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Advances in Gene Editing Technology in Biomedicine

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Abstract:

The CRISPR/Cas9 system represents an adaptive immunological defense mechanism observed in bacteria and archaea, which has evolved to protect these organisms from persistent viral and plasmid assaults. This system is comprised of clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated (Cas) proteins. By modifying the simplest type II CRISPR system and introducing a special small guide RNA (sgRNA) and Cas9 endonuclease into the cell, it is possible to cut double-stranded DNA at a specific location and realize knockout or knock-in. CRISPR/Cas9 system has many applications in biological and scientific research fields due to its efficient gene editing function, which makes up for the shortcomings of traditional transgenic technology, such as random integration and genetic instability, and has a broad development prospect. This paper comprehensively discusses the CRISPR/Cas9 technology's applications in drug discovery and the drug development, such as screening and targeted editing of functional genes, screening and validating drug targets, fabricating animal bioreactors, constructing animal models, and treating genetic diseases, etc. It also discusses the risks and prospects of CRISPR/Cas9 technology.

Keywords: Gene editing technology; CRISPR/Cas9; Gene Therapy; Base Editing technology; Transcription regulation tool

1. Introduction

The advent of gene editing technologies based on zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), CRISPR/Cas, have allowed biomedical field be able to manipulate the genome, which facilitate Drug research and development. Although homologous recombination technology can realize the functions of gene knock-in, knock-out and replacement, its application is limited by its huge cost on time and labour for screening. However, the new genome editing technologies can not only be able to knock-off any gene of interest, knock-in Exogenous genes and repair or replace gene, but also improve the efficiency of genome manipulation compared to homologous recombina-

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tion technology.[1] [2]CRISPR/Cas, ZFN and TALEN are used to achieve genome editing, which are capable to activate the two types of natural cellular repair mechanisms, non-homologous end joining (NHEJ) and homology-directed repair (HDR), through specific DNA double-strand breaks (DSBs). [3] ZFNs consist of Zinc Finger domain, and TALENs are made up of TAL and Fokl endonuclease domain. Both of ZFNs and TALENs promote the formation of dimers by FokI to cleave double-stranded DNA through the specific binding of proteins to DNA sequences recognized by the single-stranded DNA.

