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The Rising Pillar of Genome Engineering: Crispr/Cas9 System Interesting Facts and Challenges in the Development of Gene Therapy

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Precise gene editing seems to be an elegant approach for understanding target gene function and to support the development of personalized therapy for various disorders like inherited genetic diseases, metabolic disorders, viral diseases and cancer. One such versatile and technological breakthrough during past decade is CRISPR/Cas9 system which transposes the landscape of genome editing in a diverse array of cell types and organisms [1]. CRISPR/Cas9 (Clustered regularly interspaced short palindromic repeats/CRISPR associated protein), a bacterial adaptive immune system that employs a single guide RNA which identifies the target genomic sequences via Watson–crick base pairing and serve as a scaffold for Cas9 endonuclease binding. These endonucleases create double-strand breaks (DSBs) in the DNA which is then repaired by the endogenous cellular DNA repair mechanisms like non-homologous end joining (NHEJ) or homology-directed repair (HDR). Thus this technology enables the scientists to engineer any part/region of the human genome with extreme precision.

Some of the interesting and challenging facts of this facile genome editing were discussed as follows. Recently, CRISPR targeting CTLA-4 immune checkpoint significantly improved the T cell-mediated anti-tumor activity of cytotoxic T lymphocytes (CTLs). This CTLA-4 CRISPR KO facilitated apoptosis and increased the secretion of TNF-α and IFN-γ to kill the tumor cells and also repressed tumor growth efficiently and prolonged survival in xenograft mice [2]. This technology can also cleave DNA viruses' genome, for instance, Epstein-Barr virus (EBV), which is capable of undergoing episomal replication in human cells. In nasopharyngeal carcinoma C666-1 cells which are latently infected with EBV, two guide RNAs (gRNAs) were employed to edit the BART promoter region (BamHI A rightward transcript) (i.e., deletion of 558 bp) which encodes viral microRNAs, a causative region/segment for this carcinoma [3]. Yet another milestone has been stepped up against the dreadful disease, AIDS. The two different regions of the viral genome such as long terminal repeat, (LTR region) (LTR6), the structural protein matrix (MA3) or the integrase enzyme (IN5) are simultaneously targeted using a combinational CRISPR/Cas9 gene-editing approach. This can block HIV replication and ward off viral escape thereby providing a future challenge for HIV infection control [4]. However, in near future, several *ex vivo* or *in vivo* studies need to be investigated to confirm this editing strategy that would prevent viral escape. More recently, a tumor-targeted delivery system has been achieved in Osteosarcoma (OS). CRISPR/Cas9 VEGFA gRNA plasmid was encapsulated within PEG-PEI-cholesterol lipopolymer functionalized by OS cell-specific aptamer (LC09) facilitated the targeted delivery of VEGFA CRISPR in both orthotopic osteosarcoma and lung metastasis resulting in significant VEGF genome editing thereby inhibits OS malignancy, lung metastasis, reduced angiogenesis and bone lesion without any detectable toxicity [5].

Several preclinical studies have been performed using CRISPR technology to address and treat incurable degenerative diseases. Duchenne muscular dystrophy (DMD), a genetic disease that is responsible for muscle degeneration, loss of mobility and early death was corrected using CRISPR treatment in a mouse model [6]. Mutations in human disease-hemophilia B-specific iPSCs (induced pluripotent stem cells) targeting eight exons of human coagulation factor IX gene was corrected by CRISPR/Cas9 technology and these corrected cells were able to maintain the hepatic differentiation [7]. Additionally CRISPR perform gene edits that slowed retinal degeneration in rats afflicted with an incurable degenerative eye disease that often leads to blindness [8] and similarly this technology has been used to develop a new strategy for performing DNA knock-in in both dividing and non-dividing cells in rat model of retinitis pigmentosa, which resulted in improved visual function [9]. In another study, stem cells obtained from sickle cell patients, who suffer from anemia and premature death, were genetically edited and injected into a mouse model of sickle cell disease. Interestingly, CRISPR corrects the sickle cell mutation and maintains normal hemoglobin at clinical levels *in vivo* [10].

CRISPR-based gene therapies have been started its journey in clinical trials as well. The first US clinical trial utilizing CRISPR was started in June 2016 and the researchers are planned to edit T immune cells obtained from different cancers to attack the patient's cancer cells, thereby enhancing treatment outcomes [11]. Chinese scientists were the first to use CRISPR-modified immune cells by correcting programmed death protein, PD-1 gene in a clinical trial for patients with metastatic lung cancer [12] and companies like Editas Medicine ambitiously involved in developing CRISPR technology to expand its therapeutic applications in human patients [13]. In summary, CRISPR/Cas9 technology is a blessed gift from nature's mother. However, biosafety challenges and measures such as reducing off-target mutations, perfect delivery system, etc., are essentially considered to the greater extent allowing humanity to gain maximum benefits. Hope the success of this technology and future endeavors definitely revolutionize the practice of medicine and significantly improves the betterment of the human health from an array of dreadful and inherited diseases.

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