**CRISPR and gene editing :pharmacological implications**

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**ABSTRACT:**

The CRISPR-Cas system has transformed the field of gene editing due to its accuracy, efficiency, and user-friendly application. As a cutting-edge genome editing tool, it offers significant advantages over earlier technologies. This review explores its expanding role in pharmacology, including its applications in drug discovery, validation of therapeutic targets, and the creation of disease-specific models. CRISPR is also contributing to the development of innovative therapies for genetic disorders, cancer, and infectious diseases. However, challenges such as off-target modifications, effective delivery methods, and ethical concerns remain under active investigation. Ongoing advancements are focused on improving the precision and safety of CRISPR-based approaches, reinforcing its potential in personalized medicine and future therapeutic strategies. Overall, CRISPR represents a highly promising avenue in modern pharmacological research.

**Keywords:** gene, tool, genetic diseases, personalized medicine.

1. **Introduction:**

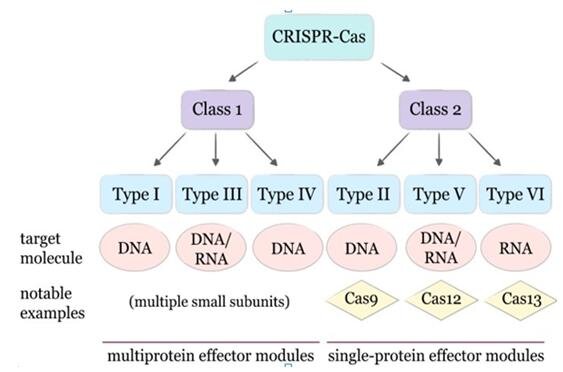
Scientific and technological progress has enabled the development of innovative treatment modalities that were once thought to be beyond reach, including gene-targeted therapies such as immunotherapy and gene therapy. Immunotherapy enhances the immune system’s ability to recognize and destroy cancer cells through strategies like cancer vaccines, checkpoint inhibitors, and adoptive cell transfer [1]. Early gene therapy approaches, however, raised concerns due to risks like insertional mutagenesis [2]. Conventionally, gene delivery has depended on viral vectors, which pose several challenges such as vector-associated toxicity, high production costs, labor-intensive processing to reduce adverse effects [3], and the body’s adaptive immune response, which can limit the effectiveness of viral delivery systems [4].

CRISPR (clustered regularly interspaced short palindromic repeats) was initially identified in Escherichia coli DNA by Ishino et al. in 1987 at Osaka University (Japan) [5]. At the time, the function and origin of these DNA sequences were unknown, and sequencing them was a time-consuming process. Despite this, researchers soon recognized the potential of CRISPR loci in medical research, particularly for genotyping bacterial strains, beginning with Mycobacterium tuberculosis [6] and later expanding to Streptococcus pyogenes [7]. Thanks to the extensive collection of bacterial strains preserved since the 1980s, researchers could map how bacteria incorporated viral DNA into their CRISPR loci as a defense against bacteriophage infections. In S. thermophilus, the integration of new spacers conferred immunity to corresponding phages, a finding that led to early patents in CRISPR technology [8] and, subsequently, to the “vaccination” of bacterial cultures using CRISPR by Danisco in 2005 [9].

1. **mechanism of CRISPER-cas system:**

The first experimental insights into the functional mechanism of the CRISPR system were reported in 2007 by French food scientists Rodolphe Barrangou and Philippe Horvath. Their research, conducted on Streptococcus thermophilus cultures used in yogurt production at the Danish company Danisco, marked a pivotal advancement in the field [10]. Currently, CRISPR–Cas systems are broadly categorized into two main classes and six types, with each type further divided into numerous subtypes. As of the time this review was written, Makarova et al. had identified more than 30 distinct subtypes [11] (Fig. 1). The primary distinction between the two classes lies in their effector modules: Class 1 systems utilize a multi-protein complex, while Class 2 systems rely on a single multidomain protein, such as Cas9, Cas12, or Cas13 [12–14].