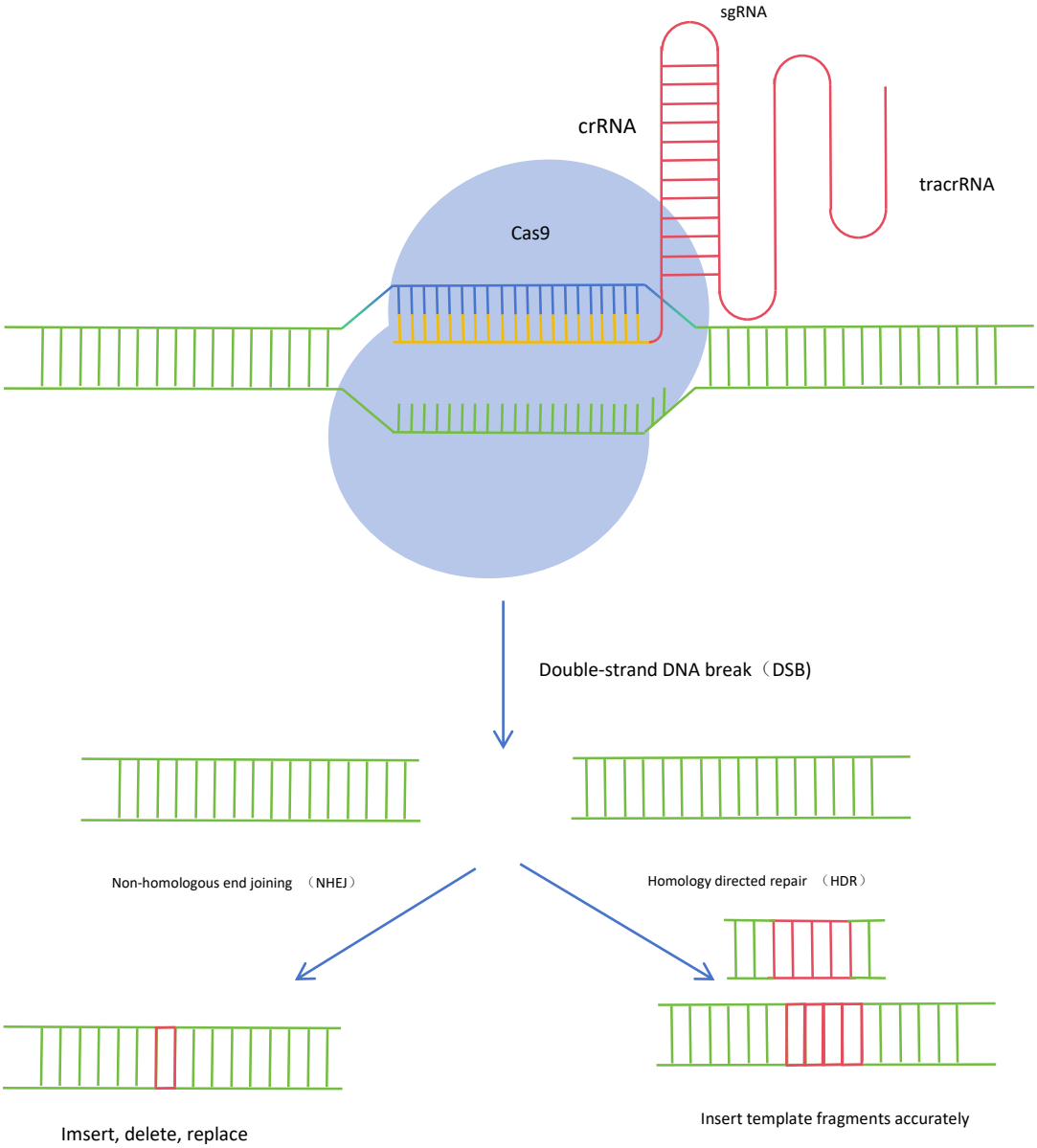

Figure 1. The principle of genome editing of ZFNs, TALENs, and CRISPR/Cas.

Compared to ZFN and TALEN, which use protein as the target, the CRISPR/Cas system uses guide RNA (gRNA) to target the gene, which is simple, fast and cheap [4]. The CRISPR/cas system, discovered in bacteria and archaeobacteria, is an acquired immune system [5] that consists clusters of regular spaced short Palindromic sequence and Cas protein. The CRISPR/cas system can recognize exogenous virus or plasmid nucleic acids and cut them, which acts as a defense against exogenous nucleic acids invasion.[6] The working process of CRISPR/Cas system starts with processing the exogenous nucleic acids into a spacer sequence of a certain length. These exogenous spacer sequences are then integrated into the repetitive sequence in a genome, forming a regular spaced short

palindromic sequence. The sequence then being transcribed and processed into mature crRNAs. Finally, the crRNAs induce the Cas protein, with the function of nucleic acids endonuclease, to cut the invading exogenous nucleic acids again. According to the structure of repetitive sequence and the subclass of Cas protein, CRISPR/Cas system is mainly divided into two classes and six types,[7] among which type II of CRISPR/Cas system is the simplest, just including crRNA, tracrRNA, Cas 9. The main function of tracrRNA is to promote the maturation of crRNA through the activity of RNase III, and crRNA recognize and guide Cas9 to bind with target DNA by using base pairing, finally, Cas9 protein use the activity of its own endonuclease to cut DNA, so as to achieve the process of target DNA editing.[8]Agricultural/*genetics</keyword><keyword>DNA</keyword><keyword>Endonucleases/*genetics</keyword><keyword>Gene Editing/*methods</keyword><keyword>Gene Targeting/methods</keyword><keyword>*Genome, Plant</keyword><keyword>Inverted Repeat Sequences</keyword><keyword>Oryza/*genetics</keyword><keyword>Protoplasts/metabolism</keyword><keyword>Triticum/*genetics</keyword></keywords><dates><year>2013</year><pub-dates><date>Aug</date></pub-dates></dates><isbn>1087-0156</isbn><accession-num>23929338</accession-num><urls></urls><electronic-resource-num>10.1038/nbt.2650</electronic-resource-num><remote-database-provider>NLM</remote-database-provider><language>eng</language></record></Cite></EndNote> Since CRISPR / Cas9 system's efficiency in editing target DNA and its ease of manual manipulation have quickly, it rapidly changed into a new technology to edit gene, becoming a new type of artificial nucleic acids endonuclease. The emergence of CRISPR/Cas9 system, which achieves the targeting gene editing technology, makes it easier to construct animal model and cell system model and significantly speed up the process of screening and validating targets, and discovering and developing new drugs. Therefore, this article mainly describes CRISPR-Cas9 technology generate DSB by specifically target genome and achieve gene integration and knock off through activating NHEJ or HR in many ways. Any phase of the cell cycle can undergo NHEJ, especially G1 phase, it repairs DNA by joining the two ends of the break, resulting in the random deletion or insertion. NHEJ can induce frame-shift mutations and destroy target genes by introducing[9, 10]; HDR pass-way occurs in late phase S or phase G2[11, 12] and performs precisely gene editing on breaking site according to a provided template, thus allowing the targeted editing of genes according to exogenously provided template.

