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CRISPR-CAS9: discovery and future therapeutic

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Abstract

CRISPR_Cas9 technology has quickly evolved into one of the most powerful tools in molecular biology, enabling precis and efficient genome editing with a wide ranging of biomedical applications. Its ability to selectively target and modif specific DNA or RNA sequences has new avenues in the treatment of cancer, inherited disorders, neurodegenerativ diseases, and viral infections. In malignant study, CRISPR has been employed to disrupt oncogenic drivers like KRAS an MYC, restore tumor suppressor pathways involving TP53 and BRCA1, and optimize immunotherapy strategies throug engineered CAR-T cells. Similarly, in genetic disorders like Duchenne muscular dystrophy and Leber congenital amaurosi CRISPR-mediated exon skipping and targeted repair approaches have demonstrated significant therapeutic potential. In th field of neurodegenerative diseases, the system has shown promise in modulating genes implicated in Alzheimer diseas particularly APP, BACE1, and APOE, offering novel insights into disease modification. Furthermore, CRISPR-based RNA targeting systems like Cas13 have been successfully applied in controlling viral pathogens, including HIV and SARS-CoV 2, not only for therapeutic interventions but also for rapid and sensitive diagnostics through platforms like SHERLOCK an SHINE. Overall, CRISPR represents a transformative advance in precision medicine. While challenges such as off-targ effects, efficiency of delivery, and ethical considerations remain, continuous progress in optimizing the technology suppor its future integration into clinical practice as a versatile therapeutic and diagnostic platform.

Keywords: CRISPR, Cas, DNA, RNA, gRNA, PAM

الملخص

تطورت تقنية CRISPR_Cas9 بسرعة لتصبح واحدة من أقوى الأدوات في علم الأحياء الجزيئي، إذ تتيح تحرير الجينوم بدقة وكفاءة مع مجموعة واسعة من التطبيقات الطبية الحيوية. وقد أتاحت قدرتها على استهداف وتعديل تسلسلات محددة من الحمض النووي الريبوزي (DNA) أو الحمض النووي الريبوزي (RNA) بشكل انتقائي آفاقًا جديدة في علاج السرطان، والاضطرابات الوراثية، والأمراض العصبية التنكسية، والالتهابات الفيروسية. وفي دراسات الأورام الخبيثة، استُخدمت تقنية CRISPR لتعطيل العوامل المسببة للسرطان مثل KRAS وMYC، واستعادة مسارات تثبيط الأورام التي تشمل TP53 وBRCA1، وتحسين استراتيجيات العلاج المناعي من خلال خلايا CAR-T المعدلة وراثيًا. وبالمثل، في الاضطرابات الوراثية مثل ضمور العضلات دوشين وعمى ليبر الخلقي، أظهرت تقنيات تخطي الإكسونات والإصلاح المستهدف بوساطة CRISPR إمكانات علاجية كبيرة. وفي مجال الأمراض العصبية التنكسية، أظهر النظام نتائج واعدة في تعديل الجينات المرتبطة بمرض الزهايمر، وخاصةً APP وBACE1 وAPOE، مما يوفر رؤى جديدة في مجال تعديل الأمراض. علاوة على ذلك، طُبّقت أنظمة استهداف الحمض النووي الريبوزي القائمة على تقنية كريسبر، مثل كاس13، بنجاح في السيطرة على مسببات الأمراض الفيروسية، بما في ذلك فيروس نقص المناعة البشرية وفيروس كورونا المستجد (SARS-CoV-2)، ليس فقط للتدخلات العلاجية، ولكن أيضًا للتشخيصات السريعة والدقيقة من خلال منصات مثل شيرلوك وشاين. وبشكل عام، تُمثل كريسبر تقدمًا جذريًا في الطب الدقيق. وبينما لا تزال هناك تحديات، مثل التأثيرات غير المستهدفة، وكفاءة التوصيل، والاعتبارات الأخلاقية، فإن التقدم المستمر في تحسين هذه التقنية يدعم دمجها مستقبلًا في الممارسة السريرية كمنصة علاجية وتشخيصية متعددة الاستخدامات.

الكلمات المفتاحيةالحمض النووي الريبوزي، الحمض النووي الريبوزي منقوص الأكسجين (gRNA)، PAM

Introduction

CRISPR technology has emerged as one of the most promising approaches in gene therapy, offering potential treatment for a wide range of diseases. In nature, many bacteria and archaea utilize clustered regularly interspaced short palindromic repeats (CRISPR) and related Cas proteins (CRISPR–Cas) to protect themselves against invading genetic elements. According to their structural composition and functional mechanisms, CRISPR–Cas systems are