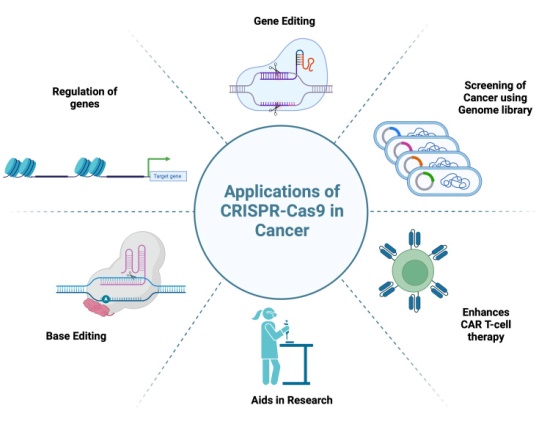
**Figure 1. classification of CRISPR**



Among the various Cas proteins identified to date, those involved in the targeted cleavage of foreign genetic material—often referred to as "genetic scissors"—are the most extensively studied. One such nuclease is Cas9, initially reported by Bolotin et al. in association with CRISPR repeat sequences [15]. A critical component of the CRISPR–Cas9 system is a small RNA molecule that arises from the transcription of the CRISPR locus. This RNA guides the prokaryotic defense machinery to invasive nucleic acids. The existence of these guide RNAs was described by a research team led by John van der Oost at Wageningen University (Netherlands), who named them CRISPR RNAs (crRNAs). They also observed that the initial transcript from the CRISPR array is a precursor molecule, termed pre-crRNA, which includes multiple spacer and repeat sequences and is subsequently processed into individual crRNA units [16]. Further research by the group led by Virginijus Šikšnys at Vilnius University (Lithuania) demonstrated that a 20-base-pair segment of the crRNA, complementary to the target DNA, is both necessary and sufficient to activate the nuclease function of the CRISPR–Cas complex, even when the original spacer sequence is longer [17].

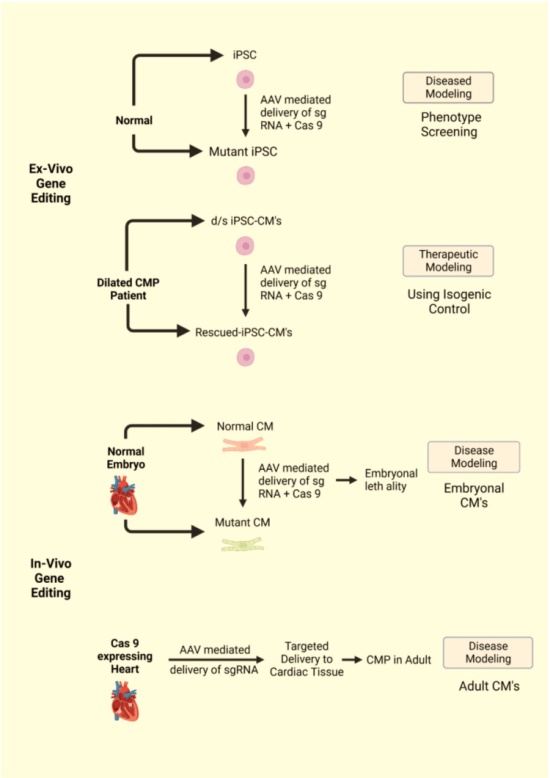
**3.pharmacological applications:**

Cancer therapy encompasses a wide array of strategies, from surgical removal of tumors and reduction via radiotherapy to the precise molecular targeting of genes implicated in tumor development [18]. Among the latest advances in molecular medicine, the CRISPR-Cas9 system stands out for its exceptional accuracy in altering gene expression and intervening in the genetic mechanisms underlying cancer progression [19]. By exploiting the cell’s natural DNA repair processes activated by CRISPR-induced breaks, scientists are now able to modify critical genetic circuits that drive tumor formation and survival. (Figure 2)

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CRISPR-Cas9 has proven to be an invaluable tool for investigating gene function and gaining deeper insights into the pathophysiological mechanisms underlying genetic cardiovascular disorders [20]. In particular, gene editing has been employed to create in vitro models of dilated cardiomyopathy (DCM), enabling researchers to replicate and study the disease process under controlled laboratory conditions [21].

