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CRISPR/Cas9-mediated gene editing: prospects and ethical concerns—an overview

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Abstract

The clustered regularly interspaced short palindromic repeats (CRISPR)-associated system (Cas), a simple and efficient tool for genome editing, have been used to modify genes in model systems including animal zygotes and human cells. CRISPR/Cas9 technology exploits the principles of bacterial immune function to target and remove specific sequences of mutated DNA. This review article discusses recent advances in CRISPR/Cas9 technology, current research, applications, ethical and biosafety concerns that it raises and prospects for gene therapy for genetic disorders in near future.

Keywords: CRISPR/Cas9; gene editing; gene therapy; ethics

Introduction

The CRISPR/Cas9 RNA-endonuclease complex, composed of the Cas9 protein and the guide RNA (gRNA) (~99 nt), is derived from the adaptive immune system of *Streptococcus pyogenes* SF370. It targets genomic sequences containing the tri-nucleotide protospacer adjacent motif (PAM) and complementary to the gRNA, and can be programmed to recognize virtually any genes through the manipulation of gRNA sequences[1,2]. Following Cas9 binding and subsequence target site cleavage, the double strand breaks (DSBs) generated are repaired by either non-homologous end joining (NHEJ) or homologous recombination directed repair (HDR), resulting in indels or precise repair respectively[2]. The ease and efficiency of the CRISPR/Cas9 system have rendered itself to many applications, including genome editing, gene function research, and gene therapy in animals and human cells[1,3,4].

The specificity of CRISPR/Cas9 is mostly guided by PAM and the 17–20 nt sequence at the 5′ end of gRNAs[4,5]. Unintended mutation in the genome can totally obstruct the application of CRISPR/Cas9, particularly in studies of development and gene therapy[6,7]. Three current studies discovered that off-target effects of CRISPR/Cas9 appeared rare in human pluripotent stem cells pointing out the risk that high frequencies of unintended targeting by CRISPR/Cas9 may be more common in cancer cell lines[8,9,10]. Lower rates of off-target effects (compared to human cell lines) have also been reported in mouse zygotes[11,12]. In spite of great strides in understanding the utilization of CRISPR/Cas9 in a variety of model organisms, much remains to be learned regarding the efficiency and specificity of CRISPR/Cas9-mediated gene editing in human cells, especially in embryos.

Background

Technologies for making and manipulating DNA have enabled progress in biology with the discovery of the DNA double helix. But introducing site-specific modifications in the genomes of cells and organisms remained obscure. Initial studies depended on the principle of site-specific recognition of DNA sequences by oligonucleotides, small molecules, or self-splicing introns. Later on, the site-directed zinc finger nucleases (ZFNs) and TAL effector nucleases (TALENs) using the

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principles of DNA protein recognition were developed. But, difficulties of protein design, synthesis and validation remained an obstacle to widespread adoption of these engineered nucleases[13].

Prokaryotes have CRISPR segments of DNA containing short repetitions of base sequences that allow bacterial cells to record DNA sequences of viruses that it has been exposed to. This enables cells to boost the immune function of progeny. The Cas9 enzyme then functions to seek out those bits of viral DNA and cut them out of sequence. The complex is programmable, allowing scientists to target and remove specific bits of DNA with great precision. The cutting-edge discovery of the CRISPR/Cas9 technology has motivated scientists forward in attempts to treat or cure diseases caused by single-gene mutations[14].

the target sequence (PAM) within the genome. This gRNA-DNA complex is specifically recognised by the cas9 protein, which induces a double-stranded break (DSB) in the DNA. Genome modification occurs, mainly through activating one of the two DNA repair mechanisms: non-homologous end joining (NHEJ) or homology directed repair (HDR). NHEJ introduces insertions or deletions within a sequence, whereas HDR requires delivery of a donor sequence through recombination with the targeting sequence can lead to point mutations or insertions.