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classified into six types. Leveraging these systems, genome editing tools have been progressed, providing unprecedented opportunities for therapeutic intervention in both genetic disorders and malignant. For CRISPR–Cas enzymes, precise control over their activity, timing, and specificity is critical to harness the full potential (Haridha Shivram et al., 2021).The CRISPR–Cas platform enables highly accurate modifications at nearly any genomic location, CRISPR–Cas components: a Cas endonuclease and a guide RNA (gRNA). Various Cas nucleases, involve Cas9 and Cas12a, differ in size and cleavage patterns and have been explored in clinical settings. The guide RNA (20–24 nucleotides) functions by guiding the Cas nuclease toward its complementary DNA target. Upon binding, the nuclease generates a double-stranded break (DSB), which is subsequently repaired by cellular repair mechanisms like non-homologous end joining (NHEJ) or homology-directed repair (HDR).The non-homologous end joining (NHEJ) pathway is rapid but inherently error-prone, often generating deletions or insertions at the cut site. On the other hand, homology-directed repair (HDR) enables template-based correction or gene insertion, offering higher accuracy though with lower potency in mammalian cells. Another pathway, microhomology-mediated end joining (MMEJ), produces larger deletions and depends on POLQ activity. Remarkably, the balance between these repair mechanisms is highly context-dependent, varying across cell types and experimental conditions, a fact sometimes overlooked in generalized descriptions. Ongoing innovations have broadened the CRISPR repertoire, introducing base editors (BEs) and prime editors (PEs), which allow precise nucleotide modifications without inducing DSBs. BEs employ a Cas9 nickase fused to a deaminase domain to mediate specific base conversions, while PEs use a reverse transcriptase fused to Cas9 nickase, guided by a prime-editing gRNA, allowing versatile edits involving all base substitutions and small insertions and deletion (indels) (Nechama Kalter et al., 2025). Furthermore, complementary advances like RNA-targeted CRISPR procedures and next-generation sequencing–based off-target mapping have improved both biosafety evaluation and mechanistic understanding. Together, these refinements demonstrate how the CRISPR–Cas platform continues to evolve toward more reliable and medically applicable approaches (Jianli Tao et al., 2023).

2. CRISPR-CAS

CRISPR–Cas systems comprise different constituents, which can impose a significant metabolic burden on host cells and, if unchecked, may pose cellular risks. Therefore, their activity is tightly regulated and typically stimulated only under specific environmental or intracellular conditions. This control represents an equilibrium between cellular energy expenditure and protective effectiveness. For example, bacterial population density influences CRISPR–Cas

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expression: low-density populations show minimal system activity because of decreased exposure to invasive components genetic, whereas densely packed cells exhibit upregulated CRISPR–Cas expression to counter aggravate threat from viral infections and horizontal gene transfer. Embedded in biofilms, cells are naturally shielded from phages; therefore, CRISPR activity is reduced to save metabolic energy. In addition, regulation is mediated with the help of intracellular signals like cyclic AMP (cAMP), which modulates CAP protein activity and consequently CRISPR demonstration. Remarkable, responses vary between species: E. coli in the human gut upregulates CRISPR under glucose-rich conditions, while Pectobacterium atrosepticum enhances expression under nutrient scarcity, likely as a defense against prophage activation. This species-specific variation underscores the ecological tailoring of CRISPR–Cas regulation. Beyond small-molecule signaling, regulatory proteins such as Csa3a and nucleoid-associated proteins (H-NS, StpA, and LRP) exert either activating or repressing impacts by altering DNA accessibility and spacer acquisition efficiency .Such fine-tuned control highlights the evolutionary necessity of balancing strong antiviral protection with the avoidance of unnecessary cellular costs. Cas nucleases generate double-strand breaks repaired via non-homologous end joining (NHEJ) or homology-directed repair (HDR), permitting targeted gene disruption or correction. Catalytically inactive Cas variants fused to transcriptional regulators allow precise control of gene expression. Cas12 nucleases provide alternative editing approaches with lower off-target impacts, while rationally designed nucleic acid blockers (SNuBs) can modulate Cas9 activity through anti-guide, anti-tracr, or anti-PAM interactions. Other strategies, like quorum-sensing interference, show promise for enhancing phage therapy efficacy by down regulating CRISPR–Cas defenses and reducing bacterial resistance. Collectively, these regulatory mechanisms illustrate the interplay between CRISPR–Cas activity, horizontal gene transfer, and bacterial adaptation, while providing a framework for therapeutic and biotechnological applications (Marta Zakrzewska & Michal Burmistrz, 2023).

3. Discovery of CRISPR

Genetic scissors: a tool for rewriting the code of life Emmanuelle Charpentier and Jennifer A. Doudna have discovered one of gene technology's sharpest tools: the CRISPR/Cas9 genetic scissors. Using these, researchers can change the DNA of animals, plants and microorganisms with extremely high precision. The first unusual repetitive sequences were described by a group of Japanese scientists in 1987 in the genome of Escherichia coli (E. coli). This technology has had a revolutionary impact on the life sciences, is contributing to new cancer therapies and may make the dream of curing inherited diseases come true .CRISPR is considered nowadays the most important discovery in molecular biology since PCR, entailing the creation of the latest and most

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successful genetic engineering technology. The Nobel Prize in Chemistry went to Emmanuelle Charpentier and Jennifer Doudna in 2020 for developing the CRISPR/Cas9 gene editing technique (A. A. Shmakova et al, 2022).

4. Types of CRISPR

CRISPR-Cas systems, naturally present in various bacterial and archaeal species, demonstrate a wide range of structural compositions and molecular mechanisms. Broadly, these systems are classified into two main categories: Class I, in which effector protein complexes consist of multiple subunits, and Class II, which relies on a single multifunctional Cas protein to execute genome editing functions.

