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The Role of CRISPR-Cas9 Technology in Genetic Editing in Medicine and Ethical Issues

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Abstract – The CRISPR-Cas system is a system found in prokaryotes and is responsible for adaptive immunity, causing a revolutionary impact in the fields of Medicine and Genetic Engineering. At its core, this system consists of a series of DNA sequences called CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) and proteins called Cas (CRISPR-associated protein). Cas proteins use CRISPR sequences as a guide to recognize and cut specific DNA strands. While genetic engineering techniques have been used throughout history, the CRISPR-Cas technology is distinct from others because it is a method that can be applied to almost any organism's genome, providing precision, rapid results, efficiency, and cost-effectiveness. These features have captured the attention of the scientific community. However, this ease of accessibility and applicability may also lead to increased potential risks.

Keywords – CRISPR, Cas9, Crrna, Genome Editing, Genetic Editing

I. INTRODUCTION

The CRISPR-Cas system is an adaptive immune system found in the majority of bacteria (~47%) and archaea (~87%), providing defense against foreign genetic elements within the cell. CRISPR consists of regularly spaced palindromic repeat clusters, while Cas (CRISPR-associated protein) enzymes are responsible for recognizing and cleaving foreign DNA within the CRISPR system [1].

One of the primary advantages of CRISPR-Cas technology is its ability to specifically modify or completely change genes. Currently, there are three next-generation genome editing technologies based on nucleases: Zinc Finger Nuclease (ZFN), Transcription Activator-Like Effector Nuclease (TALEN), and the CRISPR-Cas system. These nuclease-based genome editing technologies rely on DNA repair mechanisms induced by the cleavage of double-stranded DNA in a specific region. While ZFN and TALEN techniques have proven effective in genomic manipulations, their usage is limited due to the need for designing a specific protein for each targeted DNA region and their potential for off-target activity. In comparison, CRISPR-Cas technology offers a higher level of specificity in selecting the target region [2],[6].

II. CRISPR-CAS9: FUNCTION AND MECHANISM

CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) is a term that denotes repetitive DNA sequences within the genomes of bacteria and archaea. Its main role is to function as a defense mechanism against viral infections. Each of these repeats has the capability to cut and store viruses or foreign genetic material.

Fig. 1 Clustered Regularly Interspaced Short Palindromic Repeats [3]

CRISPR-Cas9 consists of an enzyme and a protein complex, and each component plays a distinct role:

a- Cas9 (CRISPR-associated protein 9): Cas9 serves as an endonuclease, a cutting enzyme, within the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) systems. It identifies and cleaves a target DNA sequence, providing the starting point for potential DNA repair or modification.

b- CRISPR RNA (crRNA) and TracrRNA: Cas9 enzyme requires an RNA guide to recognize and cut the target DNA. These RNA guides are integral components of the CRISPR system, existing in two main types: crRNA (CRISPR RNA) and tracrRNA (trans-activating CRISPR RNA). These RNAs play a crucial role in determining the target DNA sequence and facilitating the cutting process by the Cas9 enzyme [4], [5].

The Emergence of CRISPR-Cas9 Technology Back in 2012, under the leadership of Jennifer Doudna, a team achieved a groundbreaking feat by introducing an application capable of slicing, isolating, and modifying an organism's DNA. The key to this precision lies in the active Cas9 protein, which serves as a molecular scalpel, allowing for precise DNA cleavage. To make this happen, an RNA molecule was synthesized to match the target DNA sequence. Subsequently, this RNA molecule was merged with the Cas9 protein to form a sophisticated complex. CRISPR-Cas9 technology empowers scientists to make precise incisions at specified DNA locations [5].

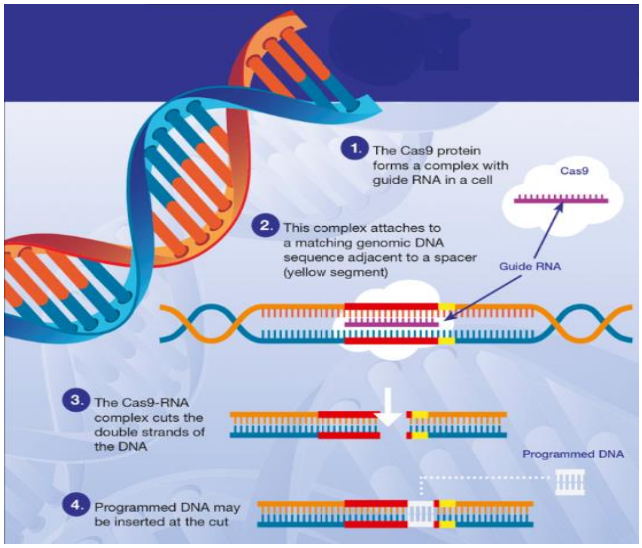

Fig. 2 [7]

