CRISPR/Cas9 technology: a revolutionary molecular scissors for genome editing and genetic research

Abstract
Targeted gene modifications to animals’ especially mammalian genomes are central to understand the role of the gene in development, physiology, and different disease conditions. Functional elucidations of a gene or genetic variants require precise editing technologies. In conventional gene targeting methods, mutations of the gene or gene has been introduced into the genome by homologous recombination in embryonic stem (ES) cells and then injected into wild-type Blastocyst to generate chimerical animal models containing the targeted gene modification. Although it is widely used approach, however, it is laborious, time-consuming, and expensive, needing well-trained researchers. Moreover, in most of the mammalian species, established ES cell lines are not available to produce chimeric animals, which limits the traditional approach. The recent discovery of new genome-engineering tools, especially CRISPR/Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR-associated) overcome much of the difficulties. CRISPR is a microbial nuclease system that is involved in defense against genome invading phages and plasmids. It has been experimentally proven that the Cas9 end nuclease from Streptococcus pyrogenes type II CRISPR/Cas system can be programmed to produce sequence-specific double strand breaks (DSBs) in vitro by providing a synthetic guide RNA (gRNA-fusion of crRNA and tracrRNA). There are lots of researchers that have illustrated that the CRISPR/Cas9 system can be used to generate different gene deletions or replacement in mammalian as well as in model organisms. In this review, we will discuss the pros and cons of the system and briefly discuss the future direction.

Keywords: crispr/cas9 technology, molecular scissors, recombinant dna technology, insertional mutagenesis, genes mutations

Abbreviations: ES, embryonic stem; DSBs, double strand breaks; NHEJ, non-homologous end joining; gRNAs, guide rnas; TALEN, transcription activator-like effector nuclease; ZFN, zinc finger nucleases; tracrRNA, trans-activating crRNA; crRNAs, crispr rnas

Introduction
The understanding of the mammalian genome has increased significantly as recombinant DNA technology has grown ever faster and cheaper over the last decade. Modulation of the genome both in vitro and in vivo is much easier now. In vivo gene modification to create transgenic animals are valuable tools for studying development and disease. Transgenic animals represent a crucial model to study cell biology, genomics, proteomics, and many other applications in biotechnology and basic science. Transgenic animals are conventionally generated by insertional mutagenesis or by gene-targeting methods. However, these methods rely on the random integration of the transgene into the genome followed by drug selection. To identify cell populations expressing the transgene is a laborious screening process in the traditional method. In the recent decade, there have been a few methods developed which expedite the pace of genome editing technology. They are ZFN (Zinc Finger Nucleases), TALEN (Transcription Activator-Like Effector Nuclease) and the type II bacterial CRISPR/Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR–Associated) technologies. Among them, CRISPR/Cas9 technology is considered as the best efficient gene-editing technology as the efficiencies of ZFN and TALEN are low. Also, ZFNs and TALENs remain somewhat difficult and expensive to design, develop, and empirically test in the cellular context. Further, with CRISPR/Cas9 technology, it is easy to design and delivery of multiple gRNAs suggests the possibility of multiplexed genome editing in a short time.

CRISPR/Cas9 is an RNA-guided immune system that protect Archaea and Bacteria cells against genome invading viruses and plasmids. The puzzling CRISPR DNA segments were first observed in 1980, but their function was not well understood for almost two decades. In general, when an unknown virus attacks, bacteria incorporate viral DNA sequences into the repetitive sequences of their genome. Thus, when the bacteria again encounter that virus, bacteria use the DNA in these repetitive sequences to synthesize RNAs that recognize the matching viral sequences. Next, a protein attached to one of these RNAs cut up the viral DNA. Two forms of gRNAs are used to facilitate Cas9-mediated DNA cleavage, these are a Cas9 RNA (crRNAs) array and tran-activating crRNA (tracrRNA) (Figure 1). This crRNA in association with tracrRNA guide the Cas9 protein to a region called the ‘protospacer adjacent motif’ (PAM), a three-nucleotide sequence (NGG) juxtaposed to the DNA complementary region in the DNA (Figure 1). In the presence of an appropriate homologous donor, the CRISPR/Cas9 system may be used to generate defined modifications and insertions at a targeted locus through the homologous DNA repair process. In the absence of a donor, single DSBs generated by CRISPR/Cas9 are repaired through the error-prone non-homologous end joining (NHEJ), which is prone to insertion or deletion mutations.
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Advantage

1. Complex and time-consuming design.
2. Potentially dangerous off-target cuts.
3. Commercial sources were expensive.
4. Although many sequences could be targeted, some could not.
5. DSBs induced by ZFN can be repaired by error-prone NHEJ resulting in mutant mice and rats.
6. The specificity of some ZFNs changes depending on which other fingers they are next to.