2. CRISPR-Cas9 system and associated technology

2.1 Base editing technology

Many diseases are caused by the alternation of base pairs in the DNA genome. Although the DNA sequence can be accurately repaired by inserting specific DNA template using HDR, this technology is restricted to dividing cell types and has limited efficiency. The base editing technology can edit base without releasing DSB on target site editing window through two inactive structural domain, which are dead Cas9(dCas9) or Cas9 nickase(nCas9n), and form fusion protein with cytidine deaminase or adenosine deaminase. The most widely used base editing technologies currently include the cytidine base editor (CBE), which can change C to T, and the adenine base editor (ABE), which can change A to G.[13, 14] CBE and ABE can effectively mediated 4 types of single nitrogen bases mutations, which account for about 30% of currently known human disease-causing genetic sites.[15] Hence, CBE and ABE can be applied to the construction of disease models and disease treatment caused by nitrogen base pair transitions in many cell types and organisms, and have been successfully used in agriculture to achieve the goals of improving reproductive performance of livestock, allogeneic organ transplantation, and improving economic shape of crops. At present, the application of nitrogen base editor is restricted by the production of by-products, including the by-products occurring on its target sites, such as transversions of target nucleotide, side editing and various indels, and by-products occurs on off-target sites and so on. Therefore, from different components of base editor system, researchers optimized and improved the system to broaden its editing domain, reduce the chance of off-targeting effects and so on. By the continuing exploration, the nitrogen base editing technology based on Cas9 protein has higher efficiency and accuracy, having a driving role in the treatment of diseases caused by point mutations, such as the treatment of cystic fibrosis and sickle cell anaemia.[16, 17]

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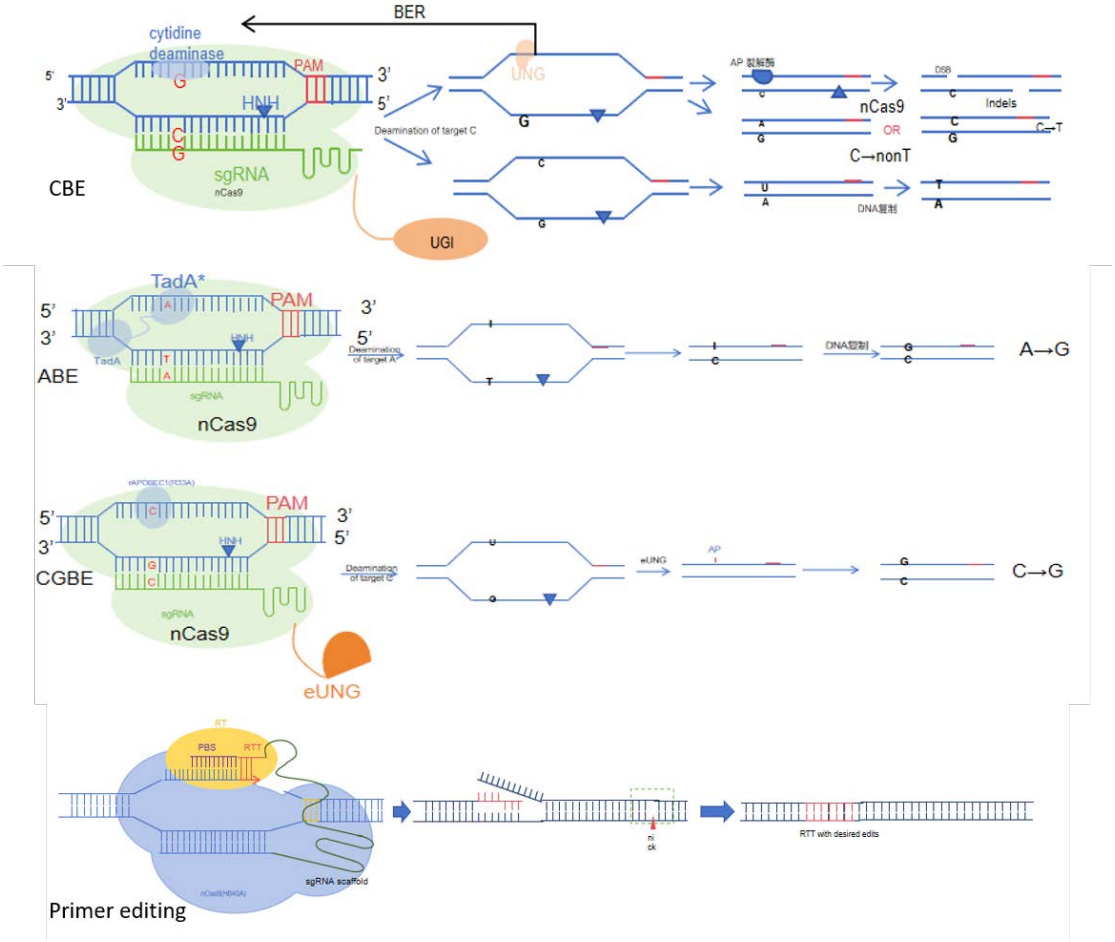

