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In vivo Human Cell Regeneration: Current Perspectives

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Editorial

The mechanism of "clustered regularly interspaced palindromic repeat" (CRISPR) was first elucidated in the year 1987. Originally, CRISPR/Cas system is an adaptive immune molecular mechanism found to occur among 40% of bacteria and 90% of archaea that resist foreign genetic plasmids and invasion by phages. They have an important role in protecting the integrity of the inherent genetic constitution and factors. This structure recognizes the DNA of the phage and binds with it through CRISPR RNA also called crRNA and then guides the CRISPR-associated protein (Cas) to recognize and cleave the exogenous DNA by trans-activation of crRNA. There are two Cas protein families, class 1 (multi sub-unit effector complexes) and Class 2 (single protein effector modules). CRISPR/Cas9 belongs to the class 2 family that requires single guide RNA (sgRNA) to cleave the target gene. Cas9-crRNA can cut the target DNA under *in vitro* conditions causing breaks that occur at three nucleotides upstream of the protospacer adjacent motif (PAM). Therefore, it can edit the genes in a test tube. Later on, the efficiency of the system was improved and applied for editing the genome of eukaryotic cells, animal models for gene therapy, and plant genetic trait improvement. Charpentier and Doudna were awarded Nobel Prize in Chemistry in recognition of their significant contributions to CRISPR/Cas9 based gene editing knowledge in the year 2020.

Structurally, the CRISPR/Cas9 functional complex consists of Cas9 nuclease and chimeric sgRNA (crRNA+tracrRNA) and can function with any gene or any region in the whole genome. Therefore, site-directed gene editing can be performed including insertion, deletion, mutation, and knockout. The sgRNA directs the Cas9 nuclease to bind to the target DNA at a site followed by a 5'-NGG-3' protospacer adjacent motif and enables cleavage of double-stranded DNA. The CRISPR-associated protein (Cas) differs in terms of its nucleic acid target that could be either DNA, RNA, single or double-stranded genetic material, linear or circular DNA. In mammalian cells, this break is repaired by non-homologous end joining (NHEJ) or homology-directed repair (HDR). HDR has high fidelity for performing precise genetic repair while NHEJ is prone to errors and inaccurate repairs and may generate random deletions or insertions at the break site. This genetic engineering tool has been developed for editing genomes of mammalian cells, vertebrates, plants, eukaryotes, and even viruses.

CRISPR technology could be used for the production of allergen-free foods, eradication of crop pests and insects, development of desirable animal breeds, and even higher production of biofuels, The technology could also be used for basic experimental research for studying the gene function, host-virus interactions, optimization of gene therapy, cell-based genetically engineered vaccine development and production with high yield with faster seroconversion and high rate of antibody positivity. Modification of human embryos for preemptive curing of congenital diseases are some of the prospects however, ethical guidelines need to be framed on common grounds.

Immense therapeutic potential in human cells using gene editing was also reported. The system has minimal molecular weight and is convenient to handle. Multiple genes can be edited that are useful for genetic therapy of complex diseases. Further, the bioinformatic approaches have enabled precise analysis of sequence data and optimization of genetic engineering methods. Genome-wide sequence analysis can help in the identification of risk loci of a disease condition. These innovations aid in analyzing the efficacy of gene editing under *in vivo* conditions by taking into account the gene expression profiles, physiological networking, intermolecular interactions, and estimation of compensation effects. Gene editing has immense relevance in not only understanding the regulatory mechanisms of the neurons but also aid in gene therapy of neurodegenerative diseases. Neurodegenerative diseases involve complex cell programming and share common pathological pathways such as mitochondrial dysfunction, cytoskeletal integrity, and defects in DNA repair. Therefore, patient genotyping is valuable for effective treatment.

CRISPR/Cas9 gene-editing technology was applied successfully to the mammalian cell genome through the modification of the bacterial CRISPR system and heterogeneous expression of the key components of the prokaryotic CRISPR system. The technology was evolved and editing of multiple target genes could be achieved by using multiple sgRNA. It has enabled biomedical scientists to engraft CCR5-knockout HSPCs into patients having simultaneous acute lymphocytic leukemia (ALL) and HIV infection. Complete remission could be achieved without any gene-editing side effects and the CD4+ cells ablated by CCR5 increased after discontinuation of the antiretroviral treatment. Similarly, the CRISPR/Cas9 genetic engineering technology is now widely applied in diverse clinical trials for the treatment of blood diseases, hereditary eye disorders, viral diseases, carcinoma, malignant glioma, metastatic lung cancer, prostate cancer, oesophageal cancer, and renal cell cancer.

The adopted immunity system of the prokaryotes that detects the foreign virus DNA or RNA and stop its action can be applied to prevent the infection of the SARS-CoV-2 virus in human lung airway epithelial cells and restrict its spread and stop the proliferation of the virus by inactivation of the specific viral protein that aid in infection and replication. The identified essential proteins for coronavirus proliferation are S, E, N, M, ORF3a, ORF6, ORF7a, ORF7b, ORF8, and ORF10 protein. The technology could be applied using an injectable gene gun or spray aerosol to deliver to its target site. This technology will also address the variability and evolution of the viral gene.

Research and development are continuing on the application of the gene-editing technology for musculoskeletal tumors, screening, identification, and knockout of oncogenes, drug-resistant genes, and identification of tumor suppressor genes. Such treatment could avoid, surgery, chemotherapy, radiotherapy, and their toxic side effects. However, the phenotype is a complex interaction of genetic,

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epigenetic, distal regulatory genes, additional genes on different locations, enhancer and repressor elements, and environmental factors, and these phenotypes interact in a complex manner via diverse and multiple physiological and biochemical exchanges. Though gene-editing is accurate and can be achieved promptly it has got its limitations when viewed from a whole-genome perspective. CRISPR/Cas system could bring in certain pleiotropic effects that are still to be understood. Gene editing in somatic or germ cells could bring about unpredictable consequences. The application of CRISPR technology to human embryos has led to ethical and legal debate. Experimentation on human embryos after 14 days is regarded as ethically intolerable. Therefore several Nations have a variable degree of restrictions on gene-editing technology and *in vitro* fertilization.