Review Article

Precision Gene Regulation Exploiting CRISPR-Cas for Targeted Epigenetic Modifications

Yasameen Waleed Al-Abedi[1], Murtadha Kadhim Hasan[2*], Alaa Abdalhadi Halboti[3], Ali Abdulhussein Saleh Alsaeedi[3], Rafed Abbas Kadhum[1]

[1]Forensic Department, College of Science, Wasit University, Iraq; [2]Department of Biology College of Science for Women University of Babylon, Iraq; [3]Medical Laboratory Technique, Kut University College, Al-Kut, Wasit, Iraq, 52001.

Abstract | Precise genome editing, which is the foundation of modern biotechnology, is uniquely capable of introducing highly specified genetic modifications into DNA with a high level of accuracy. This study is tailored to demonstrating applicability of antiviral system (Clustered regularly interspaced palindromic repeats (CRISPR) and CRISPR-associated (Cas) proteins (CRISPR-Cas) for carrying out site-specific epigenetic alterations and so uncovering the capability of its use for the upcoming revolution of genetic engineering. The study provides an outline of the CRISPR-Cas system, followed by the mechanisms of genome modification, mainly focusing on the repair mechanisms of the double-stranded breaks. This key idea becomes the starting point of the investigation for the grander pathway that leads to exploring the essence of epigenetic modifications and their role in gene regulation. The precise gene regulation has a potential to provide better understanding of the intricate process as well as the wide spectrum of biological processes. Also, it can offer novel therapeutic interventions. Epigenetic modifications are one of the most perspective field in regulating gene – with CRISPR-Cas as the instrument for the targeted modulations. Using the CRISPR-Cas system to remodel epigenetic marks, in particular DNA methylation and histone coordination, researchers are able to very precisely control expression patterns without affecting the underlying DNA sequence. To sum up this research, it shows how CRISPR-Cas technology, as a revolutionary genetic tool, has been efficient in achieving such levels of gene regulation. This study is aimed at clarifying the mechanism of action and exploring optimal cases for modified epigenetics through CRISPR-Cas integration in biological engineering and medicine. Therefore, it enriches the body of knowledge concerning the application of CRISPR-Cas in valuable discoveries in biotechnology and medicine.

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***Correspondence** | Murtadha Kadhim Hasan, Department of Biology College of Science for Women University of Babylon, Iraq; **Email:** sci131.murtdha.kadhim@uobabylon.edu.iq
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Introduction

Through our ability to edit genomes, which has been transforming life sciences in a very significant way, we seem to have entered into a completely new era of biology. Once, you needed to be a scientist to examine the epigenome because of the limitations of the methodologies. Now, we can do that with precision by engineering epigenomes of living creatures. Revolutionizing work as we know it is at the scope now and may bring benefits along with risks and additional consequences. The area of epigenetic regulation of genes one of the key mechanisms of which is the histone modifications group which represents a complex one. Case for testing and maybe reversal the biological consequence of individual histone modifications is becoming thought, and the main concern of the researchers is to establish at this point the discovery of the ways to control these specific epigenetic causes. It could even take down the maneuver of disease prevention. With the discovery of 'gene editors' we imagine ourselves with a tool to compose a guide for gene regulation. The task of gene editing could be solved with a combination of two approaches. First, we may use the tools of genetic engineering specifically targeted at replacing the message of genes thus creating a set of new editors with control over the state of the gene and the particular extent of its activity. Before this genetic engineering era a hereditary genetic disease might probably be cured but in the future genetic engineering might become a bigger toolbox that will be used to not just prevent disease but also maintain healthy physiology throughout one lifespan (Millán-Zambrano *et al*., 2022).