The CRISPR-Cas9 technology, abbreviated as "Clustered Regularly Interspaced Palindromic Repeats," facilitates precise genome modifications by altering the DNA structure within cells. This revolutionary system enables the manipulation of an organism's DNA, much like editing a Word document, allowing for the desired modifications of genes. However, the immense potential this technique offers, akin to the power of modern computers, comes with a significant caveat: the slightest error could lead to far-reaching consequences in future generations. CRISPR technology is primarily discussed in the context of two overarching objectives: "genetic therapy," aimed at rendering dysfunctional or disease-causing genes inert and halting protein synthesis, and "genetic enhancement," which involves introducing genes to bestow novel traits upon an organism. Experimental studies conducted on animals have showcased CRISPR's potential to mitigate or completely eliminate the effects of certain diseases, underscoring its utility for therapeutic purposes [8], [9].

The inspiration for the CRISPR method derives from the immune mechanisms employed by bacteria to detect and combat viruses. Some bacteria harbor repetitive sequences within their DNA. When a bacterium becomes infected by a virus, it incorporates a fragment of the virus's DNA into its own genetic material. This adaptive strategy enables the bacterium to recognize the same virus upon future encounters, effectively neutralizing the threat [10].

III. Applications of the CRISPR-Cas System

The CRISPR-Cas system is a highly efficient and precise tool for genetic modifications. It works by creating double-strand breaks in the target DNA sequence, activating genetic repair mechanisms such as NHEJ or HDR. Compared to previous methods like ZFN and TALEN, CRISPR-Cas technology is considered to be more specific and effective. It is used for various purposes, including the disruption of malfunctioning or disease-causing genes to prevent protein synthesis (gene silencing - knock out) and the introduction of new traits into an organism (knock in) [11], [12].

While gene therapy is one of the most prominent applications of CRISPR-Cas technology, it has also demonstrated potential in

several other fields. These applications range from the production of biomedical animal products to improving productivity and resilience in plant and animal agriculture. Additionally, CRISPR-Cas technology has found uses in identifying and modifying microorganisms involved in food fermentation, among many others [13].

A. *Applications in Medicine*

Gene therapy involves techniques that manipulate specific genetic components to repair non-functional genes within a patient's genome or mitigate the effects of mutant genes responsible for diseases. Genetic modification methods based on HDR are frequently employed in gene therapy to rectify mutations linked to various medical conditions [14], [15].

Recent investigations on human stem cells and animal models have indicated the versatility of CRISPR-Cas technology in treating a wide array of genetic diseases. For example, CRISPR-Cas9 technology has been harnessed to correct mutations associated with Sickle Cell Disease (SCD), a monogenic hereditary disorder [16]. Cystic fibrosis (CF), one of the most prevalent genetic diseases, arises from mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene. CRISPR-Cas9 has been employed to rectify CFTR gene mutations, exhibiting promising outcomes in tissue and animal models afflicted with CF [17]. Hemophilia B (HB), a hereditary condition characterized by abnormal blood clotting due to mutations in the Factor IX (FIX) gene, is a frequently studied target for gene therapies. Animal studies have demonstrated that CRISPR-Cas9 technology can correct FIX gene mutations by introducing corrective genes, offering encouraging prospects for treating HB [18].

Initial explorations of CRISPR-Cas technology's application in cancer treatment have been conducted in animal models. A study focused on the ASXL1 (Additional sex combs-like 1) gene associated with cancer utilized CRISPR-Cas9 technology to make modifications that restored the function of ASXL1 in leukemia cells, subsequently increasing survival rates in mice. The mutated mice subjected to this study exhibited prolonged lifespans compared to the control group [19]. In another study targeting lung cancer treatment, T cells underwent modifications through CRISPR-Cas technology to silence the "Programmed cell death protein-1 (PD1)" gene. Subsequently, these modified T cells were administered to patients for immunological evaluation [20]. These and similar clinical models underscore the promising potential of CRISPR-Cas technology in the realm of cancer treatment [21].

B. *Unlocking the Potential of CRISPR-Cas9 for Genetic Disease Treatment*

When we delve into the root causes of certain diseases in our communities, it becomes clear that genetic mutations, viruses, and enzymes often play a pivotal role. CRISPR offers a groundbreaking solution by allowing us to modify these causative factors. One of the standout advantages of this system lies in its capacity to simultaneously silence homologous genes through a single guide RNA [22], [23]. To harness CRISPR technology effectively, all that is required is a 20-nucleotide RNA sequence meticulously designed for the specific region in the genome, complemented by a DNA endonuclease known as Cas9 [24].

