RESEARCH ARTICLE

Advances in Gene Therapy for Human Genetic Diseases Based on Crispr/Cas9 Gene Editing Technology

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Abstract: The CRISPR/Cas9 system provides a powerful technique for targeted gene editing. Using sequence-specific sgRNA guidance, the CRISPR/Cas9 system can accurately introduce double-stranded incisions into the exact location of the target DNA. At present, a large number of CRISSR/Cas9 gene editing studies involving multiple species in vivo and in vitro have fully demonstrated the tremendous potential of this technology, which brings hope for disease treatment research and clinical application based on this technology. This review will summarize recent advances in the use of CRISPR/Cas9 gene editing techniques in the treatment of human genetic diseases. Progress in pre clinical research.

Keywords: CRISPR/Cas9; gene editing; genetic diseases; gene therapy

In the past decade, research on genetic diseases has focused on the focus of genetic differences, and great progress has been made[1].The emergence and development of second-generation gene sequencing technology has greatly promoted genome-level research, and a large number of genetic defects associated with major human diseases have been successfully identified. In the past[1–3], various defects of genome sequence, such as small-scale mutation, gene deletion and large-scale chromosome rearrangement, have been deeply explored and studied[2,4,5].

The type II CRISPR/Cas system encodes a marker cas9 protein with nuclease activity and is composed of relatively simple nucleoprotein complexes in three types of CRISPR/Cas systems (I~III). Therefore, much attention has been paid to its mechanism and application[6,7]. Identification and direct splicing of target genes[8–10]. Compared with ZFN and TALEN, CRISPR/Cas9 has the advantages of simpler, easier editing, higher efficiency, lower cost and the potential of multi-target simultaneous gene editing[9,11]. Gene editing studies, including Drosophila melanogaster[12], Caenorhabditis ele-gans[13,14], Danio rerio[15–17], Mus mus-culus[8,18,19], Rattus norvegicus[20] and Homo sapiens[8,11,21–23] cells, have shown interesting genome editing studies. Exciting potential.

CRISPR/Cas9 system can repair the gap by two main ways after introducing double-stranded cleavage into a specific site of the target gene. Among them, non-homologous end-joining (NHEJ) is the most active repair mechanism, which has great randomness and often leads to double-stranded gap. On the other hand, homology directed repair (HDR) provides specific reference by using exogenous repair templates, which can accurately repair the target region in accordance with the established target[11], thus providing the possibility of inserting and replacing a single base or large fragment. Perhaps. More importantly, compared with most other existing gene therapy strategies, the effect of CRISPR/Cas9 on genome modification is heritable. By removing genetic defects permanently, the disease-causing genes can be completely repaired and the treatment effect can be sustained and stable[22]. Guided gene editing technology has been gradually applied in the treatment of human genetic diseases.

This review will summarize the recent advances in CRISPR/Cas9 gene editing technology-mediated gene therapy

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for human genetic diseases, focusing on the related treatment strategies, the design of drug delivery methods, and preliminary analysis of relevant clinical treatment prospects.

1. Amendment strategy

Many related functions of CRISPR/Cas9 gene editing technology have shown tremendous potential in gene therapy research. By introducing double strand gap into target DNA, CRISPR/Cas9 gene editing system can produce different types of gene insertion, splicing or knockout by mediating NHRJ or HDR action, thus achieving the goal genome sequence. The directional or nondirectional alterations of columns create an opportunity for gene therapy research using CRISPR/Cas9 technology to repair specific gene defects.