From a scientific viewpoint, this vision has many challenges including the location and development of successive stages. The study of how the genetic material of cells is turned on and off is still in its infancy. Moreover, there is limited equipment for applying such a major science field. Just as the previous one was stating there is a fairly large variety of epigenetic mechanisms; these processes are as far from being refined as the procedures to manipulate them. Given its current importance we took much care in preferring the name "epigenetic editing", and, for now, look at our undertaking as a general one to design and invent technologies that will ensure the exact invention of epigenetic marks and gene expression which will serve as a means necessary to achieve specific therapeutic results. This commentary is centered on gene regulation as a so far beyond the horizon concept for the purpose of strengthening its research and science competitiveness and appealing the brightest and the sharpest minds in medicine to a domain that has the capacity to revolutionize human health and could compete on equal terms with gene therapy but at the current moment is shadowed by the latter (Li, 2021).

Background

In gene targeting therapy, one may want to make animal models (e.g., mice or rats) of human diseases so as to mirror human condition. Mice are a commonly used model, and the genetic changes are introduced by the ES cells in the past. For instance, a lot of other previous models use ES cells that are embryonic. This could be a mundane and inexcusable process since one can hardly genetically modify mice to possess exact mutations. Through the double stranded breaks formed at specific nucleic acid sequences, and the simplicity of the system; CRISPR-Cas has the advantage over other techniques already well established, thus being an interesting option for generating genetically modified animals. Around 2013, there were three different publications that showed the feasibility of delivering Cas9 mRNA and guide RNA directly into mouse eggs and the resulting embryos could be successfully targeted. From then on, it could be considered that CRISPR-Cas is able to modify animal genomes. Furthermore, a new kind of development for the Cas9 protein creation is a mouse where they tissue-specific expression of the Cas9 transgene, which allows tissue-specific gene editing by inducing DSBs in the genome. The first method of achieving this was by developing a transgenic line of mice that expressed Cas9 protein under human ubiquitin C promoter. Afterwards, the result was achieved more efficiently, by making an adeno-associated virus (AAV) that would reprogram the mice by delivering Cas9. They can be used in mouse models and viral nodes, and lay a foundation for in vivo gene editing, thus becoming a crucial factor for diagnosing the causes of the disease and for trial of the therapies. Such genetic engineering methods could allow scientists to create animal models that display diseased conditions like humans, and, can be used in novel gene therapy. In near future, the producer of disease-curing drug will take advantage of CRISPR-Cas9 technology that may save mice whose genetics are varied with a lot of genetic variations, which will speed up the studies more efficiently (Alghadban *et*

al., 2020).

Finding in molecular biology and genetics have made the scientists wonder the panoramas of human disease, giving place to new treatments, therapies and drugs. Subsequently, the studies followed in this regard have led to the identification of the crucial genes as well as pathways of epigenetic modifications in cases of diseases. Indeed, it is not that long a time since the comparatively fresh field of epigenetics emerged. This very crucial zone in this field contends with the attempt to identify and determine the nature of the mechanism through which critical genes can stop or start the corresponding protein accumulation and synthesis. A lot of times, gene activation is ineffective, or silencing of gene may become an issue resulting in the state of illness. When these canonical pathways are documented as well as modified, this would lead to new therapies that can reactivate genes that have been recently inhibited or suppress some genes that have been highly expressed. Concretely, the localization of the most promising method of the precise gene regulation for the next time, without doubt, is using the recent, the genome editing tool, CRIPSR-Cas, genetically, to achieve the targeted epigenetic modifications (Zhang *et al*., 2022).

