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Therapeutic potential and challenges of the CRISPR technology

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Abstract

The CRISPR technology has recently received extensive attention from the research and medical community due to its remarkable genome-editing capacity. In particular, the therapeutic potential of translating the CRISPR technology into clinical interventions for various human diseases has brought bright hopes for patients around the world. In the current Commentary, I shall scrutinize recent advances in manipulating and improving the CRISPR technology for viral diseases and genetic disorders in humans. Key challenges to realize the full clinical potential of the CRISPR technology will also be discussed. **Keywords:** CRISPR, human disease, DNA-editing, gene therapy.

Thanks to the rapid development of various sequencing platforms, the past decade has witnessed an exponential growth in our understanding of the genetic landscapes in a wide array of species, including humans. Importantly, this has enabled accurate identification and characterization of genetic factors that contribute to the etiology, biology and pathology of human diseases. However, translation of genetically relevant knowledge into medically effective therapeutics for human patients represents a daunting challenge. Fortunately, recent development of emerging genome engineering technologies, particularly the CRISPR (Clustered Regularly Interspersed Short Palindromic Repeat) system, has achieved inspiring therapeutic triumphs.

Initially identified from the adaptive immune system in prokaryotes, the CRISPR system has now become a revolutionizing genome editing tool that has attracted extensive attention from both basic research and clinical practice communities. The genome editing machinery comprises CRISPR-associated programmable nuclease Cas9 and a single guide RNA (sgRNA), which collectively generates sequence-specific double-stranded DNA breaks at targeted genomic regions. This is followed by nonhomologous end joining (NHEJ) to produce insertions/deletions (indels) mutations or by homology-directed repair (HDR) to produce specific DNA modifications based upon an exogenous DNA template. Because of its technical ease to manipulate and implement, the CRISPR system may be leveraged to target and modify virtually any genomic regions of interest for therapeutic benefits. Indeed, this has been accomplished for a number of human disorders, which will be discussed below.

HIV infection is currently affecting 1.2 million people in the United States and more than 35 million worldwide. Current avenues of treatment are paralyzed by their inability to eliminate integrated viral genomes from infected host cells to prevent latent infection and relapse. Excitingly, the feasibility of utilizing the CRISPR system to disrupt and eradicate HIV reservoirs from infected human cells has been demonstrated by several recent studies[1-4]. When incorporated with sgRNAs that specifically target HIV long terminal repeats (LTRs), the CRISPR system can effectively impair viral expression, remove integrated proviral genomes from different chromosomes of the same infected cells, and provide long-term protection against active re-infection. Notably, these studies also cast light on the therapeutic potential of using CRISPR strategy against other infectious viruses, such as type I herpes simplex virus and hepatitis B virus.

In contrast to its capacity to exterminate the pathogenic genetic elements, the CRISPR system can also be harnessed to correct

disease-inducing mutations to remedy genetic disorders. For instance, in a mouse model of hereditary tyrosinemia type I (HTI) that results from loss-of-expression mutation in fumarylacetoacetate hydrolase (FAH), hydrodynamic coinjection of vectors expressing FAH-targeting sgRNAs and Cas9 and single-stranded DNA expressing wild-type *FAH* rescued diseased mice from liver damage and weight loss[5]. Similar CRISPR-mediated repair strategies have also been evaluated targeting other genes, including *hemoglobin beta* (*HBB*) gene for β-thalassemia[6], *gamma-crystallin gene* (*Crygc*) for dominant cataract[7] and *dystrophin* (*DMD*) gene for Duchenne muscular dystrophy[8]. Collectively, these impressive studies have illustrated the therapeutic potential of the CRISPR system against genetic disorders.

In addition to its utilization in the above-mentioned monogenic diseases, the CRISPR tool can also be engineered to induce multiplex genetic alterations for complex diseases, such as cancer. Indeed, multiple recent studies have successfully manipulated the CRISPR system to establish informative animal models for cancer research. For example, CRISPR-mediated targeting of tumor suppressors PTEN and P53 in adult mouse liver results in simultaneous indel mutations in both genes, thereby inducing liver tumor development *in vivo*[9]. A mouse model for lung adenocarcinoma can also be constructed using the multiplexed CRISPR technologies, where oncogenic mutations of $Kras^{G12D}$ and indel mutations of *p53* and *Lkb1* are simultaneously introduced to initiate lung cancer[10]. Although these studies do not provide direct therapeutic benefits for cancer patients, mechanistic insights from these animal models will undoubtedly facilitate and promote future design of novel anti-cancer therapeutics.

Despite these promising accomplishments in utilizing the CRISPR system for understanding and curing human diseases, many barriers remain for translating the genome editing tool in clinical medicine. Foremost, CRISPR delivery approach, such as hydrodynamic injection used for mouse models, is associated with toxicity and low editing efficiency, and thus requires considerable improvements prior to moving to the clinic. In addition, the off-target effect as a result of nonspecific cleavage at unintentional genomic regions by the CRISPR tool poses a prohibitive risk, and needs to be thoroughly interrogated with whole-genome sequencing and exome sequencing. It should also be noted that current attempts to genetically modify human germline and stem cells with the CRISPR technology must be strictly regulated to ensure bioethical integrity. These are undoubtedly significant challenges that lie ahead and will necessitate tremendous efforts for optimization and improvement. However, we are inspired by the rapid development of the CRISPR technology that definitely holds great therapeutic promise for treating human diseases.

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