1.1 Point mutation repair

Exon sequencing and genome-wide sequencing have greatly facilitated the screening and identification of novel single gene mutations in rare genetic diseases[1,24,25]. The production of cut-or-stop signals results in the reduction or dysfunction of the corresponding mRNA or protein products. Therefore, the introduction of double stranded DNA incisions near the point mutation site by CRISPR/Cas9 system and the subsequent homologous or non-homologous repair can be used to correct and treat the mutation-induced familial genetic diseases in varying degrees. In 2014, Yin *et al*.[26] reported the use of CRISPR / Cas9-mediated HDR to repair Fah gene point mutation in the treatment of hereditary hypertyrosinaemia (HTI). Type I hereditary hypertyrosinaemia is a fatal genetic disease caused by Fah point mutation, which encodes a delayed gene. The last participant in the tyrosine metabolism pathway in vivo[26-28]. The mutation generally originates from the G_A point mutation at exon 8 of the Fah gene in HTI patients, which results in the production of unstable truncated FAH protein products by directly skipping exon 8 during gene transcription. In response to this mutation, Yin *et al*. designed a number of gRNA targeting exon 8 of Fah gene and co-expressed it with Cas9 nuclease in the same plasmid. The CRISPR/Cas9 edited plasmid mentioned above and the 199nt single containing wild-type sequence. Single strand oligonucleotides (ssODN) repair templates were injected into Fah5981SB mice (carrying the same point mutation as human HTI). To evaluate the effect of gene editing in vivo, a series of methods including gene sequencing, Fah + cell immunohistochemical analysis, mRNA quantitative PCR and weight monitoring were selected. The weight of the mice was maintained successfully after a single treatment, indicating that the treatment effectively protected the liver. At the same time, as direct evidence of successful repair, the authors successfully obtained a PCR band containing the full-length sequence of exon 8 from the CRISPR/Cas9 treated mice, and confirmed the A_G base by sequencing. This innovative study demonstrates the ability of CRISPR/Cas9 to successfully repair point mutations in vivo and provides strong evidence for CRISPR/Cas9 to treat gene-deficient diseases.

For example, adeno-associated virus vector (AAV), Yang Yang *et al*.[29] successfully repaired mouse OTC gene in vivo by intravenous injection of CRISPR/Cas9 system and DNA template respectively into newborn mice. Bassuk *et al*. will edit induced pluripotent stem cells (iPSCs) from patients with X-linked retinitis pigmentosa. The RPPR gene carried by these patients has C. 3070G T point mutation. In addition, HDR-mediated point mutation repair strategies also included beta-thalassemia[9,31], chronic granuloma[32], Duchenne muscular dystrophy (DMD)[33], sickle anemia[34] and severe combined immunological deficiency (SCID)[35]. The disease has been applied.

It is generally believed that although the efficiency of gene sequence repair is positively correlated with the efficiency of HDR, the latter is generally less likely to occur in cells (less than 10%). Therefore, it is important to design and select suitable gRNA and repair templates. In the case of Fah point mutation repair, Yin *et al*.[26] designed three gRNA targeting different locations of exon 8. The binding regions of these sgRNA directly covered the point mutation sites, thus facilitating the double-stranded incision to be more effective. Close proximity increases the accuracy of repairing with HDR and templates. Long *et al*.[33] also selected gRNA covering point mutation sites in DMD gene editing therapy studies. However, for CRISPR/Cas9 systems, the selection of gRNA is strictly constrained by PAM sequences. Therefore, in some research cases, the preferred target sites were selected. In this case, the CRISPR/Cas9 system has been shown to be equally efficient in point mutation repair. For example, Yang Yang *et al*.[29] designed three sgRNA in the range of 20-40 bp upstream and downstream of the mutation site in the OTC gene repair study. As mentioned

above, even though the CRISPR/Cas9 system has the ability to produce high shear efficiency, the incidence of intracellular HDR is usually very low. Therefore, some double-stranded incisions produced by CRISPR/Cas9 will inevitably pass through the NHEJ machine. Based on the above considerations, Yang Yang *et al*. can effectively prevent further random mutations in the coding sequence that may occur when NHEJ prevails, leading to the remainder by designing the target of sgRNA in the intron region adjacent to the repair site. On the other hand, Chang *et al*.[35] designed six gRNAs near the point mutation site of exon 14 of JAK3 gene in the gene editing study for the treatment of SCID. Four of them had a repair efficiency of 73.3%, while the other two were almost ineffective. It is suggested that the efficiency of repair may be related to the distance between the selected gRNA and the mutation site.

