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COMMENTARY

The CRIPSR/Cas gene-editing system—an immature but useful toolkit for experimental and clinical medicine

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Abstract

A Chinese scientist, Jiankui He, and his creation of the world's first genetically altered baby made headlines recently. As a newly developed gene-editing technique, the CRISPR/Cas system should not be applied to human beings for reproductive purposes until it has been extensively tested. However, numerous experimental research studies in human somatic, germline cells, and even in embryos, have been conducted, which have shown CRISPR/Cas to be a useful tool for human genome editing and a potential therapeutic method for future clinical use.

KEYWORDS
CRISPR-Cas, gene editing, gene therapy, human embryos, recombinant DNA

With the development of recombinant DNA technology, programmable nuclease-based genome-editing tools are gradually being created. Zinc finger nucleases (ZNFs)[1] and transcriptional activator-like effector nucleases (TALENs)[2] have been well studied and are already being applied in several gene therapy clinical trials for serious inherited genetic diseases, and the newly developed CRISPR/Cas9 system[3] is turning out to be a more promising tool. In nature, CRISPR/Cas systems provide bacteria with RNA-guided adaptive immunity against foreign genetic elements by creating doubled-stranded breaks (DSBs) with their directing nuclease activity.[4,5] Among them, the designated class 2 CRISPR/Cas9 systems with single guide RNA (sgRNA) are being applied to mammalian genome editing, including in human cells.[6] Besides gene editing, the derived epigenetic editing tools CRISPR interference (CRISPRi), CRISPR activation (CRISPRa), and catalytically deficient Cas9 (dCas9) have also been extensively used and modified.[7-9] Since the first application in mammalian cells in 2013, CRISPR/Cas9 systems have been researched in human somatic cells, embryonic or germline cells, and even in embryos prior to germ-layer formation. Despite its practical immaturity and drawbacks, the CRISPR/Cas system still holds great promise for disease treatment and prevention.[10-12]

1 | GENETIC SCREENING AND PROGRAMMABLE NUCLEIC ACID IMAGING

The CRISPR/Cas9-based gene-editing system has prompted functional genome-wide genetic screening, which enables the study of

gene functions and genetic interactions in complex heritable diseases. Up to now, a number of cell types have been studied.[13,14] Diseases such fragile X syndrome, type I diabetes, acute kidney injury, and murine muscular dystrophy have all been researched.[15,16] Furthermore, spatiotemporal localization of specific genomic loci can also be detected by using dCas9. Dysregulated molecular localization can result in or aggravate diseases.[17] Since this tool for rapid detection of specific loci (DNA) or transcripts (RNA) is partially adaptable for clinical use, related point-of-care diagnostics should be available in the near future. The CRISPR/Cas9 system is therefore a powerful screening tool for systematically elucidating gene function in health and disease states, which is crucial for understanding the pathogenic mechanism.

2 | GENE THERAPY WITH SOMATIC CELL GENE EDITING

The first clinical trial of conventional somatic gene therapy was allowed to proceed in the 1990s, but the ensuing issues of safety and efficacy, and several tragic medical accidents, largely delayed its translation to the clinic. Recently developed nuclease-based gene-editing technologies have already breathed new life into somatic gene therapy. CRISPR/Cas9-based gene editing, with its high efficiency, flexibility, and accuracy, can be applied to correct mutations or inactivate defective exons in animal models or human cells with pathologies such as Duchenne muscular dystrophy (DMD), amyotrophic lateral sclerosis and Huntington's disease.[18-21] Recently, researchers succeeded in eliminating an entire chromosome in different cell types, including human induced pluripotent stem (iPS) cells with trisomy 21.[22] Furthermore, on November 30, 2018, Editas Medicine announced FDA acceptance of their Investigative New Drug (IND) application for EDIT-101, a CRISPR genome-editing tool that reverses the IVS26 mutation using the CRISPR/Cas9 system to restore the function of photoreceptor cells.[23]

