Editorial: CRISPR/Cas9 Genome Editing System

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Biomedical Applications of CRISPR/Cas9 Genome Editing System

The clustered regularly interspaced short palindromic repeat (CRISPR) and associated protein 9 (CRISPR/cas9) gene editing system enables manipulation of any gene in cells and tissues originally associated with the adaptive immune system of *Streptococcus pyogenes*. CRISPR/cas9 induces double strand DNA breaks (DSB) in the genome at precise, predetermined loci. The essential CRISPR-Cas components constitute the Cas9 RNA-guided endonuclease which cuts DNA at a specific site, a single-guide RNA (sgRNA) that carries a sequence (protospacer) complementary to the DNA target and a short sequence in the target called the protospacer-adjacent motif (PAM) essential for Cas9 binding. The system enables replacement or modification of the aberrant/diseased gene by insertion or deletion in the genome at the precise position. The applications of the system, capable of simultaneous alteration of multiple genes, are immense in the areas of agriculture and biomedical fields and are of critical value in the current scenario. Thus tackling almost insurmountable, global, biomedical problems such as antimicrobial drug resistance, reversal of hereditary gene defects, and complete cure in cancers with currently no known cure, is feasible. The future envisaged is symptomatic treatment with the endpoint of a complete cure and/or reversal of the disease progression. New delivery systems to induce permanent effects safely will be a requirement for clinical applications. The drawback is the non-specific recognition and digestion of non-target sites, introducing mutations at off-target sites with untoward effects in treatment of human disease. Inroads have been made using the system towards treatment in monogenic diseases such as β-thalassemia, sickle cell anemia, Duchenne muscular dystrophy, neuro-degenerative disease including Huntington's disease, Parkinson's disease, Amyotrophic lateral sclerosis.
and multigeneic complex disease including human cancers.

The approach of ex vivo modification of cells and animal disease models, expansion of the modified cells and return to the models is currently favoured. For persistent benefit, long-term hematopoietic stem cells have been considered as the choice for targeted correction of the β-thalassemia mutations, sickle cell mutation and persistence of fetal hemoglobin. In vivo genome editing is more complicated and needs appropriate delivery systems to the target tissues.

The editorial is a brief glimpse of feasible clinical applications of the CRISPR/Cas9 system in the near future for better patient management in genetic diseases and cancer.

Clinical Application of CRISPR/Cas9
CRISPR/Cas9 system has been successfully used to correct genetic defects in monogenic diseases including β-thalassemia resulting in in vitro hematopoietic differentiation and β-globin gene expression in skin fibroblasts; cystic fibrosis with CFTR F508 del in 3D-intestinal organ cultures resulted in in vitro differentiated intestinal organoids; Duchenne muscular dystrophy cells in vitro differentiated to skeletal muscle cells with dystrophin expression.

CRISPR/Cas9 is useful in creating animal mouse models for diverse diseases such as tyrosinemia, muscular dystrophy and several inherited genetic diseases, as well as cancers. Duchenne muscular dystrophy (DMD), a fatal genetic muscle disease, is produced by in-frame deletions, affecting the dystrophin gene. Scientific teams at Duke University, University of Texas, Southwestern Medical Center and Harvard Medical School used CRISPR/Cas9 editing system for removing the deleterious DNA sequences and restoring the reading frame of Dmd gene in cardiomyocytes and muscle stem cells. The treatment resulted in Dmd expression and rescue of muscle structure and function.

CRISPR-mediated gene therapies in Huntington's disease, Parkinson's disease, and hereditary ataxias are a therapeutic option in these currently difficult to cure diseases. Huntington’s disease (HD) and spinocerebellar ataxia types 1, 2, 3, 6, 7 and 17 are prototype trinucleotide repeat disorders. The abnormal expansion of the polyglutamine region in the specific proteins has
pathognomonic consequences in neurodegenerative diseases. Parkinson’s disease, Alzheimer’s disease and amyotrophic lateral sclerosis result from accumulation of abnormal misfolded proteins. CRISPR/Cas system is used to modify the abnormal trinucleotide expansion and protein production and prevent their accumulation in the diseases. Besides correcting the abnormal production of aberrant pathogenic proteins, stable introduction of genes e.g., frataxin gene into the cells of Friederich's ataxia patient is an approach attempted recently by Vannocci and colleagues as a therapeutic modality. Genome editing opens a new avenue to block critical receptors such as coreceptors CCR5, essential for infection with Human Immunodeficiency virus.