Figure 2. The principle of BE and PE.

2.2 Primer editing

The nitrogen base editing technology have a large number of unpredictable random off-target event at whole genome and transcriptional level. This is mainly because deaminase non-specifically recognize the nitrogen base on DNA or RNA and induce the bases deaminate, which cause severe potential danger in application level.[18-21] In addition, regular base editing technology could only achieve the transition between nitrogen bases and neither could realize the base transversion nor the accurate insertion and deletion of bases.[22] Since the above defects of the base editing system, in 2019, David Liu's group successfully developed the Prime Editor[23], which accomplished any form of base substitution with the help of this brand new technological pathway, and thus pushing the accuracy of base editing technology to a new height. This system overcomes the problem that base editing technology could only achieve the transition of 4 bases, and the new system is able to accomplish other 8 kinds of base transversion and the precise insertion and deletion of small fragment bases[23, 24]. The Prime Editor (PE) represents an advanced gene editing technique that utilizes a novel protease fusion of Cas9n (H840A) and reverse transcriptase. In this system, the engineered guide RNA (pegRNA) is designed with a 3' end that incorporates both a primer binding site (PBS) and an RT template, which guide the protease target and cut the non-complementary strand of dsDNA, and PBS recognizes and pairs the complementary sequence in front of cleavage site, reverse transcriptase preform reverse transcription with transcription template sequence as a template and polymerize the target sequence on the cleavage site of DNA strand. This system enables precise insertions, deletions, and a wide range of point mutations at the target site, all without generating DSBs or DNA templates[25].

2.3 Transcription regulation tool

Through binding to or recognizing specific region of upstream genes, transcription factor regulate the recruit of RNA polymerase and finally up-regulating or down-regulating the expression of target gene[27, 28]. The transcrip-

tion factor can not only bind with promoter region, but also binding with other transcription factors which forms a complex to regulate target gene more complexly and refinedly, facilitating or inhibiting the transcription from heritage message on DNA to RNA. The CRISPR gene regulating system includes CRISPR activation system (CRISPRa) and CRISPR interference system (CRISPRi). This system integrates a transcriptional activator or repressor factor with the dCas9 protein, leveraging the precise targeting and flexible programmability of the CRISPR system. By directing the fusion protein to the specific gene of interest, it enables the activation or suppression of gene transcription.[29, 30]