Significance of precision gene regulation

Seeing that Cas technologies have expanded, we now have choice of DNA or RNA sequences that can be blocked beyond the scope of what was originally just meant for gene regulation. In contrast, research toward the regulation of the epigenome for accurate gene control has been hindered by the absence appropriate of genome-targeting technology. The fact that epigenetic marks are as vital as the DNA sequences involved commands the gene expression is something you should reflect over, the desire for strategies that precisely control the sites of epigenetic modifications to remain unabated has arisen. Taking advantage of the fact that CRISPR-Cas systems are programmable, recent work has focused on building epigenome editing tools by using either of two types of catalytically inactive Cas variants or modified Cas proteins (fusions or mutants). These proteins function as guides to install, excise or read the type, placement or combination of specific epigenetic marks at a desired genomic locus. Thus, along with CRISPR-Cas, the epigenome editing technologies provide much hope for future of gene regulation, and it is a very systematic and determinate way to find out the functional roles of epigenetic marks during this process of controlling gene expression. In spite of this, it may be possible that new methods and tactics will be developed to deal with diseases that have pathophysiological origins in the abnormalities of ep A presentation of epigenome editing using CRISPR-Cas is given in an article in the same issue of Cells (Miglani *et al*., 2020).

Importance of precision gene regulation

The most essential advantage of these systems is that the target gene's function can be studied from a biological process or a disease state perspective. In the case of many modifiers, their roles are intricate for the gene function in different cell types at different times. The selective expression of a gene therapeutic gene is involved by turning off the expression of a specific gene only in a certain type of cell. While such presence therapeutic genes could be an apoptosis promoter for the aforementioned destruction of cancer cells, they could also take the form of the up-regulation of a metabolic enzyme in a genetic condition. A precise and reversible modulation of these genes is absolutely wanted to prevent the doing harm to other genes or tissues. For this reason, fast-running development of new approaches for the precise control of gene expression gained its popularity due to potentially positive results in this matter. This is often applied to the techniques permitting for the reversible gene regulation (Van *et al*., 2020).

Genes regulation is the complicated protein to make the cells of our body switch on or off their genes. In the case of a gene, it can be switched on, switched off, or be amplified or attenuated as far as how much protein is made. These ways provide the cell the necessary information of the environment and cement it to a particular, specialized type of cell. Such process is normally very stringently regulated and its profile usually changes or not be present in many disease states, for instance, cancer. With knowledge of and manipulation of regulon, it is possible to achieve radical cure of maladies. There have been many different ways to regulate the gene, as it takes different dictates. Earlier studies had been based on interrupting or stimulating some genes at an individual level usually by introducing genetic mutations or by activating specific protein inhibitors. However, these studies are limited for revealing the functions of these specific genes, which fail to investigate the endogenous regulation of these particular genes. Omics method including genomic mutation are usually meliorated by

using miRNA interference techniques which suppress the expression of specific genes. These means, while working accurately for gene specificity, are still off-target and not so exact in gene expression control in vivo. To begin with, this often-ambiguous regulation of genes has led to a great frustration n doctors since the doctor has no effective way of regulating the endogenous gene expression with the highest measure of precision, accuracy and reversibility (Corchete *et al.*, 2020).

CRISPR-Cas System

Overview

CRISPR-Cas is not only a distinctive immune system present in nearly all bacteria and archaea, but it represents an adaptive measure for the organism's defense against phages, viruses, and other foreign elements by a form of specific gene editing (Deveau *et al.*, 2010; Horvath and Barrangou, 2010). It consists from of a CRISPR array of repeats-spacers, which can be subsequently transcribed into a crRNA, and a tracrRNA, as well as a set of cas genes that encode Cas proteins with nuclease activity (Koonin *et al.*, 2009). When an invader causes injury to the target prokaryote, the invading foreign genetic materials are subsequently cut by Cas proteins into fragments that are complementary to the foreign DNA, then the short genomics sequences (CRISPR array) will be modified by incorporating the new spacers (Makarova *et al.* 2011). The invader is the same as before; upon cleavage, crRNA will bind to the target DNA sequence. Through this arrangement, Cas protein will locate the particular foreign DNA sequence and then it will break. The invader will be eliminated from the host. Therefore, cellular immunity will be rebuilt, resulting in a host response (Makarova *et al.* 2011).