Programmed Death 1 (PD-1), an inhibitory receptor in T-cells is a therapeutic target blocking interaction between PD-1 and anti-PD-1 antibody, clinically tested to suppress tumors, treatment approved US Food and Drug Administration for treat melanoma.

CRISPR/Cas9 has been applied in conjunction with cancer immunotherapy wherein autologous T-cells are engineered in vitro to express chimeric antigen receptors (CARs) that recognize cancer cells. The approach is promising in treating lymphoma, leukemia and melanoma in mice.

**Genome editing in cell-based immunotherapies**

Recently, National Institutes of Health, USA, approved use of CRISPR/Cas9 in human clinical trials to edit the genome of T cells and augment cancer therapies. CRISPR/Cas9 was used to inactivate the gene encoding programmed cell death protein PD-1, enhancing effectiveness of chimeric antigen receptor (CAR) T-cell therapies. The CAR T-cell approach, pioneered at Memorial Sloan Kettering, New York, US, equips a patient’s T-cells with receptors that recognize the cancer cells and initiate an immune response to destroy the cells.

**Genome editing in embryonal cells**

Besides modification of diseased cells, changes in the genomes of human embryos is considered for persistent and permanent correction of the gene, and will be applicable in inherited genetic diseases, although currently attempted for research purposes. The diagnostic and therapeutic protocols of preimplantation genetic diagnosis with negative selection and implantation of the normal embryo is
used to ensure normal birth post \textit{in vitro} fertilization. The principle is extended using CRISPR/Cas9 for editing embryonal cells with a group of scientists in China who have initiated editing of embryonal cells and published their experimental studies.

Several labs in the US, China and UK, have stated their current research and future plans to apply CRISPR to human embryos.

**Genome editing in cancer**

Lu You and his team at Sichuan University in Chengdu delivered CRISPR/Cas9 modified cells into a patient with aggressive lung cancer as part of a clinical trial at the West China Hospital. The team of clinical oncologists disabled PD-1 gene inhibiting PD-1 expression and consequent immune response of the cells. The edited cells were cultured \textit{in vitro} and injected into the patient with metastatic non-small-cell lung cancer to destroy the cancer cells. The team plans to treat a total of ten patients who will each receive either two, three or four injections. It is primarily a safety trial, and participants will be monitored for six months to determine for serious adverse effects. The team will also monitor further duration or benefit from the treatment. Dr. Carl June, University of Pennsylvania in Philadelphia, proposes CRISPR system to target various cancers. A group at Peking University in Beijing has proposed initiation of clinical trials using CRISPR against bladder, prostate and renal cell cancers, shrink the aggressive tumors and increase survival rates of cancer the patients. In December 2015, an International Summit on Human Gene Editing comprising esteemed members of National Scientific Academies of America, Britain and China agreed to support basic and clinical research under appropriate legal and ethical guidelines. On the other hand, a worldwide moratorium on applying CRISPR to the human germline, especially for clinical use, has also been declared. The safety, short term and long term consequences of such modifications, and appropriate use in correction of diseases/disorders rather than evolution of a new breed of designer babies needs to be considered.

**CRISPR/Cas Evaluation**

CRISPR/Cas system cannot be slighted for gene editing and the future will confirm the dues to the technology for unbelievable strengths for mankind with tremendous therapeutic benefits. A look
into the past imprints the letters on the wall. The use of CRISPR/Cas9-gRNA complex for genome editing was the AAAS's choice for breakthrough of the year in 2015. In 2015 CRISPR was the winner of Science Magazine's Breakthrough of the Year award. CRISPR was named as one of MIT Technology Review's ten breakthroughs technologies in 2014 and 2016. In 2015, the journal Science chose CRISPR-Cas9 technology as the most important technological advance of science in the last years.