When targeting dCas9 protein upstream of the target gene, the spatial blocking effect of dCas9 protein on RNA polymerase can inhibit gene expression to a certain extent [31], but this inhibit effect only have high efficiency in prokaryotic cells, it has low inhibition efficiency in eukaryotic cells due to the complex internal regulation mechanism[32]University of California, San Francisco (UCSF. Fusing the dCas9 protein with transcriptional repression domains such as KRAB[33], MXI1[33] and TUP1[34] can enhance the efficacy of the CRISPR interference (CRISPRi) system in eukaryotic cells. Among them, the KRAB zinc finger protein (KRAB-ZFP) belongs to the largest family of transcriptional regulators in mammalian cells[35], which can repress gene transcription by inducing changes in the chromatin structure of target sequences[36]. The fusion of KRAB with dCas9 is an effective strategy for silencing specific genes or non-coding RNAs. This approach works by directing the KRAB-dCas9 fusion to various regulatory regions, including promoters, 5′ untranslated regions (UTRs), and both proximal and distal enhancer elements.

[37-39]. In 2018, Yeo and others[40] characterized the repression efficiencies of more than 20 known transcription factors capable of inducing gene silencing, They identified the six most effective repressors—MeCP2, SIN3A, HDT1, MBD2B, NIPP1, and HP1A—and combined each with a general repressor to create dual repressors. These were then fused with the C end of the dCas9 protein to express and characterize the inhibition efficiency of the CRISPRi system, and finally obtained the dual repressors fusion protein dCas9-KRAB-MeCP2, which had 60-fold increase in the repression efficiency than that of dCas9 alone.

When fuse dCas 9 with different transcription activator, it allows the precise activation of target gene by targeting, which forms a system called CRISPRa. The dCas9 in the initial CRISPRa system only fuse with a single trans-activation factor p65 or VP64, collectively, these former work constitute into the initial editable dCas9 gene activate toolkit, however, the results were unsatisfactory[41, 42]. In order to keep increasing the activate efficiency of CRISPRa system, researchers attempt various ways to apply more complex or muti-duplicated activation structural region to CRISPRa. For example, the construction of CRISPR mediated protein tag signal amplification system, which is introducing SunTag system into CRISPRa and CRISPRi system and optimizing the skeleton length and transcriptional factors, which successfully increase the target gene transcription level in Saccharomyces cerevisiae by 34.9 fold and are subject to almost complete repression, respectively. The Suntag system is composed of several duplicated GCN4 peptides and the single-strand variable fragment of anti-GCN4 antibody scFv[43], the muti-duplicated GCN4 peptide which fuse with the dCas9 regulate transcription by recruiting a multicopy fusion protein of the transcription factor and the GCN4 antibody tag. Thus, based on the fusion of dCas9 and transcriptional factors, CRISPRa and CRISPRi system can increase regulation efficiency by utilizing the fusion of multiple structural domains or the fusion of protein backbone systems.

3. The application of CRISPR-Cas technology in biomedical field.

The CRISPR-Cas9 system, known for its powerful gene editing capabilities, has been employed in various applications, including drug development, the construction of animal models as well as bioreactor, and the gene treatment in many fields, which lay a good foundation of the development of new drugs and new treating strategy.

3.1 The screening and editing of functional gene.

The function of human genome is not fully shown, there are many diseases have yet to find the suitable drug target. The inconvenience in operating genome has greatly restricted the revealing of disease mechanism and the screening research on drug targets. The CROISPR/Cas9 gene editing technology only needs to set up a library of sgRNAs and introduce it into the cell, and then through the evaluation of related phenotype, it can realize the large-scale targeted editing of genomes, thus further revealing the physiological functions of genes and providing reliable targets for new drug development. Koikeyuse and his group create a library of sgRNAs which could target all genes in the mouse genome, it uses Lentivirus to introduce these sgRNAs into the stem cell of small mice which can constitutive expressed Cas9 protein, and then genetically screen the mutant stem cell library, from which they

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found 27 known resistant genes and 4 unknown resistant genes[44]. Wei [45] and others created a targeted lentiviral library in human cells and developed a functional gene screening platform using the CRISPR/Cas 9 system, besides he combined with deep sequencing technology and successfully identified the host genes critical to the toxicity of Anthrax and diphtheria toxins, which will provide target points on further developments of new anti-bacterial drugs. For genes transcribing non-coding RNAs, insertion or deletion mutations alone are likely to have no significant effect on the phenotypes of the transcription products, and are therefore difficult to screen. Liu and others[46], using a paired guide RNA (pgRNA)-based approach, constructed a pgRNA library with lentivirus as a vector, and performed a genome-wide functional screening of nearly 700 long-chain non-coding RNAs (lncRNAs) related to cancer and other diseases in the human liver cancer cell line Huh7.5OC.