Figure 3 demonstrates how CRISPR technology is applied in the development of DCM disease models.



**infectious** **Diseases**: **CRISPR** **in** **COVID**-**19** **Detection:**

A novel advancement in CRISPR-based diagnostics was introduced through the development of a “one-pot” testing method during the enhancement of COVID-19 detection technologies. This technique eliminates the need for RNA purification from patient samples. Instead, the entire detection process occurs within a single reaction tube. The method, later named SHERLOCK Testing in One Pot (STOP), uses loop-mediated isothermal amplification (LAMP) to amplify viral RNA and integrates the AapCas12b enzyme, which remains active at the LAMP-required temperature of 60 °C. With the addition of viral lytic agents to saliva or throat swab samples containing SARS-CoV-2, viral RNA can be detected directly—without separate RNA extraction steps [22].

**Neurodegenerative** **Disorders:**

Neurodegenerative diseases such as Alzheimer’s disease (AD) and Parkinson’s disease are prevalent and progressive, leading to cognitive or motor dysfunction [23] . Advances in gene editing have supported the study of these disorders, including research on basal forebrain cholinergic neurons (BFCNs) derived from induced pluripotent stem cells (iPSCs). These iPSCs were generated from fibroblasts of individuals carrying the PSEN2 mutation [24]. In PSEN2N141 iPSC-derived BFCNs, impaired electrophysiological properties were observed; however, CRISPR/Cas9-mediated correction of the PSEN2 mutation led to significant improvements in neural activity and in the amyloid-beta peptide ratio [25].

**Duchenne** **Muscular** **Dystrophy** **(DMD)**

DMD is a severe X-linked recessive disorder caused by mutations in the DMD gene on chromosome 21, resulting in reduced production of dystrophin, a protein essential for maintaining muscle structure and function [26]. Both induced pluripotent stem cells (iPSCs) and embryonic stem cells (ESCs) can differentiate into large populations of skeletal muscle progenitors and exhibit regenerative potential and dystrophin synthesis capability [27,28]. ESCs, in particular, are ideal candidates for CRISPR-based genome editing aimed at alleviating DMD symptoms [27]. Therapeutic strategies under development include both anti-inflammatory approaches and gene correction or replacement techniques, such as cell-based therapies to restore dystrophin expression [29].

**4. Safety, Limitations, and Challenges:**

Despite the proven versatility and effectiveness of the CRISPR/Cas9 system across numerous research and therapeutic domains, significant safety concerns remain—particularly related to off-target mutations, which could hinder its future clinical utility. Multiple studies have confirmed that unintended edits introduced by CRISPR/Cas9 are a major limitation, irrespective of the targeted gene or the type of cell used [30,31,32,33,34,35]. Furthermore, research has shown that gene therapies involving CRISPR/Cas9 often result in a high frequency of off-target activity, primarily due to double-strand breaks (DSBs) and reliance on the non-homologous end joining (NHEJ) repair pathway [36].

To address these limitations, several strategies have been developed to enhance CRISPR specificity and promote homology-directed repair (HDR) over NHEJ, thereby minimizing the risk of unintended mutations. For example, exposing minicircle iPSCs to cold shock following CRISPR/Cas treatment has been shown to improve HDR efficiency, though it does not significantly alter the rate of insertions or deletions (indels) [37]. Another approach involved modifying the ratio of single guide RNA (sgRNA) to Cas9 protein; studies indicate that increasing the sgRNA-to-Cas9 ratio can significantly reduce off-target activity [38].

