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Extended Abstract

CRISPR/Cas-Based Therapy Development for Rare Genetic Diseases: From Shakedown to Maiden Voyage

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Abstract

The long held curiosity about how to correct a variety of human diseases and defects, as well as the quest to address disturbing and obscure solution to misfortune chance of extra chromosomes in rare diseases such as Down syndrome has deem the light of scientist's search to the field of gene therapy. The discovery of the Clustered Regularly-Interspaced Short Palindromic Repeats (CRISPR), the mechanism of the CRISPR-based prokaryotic adaptive immune system (CRISPR-associated system, Cas), and its repurposing into a potent gene editing tool has revolutionized the field of molecular biology and generated excitement for new and improved gene therapies.

Additionally, these advances in genome editing with programmable nucleases have opened up new avenues for multiple applications, from basic research to clinical therapy. However, there are still many unknowns in the therapy development for rare diseases based on CRISPR/Cas technology. But with the field developing at a staggering pace, it is unclear how well current cutting-edge technology in terms of off-target effects, efficiencies of delivery, and of cell-autonomous correction will be addressed in the near future. Herein, highlight of history and basic mechanisms of the CRISPR/Cas9 system and its application in rare genetic diseases, lessons learned from past human gene therapy efforts are done. The challenges, ethical issues, and future prospects of CRISPR-based systems for human research are also discussed.

The Principle of the CRISPR genome editing tool

Over the past decades, genome editing technologies have been composed of zinc-finger nucleases (ZFNs) and transcriptional activator-like effector nucleases (TALENs), empowering scientific results at both the basic and clinical level (1,2). Despite the advances that have been reported in the field of genomic engineering, the use of ZNF or TALEN nucleases is associated with several obstacles.

The discovery of a novel RNA-guided endonuclease-relied genome editing technology called the Clustered Regularly-Interspaced Short Palindromic Repeats (CRISPR) (3) their role as part of an adaptive prokaryotic immune system (CRISPR-associated system, Cas) (4, 5), and subsequent development into a genomic editing tool (6, 7), has revolutionized the field of molecular biology. The ease of use of the technology—and particularly clustered regularly interspaced short palindromic repeats (CRISPR)—will allow us to improve our understanding of genomic variation in disease processes via cellular and animal models. However, much of the excitement of this technology lies at the core center of its potential in treating human disease and editing the human genome.

In just one decade after these tragedy, great progress has been made in advancing gene therapy technologies, leading to renewed enthusiasm in the promise of broad-spectrum treatment of genetic diseases. An important step towards the understanding of the role of CRISPRs was the

identification of four genes closely located to the CRISPR arrays (8), which were termed CRISPR-associated (cas) genes 1-4 which were only present in genomes containing CRISPR arrays and their predicted functions included helicase and nuclease activities, which led to the hypothesis that CRISPR/Cas systems might be involved in DNA repair (9). The CRISPR system was further simplified, based on its ability to interfere with and participate in bacterial adaptive immunity, comprising Cas nuclease and single-guide RNA (sgRNA), consisting of critical elements: a CRISPR array, an upstream leader sequence and the cas genes.

The mechanism of the pioneer CRISPR approach is based on RNA-DNA interaction, whereas previous genome editing tools (ZNFs and TALENs) were based on protein-DNA associations (10). Furthermore, the properties of the CRISPR system that render it amenable is reported to be as a result of its simplicity in constructing the Cas9 nuclease and its capacity to target many genomic loci simultaneously. Notably, the CRISPR system has been distinguished over other approaches, as it enables the simultaneous study of numerous genetic modifications in one step, based on the method of multiple target recognition, which uses many sgRNAs at the cellular level.

The CRISPR system has proven to be efficient in inducing a wide variety of genetic modifications, ranging from the elimination and mutations of genes to genomic insertions (11,12), inversions (13) and translocations (14). For instance, the insertion of one specific DNA template can be accomplished using homology directed repair with duplex DNA templates or single-strand oligonucleotides or viral encoded templates. The numerous capacity of the CRISPR system is invaluable in studying the underlying molecular mechanisms that are implicated in tumor progression, which involves the accumulation of genetic changes, such as mutations, genome rearrangements and epigenetic alterations.

Evidently, CRISPR/Cas technology has moved beyond gene editing and allows flexible and precise modification of the epigenome and the transcriptome. Its versatility and developments such as the use of dCas9 or small Cas13 derivatives in combination with additional functional domains will accelerate basic research into transcriptional, epigenome, and RNA-level regulation of gene expression, besides contributing tools for translational medicine. The early findings discussed here showcasing such modular use of CRISPR/Cas technology demonstrate that therapy of rare diseases will have its fair share of benefit, but that certain aspects such as efficiency and permanence of treatment still need to be addressed.