CRISPR-Cas systems are either classified into Class 1 and Class 2 or Type I to Type IV andType V and Type VI respectively. It is the specific effector proteins that differ between Class 1 systems (which have mulitple effector protein complexes) and Class 2 systems (with a single effector protein) (Koonin *et al.*, 2017). Moreover, as shown in the Table 1 (Jiang and Dounda, 2017) there is the description, a typical value and an exemplary value of each CRISPR-Cas system, their classification, their members and the characteristics of each CRISPR-Cas system are summarized in Table 1(Jiang and Dounda, 2017).

Table 1: *CRISPR- Cas system illustration classification, representative members, and typical characteristics of each (Jiang and Dounda, 2017).*

Class	Type	Subtype	Effector	Target	Nuclease domains	TracrRNA requirement	PAM/PFS
1	I	A, B, C, D, E, F, U	Cascade	dsDNA	HD fused to Cas3	No	–
1	III	A, B, C, D	Cascade	ssRNA	HD fused to Cas10	No	–
1	IV	A, B	Cascade	dsDNA	unknown	No	–
2	II	A	SpCas9	dsDNA	RuvC, HNH	Yes	NGG
2	II	A	SaCas9	dsDNA	RuvC, HNH	Yes	NNGRRT
2	II	B	FnCas9	dsDNA/ssRNA	RuvC, HNH	Yes	NGG
2	II	C	NmCas9	dsDNA	RuvC, HNH	Yes	NNNNGATT
2	V	A	Cas12a (Cpf1)	dsDNA	RuvC, Nuc	No	5′ AT-rich PAM
2	V	B	Cas12b (C2c1)	dsDNA	RuvC	Yes	5′ AT-rich PAM
2	V	C	Cas12c (C2c3)	dsDNA	RuvC	Yes	5′ AT-rich PAM
2	VI	A	Cas13a (C2c2)	ssRNA	2xHEPN	No	3′PFS: non-G
2	VI	B	Cas13b (C2c4)	ssRNA	2xHEPN	No	5′PFS: non-C; 3′PFS:NAN/NNA
2	VI	C	Cas13c (C2c7)	ssRNA	2xHEPN	No	–
2	VI	D	Cas13d	ssRNA	2xHEPN	No	–

Type II- SpCas9 system which has been originated from Streptococcus pyogenes (SpCas9) from among a group of widely used CRISPR-Cas systems (Jiang and Dounda, 2017) is one of the the best characterized and most commonly used category. The main components of CRISPR-Cas9 system are RNA-guided endonuclease Domains of Cas9 protein have two nuclease names as HNH enzymatic domain and RuvC, and the cleavage occurs on one strand of target double-stranded DNA. The sgRNA, formed from the merging of the two ancestral crRNA and tracrRNA, is a simplified and more efficient version of the older sgRNA. The Cas9 nuclease and sgRNA become a Cas9 ribonucleoprotein (CRNP), and this CRNP complex can interact with and cut the particular DNA target. Additionally, Cas9 protein is bonded to the target DNA through the protospacer adjacent motif (PAM) sequence which creates an R-loop and two double-stranded DNA breaks (Jinek *et al.*, 2012).

During the gene editing, sgRNA binds Cas9 endonuclease specifically at an exact base pair on the genome to create a double-stranded break (DSB) which can be repaired by two endogenous self-repair mechanisms, the non-homologous end joining (NHEJ) pathway that is not precise or the precise homology-directed repair (HDR). Generally speaking, NHEJ is more efficient side of the HDR; it operates in approximately 90% of the cell cycle and does not need to be neighboring homology donor. One peculiar trait of the Homologous Recombination- like pathway (NHEJ) that describes the repair mechanisms occurring in the case of double strand breaks of the DNA, is the fact that the cleavage sites can have

either addition or deletion of bases (indels) in them, which then results in the creation of the frameshift mutations or stop codons (premature or otherwise) within the open Oppositely, HDR could give you to the maximum precise modification in the gene's site by using homologous recombination template (Figure 1) Besides, the simultaneous deletion of a gene's large fragment, or the knocking out of many genes at once, could be achieved by using two or more different sgRNAs that target one single gene or more (Cong *et al.*, 2013).