4.1 CRISPR Cas3: A Multifunctional Nuclease-Helicase for Extensive Genome Remodeling Cas3 represents the signature nuclease-helicase of Type I CRISPR-Cas systems, which are the most abundant and evolutionarily ancient among CRISPR variants. Unlike Class II effectors like Cas9 and Cas12, which function as individual multidomain proteins, Cas3 operates within a Class I multi-protein assembly termed Cascade. The CRISPR-associated complex for antiviral defense (Cascade) is a multiprotein assembly that functions together with a guide crRNA to identify foreign DNA sequences adjacent to a protospacer adjacent motif (PAM), usually 5′-AAG or subtype-specific variants. Once a complementary sequence is detected through Watson–Crick base pairing, the Cascade–crRNA complex undergoes a structural rearrangement, exposing the DNA strand and enabling the recruitment of Cas3. This enzyme contains both a Superfamily 2 (SF2) helicase domain and an HD-type nuclease domain, allowing it to unwind DNA in the 3′–5′ direction while degrading the displaced strand. Unlike Cas9 or Cas12, which generate precise double-stranded breaks, Cas3 performs extensive and processive DNA degradation, effectively removing large genomic regions and serving as a key defense strategy against bacteriophages and plasmids. Recent advances in molecular engineering have made it possible to reconstitute the Cascade–Cas3 machinery in mammalian systems, thereby enabling programmable large-scale deletions of repetitive sequences, non-coding elements, and pathogenic expansions. Applications include modeling repeat-associated disorders such as Huntington's disease, minimizing synthetic genomes, and dissecting chromatin features like topologically associating domains (TADs). Beyond DNA cleavage, Cas3 has been adapted for innovative functions by fusion with enzymatic effectors. For instance, linking Cas3 to cytidine deaminases has generated tools for localized hypermutation, capable of inducing high-frequency mutations across extended genomic regions, including entire operons. This strategy achieved substantial increases in mutation rates—sufficient to drive adaptive evolution, functional pathway redesign, and combinatorial mutagenesis in microbial and synthetic systems. Furthermore, Cas3's helicase activity provides opportunities for integration with base editors, prime editing systems, and

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epigenome modifiers, where efficient DNA unwinding is critical. Although challenges remain regarding delivery efficiency and specificity, Type I CRISPR systems incorporating Cas3 are increasingly recognized as versatile platforms for genome engineering, particularly in contexts requiring high-throughput, long-range, or multiplexed editing (Douglas M. Ruden, 2025) .

4.2 CRISPR Cas9: The Streptococcus pyogenes-derived CRISPR/Cas9 system, a Type II CRISPR platform, has become the most prevalent tool for genome editing to date. The Cas9 enzyme family relies on the formation of a base-paired structure between the activating tracrRNA and the targeting crRNA to facilitate the cleavage of the double-stranded DNA (dsDNA) at the target site. After recognizing its DNA target, SpCas9 typically induces a blunt double-strand break (DSB). SpCas9 directs DNA targeting through a 20-nucleotide guide sequence and requires a 5'-NGG PAM sequence adjacent to the target, with "N" indicating any nucleotide. Cas9 systems are guided by dual RNAs: the CRISPR RNA (crRNA), which directs DNA targeting and hybridizes with the transactivating crRNA (tracrRNA). The tracrRNA facilitates the assembly of the Cas9 complex. The functions of both the crRNA and tracrRNA can be integrated into a synthetic guide RNA (sgRNA). Compared to alternative gene editing instruments, Cas9 is an RNA-guided nuclease whose sequence specificity is contingent upon the Watson-Crick base pairing between the target DNA sequence and its guide RNA (gRNA), in addition to the direct interaction between Cas9 and the protospacer-adjacent motif (PAM). The Cas9 protein itself remains unchanged during different DNA targeting; only the short sequence of the gRNA (guide RNA) needs to be altered to re-target the Cas9 complex to a specific DNA sequence. This design makes the CRISPR/Cas9 system highly flexible and versatile. Conformational changes in the Cas9 protein are critical for its DNA shearing activity. The conformational state of the HNH nuclease structural domain directly controls DNA shearing activity. The relative orientation of the Cas9 catalytic structural domain upon binding to DNA was examined by Forester resonance energy transfer (FRET) experiments, and it was found that the DNA shearing efficiency is directly proportional to the degree of sampling of the activated conformation of the HNH structural domain. Cas9 proteins exhibit different conformational states in the absence of RNA, when sgRNA is bound and sgRNA/DNA is bound, and these conformational changes are essential for understanding the DNA shearing mechanism of Cas9. The conformational dynamics of the HNH structural domain may impact the shearing specificity of the Cas9-sgRNAase complex (Shuqi Pang et al, 2025).

4.3 CRISPR Cas13a: (also known as C2c2) belongs to the Type VI CRISPR-Cas system. Unlike the widely used Type II systems (e.g., Cas9), which target DNA, the Cas13 system targets RNA. Cas13's ability to specifically split

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single-stranded RNA (ssRNA) targets has been used in the evolution of RNA-targeting tools and nucleic acid detection systems. The system's RNA-guided RNase functionality allows for the specific cleavage of complementary primary spacer sequences, making it a viable option for sequence-specific RNA manipulation. Moreover, Cas13's "side effect"—non-specific cleavage of nearby RNA after target recognition—has been applied to create sensitive diagnostic tools. The functional mechanism of Cas13 relies on two higher eukaryotic and prokaryotic nucleotide-binding (HEPN) domains that are activated upon recognition of a target RNA. Guidance toward the RNA transcript is mediated by a CRISPR RNA (crRNA), which aligns with its complementary sequence through the spacer region. Once the HEPN domains are triggered, Cas13 induces specific Strand scission of the target RNA. This activation of Cas13 leads to the Strand scission of target ssRNAs (cis-cleavage) as well as the Strand scission of non-specific bystander ssRNAs (trans-cleavage), a property that has been utilized for in situ detection of viral RNAs. Recent innovations have expanded Cas13 beyond detection into RNA editing. To illustrate, catalytically inactive Cas13 (dCas13) fused with adenosine deaminase has facilitated targeted A-to-I (adenosine to inosine) RNA conversions, effectively altering codons without permanent genomic modifications. This feature highlights Cas13 as a promising RNA-centric platform with potential roles in translational medicine, antiviral strategies, and temporary genetic regulation. Cas13 therefore stands out within the CRISPR family not only for its structural divergence but also for its unique functional versatility in RNA biology. Unlike the Cas9 and Cas12 systems, the Cas13 system exhibits substrate RNase activity activated by the target RNA. Through its unique structural and mechanistic principles, the Cas13 system provides a powerful platform for RNA targeting, editing, detection, and imaging, and shows potential for biotechnological and therapeutic applications .CRISPR-Cas9 exhibits variable off-target rates (0.1% to >50%), strongly influenced by gRNA design and chromatin accessibility . While high fidelity variants such as HiFi-Cas9 reduce off-target editing by 100-fold, they incur a ~30% loss in on-target efficiency. For RNA-targeting systems, Cas13a's collateral cleavage of bystander RNAs-useful in diagnostics, poses therapeutic risks. However, recent engineered mutants (e.g. dCas13a-ADAR) achieve 95% suppression of trans-activity while maintaining target specificity, offering a safer alternative (Shuqi Pang et al, 2025).