Applications

CRISPR/Cas9 provides immense capacity to make remarkable progress in biotechnological, basic biological and medical research fields. Its application in genome studies will enable large-scale screen-

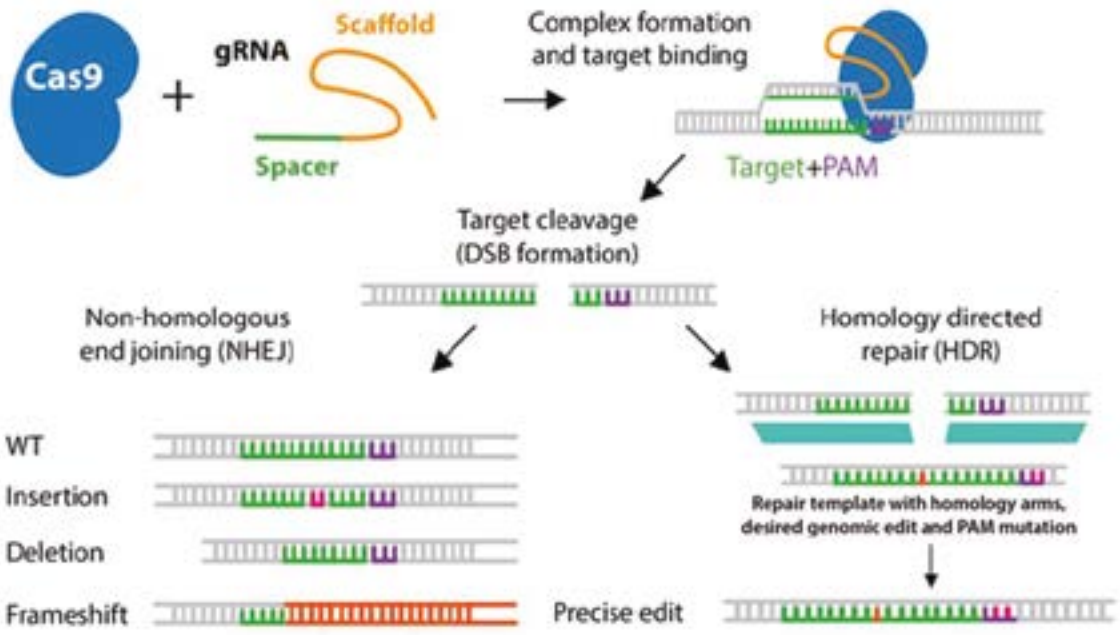

Fig. 1. Mechanism of CRISPR-Cas 9 gene editing (Source: www.addgene.org)

In genome editing by crispr/cas 9 mechanism, the genome of a target cell can be modified by expression of the double-stranded DNA endonuclease Cas9 and a guide RNA (gRNA). Expression of these components can be achieved by transfection with plasmids carrying genes for Cas9 and g RNA or by direct injection of Cas9 mRNA and gRNA. The gRNA (DNA-binding-domain) binds

ing for drug targets and other phenotypes and will aid the production of engineered animal models that will promote pharmacological studies and the understanding of human diseases. CRISPR-Cas9 applications in plants and fungi also guarantee to alter the pace and course of agricultural research. CRISPR/Cas9 finds its application in generation of genetically modified (GM) mouse model of