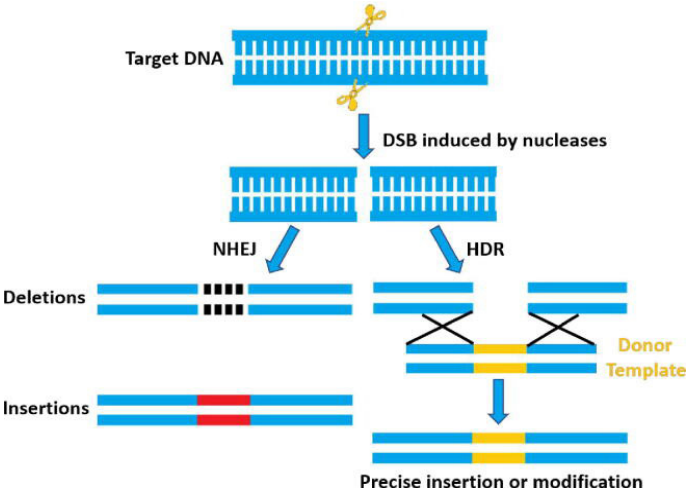

Figure 1: *The genome editing mechanism.*

Mechanism of CRISPR-Cas genome modification/ editing

The mechanism of CRISPR/Cas-9 genome editing can be generally divided into three steps: Recognition, cleavage, and repair (Shao *et al.*, 2016) The sgRNA which is specially built directs Cas-9 and targets the select gene by its mt portion of crRNA that matches the target sequence preserving the crRNA complements. The Cas-9 protein left incapable of acting in the situation when there does not consist of a sgRNA. The given Cas-9 nuclease makes double-targeted breaks (DSBs), for instance, at a position 3 base pairs upstream to PAM (Ceasar *et al.*, 2016). The PAM sequence is short, 2–5 base pairs length and just downstream to the cutting site. Furthermore, its size is a fluctuation from one bacterial organism to other. The Cas-9 nuclease which is the most used nuclease in the gene-editing tool, and it recognizes the specific target sequence which is a 5′-NGG-3′ at the PAM (the first N can be either of the two bases adenine or cytosine, while second N can be any of the base thymine or guanine). Therefore, a question that has not been answered yet is how Cas-9 protein carries out its function of melting the DNA double helix at the target site with a 3' end protospacer adjacent motif (PAM). Finally, Cas-9 complex recognizes the coding part of DNA as target sequence and cuts the DNA. The resulting covalent complex ensures the intervention of RuvB domain, which cleans D-loop by inducing passage of DNA helix through Claspin domain. This step is the last. The DSB is repaired by the machinery of the host cellular again (Mei *et al.*, 2016).

Double-stranded break repair mechanisms

There are two mechanisms to repair DSBs, the NHEJ (non-homologous end joining), which involves a series of enzymatic processes that joins DNA fragments in the absence of exogenous homologous DNA and works throughout the cell cycle. It plays the lead and most effective repair mechanism, active in the cells, but errors being inevitable, there may be insertion or deletion (indels) at the cleavage site resulting in frameshift mutation or stop codon right along the reading frame of a gene (Yang *et al.*, 2020). HDR, unique for this method, is highly accurate while homologous DNA is the template. It is the highest at the beginning of the cell cycle when the cell is proceeding through the S-phase or in the G2 phase. HDR is the CRISPR-gene editing subtype that requires a big portion of donor (exogenous) DNA templates game to code a sequence that is wished to be introduced to DNAs. HDR carries out the disposal of gene copying or replacement by attaching a gene carrier with known sequence homology to NHEJ site (Liu *et al.*, 2019; Yang *et al.*, 2020).