4.4 CRISPR Cas 12a: Is a nuclease in the CRISPR-Cas system, which belongs to the Class 2 type V system. Cas12a (also known as Cpf1) is the first member of the Cas12 family to be discovered. It is a single-component RNA-guided DNA nuclease that is capable of crRNA processing and DNA targeting independently of tracrRNA cleavage, this is because Cas12a does not require additional transcriptionally activated crRNA (tracrRNA) to process the crRNA arrays. Cas12a generates staggered cuts at DNA target

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sites with prominent 5' ends and does not utilize transcriptionally activated crRNAs. The staggered ends generated by Cas12a may prove more advantageous for applications such as precisely targeted integration of DNA sequences in comparison to the flat ends generated by Cas9. Furthermore, Cas12a is capable of cleaving crRNA arrays to generate its own crRNAs, thereby enabling the simplified editing of multiple genomes using a single customized crRNA array. Recently, engineered variants of Cas12a have been developed to expand its targeting range and improve its genome editing activity. These variants, like enAsCas12a, exhibit two-fold higher genome editing activity on sites with canonical TTTV PAMs compared to wild-type AsCas12a and can target many previously inaccessible PAMs. This advancement allows for more efficient multiplex gene editing, endogenous gene activation, and C-to-T base editing. Additionally, a high-fidelity version of enAsCas12a (enAsCas12a-HF1) has been engineered to reduce off-target impacts. These engineered variants of Cas12a not only broaden the applicability of the CRISPR-Cas system but also demonstrate the potential for further optimization of CRISPR nucleases for various genetic and epigenetic editing applications (Shuqi Pang and Jingran Qu, 2025).

5. Gene regulation and CRISPR

In eukaryotic cells, gene expression is regulated by intricate associations between cis-regulatory elements (CREs)—like promoter regions—and transacting consistent, involving transcription factors and chromatin-modifying proteins. These connections help determine cell-specific transcriptional patterns and allowed cells to adaptively respond to signals from their external environment. The three-dimensional architecture of the genome, organized into topologically associating domains (TADs) and sub-TADs, plays an essential role in this process by facilitating selective contacts between CREs and their target promoters, while insulating unrelated genes from aberrant activation. Certain structural proteins, including CTCF, serve as key architectural regulators by defining genomic boundaries that ensure genes are expressed with high specificity. Disruption of CRE–trans factor coordination, whether through genetic variation or epigenetic changes, can alter transcriptional control and contribute to disease susceptibility. This has been exemplified in immune-related loci, like the cluster containing CD28, CTLA4, and ICOS on chromosome 2q33.2. Although these genes share evolutionary origins, they display distinct expression dynamics across T cell subsets and activation states, reflecting the intricate regulation of costimulatory mechanism in immune homeostasis. CRISPR-based functional genomics has appeared as a powerful strategy to dissect these regulatory networks. While annotation approaches like ChIP-seq and ATAC-seq provide valuable insights into CRE localization and chromatin

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accessibility, they cannot confirm functional relevance. In contrast, CRISPR interference (CRISPRi) and CRISPR activation (CRISPRa) allow systematic perturbation of regulatory elements in their native genomic context, enabling direct linkage between CREs, transfactors, and their target genes. Recent studies applying these techniques to primary human T cells have revealed cell type–specific CREs within the CD28–CTLA4–ICOS locus and highlighted the role of CTCF in maintaining regulatory boundaries. These studies underscore CRISPR's utility in decoding the regulatory logic of complex genomic regions and its potential to illuminate disease mechanisms driven by deregulated gene expression (Cody T. Mowery et al, 2024).