Additional technical hurdles include the use of plasmids with low targeting specificity, which may integrate randomly into the genome. Such random insertions complicate downstream analysis and tracking of editing outcomes [39].

**5. Regulatory and Ethical Landscape:**

**1. Regulatory Landscape**

CRISPR regulation differs significantly across countries, reflecting variations in legal structures, cultural values, and ethical viewpoints.

A) United States

CRISPR-related research and applications are overseen by key federal agencies:

The Food and Drug Administration (FDA)

The National Institutes of Health (NIH)

The United States Department of Agriculture (USDA), especially for agricultural applications

Currently, human germline editing is prohibited for clinical use, while somatic gene editing may be pursued in clinical trials with appropriate FDA approval.

B) European Union

The EU maintains a highly cautious approach, particularly regarding germline interventions.

In 2018, the Court of Justice of the European Union (CJEU) ruled that gene-edited organisms fall under the same stringent regulations as genetically modified organisms (GMOs).

The precautionary principle underpins EU policy, emphasizing risk avoidance in uncertain scientific areas.

C) China

China has adopted a more permissive stance toward CRISPR research, although high-profile controversies, such as the 2018 case involving gene-edited babies, prompted global criticism.

Following this, China implemented stricter ethical review systems and enhanced regulatory oversight to ensure responsible scientific conduct.

D) India

CRISPR research is regulated by:

The Department of Biotechnology (DBT)

The Indian Council of Medical Research (ICMR)

Human germline modification is prohibited, while agricultural gene editing is allowed under carefully controlled conditions and government guidelines.

**2. Ethical Considerations**

A) Germline vs. Somatic Editing

Somatic cell editing is generally considered more ethically acceptable since its effects(40,41,42)

**6. Future Directions:**

Ongoing advancements in next-generation CRISPR technologies, such as the CRISPR-MAD7 system and modified nucleases like Cas12a, are enhancing the precision, scope, and potential applications of gene editing tools. Innovations including engineered variants—MAD7-RR, MAD7-RVR, and M-AFID (a MAD7-APOBEC fusion protein)—have extended the targeting capacity of MAD7. These versions enable precise deletions between 5′-deaminated cytosines and the MAD7 cleavage site. Experimental studies have shown that this system can achieve editing efficiencies of up to 65.6% in rice and wheat models [43].

The MAD7 system further enriches the CRISPR toolbox by offering efficient gene disruption and insertion capabilities, broader compatibility with various protospacer adjacent motifs (PAMs), and requiring only small-guide RNAs, which makes it especially adaptable for diverse genome engineering tasks [44].

Additionally, recent progress in other CRISPR/Cas systems—such as Cas3, Cas12, Cas13, and Cas14—has significantly improved editing accuracy and enabled simultaneous modifications at multiple sites, particularly in viral genomes. This multiplexing approach, using single-guide RNAs targeting different genetic regions, offers a promising strategy to enhance broad-spectrum antiviral resistance, minimize the emergence of escape mutations, and better control infectious diseases in agriculture and medicine

**7. Conclusion:**

While CRISPR/Cas9 has shown remarkable potential in gene editing, its impact on human biology still requires extensive research. Notably, human and mouse induced pluripotent stem cells (iPSCs) exhibit differing responses depending on the specific CRISPR variant employed. One of the key limitations remains the formation of insertions and deletions (indels) resulting from non-homologous end joining (NHEJ), which contributes to unintended off-target effects. To address this, current research is focused on optimizing CRISPR systems to favor homology-directed repair (HDR), thereby reducing mutation rates and enhancing editing precision.

Although earlier gene editing tools such as zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs) marked important milestones, their broader applicability is constrained by technical limitations. In contrast, CRISPR technology offers higher efficiency, greater ease of use, and reduced off-target activity, making it a preferred platform for developing gene-based therapies. Nevertheless, mitigating unintended genomic alterations remains a major challenge. Future studies should prioritize improving CRISPR system fidelity and safety to fully harness its potential in human therapeutic applications.

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