Epigenetic modifications overview and definition

An epigenetic modification is a natural and heritable transcriptional event that lies outside the scope of the DNA sequence and alters gene expression through epigenetics. Hence, nowadays scientific research findings on population genetics frequently are targeted at establishing a clearer connection between the hereditary traits of an individual and that person's genetic makeup. And the one we know best is that the DNA which is made of the sequence of deoxyribonucleic acid is nothing but an instructional manual for the building and sustaining of a living organism and each gene in the DNA provide different instructions. When a state reaches a point of ruins, this often provides a strong symbol of the whole process. In the same way a house is a good image of a state that could fall. The house structure of a new real estate project is designed to be standardized, but every

family will have different furniture and appliances. But some of the houses will be single-story and the others shall be two-story. Some can have everything from a pool and fireplace to an entertainment system that is already built in. That is same with the human settlements as the huts that the different members of the community stay and the way the latter function are similar to other organism belonging to the same species that look and function in the same manner. Here, the interests of the architects coincide with the requirements of the organism with different levels of gene expression (Kumar *et al*., 2020).

Literature shows a deficiency of information on epigenomic and epigenetic, the latter frequently appearing as a substitute term for diverse processes of gene regulation. This semantic confusion has been harming, to a quite an extent, the general perception of epigenetics. The subsequent point supported by the Worden *et al*. article is a complete list of gene regulatory mechanisms per category and whether they truly deserve to be called epigenetic or not. The part where she said "genetic factors that sustain cellular lineages" could either be developmental control involving genetic mechanism or disregard the epigenetic mechanism's part totally. Conversely, with regards "to the formation of positive or negative complexes for gene activity or silencing", this is a general way of transcriptional control and not epigenetic regulation. Lastly, "chromatin protein modification to the histone code, which signifies the functional status of the underlying DNA" is, no doubt, a prototype of a pivotal example of epigenetic gene regulation, and it is going to be considered globally in this discussion. The other authors of the article have been aware of the fact that a unified definition of epigenetics is yet to be found as the pace of discoveries is increasing in this field leading to the alterations in the conceptual approach to this field. Nevertheless, they have proposed a contemporary working definition that encompasses broad epigenetic themes: "Epigenetics are actually the research of changes in the usual point of gene function which don't bring about a change in DNA sequence but which are passed on both mitotically and meiotically". Unlike the one provided by Goldberg before, epigenetics is now defined as (the) "occurrence of the processes that trigger genes to change their activities, normally with the chance of heritance, without the need for DNA sequencing amendment", so the dynamics of epigenetics are more uniform now. One of the more important things to mention is that the new definition of epigenetics is in the same line as the notion of a system of inheritance, which the epigenetic information must pass down from one cell to its daughter cells (Zhang *et al*., 2021; Neganova *et al*., 2022).

CRISPR-Cas based targeted epigenetic modifications

The epigenetic modifications may be considered as another important factor shaping gene expression regulation, thus, conveying more information and functional complexity to the interpretation of the genetic content. The major difference between the modification of sequence in DNA and epigenetic alterations is that the former can be reversed, whereas the latter can be impacted by various environmental factors. In this article, we will consider the role epigenetic modifications are playing in gene expression regulation and be looking at their effect on cells processes and human health (Portela and Esteller, 2010).

Epigenetics represents the chemical changes that act upon DNA or histone proteins which are responsible for the packaging of the DNA molecules, without actually altering the DNA code sequence. There are two principal types of epigenetic modifications known as DNA methylation and histone modifications. DNA methylation generally involves the modification of cytosine residues by methylating them, mainly through the use of the CpG dinucleotide. This suppression is commonly brought about by the inability of the regulating proteins and transcription factors to bind to targeted DNA sequences of the affected genes (Jones and Baylin, 2002).