6. Gene editing technology

Gene editing technologies have undergone significant evolution, providing exact tools for targeted genomic alterations. One of the most widely utilized approaches including homology-directed repair (HDR)-mediated genome editing, which leverages the cell's endogenous repair machinery to incorporate exogenous DNA templates at specific genomic loci. Initial approaches for HDR-based editing relied on introducing double-stranded DNA (dsDNA) templates into cells, where occasional recombination with the target locus allowed precise modifications, successfully generating knock-in cell lines and genetically modified animals. However, in the absence of targeted double-strand breaks (DSBs), the frequency of homologous recombination was exceedingly low, limiting the therapeutic applicability of these methods. The efficiency of HDR increases dramatically when DSBs are induced at the target site, underscoring the importance of nuclease-mediated DNA cleavage for precise genome editing. Several classes of site-specific nucleases have been developed to facilitate HDR. Zinc-finger nucleases (ZFNs) are chimeric proteins combining the DNA-binding specificity of zinc-finger domains with the FokI nuclease. Tandem arrays of zinc-finger domains recognize specific DNA sequences, and FokI dimerization upon binding induces site-specific DSBs. While ZFNs enabled precise genome targeting, challenges such as complex design and limited target site flexibility restricted their widespread adoption. Transcription activator-like effector nucleases (TALENs) overcome some of these limitations by utilizing TALE DNA-binding repeats fused to FokI, allowing rapid and flexible targeting of extended DNA sequences, although repetitive TALE structures pose delivery challenges. The CRISPR/Cas9 system has emerged as a highly adaptable and efficient gene-editing tool. By programming a single guide RNA (sgRNA) to match a 20 bp target sequence adjacent to a protospacer adjacent motif (PAM), Cas9 introduces DSBs with high specificity. This system enables multiplexed genome editing and can be optimized to minimize off-target effects through engineered Cas9 variants, nickases, or fusion with FokI

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nuclease domains. Moreover, smaller Cas variants, such as Cas12f, facilitate packaging into viral vectors for efficient delivery alongside donor templates, further broadening their applicability. The HDR pathway repairs DSBs using homologous donor templates, producing precise edits. Key proteins, including the MRN complex, ATM kinase, BRCA1/2, RAD51, and DNA polymerases, coordinate the recognition, strand resection, and synthesis processes that result in either crossover or non-crossover outcomes, depending on the sub-pathway employed (DSBR or SDSA). HDR efficacy is cell cycle-dependent, occurring predominantly during S/G2 phases, and is influenced by factors like donor template length, target locus, and the relative activity of competing repair pathways, involve non-homologous end joining (NHEJ). Despite its precision, HDR-mediated genome editing faces limitations: lower efficiency compared to NHEJ, dependence on proliferative cell phases, potential for unintended genomic alterations from DSBs, and challenges in delivering nuclease and donor templates in vivo. Strategies to enhance HDR outcomes include NHEJ inhibition, HDR pathway activation, donor template modifications, and optimization of nuclease delivery systems Figure 1. Collectively, these advancements underscore the versatility and growing therapeutic potential of gene editing technologies, establishing a robust foundation for future research and clinical applications (Yanjiang Zheng et al, 2024).

gene transfer to precise genome-editing systems based on engineered nucleases. Traditional site-specific editing involves generating double-strand breaks at targeted DNA loci, followed by repair through endogenous cellular mechanisms, enabling mutation correction or insertion/deletion of specific sequences. While technologies such as ZFNs and TALENs offer certain advantages, their complexity, variable efficiency, engineering challenges, limited multiplexing potential, and off-target activity have constrained widespread adoption. The CRISPR/Cas system has emerged as a highly versatile tool for genome manipulation across diverse organisms, offering simplicity in design, cost-effectiveness, high accuracy, and the ability to target many loci simultaneously. Delivery of CRISPR components can be achieved without viral vectors via plasmid DNA encoding Cas protein and gRNA, direct RNA delivery for cytoplasmic translation, or ribonucleoprotein (RNP) complexes that provide rapid and transient expression, minimizing off-target effects. Engineered carriers play a critical role in enhancing the safety, efficiency, and specificity of CRISPR-based therapies. These carriers improve the transport of nucleic acids and proteins to target cells while decreasing side effects. Current platforms involve biological and metallic nanoparticles, hybrid nanoparticles, virus-like particles, and exosomes. They protect cargo, enable targeted delivery, and accommodate

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large payloads such as RNPs or multidomain proteins. CRISPR delivery strategies are broadly classified into viral, non-viral, and physical methods. Viral and non-viral carriers are commonly applied in vivo, whereas physical approaches used in vitro to introduce CRISPR components directly into cells, such as electroporation, microinjection, and hydrodynamic injection, are employed typically. Each method provides varying degrees of precision, efficiency, and suitability for clinical applications. Viral vectors—including adeno-associated viruses, lentiviruses, and adenoviruses—offer targeted delivery and stable expression but may face limitations in cargo capacity or provoke immune responses. Non-viral platforms such as virus-like particles and exosomes provide safer, customizable alternatives with high biocompatibility, efficient payload delivery, and reduced immunogenicity. Exosomes, in particular, possess prolonged circulation, the ability to cross the blood–brain barrier, and natural biocompatibility, making them a highly promising platform for CRISPR/Cas-mediated gene therapy (Neda Rostami et al, 2024).

8. Viral vectors

Viral vectors are engineered viruses used as tools to deliver therapeutic genes into target cells, offering promising strategies for treating genetic disorders, cancers, and infectious diseases. AAV Vectors Adeno-associated virus (AAV)-mediated gene transfer holds significant prospective as a therapeutic approach. Most of the currently developed AAV vectors are directed toward monogenic diseases, which fall under the category of rare diseases. Gene therapy products based on AAV vectors have received regulatory approval, such as LUXTURNA for patients with confirmed biallelic RPE65 mutation-associated retinal dystrophy and ZOLGENSMA for pediatric patients under two years with spinal muscular atrophy (SMA) caused by bi-allelic SMN1 mutations. Recombinant adeno-associated virus (rAAV) serotypes with defined tissue tropisms are being actively investigated as potential vectors for localized antitumor therap. Notably, EBV-positive B lymphocytes demonstrate enhanced susceptibility to rAAV6.2 infection, raising the possibility of utilizing this vector to deliver suicide genes for the treatment of EBV-driven B-cell lymphoproliferative disorders. Likewise, intracranial administration of interferon-β (IFN-β) via AAV vectors has yielded promising outcomes in preclinical models of orthotopic glioblastoma and infiltrating tumors. Several unique features render AAV vectors attractive for cancer gene therapy. The replication-incompetent parvoviruses normally require helper viruses for efficient replication; however, the development of helper-free production systems has streamlined their clinical application. Importantly, AAVs are considered non-pathogenic and have not been associated with human disease, supporting their safety profile in therapeutic contexts. There are at least 12