Technological advances are the current scientific achievements and the applications in both basic and applied sciences are stupendous. A critical application in biomedical science is use of the technology in understanding the biology of diseases. In the current issue of the Biomedical Research Journal, Dr. Prasad Pethe, at Sunandan Divatia School of Science, NMIMS (deemed-to-be) University in his review ‘Polycomb Group Proteins: Emerging Players in Neurogenesis’ has attempted to better understand Neuro-developmental and neuro-pathological disorders, a difficult to fathom multi-factorial process governed by an intricate interplay between developmental genes, promoters, transcription factors, and epigenetic modifiers that act as transcription activators or silencers. In the current review, Dr. Prasad takes us succinctly through the various processes of early development and maturation highlighting the activity of Polycomb group (PcG) proteins in neurogenesis.

On the other hand the review on ‘Lithium induced neural plasticity’, by Dr. Rita Mukhopadhyaya, Molecular Biology Division, Bhabha Atomic Research Centre, Mumbai, and Dr. Medha S. Rajadhyaksha, Sophia College, Bhulabhai Desai Road, Mumbai, reviews lithium as a drug for treating mood disorders including bipolar disorder. The mini review, aims to present and comprehend lithium induced neuroplasticity through molecular pathways. The authors indicate and support the positive impact of lithium on neurite extensions, subtly presenting the negative effects of lithium on dendrites. The review details the regulation of neural plasticity by lithium, effect on long term potentiation, learning and memory, and neurotransmission along the signaling mechanisms.

The article on ‘Recent Advances in Nanomedicine for Antimalarial Drug delivery’ by Dr. Manashjit Gogoi,
Department of Biomedical Engineering, North-Eastern Hill, University, Shillong, Meghalaya, covers a critical disease – malaria, of high incidence and mortality in tropical and subtropical countries. A majority of the problems in battling communicable infectious diseases today is drug resistance and optimal drug delivery to the appropriate cells. The author has detailed various nano drug delivery systems such as liposomes, solid lipid nanoparticles, dendrimer, nano-emulsion and polymeric nanoparticles for treating malaria.

In the same vein, the review article on 'Preclinical PET, SPECT, CT, MRI and Optical Imaging in Cancer Research: An Overview' by Dr. Pradip R. Chaudhari, Advanced Centre for Treatment, Research and Education in Cancer (ACTREC), Tata Memorial Centre, Navi Mumbai, emphasizes the comparative clinical picture of various imaging technologies. The review focuses on the dedicated preclinical imaging modalities such as Positron Emission Tomography (PET), Single Photon Emission Computed Tomography (SPECT), X-ray Computed Tomography (CT), Magnetic Resonance Imaging (MRI), optical imaging and Cerenkov imaging. The review highlights the structural, functional and molecular processes for evaluation of the suitability of target molecules in clinical trials.

The final article in the Journal on ‘Role of Anti-aging Gene Klotho in Oral and Gastrointestinal Cancers’ by Dr. Gauri Pathare and Dr. Kavita Shalia, Sir. H. N. Hospital and Research Centre, Mumbai, comprises an in-depth review on the functions, mechanism of action and effect of Klotho genes on oral and gastrointestinal cancer. Klotho, the anti-aging gene, also functions as a tumor suppressor gene, with the tumor suppressor effects of Klotho attributed to its ability to modulate deregulation of insulin/Insulin Growth Factor-1 (IGF-1), Wnt and fibroblast growth factor (FGF) signaling. Klotho gene is primarily silenced through promoter hypermethylation. The authors indicate that the ectopic expression or the restoration of Klotho directly correlated with reduction in cancer. The details of the ectopic action or restoration of Klotho gene function for clinical management of the cancers is elaborated, however, confirmation and revalidation of the protocols is necessary.