Unlike histone acetylation or methylation, histone modifications involve processes where various chemical groups of acetyl, methyl or phosphate groups are added or removed to histone proteins. This way scientific will be manipulating the structure of chromatin which then makes the DNA either looser or more tightly packed, and this way eventually, the expression of genes could be affected. In particular, acetylation of histones is commonly associated with transcriptional initiation, however, methylation can either activate or repress the transcription depending on the site and context (Wang *et al*., 2009).

Epigenetics play a key role in many cellular processes aside from development, differentiation, and serving as a specific identity tool. In the process of multicellular

organism embryo development, epigenetic alterations perform an integral role in the selection and diversification of stem cells into the specific cell types. They play a critical role in regulating the conveyance of specific genes among cells by dropping the urinary tract infections (Jaenisch and Bird, 2003).

Also, epigenetic modifications can be considered to be influenced by environmental conditions like energy intake, the practitioner lifestyle, and the number of poisonous agents. Besides these, these factors may result in alteration of epigenetic marks and as a consequence gene expression pattern emerging, eventually evolving into some disease types. Thus, epigenetic dysregulation involving abnormal DNA methylation patterns has been detected in several kinds of cancer, e.g., colorectal, breast, and lung cancer. Additionally, epigenetic variations are associated with other types of diseases, including neurological and neurodegenerative diseases, cardiovascular diseases, and autoimmune disorders (Allis and Jenuwin, 2016).

Conclusions and Recommendations

Molecular biology and biotechnology are facing the test of time in this speeding up world of genetic engineering. Where the precision gene regulation is the front-line defense of exploration about genetic mechanism; while at the same time, holds vast potentials for targeted solutions. This research has shown the amazing capability of CRISPR-Cas genes' technology benefit in lung cancer with such precision which has never been done before and give us insight into the future of genetic therapy.

CAPE-CasPR elucidation is based on its double-strands breaking repair mechanism, which has helped researchers to lay strong platforms which can be used to regulate the genes. Through the use of CRISPR-Cas's modifiable and accurate feature genes expression has been edited with an un-anticipated level of specificity, clearing a path for convergent developments - for example in biomedicine.

To have an accurate regulation of target genes is more than indispensable. It not only is a tool to enhance our knowledge of basic biology but at the same time, there are lots of possibilities to create more complex genetic conditions and declare the new trends in personalized medicine. The CRISPR-Cas system, with its ability to directive specific site modification and epigenetic mark manipulation, provides scientists with a tool that is both versatile and flexible to approach the complexities of gene regulation as well as to create targeted approaches for therapeutic interventions.

Among the exploding amazing sectors in the precision gene regulation, the most novel one is targeted epigenetic modifications. The application of CRISPR-Cas tech to epigenetics to promote methylation or histone modification is this way. This would allow the researchers to decide which genes will be turned off or on through a sequence. This approach no only creates new ways for perceiving the role of epigenetics in health and disease but also ensures that we will have possibilities to develop innovative approaches for treatment of almost all disorders- from cancer to neurodegenerative diseases to name just a few.

As technology advances in the future, it is going to stand out more and more on the historical background of molecular biology and biomedicine which are still being shaped. While gene editing has undoubtedly the potential to transform and improve the lives of humanity, the ethical, social and regulatory consequences of gene editing science should also be existing. Dedicated and spululate counseling on precise gene engineering and the purpose of broad repercussions of these technologies are of utmost importance as it will ensure that such technologies are used effectively and ethically to the benefit of society.

The later research reiterates a fact that is so real that the CRISPR-Cas technology could be a paradigm shift in precise regulation of gene and the level, reliability and understanding of genetics and human health could be redefined by this ultimate revolution in genetics. Through the explanation of its mechanism, its spell in the science and medicine, and providing a broader perspective on its moral impact, we conquer the question of how CRISPR-Cas could be used for revolutionary advances in biotechnology and medicine while taking into consideration the ethical distribution of its benefits and ensuring nothing bad happens.

Author's Contribution

All authors contributed equally in the manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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