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natural serotypes with varying tissue tropisms, determined by differences in receptor binding. For example, AAV-2 primarily binds heparin sulfate proteoglycan, whereas AAV-3 relies on heparin, heparin sulfate, and FGFR-1 .AAV vectors deliver DNA without integrating into the host genome, forming episomal concatemers in the nucleus that enable prolonged transgene expression in non-dividing cells, like neurons and cardiomyocytes. Integration frequency increases under certain conditions, such as high multiplicity of infection or adenoviral co-infection. High copy numbers of some AAV vectors, like AAV9, can induce toxicity in animal models. Despite limitations, AAV vectors are favored for in vivo gene treatment due to their ability to transduce both dividing and quiescent cells, robust transduction efficacy, prolonged expression, tissue-specific tropism, low immunogenicity, and overall clinical safety. Challenges include the limited insert size, complex production processes, low viral yield, high cost, and restricted targeting of certain organs. Strategies to overcome these obstacles include splitting large genes across multiple vectors, removing non-essential viral sequences, improving transfection and production methods, and exploring new payment mechanisms for high-cost therapies. Despite these challenges, ongoing AAV-based gene therapy trials are expected to yield novel clinical products in the future. Adenoviral Vectors (Ad Vector) Adenoviruses are large, non-enveloped, double-stranded DNA viruses with icosahedral capsids, capable of accommodating 26–45 kb of DNA. Over 100 serologically distinct adenovirus types exist, with 49 infecting humans. Adenoviruses bind various cell surface proteins to facilitate entry and are highly efficient for gene delivery across a broad range of cells and tissues. They do not integrate into the host genome, and their well-characterized biology, large transgene capacity, genetic stability, and scalable production make them valuable for gene therapy. Adenoviral vectors can be replication-deficient or replication-competent oncolytic viruses. The commonly used vector is human Ad serotype 5, which is induced replication-defective via deletion of the E1 and E3 genes. Successive generations of adenoviral vectors have improved safety, increased transgene capacity, and reduced immune responses while maintaining high transduction efficiency. Ad vectors are widely used in cancer gene therapy and vaccine development, including vaccines against COVID-19. Limitations include pre-existing immunity, innate immune responses, and potential liver toxicity. Strategies to overcome these include using non-human adenovirus vectors or sequential administration of antigenically distinct viruses. Lentiviral Vectors (LVs) Lentiviruses belong to the retrovirus family and include primate and non-primate types. They are spherical, approximately 100 nm in diameter, and contain a diploid single-stranded RNA genome.

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Lentiviral vectors typically include the 5′ long terminal repeat (LTR), packaging signal; central polypurine tract, Rev-responsive element, and 3′ LTR with polyadenylation signal. Four generations of lentiviral vectors have been developed. First-generation vectors contained much of the HIV genome, including accessory and regulatory genes. Second-generation vectors removed accessory genes to improve safety. Third-generation vectors are replication-incompetent and self-inactivating, using heterologous envelope proteins for broad host tropism. Fourth-generation vectors address residual recombination risks through codon optimization and heterologous sequence replacement. Lentiviral vectors offer unique advantages, including rare neutralizing antibody generation, prolonged and stable gene expression, and the ability to infect dividing and non-dividing cells, making them suitable for pediatric and neurological applications (Xuedan Li et al, 2023).

9. Application of gene-editing tool

The CRISPR-Cas9 system has emerged as a transformative tool in genome engineering, providing unprecedented precision and efficacy in DNA modification. Its versatility has enabled applications across diverse fields, extending beyond medical research to agriculture, environmental sciences, biotechnology, and aquaculture. In agriculture, CRISPR-Cas9 offers the capability to directly manipulate plant and livestock genomes, expediting the introduction of traits like disease resistance, enhanced yield, enhanced nutritional value, and tolerance to abiotic stress. By passing multiple generations, cross-targeted gene editing considerably reduces crop improvement timelines. By way of example, editing susceptibility genes in rice has conferred resistance to bacterial blight, while modifications impacting grain size regulation have augmented yields. Livestock applications are progressing toward improved meat quality, disease resistance, and animal welfare, although public acceptance and regulatory considerations remain fundamental factors. In the biomedical sciences, CRISPR-Cas9 is a cornerstone of precision medicine research, enabling the correction of pathogenic mutations, the disruption of viral entry mechanisms, and the enhancement of immune cell function. Pivotal studies involve editing the CCR5 gene in hematopoietic stem cells to confer HIV resistance and modulating oncogenes in neoplasm cells for targeted therapy. Furthermore, ex vivo editing of patient-derived cells is advancing in clinical studies, particularly for hematological and metabolic disorders. Nevertheless, concerns regarding off-target impacts, delivery capability, and long-term safety remain central barriers to translational research, with germline editing continuing to raise profound ethical debates. Environmental significance focuses on conservation biology, ecosystem monitoring, and invasive species control. CRISPR-Cas9 has