In addition, in HDR-mediated point mutation repair, template design strategies, such as the length of homologous arms at both ends and the type of template, are also closely related to the efficiency of RISPR/Cas9-mediated point mutation repair. For example, Yin *et al*.[26] used 199 NT long ssODN containing wild-type sequences and homologous arms; Bassuk *et al*.[30] used 162 NT long ssODN as a template for HDR-mediated RPGR gene repair; Yoshimi *et al*.[2] used shorter 88 NT ssODN to repair point mutations in the TyrC gene. In addition, dsODNs and DNA plasmids are also used as repair templates to participate in the HDR process. For example, Huang *et al*.[34] used plasmid templates to provide wild-type HBB gene sequences to treat SCID, Song *et al*.[9] constructed a 5 KB left arm long and 3 KB right arm long model. Plasmid templates repair HBB genes. Normally, donor plasmids need to be linearized by restriction endonucleases before being introduced into cells to perform template function. Meanwhile, because it is more difficult to insert large fragments into the target region than small fragments, and the repair strategies of each gene are different, the selection of repair templates for HDR still depends on the specific situation.

1.2 Gene deletion repair

In addition to point mutation, another important cause of genetic disease is the deletion of bases in different segments of the genome, ranging from a single base to the entire exon level. Similar to point mutation repair, CRISPR/Cas9 gene editing techniques have also been used in the treatment of this type of genetic disease by utilizing the HDR effect of the cell and combining with appropriate repair templates.

One typical example is the treatment of DMD, a serious muscular degenerative disease caused by mutations in the Dmd gene on the X chromosome[39,40]. Disabled dystrophin[41]. Although methods including AAV transporter[42], lentivirus transporter[41] and Sleeping Beauty transposon[43] have been used in the study of gene therapy for DMD, the large sequence length of Dmd gene hinders the therapeutic effect to a great extent. Effective delivery of genes. Therapeutic efforts to restore the full-length atrophic protein coding sequence remain challenging[44]. This finding also provides a potential strategy for the treatment of DMD[45]

Therefore, the use of genetic editing techniques with heritable effects to repair the genetic defects involved in DMD has been widely considered an ideal treatment and has been tried by several research teams. For example, Li *et al*. knocked out the full-length sequence encoding exon 44 of the Dmd gene using the HDR function mediated by the CRISPR/Cas9 system. In order to replace the original sequence in the genome and restore the protein coding region in the patient-derived iPSCs cells, a double-stranded incision was first introduced into exon 45, and then repaired by a vector consisting of two homologous arms and a complete sequence fragment of exon 44. After analyzing the skeletal muscle cells differentiated from the edited iPSC, the authors detected the mRNA of myotrophic protein with intact and non-mutated exon 44 sequence, which confirmed that the inserted exon 44 and the subsequent exon 45 were expressed in normal sequence, demonstrating the potential of this strategy in the treatment of DMD.

Gene knock-in repair based on CRISPR/Cas9 has also been used in the treatment of other genetic defects. Schwank *et al*. used CRISPR/Cas9-mediated HDR to repair the CFTR gene in intestinal stem cells derived from patients with cystic fibrosis. Mutations in the CFTR gene lead to phenylpropanoid in exon 11. In order to repair the above 3 bp deletion of CFTR gene, different sgRNA targeting exon 11 or intron 11 were introduced into the template encoding the wild-type CFTR sequence. Similarly, the use of CRISPR/Cas9 to repair a 4 bp deletion in the HBB gene that causes beta-thalassemia[31,37], and Wu *et al*.[23] to repair the Craigc gene that causes cataract, were reported by two independent research groups. The deletion of 1 BP in exon third.