Disadvantage

1. TALEN proteins difficult to design and produce.
2. Off-target cuts are a problem.
3. TALENs are large proteins that are cumbersome to work with and deliver into cells.
4. DSBs induced by nucleases can then be repaired by error-prone NHEJ resulting in animals.
5. Affected by cytosine methylation and chromatin structure

Table 1 Clinical and biochemical variables of individuals with overweight-obesity

| Present gene editing options | Details | Advantage | Disadvantage |
|-----------------------------|---------|-----------|--------------|
| Zinc Finger Nuclease (ZFN)  | A protein consisting of a DNA-cutting enzyme and a DNA-grabbing region that can be tailored to recognize different genes. | It was the first programmable genome-editing tool and faster and cheaper than the traditional methods. | 1. Complex and time-consuming design. |
| TALEN (Transcription Activator-Like Effector Nuclease) | Protein is containing a DNA-cutting enzyme and a DNA-grabbing region that can be programmed to recognize different genes. | This is easier to design than ZFN. It also is cheaper than ZFN. | 2. Potentially dangerous off-target cuts. |
| CRISPR/Cas9 | A DNA-cutting protein guided by an RNA molecule that can find the specific gene of interest. | This technique is affordable, easy to use, and it works for high throughput multi-gene experiments. CRISPR also give scientists a precise way to delete and edit specific bits of DNA-even by changing a single base pair. | 3. Commercial sources were expensive. |
|                   |         | It can make off-target cuts. However, improved engineered Cas9 protein reduces the chance off-target cuts. | 4. Although many sequences could be targeted, some could not. |

SD, standard deviation; BMI, body mass index; WC, waist circumference; AC, abdominal circumference; HC, hip circumference; RER, respiratory exchange ratio; HR, hear rate

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The genome editing technology is advancing at an exceptionally rapid pace but there remains some key issues regarding development of appropriate assays to evaluate off-target effects and establish safety. Thus, off-target errors are the greatest disadvantage of CRISPR/Cas9 system. Recently, different research groups optimized CRISPR/Cas9 component to drive down its error rate.21–30 Different genetically altered Cas9 enzyme were synthesized that reduced off-target errors at least tenfold compared with unaltered Cas9 enzymes.31 In this direction, various groups are working to reduce further off-targets cut by Cas9 enzyme.

Future direction and conclusion

Animal models are widely used to understand the complex process of development and disease. Reporter transgenic animals are critical tools to study specific cells or organs. As CRISPR-Cas9 is so quick, cheap and easy to use, the creation of more and more reporter transgenic animals will help us to understand the development and disease more precisely. One of the most important things of this technology is to make multiple genetic changes at once. In this context, the study of multiple genes at a single time in the same animal is possible. Further, this technology might be utilized for the complex human genetic disease such as autism, sickle-cell anemia, HIV, cystic fibrosis or schizophrenia. However, there remains a significant amount of research that needs to be done in order to utilize CRISPR/Cas9 for the benefits of the patients. We still do not know what the potential effects and consequences could be if we correct the mutated DNA in sickle-cell patient’s stem cells and put back into the patient; what will be the consequences. However, improvement of this technique could be deployed in the coming future to treat deadly genetic diseases. In this direction research works are going on and hopefully, we will get an answer soon.

Summary

In summary, CRISPR/Cas9 technology is an efficient and simple method of generating sophisticated genetic modifications in one step. We need to take maximum caution for therapeutic application of this technology to make sure that the enzyme does not cause unwanted genetic modifications. Recently, researchers from the United States of America, United Kingdom, and China were convening in Washington DC to discuss the different ethical issues of gene editing technologies.30

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Conflict of interest

The author declares no conflict of interest.

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