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been explored to augment genetic resilience in endangered species, like conferring resistance to fungal pathogens within bat populations. Analogously, genetic manipulation techniques are being developed to limit the reproductive success of invasive organisms. CRISPR-based biosensors also show promise for detecting pathogens and pollutants in environmental samples, contributing to early warning systems and sustainable ecological management. In industrial biotechnology, CRISPR–Cas9 facilitates the generation of genetically engineered microorganisms for high-yield biosynthesis, synthetic biology innovations, and precision regulation of metabolic pathways. Its applications extend to translational study, where animal models like genetically edited pigs provide valuable platforms for studying metabolic and cardiovascular disorders. In aquaculture, the technology is being optimized to improve fish growth rates, stress tolerance, and pathogen resistance, while also offering genetic approaches for invasive species control to protect biodiversity. Nevertheless, responsible implementation requires careful ecological risk assessment and the establishment of robust regulatory frameworks. Overall, CRISPR–Cas9 has redefined genetic engineering across multiple fields. It's a responsible application; only through responsible implementation can its dual promise—scientific advancement and societal benefit—be fully realized (Arif NM Ansori et al, 2023).

10. Application to treat disease

10.1 Applications of CRISPR/Cas9 in Oncology: The CRISPR/Cas9 platform has emerged as a powerful genome-editing tool with significant potential in oncology, particularly through the modulation of genes that regulate neoplasm initiation and progression. By either inactivating oncogenic drivers or reactivating tumor suppressor pathways, CRISPR/Cas9 enables precise genetic manipulation of cancer cells. For example, targeting well-characterized oncogenes such as MYC, KRAS, and EGFR has provided insight into novel therapeutic avenues. Experimental disruption of constitutively active fusion oncogenes, including BCR-ABL1 in leukemia and EWSR1-FLI1 in Ewing sarcoma, has been shown to induce apoptosis, restrict leukemic stem cell proliferation, and reduce overall tumor burden .Mutations in KRAS, a frequently altered gene in human malignancies, represent a key focus of CRISPR-mediated interventions. In both in vitro and in vivo models, deletion of mutated KRAS suppressed colorectal cancer cell growth, highlighting its translational relevance. Beyond classical oncogenes, novel cancer-promoting mediators such as interleukin-30 (IL27/p28) have also been successfully targeted using CRISPR/Cas9, leading to reduced stem cell expansion and altered tumor microenvironments in colorectal, breast, and prostate cancers. IL-30, a cytokine expressed by malignant cells and infiltrating

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immune cells, is thought to enhance disease progression through pro-inflammatory and oncogenic signaling cascades. Its deletion using CRISPR/Cas9 approaches has been associated with reduced tumor growth and improved survival in xenograft systems .Moreover, nanoparticle-based delivery systems, such as immunoliposomes carrying Cas9-gRNA complexes, demonstrated effective inhibition of tumor proliferation in prostate cancer models without notable systemic toxicity, thereby underscoring the therapeutic potential of genome editing in precision oncology .CRISPR/Cas9 presents a potential strategy to correct mutations or deletions in neoplasm suppressor genes, including TP53, BRCA1, RB1, and PTEN, thereby restoring their normal function and halting disease progression. Despite this promise, implementing CRISPR-mediated gene repair as a treatment approach faces limitation like limited efficacy of Homology-Directed Repair (HDR), dependence on the cell cycle, and the need for highly specific single guide RNAs (sgRNAs) to prevent unintended off-target modifications .In addition, Epigenetic alterations often cooperate with genetic changes to drive oncogenesis. CRISPR-based epigenetic editing, through fusion of a catalytically inactive dCas9 with epigenetic modifiers like Dnmt3a or Tet1, allows modulation of gene expression without altering the DNA sequence. These approaches can add or remove epigenetic marks, reversing malignant -associated changes. Epigenetic interventions have enhanced immune checkpoint therapy efficacy in metastatic non-small cell lung cancer; however, CRISPR/dCas-based strategies for epigenetic editing require further evaluation, particularly in combination therapies, given the interplay of genetic and epigenetic alterations in cancer. CRISPR/Cas9 can enhance T lymphocyte cytotoxicity against cancer by engineering cells to express chimeric antigen receptors (CAR-T). Bispecific T cells can be generated via genes encoding TCRs and CARs with tumor-specific affinities. Successful CRISPR-mediated CAR expression targeting CD19 in B-cell lymphoma patients has yielded durable remissions. Additionally, CRISPR has enabled the progress of non-autologous CAR-T cells usable across patients without HLA matching, lowering costs and improving accessibility (Emma Di Carlo and Carlo Sorrentino, 2024). Deletion studies of enhancer elements, such as IkE120 upstream of Ikzf1, highlight the regulatory importance of distal enhancers in T-cell development, immune homeostasis, and leukemogenesis (Alomairi et al., 2020). CRISPR/Cas9 facilitates knockout of genes encoding immunological checkpoint proteins, like PDCD1, enhancing the function of T and NK cells against neoplasm. Interruption of PD-1/PD-L1 signaling enhances CAR-T cell efficacy within immunosuppressive tumor microenvironments (Emma Di Carlo and Carlo Sorrentino, 2024).

10.2 Applications of CRISPR/Cas9 in Genetic Disorders: Duchenne Muscular

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Dystrophy (DMD), a monogenic X-linked disorder caused by dystrophin mutations, represents an ideal target for CRISPR/Cas9-based therapy. Initial experiments in mdx mice demonstrated that deletion of mutant exon 23 restored dystrophin expression and improved muscle function. The sgRNA screening enables targeted exon skipping for the majority of DMD mutations, restoring dystrophin in gene-edited induced pluripotent stem cells (iPSCs). The FDA-approved first gene therapy delivering anti-dystrophy protein underscores the potential for CRISPR-based treatments. Leber Congenital Amaurosis (LCA), associated with CEP290 and RPE65 mutations, has also been targeted with CRISPR/Cas9. Editing CEP290 in LCA10 models restored gene expression, while RPE65-targeted editing improved retinal function in mice without notable adverse effects. Subretinal ABE targeting of KCNJ13 similarly restored vision function (Yangsong Xu et al, 2025).