Cancer is characterized by multiple genetic alterations leading to malignant cell proliferation. Newly developed cancer immunotherapies, such as chimeric antigen receptor T-cells (CAR-T), have shown striking efficacy against multiple cancers in clinical trials. Currently, both CRISPR-Cas9 and TALENs gene-editing systems are being applied in T-cell engineering.[24] In 2015, oncologists at Sichuan University injected CRISPR-Cas9-modified T cells into patients with aggressive lung cancer at the West China Hospital, in the first CRISPR-Cas9 application in a clinical trial.[25]

3 | EPIGENETIC-EDITING AND RNA-TARGETING CRISPR SYSTEMS FOR CLINICAL USE

Epigenetic editing does not induce DSBs, so it turns out to be safer than direct gene editing. dCas9 is the most useful scaffold for incorporating multiple modulators to perturb transcription without permanent DNA alternation. Chromatin and histone modifications are the most direct way to regulate inherited gene expression.[26,27] Moreover, based on the dCas9 platform, targeted DNA methylation and demethylation, and deployment of long non-coding RNA (ncRNA) to ectopic genomic sites can be used in both the study of gene expression and regulation, and also potential therapeutic strategies.[28,29]

Posttranscriptional engineering mainly focuses on RNA. It can be used for eliminating pathogenic RNA molecules, adjusting aberrant mRNA expression and splicing. Nowadays, artificial RNA-targeting Cas9s have programmable RNA modulating activity independent of Protospacer Adjacent Motif (PAM)-presenting oligonucleotides (PAMmers).[30] CRISPR-Cas13, another class 2 type VI CRISPR-Cas system, specially targets RNA molecules and can be used for targeted gene knockdown in human cells.[31,32] Although the CRISPR-Cas13a system is currently only in its infancy, we still expect it to be applicable in anti-virus prophylaxis and other disease treatments.

4 | GENE EDITING IN HUMAN GERMLINE CELLS AND EMBRYOS, AND RELATED ETHICAL ISSUES

Apart from gene editing in somatic cells, the CRISPR/Cas9 system has been applied in human germline cells and embryos. Recent research has proved that this system is effective in HBB and G6PD point-mutation correction in human zygotes.[33] In 2017, Dr Shoukhrat Mitalipov and his team claimed success in correction of the heterozygous MYBPC3 mutation in human preimplantation embryos using a CRISPR/Cas9 system.[34] However, the relevant safety and efficacy issues of gene editing in human embryos are still being debated.[35-37]

Surprisingly, and unfortunately, Dr Jiankui He from the Southern University of Science and Technology of China recently announced that he has created the world's first gene-edited babies using CRISPR-Cas9 technology. This breaking news incurred world-wide criticism and controversy. In the current circumstances, and in agreement with the Chinese Academy of Medical Sciences response,[38] we are opposed to any clinical application of human embryo genome editing for reproductive purposes, which, in the absence of full scientific evaluation, is in violation of laws, regulations, and ethical norms.

5 | SAFETY

Safety is the prerequisite for every therapeutic technique. Cas9-mediated single-base editors provide the potential to correct point mutations of disease-related genes without inducing DSBs, and thus without small insertions or deletions.[39,40] Off-target effects of the Cas endonucleases are also an important concern of this newly developed gene-editing tool. Recent research has revealed that, with appropriately designed sgRNA, and using a highly sensitive strategy for identifying such effects, the CRISPR/Cas9 system can achieve in vivo editing without detectable genome-wide off-target

mutations.[41] These studies all indicate that the CRISPR/Cas9 system can be further improved and finally become clinically applicable.

In conclusion, research into the CRISPR/Cas9 system has shown that, although the system is still not mature enough for clinical application in humans, steady progress has been made. While we should undoubtedly condemn Jiankui He's irresponsible actions, at the same time we should not deny that the CRISPR/Cas gene-editing technique has a bright future.

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CONFLICT OF INTEREST

None.

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