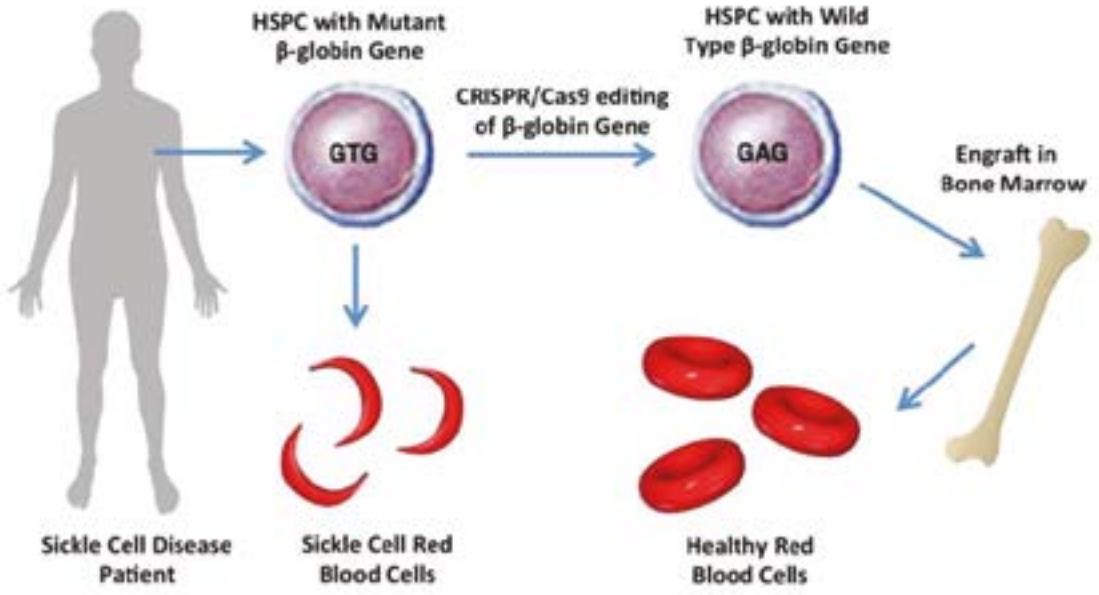

Fig.2 CRISPR/Cas9 technology in the study of sickle cell anaemia. (Source:cen.acs.org/articles/94/i41/CRISPR-edits-sickle-cell-mutation.html)