3.1.1 The screening and testing of drug targets

In drug development, the verification of the interaction between drug and its target is necessary. Finding drug resistance mutations is a must for target identification, and introducing resistance mutations into cells with a wild-type background is the final critical step in target validation, for example the discovery of the important drug-targeting combination of rapamycin-TOR1[47]. Integrating CRISPR/Cas9 technology with whole genome sequencing and drug resistance mutation screening allows for more efficient target verification.[48]. Specific macrophage models were constructed by combining CRISPR/Cas9 and iPSC technologies, demonstrating that the IRF5 and IL-10RA genes in the immune system play key roles in the resistance to chlamydial infections, which identifies new drug targets for the treatment of chlamydia-induced diseases[49]. Currently, the screening strategy which uses CRISPR/Cas9 technology is mainly to target and induce mutations of the 5' end exons on the candidate genes, the flaws of this strategy is that it is likely to generate in-frame variants which remain part of its function, even though a strong genetic correlation exists, no significant phenotypic differences may be observed. To overcome this problem, researchers[50] specifically mutates the exons of encoding functional domains of proteins, suggesting considerable room for optimizing CRISPR/Cas9 technology in the screening and validation of cancer drug targets.

3.1.2 The construction of animal model

The construction of animal diseases plays a crucial role in the research of disease mechanisms and developing effective prevention and treatment strategies to disease. Besides, it is instrumental in drug development, organ transplantation, and other medical advancements.The construction of traditional mouse model is mainly based on the homologous recombination and anti-biotic screening, and usually need the use of embryonic stem cell as a transition, this process is complicated and time consuming. While the usage of CRISPR/Cas9 technology significantly reduced time and money associate with the construction of animal model. Le and others[51]t' introduced CRISPR/Cas9 system into mouse eggs using RNA injection technology, which compared to DNA injection, this method can generate a more effective point mutation in mouse embryo and is not limited by the genetic strain of mice. Until now, researchers already used CRISPR/Cas9 system to construct the mice cancer model[52], such as human liver cancer, lung adenocarcinoma and construct mice disease model such as type B Hemophilia, heart failure and so on. Besides, Yan[53] and others generate target insertion human-derived mutation gene HTT into the HTT gene base of pigs, which successfully produced gene edited pig which can express human full length mutated HTT gene. This model can analog the characteristic of human neurodegenerative diseases, providing a foundation for further investigation into the pathogenesis and therapeutic strategies for neurological diseases in large mammals.

3.2 Construction of animal bioreactors

The advancement of animal bioreactors has opened up new horizons for the production of pharmaceutical proteins. We are now able to successfully express exogenous active proteins using these bioreactors. For example, the CRISPR/Cas9 gene-editing tool was utilized to genetically edit the genome of a fertilized pig egg to knock in the human albumin-producing gene, and the resulting piglets all carried the expected knocked-in gene, and human albumin could be detected in their blood [54]. Considerable progress has also been has been achieved in utilizing salivary gland bioreactors for obtain therapeutic proteins expressing human genes. zeng et al. [55] constructed a transgenic mouse with salivary gland-specific expression of human nerve growth factor (hNGF), and were able to use the salivary glands of this transgenic mouse as a bioreactor to produce hNGF proteins from the saliva of the transgenic mouse. zeng et al successfully prepared a transgenic human nerve growth factor (hNGF) transgenic protein in a mouse with the expected knock-in gene, and were able to detect human albumin in their blood. Zeng et al. [56] successfully prepared a pig transgenic for human nerve growth factor (hNGF) and synthesized hNGF from the saliva of transgenic pig for the first time by using the salivary gland of transgenic pig as a bioreactor. Compared with other bioreactors such as mice, the total amount of

protein expression and activity in the porcine salivary gland bioreactors developed and applied in recent years is higher, which makes an important contribution to promoting the production and application of recombinant drug proteins.