Gene therapy, defined as the transfer of genetic material to cells with the aim of treating diseases or enhancing a patient's clinical condition, represents a beacon of hope. The primary objective of gene therapy revolves around delivering a therapeutic gene to target cells through a vector [25]. Furthermore, this therapeutic approach strives to restore normal cellular functions, replace malfunctioning or missing genes with healthy counterparts, and guide expression towards producing normal proteins [26]. The linchpin of successful gene therapy hinges on the development of safe and efficient gene delivery tools [27]. In essence, gene therapy encompasses the potential to complete a deficient gene for the expression of the essential protein or serves as a field-specific gene development capacity for therapeutic purposes [28], [29].

Gene therapy encompasses various strategies, including:

- Replacing a faulty gene with a healthy one,
- Introducing new genes to combat diseases,

- Repairing defective genes through selective reverse mutations,
- Adding a healthy gene to a non-specific location within the genome, the most widely accepted approach among common strategies [30], [31].

Sickle Cell Anemia (SCA), a blood-related disorder stemming from a mutation causing the formation of abnormal hemoglobin S (HbS) protein in the β-globin gene, serves as a testament to the promise of CRISPR-Cas9 technology. Through CRISPR-Cas9-based correction of the HbS gene in hematopoietic stem and progenitor cells (HSPCs) sourced from patient blood, normal functional hemoglobin has been successfully restored.

CRISPR-Cas9-based genome editing is emerging as one of the most auspicious tools for treating various human genetic diseases, encompassing cardiovascular conditions, neuronal disorders, and cancers. Genome editing stands as a potent instrument for both understanding and treating genetic disorders, with a particular focus on those linked to point mutations [32]. Furthermore, CRISPR-Cas9 has shown its potential by inducing mutations in the LTRs of HIV-1 proviral DNA, leading to the degradation of latent HIV-1 provirus. This gene editing method has been reported to thwart multiple stages of HIV-1 infection [33].

Utilizing CRISPR-Cas9 in Managing Genetic Disorders

Cystic Fibrosis (CF): CF, an inherited ailment characterized by mucus buildup in the lungs and various organs, witnessed a noteworthy breakthrough when genetic mutations in a CF patient residing in Scandinavia were rectified through the application of CRISPR-Cas9. This success underscored the potential of genetic modifications in disease management.

Beta-Thalassemia: Beta-thalassemia, stemming from a group of inherited disorders hindering the customary oxygen transport by blood, saw Chinese scientists embark on studies employing CRISPR-Cas9 to rectify beta-thalassemia-associated gene mutations in patients.

Sickle Cell Anemia: The hereditary condition known as sickle cell anemia, marked by the abnormal deformation of red blood cells, is currently the subject of extensive research across various laboratories. Scientists are exploring the use of CRISPR-Cas9 for genetic modifications aimed at treating this ailment.

Retinitis Pigmentosa: This eye disorder, potentially leading to blindness, may find a solution through CRISPR-Cas9. The technology holds promise for correcting genetic mutations responsible for retinitis pigmentosa.

Myopathy: Myopathy, an encompassing term for a cluster of inherited diseases causing muscle weakness, is currently under investigation for genetic mutation correction using CRISPR-Cas9 techniques.

Fig. 3 [34]

IV. Risks Associated with CRISPR/Cas9

The utilization of the CRISPR/Cas9 method comes with inherent risks, primarily centered around the potential for off-target mutations. Research findings have shown that while off-target mutations exist at low but significant rates in zebrafish and mice, interventions on human embryos have demonstrated considerably higher frequencies of off-target mutations [35]. Despite suggestions that using more appropriate Cas9 proteins or conducting studies on normal embryos could enhance success rates, current research indicates that the technology achieves a success rate as low as 50%, underscoring the existing limitations in precision when applying CRISPR/Cas9 to human embryos [36].

Given the abundance of nearly identical or closely related genetic coding sequences within DNA, there is a substantial risk of unintended interference with incorrect genetic segments due to CRISPR/Cas9's gene-editing techniques. Consequently, it is contended that utilizing the technology on human embryos, before robust risk

mitigation strategies are developed, could lead to irreversible and off-target mutations, potentially resulting in the emergence of entirely new hereditary genetic disorders [35].