In the above study, the target gene was normally expressed through homologous repair mediated by gene editing, resulting in complete functional recovery. Regardless of the size of the gene fragment to be knocked in, a single sgRNA was sufficient to introduce the double-stranded notch and insertion deletion (indel) into the desired site to induce subsequent HDR in the CRISPR/Cas9 system. On the other hand, as mentioned earlier, for HDR templates, plasmid DNA and ssODN have their advantages and disadvantages[2]. It is noteworthy that genes of different lengths are introduced from the point of view of gene delivery. In knock-in studies, ssODN is more often used in research methods involving direct microinjection of fertilized eggs or embryos, but less involved in transduction methods. Several studies have reported that ssODN-like donor templates are used in HDR-mediated gene knock-outs in mouse gene editing studies using CRISPR/Cas9 system. However, there is still a lack of systematic comparative studies to analyze the two templates in CRISPR/Cas9 system, especially the genes with different fragment lengths. Compared with point mutation, it is more difficult and complex to integrate DNA fragments with several, dozens or even hundreds of bases into target sites by HDR, so template selection is needed according to actual conditions. The mechanism of action of A in repairing double-stranded incision is probably different[48,49]. Therefore, more systematic studies on ssODN and dsDNA are needed to analyze the advantages and disadvantages of the two, and to make more authoritative and objective judgments.

1.3 Exon excision

The CRISPR/Cas9 system can also perform large fragment excision of the target gene by introducing multiple gRNA into multiple double-stranded incisions at the same time. In special cases, exon excision using CRISPR/Cas9 technology can also provide a unique therapeutic idea for gene therapy of related diseases.

DMD provides a good example of this strategy in the treatment of hereditary deficiency disorders. As mentioned above, DMD is a serious X-linked disease leading to progressive muscular dystrophy. In addition to the strategy of knocking in deleted Dmd fragments, DMD is also one of the most important examples of functional repair in single-gene genetic diseases by cutting out unimportant regions within the gene[45,50]. For example, the deletion of gene sequences in the common regions of Dmd mutations in exons 45-55 Loss leads to truncated, but partially functional, myotrophic proteins[40]. Patients with this type of mutation are usually asymptomatic or have mild symptoms like Baker's Muscle Dystrophy, which is much less severe than DMD[40]. This finding prompts researchers to develop exon-based jumps. This strategy intentionally "skips" the transcription of a specific exon by regulating the mRNA splicing behavior, restoring the readable frames to normal order at the mRNA level, thereby transforming DMD symptoms into lighter Becker-like phenotypes[50]. Based on this concept, Ousterout *et al*. attempted to use CRISPR/Cas9 directly. Gene editing technology, which treats DMD by direct excision of related exons, has designed two sgRNA for identifying the lateral intron region of exon 45-55 to edit skeletal myoblasts from DMD patients. The system has successfully produced a large segment of genome deletion in the corresponding region and cut it off. The sequence length was 336 kb, covering all the common mutation hotspots in exons 45-55, effectively resolving more than 60% of DMD gene mutations[45,51,52]. Although the treatment strategy resulted in deletion of exon 45-55 of myotrophic protein, it effectively restored the expression of myotrophic protein in cells, and still remained. By enriching the edited cells and implanting them into immunodeficient mice, the expression of human amyotrophic protein can be successfully observed in vivo, suggesting obvious therapeutic results. Similarly, Xu *et al*.[53] also successfully used a pair of gRNA which recognized the introns 20 and 23 of the mouse Dmd gene, respectively, to achieve in vivo expression of human amyotrophic protein. Total excision of exons 21 (181_bp), 22 (146_bp) and 23 (213_bp) was performed in the model mice. In addition, the expression of atrophic protein in the sarcolemma of skeletal muscle was successfully restored by excision of the 23_kb fragment of the X chromosome in the DMD model mice.

These studies have successfully demonstrated a new approach to DMD treatment by removing the entire exon region of the genome on a large scale. Compared with the strategy of producing indel via NHEJ or HDR to repair gene defects, the main advantage of this approach is that the protein products edited by the target gene are Predictable, and confirmed in the transition-inducing study of Baker's muscular dystrophy. Compared with indel-mediated repair, this strategy effectively avoids the creation of new and unpredictable phenotypes in the target area during each edit-repair process. In addition, this method does not require any form of donor template. However, on the other hand, the intro-

duction of multiple double-stranded incisions inevitably increases the strict requirements for the accuracy of the gene editing system to reduce the probability of accidental chromosome rearrangement.