3.3 Gene therapy

With the rapid development of genomics, novel gene modifications with the advantages of target specificity and high precision have become powerful tools in the field of gene therapy, and have been widely utilized in the treatment of hereditary diseases, infectious diseases, and cancer treatment, etc. Niu et al [57] successfully accomplished precise gene targeting in crab-eating monkeys by co-injecting Cas9 mRNA and sgRNA into single-cell-stage embryos, This achievement represents landmark significance for the gene therapy in human hereditary diseases. The CRISPR/Cas9 gene modification system can effectively edit genes in stem cells and immune-responsive cells. Liao et al. designed a sgRNA sequence targeting the human immunodeficiency virus (HIV), and induced the Cas9 proteinbto shear the DNA double strand of the HIV virus at a specific location, inactivating the HIV virus in 18% to 72% of the infected cells in the human body[58]. Bogerd et al. used CRISPR/Cas9 to construct specific transcriptional activators that induced HIV-infected human cells to express the deficient restriction factors APOBEC3G and APOBEC3B, thereby blocking intracellular replication of Vif-deficient HIV-1 and partially inhibiting the infectivity of wild-type HIV-1 [59]. Kaminski et al. [60] utilized a specific CRISPR/Cas9 system to specifically target HIV-1 proviral DNA, which are able to effectively and safely remove the HIV virus from human CD4+ T cells cultured in vitro, and these T cells, which had been eradicated of HIV-1 DNA, were able to grow and function normally. These results hold promise for future clinical applications in the treatment of HIV infection.

In cancer immunotherapy, targeted knockdown of programmed death receptor-1 (PD-1) by CRISPR/Cas9 system and then transplanting the engineered cells back into the body can significantly improve its cytotoxicity against cancer cells[61], and the clinical trials of this technology for liver, lung and prostate cancers are underway and are expected to achieve greater breakthroughs. Koo et al.[62] co-delivered plasmids encoding Cas9 protein and EGFR mutation-specific sgRNA via adenovirus and inhibited tumor growth in xenografted mice. Lu et al.[63] employed CRISPR/Cas9 technology to knocked down the immune inhibiting PD-1 gene in T cells and expanded T cells in vitro, subsequently expanding these cells in vitro. Once suffcient number of cells was achieved, they infused the modified T cells back into Non-small cell lung cancer (NSCLC) patients as a treatment for tumors. Researchers [64] used the multifunctional polymer RGD-R8-PEG-HA to modify an artificial virus, and delivered a safe CRISPR/Cas9 system, RRPHC/Cas9-hMTH1, which targets the MTH1 gene in breast cancer, to a mouse tumor model and effectively inhibited the tumor growth, which provided a novel way to introduce CRISPR/Cas9 plasmids into the body.

In addition to the anti-cancer field, which is currently the subject of much research, the CRISPR/Cas9 system also holds substantial promise for the therapeutic development of various hereditary diseases (e.g., β-thalassemia, tyrosinemia). Yin et al [65] performed in vivo gene editing on a mouse model with hereditary tyrosinemia I using CRISPR/Cas9 technology. Hereditary tyrosinemia I is an inherited fatal disorder caused by mutations in the fumonisoylacetoacetate (FAH) gene. These mutations lead to the accumulation of the toxic metabolite fumonisoylacetoacetate in hepatocytes, resulting in severe liver damage. After analyzing by AST and ALT, it was found that mice with hereditary tyrosinemia I did not show abnormalities in liver function after treatment with CRISPR/Cas9 technology. This finding indicates a significant improvement in liver function after treatment with CRISPR/Cas9 technology. Canver et al[66] employed CRISPR/Cas9 technology to remove the red lineage enhancer controlling the molecular switch BCL11A in human hematopoietic stem cells, which resulted in a notable increase in fetal hemoglobin (HbF) and a relative decrease in adult hemoglobin (HbA) after hematopoietic stem cell maturation, and HbF was able to counteract the effect of the sickle cell mutation. Wang et al.[67] addressed genetic diseases linked to β-globin mutations by editing or repairing the mutated HBB gene at the BCL11A enhancer locus in hematopoietic stem cells from patients with β-thalassemia and sickle anemia, and by transplanting autologous hematopoietic stem cells so that they could differentiate and produce normal erythrocytes in vivo. Yeh et al [68] successfully cured deafness caused by recessive mutations in the Tmc1 gene in mice by delivering the CBEMax system through dual AAV, enabling them to respond to moderate sounds again. Genomic targeted modification technology demonstrate great potential for treating genetic diseases, and if we can set up a safe detection method for the adverse reactions caused by artificial nucleic acid endonucleases and reduce the cytotoxicity, we will continue to expand the scope of application of gene therapy.