Another critical concern surrounding genetic interventions on human embryos pertains to the potential to perpetuate class disparities and eugenics in the long term [37]. While contemporary methods like preimplantation genetic diagnosis and selective abortion fall under the category of "negative eugenics" by preventing undesirable outcomes, technologies like CRISPR/Cas9, classified as "positive eugenics" for enhancement and improvement, have sparked ethical divisions within the scientific community [37].

Furthermore, ethical implications extend to the disruption of ecological balance due to genetic alterations within the human genome. Especially when off-target mutations occur during hereditary genetic interventions, it is emphasized that these genetic anomalies may be inherited by subsequent generations, potentially giving rise to entirely new hereditary genetic conditions on a societal scale through reproductive processes. Additionally, it is highlighted that newly emerging disorders resulting from off-target mutations may not be solely genetic in nature. For instance, if human prenatal genetic intervention leads to the development of a trait in which the human body entirely eliminates a particular type of bacterium or virus, this could inadvertently trigger the proliferation of new diseases caused by other viruses or bacteria that were initially suppressed, potentially leading to regional or even continental-scale epidemics [35].

V. CRISPR'S BOUNDARIES AND ETHICAL DILEMMAS

While CRISPR-Cas9 technology has shown promise in saving lives through a limited number of treatments, the potential application of CRISPR-Cas9 and similar gene-editing methods to germline cells has ignited extensive ethical debates. The modification of germline cells, which are passed from one generation to the next, raises profound ethical concerns.

Although the CRISPR method has gained significant attention and even earned Nobel Prizes, concrete outcomes remain elusive. While experiments involving human stem cells and CRISPR/Cas9 are ongoing, the technology has not yet been employed in humans due to factors such as unpredictability in outcomes and a success rate ranging from 20% to 30% in primates.

The first instance of genetic intervention on human embryos occurred in April 2015 in the People's Republic of China, marking the world's first public announcement of genetic alterations capable of creating hereditary changes [39]. Subsequently, in April 2016, permission was granted in London, UK, to conduct research involving hereditary modifications on human embryos using the CRISPR/Cas9 system, leading to intense ethical deliberations within the scientific community [40].

Ethical debates concerning prenatal genetic interventions typically involve:

- Concerns that genetic intervention could assume a god-like role.
- Struggles to strike a balance between risks and benefits.
- Worries that applications beyond therapy could lead to class distinctions and eugenics.
- The potential disruption of ecological balance when altering hereditary genes.
- The inability to secure consent from future generations.
- The possibilities of creating human/animal chimeras [41].

Another critique related to genetic interventions on human embryos revolves around the potential risk of perpetuating class distinctions and eugenics. As CRISPR/Cas9 technology advances, accumulating knowledge, and the potential for misuse, concerns arise that humanity could revisit the 20th-century aspirations of eugenics and the creation of a superior race [42]. While contemporary methods like preimplantation genetic diagnosis and selective abortion serve as tools to prevent unwanted outcomes, collectively referred to as "negative eugenics," technologies like CRISPR/Cas9, categorized as "positive eugenics" for enhancement and improvement, have created an ethical divide within the scientific community.

One of the ethical discussions stemming from the potential outcomes of genetic interventions involves the concept of the resulting organism

possibly becoming a "chimera" if it incorporates genetic material from different species [43].

In somatic gene therapy, where a better balance between risks and benefits has been achieved, the process involves obtaining cells from the patient (e.g., blood or bone marrow cells), conducting genetic modifications outside the patient's body, and externally monitoring measurable success. In this approach, since germline cells remain unaffected, there is no risk of the patient passing these modifications on to future generations.

Conversely, in hereditary genetic interventions, direct genetic modifications are made to a person's germline cells or embryos, fundamentally altering the genetic makeup of future offspring. This type of genetic intervention, which can be applied for therapeutic purposes or, conversely, to interfere with the "natural" genetic makeup of a healthy embryo to enhance its "desirable" traits, raises significant ethical concerns [44].

VI. RESULTS

CRISPR-Cas, a versatile genome editing technique applicable to various genome types, has evolved into a potent instrument in contemporary medicine. The once abstract notions regarding genome editing's potential at the inception of CRISPR technology have now transformed into tangible achievements. The treatment of genetic disorders stands as one of CRISPR technology's most promising applications, ushering in a realm of ongoing clinical trials encompassing a spectrum of ailments, spanning from cancer to neurological conditions. The utilization of CRISPR-Cas technology has gathered momentum in the development of animal models to scrutinize genetic disorders, particularly those arising from mutations, illuminating hitherto unexplored facets of these maladies. However, challenges such as cellular toxicity, immunogenicity related to CRISPR-Cas components, and the possibility of off-target effects remain outstanding concerns that may limit the widespread clinical adoption of this technology.

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