1.4 Repair chromosome rearrangement

Chromosome inversion and rearrangement are also important causes of genetic diseases in addition to point mutation, gene deletion and exon dysfunction. The CRISPR/Cas9 gene editing system has also been used to correct and repair this type of genetic defect. The most representative example is the treatment of hemophilia type A. Hemophilia type A is In most severe cases of hemophilia A, nearly one-second of the mutations are due to two large fragments (140 and 600 kb) in introns 1 and 22 of the F8 gene. Chromosome fragment rearrangement occurred[54,55]. The rearrangement originated from the accidental mistake of nonallelic recombination in the repair of DNA double-stranded gap. To overcome this genetic defect, Park *et al*. used a CISPR/Cas9 system containing two sets of gRNA to identify the two large rearrangement regions in iPSC from hemophilia A patients. The results showed that the endothelial cells differentiated from the edited iPSC cells successfully expressed the F8 gene in vitro. At the same time, the F8 gene defect in the hemophilia mouse model was effectively repaired, which demonstrated the theoretical feasibility of gene edited cell therapy.

CRISPR/Cas9-mediated chromosome rearrangement was first used in the establishment of animal models of human diseases before it was applied to gene therapy research. Real reproduction of chromosome fusion or rearrangement in mice, which led to tumorigenesis and development, will provide a strong support for the effectiveness and sensitivity of clinical drugs[56–58]. Recently, two independent research groups led by Maddalo and Blasco[57,59] successfully established lung cancer models driven by the rearrangement of the Eml4-Alk gene in mice by CRISPR/Cas9 gene editing. Choi and Meyerson[60] also successfully reproduced in mice including CD74-ROS1, EML4 using CRISPR/Cas9 technology. Chromosome rearrangements, including - ALK and KIF5B - RET, drive the development of lung cancer. These studies provide a more flexible and convenient strategy for establishing accurate disease gene models and methods for studying gene defects. Editorial techniques can effectively repair chromosomal inversion or large segment rearrangement in patient-derived iPSC cells. The results provide potential tools and new hope for the treatment of other genetic diseases, such as Hunt's syndrome[61] and cancer[62].

2. CRISPR/Cas9 system import strategy

In addition to therapeutic strategies, successful and efficient CRISPR/Cas9 system delivery pathways also play a decisive role in the treatment of related diseases. Up to now, several studies have successfully demonstrated the use of different means of transmission for various forms of CRISPR/Cas9 gene editing systems, with varying degrees of success. Therapeutic effects. In these studies, the application of three strategies, i.e. editing and induction of iPSC cells, systemic transmission in vivo and microinjection of fertilized eggs, has received considerable attention.

2.1 Gene editing based on iPSC cells

The emergence and rapid development of iPSC technology have brought new hope for individualized cell therapy[63–67].As a unique and differentiatable source of multifunctional cells, iPSC can repair damaged or diseased tissues through its potentially powerful differentiation ability, and thus achieve the treatment of a wide range of diseases[68,69]. Therefore, the combination of iPSC and CRISP/Cas9 technology also brings new ideas and hopes for the treatment of genetic diseases[70]. Editing and preparing individual-specific iPSC cells as individualized regenerative medicine. Long-term goals will provide a unique platform for physiological function research, drug screening, therapeutic evaluation, and gene repair-based cell replacement therapy[71,72].

Recent studies have shown that gene-edited patient-specific iPSC provides innovative strategies for the treatment of genetic diseases such as beta-thalassemia, SCID, retinitis pigmentosa and hemophilia A. For example, Xie *et al*.[31] used CRISPR/Cas9 technology to repair the process of HBB gene in iPSC cells derived from beta-thalassemia patients. By screening the edited iPSC and further differentiating it into red blood cells, the obtained cells showed normal expression of HBB gene. Meanwhile, iPSC cells from the patient's own somatic cells provided abundant cell sources for

gene editing and repair, and the obtained iPSC could be further differentiated into self-usable cells. Hematopoietic stem cells and progenitor cells (HSPC) transplanted in vivo provide a good basis for subsequent in vivo studies. This method will effectively avoid such safety problems as immune rejection caused by allogeneic transplantation or gene integration introduced by viral vectors[31].