Challenges and ethical issues

As exciting as CRISPR sounds, a variety of concerns have been expressed about this technique. Amongst them are the unpredictable repair and unwanted effect of this technology. Its quite noted that CRISPR–Cas9 uses a small strand of RNA to direct the Cas9 enzyme to a site in the genome with a similar sequence in which the enzyme then cuts both strands of DNA at that site, and the cell's repair systems heal the gap. However, most times when the edit occur, the cell seals up the cut using an error-prone mechanism that can insert or delete a small number of DNA letters. To be sure, previous work using CRISPR in mouse embryos and other kinds of human cell had already demonstrated that editing chromosomes can cause large, unwanted effects.

Future prospect

The biggest question: Is the world ready for unprecedented power of CRISPR?

Although CRISPR/Cas-related researches and publications are increasing, and deployment in a commercial scale is a promising approach. Also, plethora of tools based on CRISPR/Cas technology have already been developed, which allow control of gene expression, and transcriptional regulators as well as RNA editors have already been used towards rare disease therapy.

Despite the sizeable scientific barriers to heritable gene editing, the more difficult issues are likely to be ethical and social. Although, connsultations have been ongoing, and reports and position statements have been pouring in from scientific societies around the world which is very critical in other to explore the full benefit of this technology.

Reference

1. Bedell VM, Wang Y, Campbell JM, Poshusta TL, Starker CG, Krug RG, II, Tan W, Penheiter SG, Ma AC, Leung AY. In vivo genome editing using a high-efficiency TALEN system. Nature. 2012;491:114–118. doi: 10.1038/nature11537.

2. Urnov FD, Miller JC, Lee YL, Beausejour CM, Rock JM, Augustus S, Jamieson AC, Porteus MH, Gregory PD, Holmes MC. Highly efficient endogenous human gene correction using designed zinc-finger nucleases. Nature. 2005;435:646–651. doi: 10.1038/nature03556.

3. Ishino Y, Shinagawa H, Makino K, et al. (1987). Nucleotide sequence of the iap gene, responsible for alkaline phosphatase isozyme conversion in Escherichia coli, and identification of the gene product. J Bacteriol 169:5429–33.

4. Bolotin A, Quinquis B, Sorokin A, Ehrlich SD. (2005). Clustered regularly interspaced short palindrome repeats (CRISPRs) have spacers of extrachromosomal origin. Microbiology 151:2551–61.

5. Van der Oost J, Jore MM, Westra ER, et al. (2009). CRISPR-based adaptive and heritable immunity in prokaryotes. Trends Biochem Sci 34:401–7.

6. Cong L, Ran FA, Cox D, et al. (2013). Multiplex genome engineering using CRISPR/Cas systems. Science 339:819–23.

7. Jinek M, Chylinski K, Fonfara I, et al. (2012). A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. Science 337:816–21.

8. Jansen R., Embden J.D., Gaastra W., Schouls L.M. Identification of genes that are associated with DNA repeats in prokaryotes. Mol. Microbiol. 2002;43:1565–1575.

9. Makarova K.S., Aravind L., Grishin N.V., Rogozin I.B., Koonin E.V. A DNA repair system specific for thermophilic Archaea and bacteria predicted by genomic context analysis. Nucleic Acids Res. 2002;30:482–496. doi: 10.1093/nar/30.2.482.

10. Christian M, Cermak T, Doyle EL, Schmidt C, Zhang F, Hummel A, Bogdanove AJ, Voytas DF. Targeting DNA double-strand breaks with TAL effector nucleases. Genetics. 2010;186:757–761. doi: 10.1534/genetics.110.120717.

11. Cong L, Ran FA, Cox D, Lin S, Barretto R, Habib N, Hsu PD, Wu X, Jiang W, Marraffini LA, et al. Multiplex genome engineering using CRISPR/Cas systems. Science. 2013;339:819–823. doi: 10.1126/science.1231143.

12. Torres R, Martin MC, Garcia A, Cigudosa JC, Ramirez JC, Rodriguez-Perales S. Engineering human tumour-associated chromosomal translocations with the RNA-guided CRISPR-Cas9 system. Nat Commun. 2014;5:3964. doi: 10.1038/ncomms4964.

13. Lupiáñez DG, Kraft K, Heinrich V, Krawitz P, Brancati F, Klopocki E, Horn D, Kayserili H, Opitz JM, Laxova R, et al. Disruptions of topological chromatin domains cause pathogenic rewiring of gene-enhancer interactions. Cell. 2015;161:1012–1025. doi: 10.1016/j.cell.2015.04.004.

14. Ghezraoui H, Piganeau M, Renouf B, Renaud JB, Sallmyr A, Ruis B, Oh S, Tomkinson AE, Hendrickson EA, Giovannangeli C, et al. Chromosomal translocations in human cells are generated by canonical nonhomologous end-joining. Mol Cell. 2014;55:829–842. doi: 10.1016/j.molcel.2014.08.002.