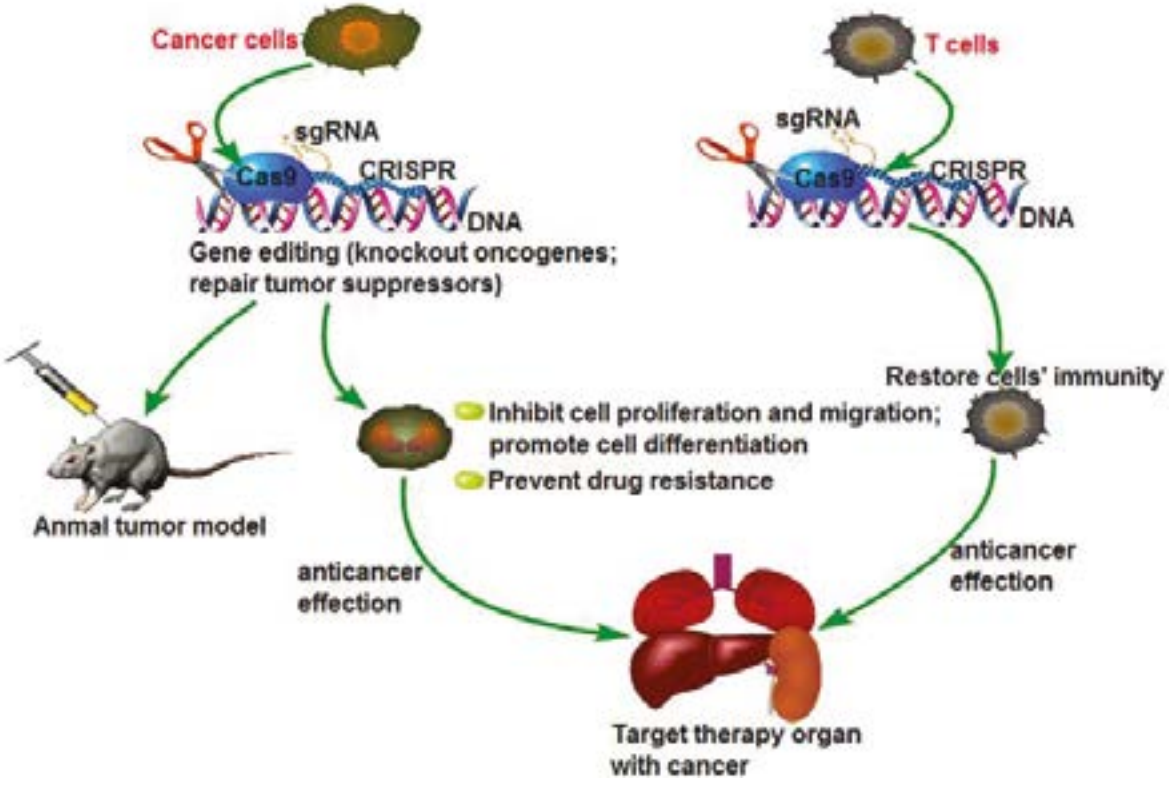

Fig.3. CRISPR/Cas9 technology in the treatment of Tumour. (Source: onlinelibrary.wiley.com/doi/pdf/10.1111/cge.13589)

human diseases, genome editing in specific tissues such as liver and brain, simultaneous generation of multiple gene mutations, flexible manipulation in epigenome for regulating the expression of specific genes and novel potentiality in RNA editing[15]. The therapeutic application of CRISPR-Cas

9 system has been reported to be successful in preclinical studies. This targeted gene therapy has shown therapeutic potential to tackle latent HIV infection, for treating cancer and for the prevention of cardiovascular diseases. Eye disorders like cataract, Leber congenital amaurosis and retinitis pigmentosa can be corrected by CRISPR-Cas 9 system. Haematological diseases like sickle cell anaemia, Haemophilia and thalassemia can be prevented by this gene therapy. Point mutations leading to SCID (Severe Combined immunodeficiency) can be corrected. This gene therapy system has the potential for treatment/ prevention of inborn errors of metabolism (eg: Ornithine transcarbamylase deficiency), muscular dystrophy, neurological disorders (cystic fibrosis) and skin diseases (dystrophic epidermolysis bullosa)[16].

Areas to be explored

Although the CRISPR/Cas9 technology has immense prospective in treating genetic disease, more research is needed before the science community begins human clinical trials. In a 2015 study by Liang et al. that was the first of its kind, CRISPR/Cas9 was used on tripronuclear (3PN) zygotes to better understand its effects in preimplantation embryos[17]. While targeting the β-globin gene (HBB), the CRISPR/ Cas9 complex also produced off-target effects. The efficacy of homologous recombination directed repair of HBB was low and produced mosaic embryos. In several of the 3PN zygotes, there were high rates of DNA repair using endogenous sequences instead of the therapeutic template. This is a major obstacle that must be overcome if the CRISPR/ Cas9 system is to be used therapeutically. Researchers must overcome a number of obstacles, such as the reaction of the human immune system, efficient modes of delivery, determining how to ensure that a corrected copy of DNA is inserted into the sequence, safeguarding against Cas9 proteins cutting at incorrect loci, and understanding and controlling off-target effects[18].

Ethical concerns

There are grave concerns regarding the ethical and safety implications of CRISPR/Cas9 research. Recently, a paper reporting gene editing in human embryos was published in the journal Protein & Cell, which raised concerns about the ethics of employing the CRISPR/Cas9 system[17]. Subsequently, both the editorial team of Nature and Science announced that although the CRISPR/ Cas9 system shows huge potential for genome editing, its use for modifying human germline cells should be considered very seriously, and progressive policy on this issue should be developed[19]. At the same time, Emilie Marcus, the editor-in-chief of Cell, stated that the journal would consider publication of manuscripts describing human germline modification, if they met high technical and ethical standards. The acceptance of this article should not be considered as endorsement or encouragement of modifying human germline cells, but should be viewed as a point to start the discussion about the human germline editing. Thus, embryo editing or engineering of human fetuses is becoming increasingly controversial among scientists. Some countries have already restricted CRISPR/Cas9 technology, by completely banning its use in humans. To address such a complicated debate, positive and negative aspects of germline editing should be weighed by an authoritative national agency, and both the scientific and social ethical concerns should be taken into consideration simultaneously[19]. If CRISPR/ Cas9 happens to be a common practice to edit genes for genetic improvement, the apprehension is that eventually those that choose not to or cannot afford the technology would be stigmatized. Once the precision of CRISPR/Cas9 comes close to that of other techniques approved for use on human embryos, it would become acceptable from a safety standpoint to move forward with human embryo studies.