4 Prospects and Conclusion

Although CRISPR/Cas9 technology is very efficient and

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widely used, it also has certain limitations, only when there is a PAM sequence near the target site, Cas9 protein can be cut accurately [69, 70], and its off-target effect may cause changes in genes other than the target gene. These problems deeply affect the safety and efficacy of this technology in therapeutic applications, and therefore it still needs to be improved. For example, Chiang [71] combined an integrated Cas9 D10A-transcriptase vector with fluorescence-activated cell sorting and enrichment, based on high-throughput genotypic and phenotypic monoclonal screening. This approach enabled high-efficiency knockdown and knock-in while meeting low off-target rates. Currently, ways to mitigate the off-target effect of CRISPR/Cas9 also include altering the secondary structure of sgRNAs [72], shortening the length of sgRNA sequences [73]Charlestown, MA, USA.
Center for Computational and Integrative Biology, Massachusetts General Hospital, Charlestown, MA, USA.
Center for Cancer Research, Massachusetts General Hospital, Charlestown, MA USA.
Department of Pathology, Harvard Medical School, Boston, MA 02115 USA.</auth-address><titles><title>Improving CRISPR-Cas nuclease specificity using truncated guide RNAs</title><secondary-title>Nat Biotechnol</secondary-title></titles><periodical><full-title>Nat Biotechnol</full-title></periodical><pages>279-284</pages><volume>32</volume><number>3</number><edition>20140126</edition><keywords><keyword>Bacterial Proteins/*genetics</keyword><keyword>Base Sequence</keyword><keyword>CRISPR-Associated Proteins/*genetics/metabolism</keyword><keyword>DNA/chemistry/genetics/metabolism</keyword><keyword>Endonucleases/chemistry/*genetics/metabolism</keyword><keyword>Humans</keyword><keyword>Molecular Sequence Data</keyword><keyword>RNA, Guide, CRISPR-Cas Systems/chemistry/*genetics/metabolism</keyword></keywords><dates><year>2014</year><pub-dates><date>Mar</date></pub-dates></dates><isbn>1087-0156 (Print, utilizing FokI-Cas9 fusion ribonucleases, purified Cas9 ribonucleoproteins, and paired-catalytic mutant Cas9 notases, etc.[74], which can achieve a lower off-target rate but also reduce the targeting efficiency to a certain extent.

To improve the accuracy and controllability of gene editing while reducing off-target effects, Liu et al [75] fused a Cas9 mutant, iCas9, to the hormonebinding domain of the estrogen receptor (ERT2), which could enable the nuclease activity of iCas9 to acquire 4-hydroxytamoxifen (4-HT) inducibility, thus rapidly and reversibly controlling the role of this system in genome editing functions. Shin et al. [76] discovered the inhibitory effect of the anti-CRISPR protein AcrIIA4 on the CRISPR/Cas9 system, providing a means to artificially manage the the risks of the CRISPR system when it is used as a therapeutic. Jain et al. [77] fused AcrIIA4 with dihydrofolate reductase (DHRF) to inhibit the binding ability of Cas9 and DNA only in the presence of trimethoprim (TMP), which enabled chemical regulation of Cas9 editing and dCas9 genome perturbation. The chemical regulation of Cas9 editing and dCas9 genome perturbation has enhanced the specificity and biosafety of the CRISPR/Cas9 system. Hu et al. [78] revealed the molecular mechanism of chromosomal translocations caused by the CRISPR/Cas9 system during gene editing, and then optimized human TREX2 protein was coupled with Cas9 to generate Cas9TX, which can inhibit chromosomal translocations, large segment deletions and other chromosomal structural abnormalities in the process of gene editing, thereby greatly enhancing the safety of gene editing using CRISPR/Cas9. The safety of CRISPR/Cas9 gene editing is greatly improved. Gene editing technology is essential for studying genetic functions and controlling genes, and has greatly promoted the development of related disease treatment fields. However, the difficulties in tissue targeting and off-target effects continue to constrain clinical application of this technology to a certain extent, so enhancing the efficiency and specificity of drug delivery is the main research direction in the future.

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