done in humans where the genetic modification will not be passed down through the generations, or in embryos used in research with no intention of being implanted. The topic of Justice has been brought up, there is concern that genome editing will only be available to the wealthy for enhancement purposes. This will create an even larger gap in the healthcare system. Many fear that if germline modification is followed to its logical conclusion, it would create classes of people characterized by the quality of their modified genome. ("What are the Ethical Concerns of Genome Editing?", n.d.)

The safeness of this technology is another factor to consider. Off-target effects are a major concern. A mutation in the wrong location might happen when cutting at the wrong sight. Off-target effects consist of unwanted phenotype, cancer cells, and even extreme outcomes such as loss of gene activity that leads to death.

Future enhancement:
Given that CRISPR-Cas9 is a relatively new finding in biology, researchers have barely scratched the surface of the potential role it could play in the future of curing diseases. This is just the beginning of employing CRISPR technology as a therapeutic tool. The many unknowns about the possible danger of CRISPR therapy are one of the most significant obstacles to turning this research into cures for different disorders. Some experts are concerned about unintended consequences such as off-target gene editing. Scientists are trying a wide range of techniques to adjust and improve CRISPR to maximize its efficacy and safety in treating humans. ("What is CRISPR-Cas9?", n.d.)

The CRISPR-Cas9 system holds a lot of hope for deadly diseases, for instance, cancer, AIDS, and Muscular dystrophy. If scientists have created effective strategies and tools for distributing the technology to the target cell or tissue and devise functional methods and directions for guiding and eliminating the technology's off-target effects, it will have an impact on different areas such as oncology as a whole in the future. CRISPR-Cas9 technology has great hopes of providing a technique to target and eliminate complicated diseases in the near future.

Conclusion:-
As CRISPR-Cas9 technology is becoming more common in the world, it is very vital that we educate ourselves about how our lives will be affected. CRISPR acts like a pair of scissors that modifies our genome. If used without consideration about ethics, the world could change for the worse. However, CRISPR-Cas9 has its own set of advantages. It has the potential to save many lives, improve medical treatment, and aid in the development of vaccines and disease. If scientists and medical professionals infuse them with caution and conduct further research. It would be safer and easier to access, resulting in significant changes in the medical field.

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