Future prospects

The CRISPR/Cas9 technology is very popular because of its affordability and potential applications in curing genetic disease. In the last few

years, several animal studies have been published demonstrating its powerful gene editing capabilities. CRISPR/Cas9 mediated gene-editing was confirmed to be possible in adult animals, and was curative for the hereditary, single-gene mutation condition tyrosinemia[20]. Other studies have examined the effectiveness of CRISPR/Cas9 in treating single gene disorders such as Duchenne muscular dystrophy, as well as eye conditions like retinitis pigmentosa and Leber congenital amaurosis (LCA)[18,21]. A number of companies have invested in commercialising CRISPR technology. Corporate research companies such as Editas advocates that the first human clinical trials using CRISPR/Cas9 will aim to treat LCA. The eye presents an ideal testing location, as it is contained, immunologically isolated from the rest of the body, easily monitored externally, and can be measured using established standards of function. CRISPR/Cas9 technology has the potential for use in diseased individuals, as well as IVF embryos prior to implantation. In the case of diseased individuals, CRISPR/Cas9 can reduce the mutation load, which may subsequently decrease symptoms and the burden of disease. Application of CRISPR/Cas9 therapy with adult patients is less controversial than its use with embryos, as adults are able to provide informed consent. Furthermore, many patients with incurable diseases and compromised quality of life are eager to participate in clinical trials of new therapies, as there are no viable alternative treatments and it offers hope for a cure[14]. After solving the current challenges, the CRISPR-Cas9 system can be applied for clinical applications in patients. This system has been approved by an advisory committee at the US National Institute of Health as the first clinical trial to attack cancer cells[22].

Conclusion

There is a need to further improve the reliability and specificity of the CRISPR/Cas9 platform, a prerequisite for any clinical applications of CRSIPR/Cas9-mediated editing. The CRISPR/Cas9 genome editing system, with its accelerated development and expanded applications, is an indispensable tool for precise and efficient genome editing, but some related problems need more attention. First, the current knowledge of the CRISPR/Cas9 system at the biochemical and crystal structural levels is insufficient and requires additional research, including a deep analysis of the Cas9 protein, one of the main components in the CRISPR/ Cas9 system. The natural variation in Cas9 proteins isolated from different species might provide new Cas9 proteins with higher efficiency and thereby broaden the choices available for precise genome editing[23]. Moreover, specific modes for delivering Cas9, gRNA, and donor oligos to cells and tissues have been developed in numerous species, such as mice, Drosophila, zebrafish, worms, and humans. For example, the CRISPR/Cas9 system being used to create transgenic mice could be fused with other proteins or effectors to control or stimulate the expression or initiation of the CRISPR/Cas9 system in vivo. The off-target mutation rates of diverse CRISPR/Cas9systems, nevertheless, remain a challenge. Last, but certainly not least, the direct and precise genome editing raises ethical concerns, such as gene modification of human germline cells using the CRISPR/Cas9 system to create ‘designer babies’, which initiates arguments and queries among scientists and the public[17]. It is urgent that the government and related social organizations formulate and enact a series of laws and regulations to enable the safe and ethical application of the CRISPR/Cas9 system in basic research and clinics. There should be a clear distinction between genome editing in somatic cells and in germ cells. A voluntary moratorium in the scientific community could be an effective way to discourage human germline modification and raise public awareness of the difference between these two techniques. Legal concerns regarding the safety and ethical impacts of germline editing must not hinder the significant progress being made in the clinical development of approaches to potentially cure serious diseases[24]. A bright future is foreseen in which the CRISPR/Cas9 system will facilitate revolution and improvement of genome, RNA, and epigenome editing.