Similarly, Li *et al*.[44] made three different gene editing attempts in iPSC from DMD patients, and eventually succeeded in obtaining skeletal muscle cells that expressed full-length atrophic protein. Similar gene therapy strategies have also been successfully applied to iPSC isolated from patients with hemophilia type A[55] and retinitis pigmentosa[30]. The above research based on RISPR/Cas9 provides a basis for more advanced cell and gene therapy using patient-derived iPSC cells. This therapy can not only save the use of immunosuppressive drugs, but also achieve the precise repair of defective genes and functions through the combination of multiple gene repair strategies mediated by CRISSPR/Cas9 technology, and achieve the "superimposed" treatment effect[71].

2.2 Systemic transmission in vivo

In order to achieve in vivo gene editing based on CRISSPR/Cas9 technology, techniques including intravenous injection[73], intrapulmonary injection[59], retrophthalmic intravenous injection[74] and prefrontal cortical injection[75] have been used for in vivo gene editing component delivery. These results further promote the systematic transmission of CRISPR/Cas9 system in vivo to treat human genetic diseases.

As mentioned above, hereditary hypertyrosinemia type I is a genetic disease caused by the deficiency of FAH protein. This disease leads to the accumulation of toxic metabolites in hepatocytes, resulting in severe liver injury[28]. Gene repair, however, is an unavoidable safety issue with regard to genome integration involved in its transporter AAV[28]. This suggests that Yin *et al*.[26] transfected Fah gene-targeted plasmids of the RISPR/Cas9 system and corresponding ssODN repair templates into tyrosinemia mice (Fah mut/mut) by tail vein injection with high pressure for gene repair in the liver. Analysis of serum markers including aspartate aminotransferase and alanine aminotransferase showed that CRISPR/Cas9 system successfully repaired Fah mutation in the liver and achieved effective treatment for liver injury caused by deficiency of FAH protein. In this study, delivery of CRISPR/Cas9 system in vivo was injected via caudal vein hypertension. In recent years, the mechanism of transporting therapeutic genes to liver tissue by tail vein hypertensive injection has been deeply studied and has been applied to include HBV.[78] and hemophilia[79], among others, plasmid DNA-based gene therapy research. In Yin *et al*., the tail vein high-pressure injection method promoted the effective delivery of CRISPR/Cas9 plasmid complex to liver tissue in mice, achieving 0.40%+0.12% in vivo gene repair rate, showing a certain application potential. The feasibility of tail-vein hypertensive injection with systemic blood circulation in humans is low, because excessive loading on human blood circulation is liable to damage cardiac function and lead to transient heart failure[77]. In another in vivo treatment study, Yang Yang *et al*.[29] passed the CRISPR / Cas9 system, which targets OTC genes, to correct similar mutations for therapeutic purposes. In this study, they integrated the Staphylococcus aureus-derived RISPR/Cas9 system (SaCas9) into two AAV8 vectors, one for SaCas9 expression and the other for gRNA and repair templates. In this study, the authors used adeno-associated virus type 8 (AAV8) as a vector to test the therapeutic effect of gene repair in newborn mice. Adeno-associated viruses of different serotypes can be used as gene delivery vectors for different human tissues to provide long-term and powerful gene delivery capacity[80–82].At present, some gene therapy products based on AV have been approved for clinical use[83,84]. After irradiation, the AAV8 virus vector was reported to infect 90%~95% of the cells in the liver of mice[84].By using a highly liver-affinity AAV8 vector, Yang Yang Yang and his colleagues achieved a breakthrough in gene therapy for liver metabolic diseases using CRISPR/Cas9 technology. In addition to the above studies, AAV vectors have been successfully used in many CRISPR/Cas9-mediated in vivo gene editing studies, showing impressive results and potential[73,85,86]. For example, several preclinical and clinical studies have shown that the gene transfer efficiency of AAV8 vector in mouse liver is 20 times higher than that in human body, and it is inferred that the application in human body may not achieve the same ideal effect as animal model[87–90]. Even so, these studies have successfully provided convincing theories and insights into intravenous CRISPR/Cas9 system-mediated therapy for lethal metabolic diseases in humans. Practice basis.