10.3 Applications of CRISPR/Cas9 in Neurological Disorders (Alzheimer's Disease) : CRISPR/Cas9 offers potential for targeting central nervous system cells to correct genetic defects in diseases like Alzheimer's disease (AD), characterized by amyloid-beta (Aβ) plaques, tau hyper-phosphorylation, and neuronal loss. Mutations in APP, PSEN1, and PSEN2 are primary contributors. CRISPR-mediated reduction of APP and BACE1 expression decreases Aβ production. Correction of PSEN2N141I mutations in patient-derived neurons normalized the Aβ42/40 ratio and electrophysiological deficits. Additionally, CRISPR strategies targeting APOE ε4 and MAPT aim to reduce AD risk and tau pathology, respectively (Amna Akbar et al., 2025).

10.4 Applications of CRISPR/Cas13a in Infectious Diseases: HIV, which targets CD4+ T cells, can be suppressed using CRISPR-Cas13a, which cleaves viral RNA and reduces gene expression, effectively inhibiting infection (Yue Zhang et al., 2024). SARS-CoV-2 Cas13-based platforms, such as PAC-MAN, target conserved viral RNA regions, achieving >95% inhibition of multiple strains including Alpha, Beta, Delta, and Omicron variants. Techniques integrating Cas13 with bioinformatics and delivery systems, including SHERLOCK, CREST, and SHINE, have enabled rapid, sensitive, and scalable viral detection, improving early diagnosis and containment of COVID-19 (Xiaoying Tan et al, 2025).

11. Challenges of CRISPR

A major limitation with the CRISPR-Cas system lies in its overall effectiveness. While the technology can accurately identify and modify numerous mutations and DNA sequences in plants, animals, and microorganisms, several factors influence its effectiveness. Limitations related to PAM site availability, the expression of multiple gRNAs within a single vector, Cas9 fidelity, and off-target effects all restrict its potential. PAM dependency confines editing to genomic

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regions containing specific motifs, reducing flexibility, while simultaneous targeting of multiple loci can create competition for Cas9 binding and diminish activity. Over and above that, off-target interactions may introduce unintended mutations, compromising the specificity and trustworthiness in of outcomes. CRISPR relies on two primary DNA repair pathways: non-homologous end joining (NHEJ), which functions across all cell cycle phases, and homology-directed repair (HDR), which occurs only through the S and G2 phases. This limits HDR's relevance, though approaches like using nano-carriers to co-deliver HDR templates with CRISPR component can achieve high accuracy. Another obstacle is the inconsistency in sgRNA activity, necessitating extensive screening to identify optimal sequences. Not all genetic conditions can be addressed during simple deletions or sequence edits, and some require more advanced modification genome. Beyond these technical challenges, CRISPR also raises major ethical limitations that are closely tied to its technical capabilities. To illustrate, advances that improve accuracy and broaden editing scope inevitably increase the feasibility of human germ line modification—changes that are heritable and could influence future generations. This possibility raises concerns over inequality, genetic discrimination, and the moral implications of selecting traits in embryos. Likewise, using CRISPR to alter organisms like plants, animals, or insects may disrupt ecological balance, illustrating how technical progress and ethical responsibility are inseparably linked. While these challenges underscore the complexity of CRISPR, they also emphasize the importance of sustained research to enhance safety, and social regulation (Mohammad Ali Karimi et al, 2025).

12. Conclusion

The CRISPR-Cas9 technique is a groundbreaking genetic editing tool that has been utilized in various scientific study fields, particularly in applications in biomedical and biotechnology. It can conduct genetic engineering with marked efficiency and specificity, and has opened new pathways for research to correct genetic defects, gene function, and discover new therapies for gene defects. The platform holds very high potential in medicine, biotechnology, and agriculture. Although promise, the technology is accompanied by substantial ethical concerns, particularly regarding germline modifications. Permanent and heritable edits raise questions about equity, genetic determinism, and societal consequences that extend beyond individual patients. Therefore, the adoption of CRISPR must be guided by careful ethical deliberation, transparent regulations, and multidisciplinary dialogue. Future perspectives emphasize the importance of integrating CRISPR into responsible innovation frameworks, ensuring its powerful potential is directed toward food security, improving health, and sustainability without compromising ethical boundaries.

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Table 1: types of CRISPR-Cas systems (Shuqi Pang and Jingran Qu, 2025).

	Types	Description
1	Cas3	Helicase-nuclease that shreds ssDNA unidirectional after Cascade complex binding.
2	Cas9	Cuts double-stranded DNA (dsDNA) guided by single guide RNA (sgRNA).
3	Cas12a	Staggered dsDNA cut guided by CRISPR RNA (crRNA); recognizes T-rich PAM; collater RNA activity.
4	Cas 13a	Single-stranded RNA (ssRNA) cleavage via crRNA; collateral RNA activity; can be used fo rapid detection of RNA viruses

Figure 1. The Cas9 enzyme creates a double-strand break, and either the NHEJ or the HDR pathway is used to repair the DNA, resulting in an edited gene sequence (Hoang Ha ،2017).