References

1. Cho S.W., Kim S., Kim J.M. and Kim J.S., 2013. *Nat Biotechnol*., 31, 230–232.
2. Jinek M., Chylinski K., Fonfara I., Hauer M., Doudna J.A. and Charpentier E., 2012. *Science*., 337, 816–821.
3. Jinek M., East A., Cheng A., Lin S., Ma E. and Doudna J., 2013. *Elife* 2:E00471.
4. Wu Y., Zhou H., Fan X., Zhang Y., Zhang M., Wang Y., Xie Z., Bai M., Yin Q. and Liang D., 2014. *Cell Res*., 25, 67–79.
5. Cong L., Ran F.A., Cox D., Lin S., Barretto R., Habib N., Hsu P.D., Wu X., Jiang W. and Marraffini L.A., 2013. *Science*., 339, 819–823.
6. Hsu P.D., Lander E.S. and Zhang F., 2014. *Cell*., 157, 1262–1278.
7. Sander J.D. and Joung J.K., 2014. *Nat Biotechnol*., 32, 347–355.
8. Smith C., Gore A., Yan W., Abalde-Atristain L., Li Z., He C., Wang Y., Brodsky R.A., Zhang K. and Cheng L., 2014. *Cell Stem Cell*., 15,12–13.
9. Suzuki K., Yu C., Qu J., Li M., Yao X., Yuan T., Goebl A., Tang S., Ren R. and Aizawa E., 2014. *Cell Stem Cell*., 15, 31–36.
10. Veres A., Gosis B.S., Ding Q., Collins R., Ragavendran A., Brand H., Erdin S., Talkowski M.E. and Musunuru K., 2014. *Cell Stem Cell*., 15, 27–30.
11. Wu Y., Liang D., Wang Y., Bai M., Tang W., Bao S., Yan Z., Li D. and Li J., 2013. *Cell Stem Cell*., 13, 659–662.
12. Yang H., Wang H., Shivalila C.S., Cheng A.W., Shi L. and Jaenisch R., 2013. *Cell*., 154, 1370–1379.
13. Doudna J.A. and Charpentier E., 2014. *Science;* 28, 346 (6213): 1258096
14. Fogleman S., Santana C., Bishop C., Miller A. and Capco D.G., 2016. *Am J Stem Cells*., 5(2), 39-52.
15. Mei Y., Wang Y., Huiqian Chen., Sun Z. and Xing-Da Ju., 2016. Recent Progress in CRISPR/Cas9 Technology. *J Genet and Genomics*., 43, 63-75.
16. Mollanoori H. and Teimourian S., 2018. *Biotechnol. Lett*. 40 (60), 907-914.
17. Liang P., Xu Y., Zhang X., Ding C., Huang R., Zhang Z., Lv J., Xie X., Chen Y., Li Y., Sun Y., Bai Y., Songyang Z., Ma W., Zhou C. and Huang J., 2015. *Protein Cell*., 6, 363-372.
18. Han A., 2016. *Look for CRISPR/Cas9 to treat eye diseases first, scientists say*. Genome Web.
19. Baltimore D., Berg P., Botchan M., Carroll D., Charo R.A., Church G., Corn J.E., Daley G.Q., Doudna J.A., Fenner M., Greely H.T., Jinek M., Martin G.S., Penhoet E., Puck J., Sternberg S.H., Weissman J.S. and Yamamoto K.R., 2015. *Science*., 348, 36-38.
20. Yin H., Xue W., Chen S., Bogorad R., Benedetti E., Grompe M., Koteliansky V., Sharp P.A., Jacks T. and Anderson D.G., 2014. *Nat Biotechnol*., 32, 551-553.
21. Regalado A., 2015. *CRISPR gene editing to be tested on people by 2017, Editas*. MIT Technology Review.
22. Reardon S., 2016. First CRISPR clinical trial gets green light from US panel. *Nat Methods*., 5, 374–375.
23. Ran F.A., Cong L., Yan W.X., Scott D.A., Gootenberg J.S., Kriz A.J., Zetsche B., Shalem O., Wu X., Makarova K.S., Koonin E.V., Sharp P.A. and Zhang F., 2015. *Nature*., 520, 186-191.
24. Lanphier E., Urnov F., Haecker S.E., Werner M. and Smolenski J., 2015. Don't edit the human germ line. *Nature.*, 519, 410-411.

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