2.3 Microinjection of fertilized eggs

Microinjection of fertilized eggs has been widely used in the modeling of various animal models. At present, most transgenic animals are realized by introducing the target gene into the fertilized eggs. Relevant technologies and supporting disciplines have also been greatly developed[91]. To edit the genome of all cells in an individual organism, including germ cells[22]. In theory, this method can achieve permanent changes to all somatic and germ cells, thus successfully passing on the edited genotype to the offspring. SPR/Cas9 technology in animal modeling and in vivo gene therapy.

For example, Wu *et al*.[23] introduced the CRISPR/Cas9 system into mouse fertilized eggs by microinjection in the form of a complex of mRNA and gRNA in order to repair the mutation in the Crygc gene. In the treatment of DMD, Long *et al*.[33] injected the mRNA, gRNA and ssODN homologous repair templates that comprise the CRISPR/Cas9 system into mice. In fertilized eggs, Dmd gene mutations were corrected on the chromosomes of mdx-deficient mice. Of the 11 offspring obtained, 7 mice were detected to have HDR-mediated gene repair on exon 23 of Dmd, and 4 mice had NHEJ-mediated stop codon removal in the readable frame. The results showed that CRISPR/Ca S9-mediated gene editing of fertilized eggs produces offspring of different genotypes with a Dmd repair rate ranging from 2% to 100%. The above studies show that the CRISPR/Cas9 system for gene editing of fertilized eggs is safe and effective in gene therapy of related diseases.

Therapeutic bases for some inherited genetic diseases characterized by systemic manifestations (such as cystic fibrosis or hereditary mitochondrial diseases), monogenic disorders that affect a wide range of inherited genetic diseases (such as muscular dystrophy) or inherited genetic diseases (such as the basal ganglia involved in Huntington's disease) that are difficult to reach by treatment Gene editors for fertilized eggs or embryos are likely to offer better options for the treatment of these diseases.

However, even if ethical considerations are put aside, several key issues still limit the implementation of fertilization-based treatment in humans. Gene editing of eggs. For example, studies have shown that genotypic heterogeneity may still occur between the genomes of the offspring of cells that have been microinjected and genomically edited for further mitosis[33,92], which makes the phenotype of offspring produced by gene editing reasonable. At the same time, although the CRISPR/Cas9 system can be successfully imported and edited by microinjection, its editing efficiency is not fully controlled, resulting in the generation of chimeras, i.e., some somatic and germ cells may not have the edited genome[92]. Some scholars reported that direct injection of CRISPR/Cas9 into fertilized eggs may reduce the probability of healthy offspring[23]. More importantly, genome editing in human germ cells or fertilized eggs is currently widely prohibited[33,92]. The impact of genome editing on society is discussed from a variety of perspectives[33]. However, existing studies on gene editing based on mouse fertilized eggs still show great potential for CRISPR/Cas9-mediated gene therapy if viewed from a technical perspective alone.

3. Prospects

CRISPR/Cas9 technology provides a powerful tool for editing specific genes and has shown tremendous potential in the treatment of genetic diseases. Whether it is through systemic in vivo administration (such as using disease animal models) or in vitro delivery (such as in vitro iPSC cell editing), CRISPR/Cas9 technology is used to correct genetic defects. Significant progress has been made both in vivo and in vitro, bringing more hope to the clinical application of gene editing in gene therapy of genetic diseases. These challenges include, but are not limited to, the development of better gene delivery systems for in vivo delivery of CRISPR/Cas9 systems, the further exploration of the potential applications of AAV and novel non-viral gene vectors, and the improvement of HDR-mediated gene repair in the design of CRISPR/Cas9 systems. In addition, preclinical studies based on gene editing should pay special attention to the miss-target problem of the CRISPR/Cas9 system. Despite these challenges, CRISPR/Cas9 gene editing The rapid development of technology will ultimately promote the progress of gene therapy for human genetic diseases.

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