REVIEW ARTICLE

CRISPR/cas9: An Emerging Revolution in Therapeutics

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Abstract

Clustered Regular Interspaced Short Palindromic Repeats also known as CRISPR, the most rapidly adopted genome editing tool was discovered through studies on bacterial immunity system. As genome editing and studies related to its functionality requires to manipulate DNA and modify genomes. Already available genome editing tools like HR-mediated targeting, ZFN, TALEN and Cre-lox etc have certain limitation but the challenge had overcome by CRISPR combined with cas9 protein. Because of the fact that future of medicinal and therapeutics seems to reside in adjustments at molecular level by genetic modifications and genome editing in cells. Alteration of a gene expression, inhibition of a gene through advanced methodologies of CRISPR/cas can provide insight to the key cause of many globally recognized diseases. This review is brief overview of highlighted present day exigent assignments of this technology in various medical and biotechnological areas to explore further horizons for therapeutic domain of research.

Keywords: Genome editing, Therapeutic, CRISPR, cas9, ZFN, TALEN

Introduction

With the vast advancement in Allied Health Sciences, future of medicinal and therapeutics seems to reside in adjustments at molecular level by genetic modifications and genome editing in cells. CRISPR/cas technology works in quite a specific manner, editing only the sequence that is targeted and guided by guideRNA (gRNA). Besides CRISPR, other genome editing tools include Double Stranded Breaks mediated Zinc Finger (ZF) motifs and Transcription activator like Effectors (TALEs), but unlike CRISPR both require extensive protein engineering as these systems work through nucleases that make site specific Double stranded breaks (CRISPR Handbook. 2016). Another breakthrough brought by CRISPR mediated system is editing of multiple genes simultaneously. Knowledge of this editing tool came up in 2013, from observations on S. Thermophilus and S. Pyogenes (CRISPR Handbook. 2016).

CRISPR/cas works as an adaptive immune system in bacteria and archaea against certain Mobile Genetic Elements (MGE), such as plasmids or viral nucleic acids (Blenke et al, 2016). Depending on the type of endonuclease, CRISPR/cas system is divided into two classes. Class-1 comprises types I, III and IV while class-2 contains types II, V and VI, where the differences lie in specific guide RNA molecule and endonucleases. Once the spacer sequence against the nucleic acid of MGEs (e.g, phages), is generated, components of CRISPR i.e guide RNA molecule and CRISPR associated endonuclease protein (cas), the catalytic core, are activated to inhibit gene expression of invading genetic element.

The short guide RNA (gRNA) guides cas9 endonuclease towards the target sequence of DNA (van de Sluis, 2016). Further response is carried out in three the key genome editing tool of modern day.

It has been utilized in targeting and modifying the genetic sequence, responsible for diseased condition of an individual or genes facilitating an infection. Unlike conventional gene editing tools (ZFN and TALEN), strategy relying on RNA-DNA distinct phases; acquisition, expression and interference (Blenke et al, 2016). This RNA guided nuclease (RGN) approach of CRISPR/cas technology is recognition in CRISPR/cas9 is simple and easy to be designed, working to confer multiple genes' modification i.e, multiplexing (Wen et al, 2015).

Present outcomes of CRISPR/cas based gene editing technologies in medical interventions and therapeutic procedures have revolutionized and accelerated the curative stratagem. In treatment for Duchenne muscular dystrophy (DMD), CRISPR/cas based therapy has been employed and promising results were achieved (Mendell et al, 2016). Resistance to antibiotics as well as anti malarials is another critical issue to be concerned about as the existing drugs, regardless their potent activities, are claimed to be impractical due to single nucleotide polymophism in gene sequence of pathogenic microbes.

CRISPR/cas based Immunity: Beyond Adaptivity

An individual's adaptive or acquired immunity is third line of defense against pathogens, after innate immunity and skin. Discovery of CRISPR/cas system and its advancement as a gene editing tool, for enhanced activity of adaptive Mutations conferred by gene editing tool has made it easy to increase the susceptibility of malarial parasite towards the drugs of first choice (Ng et al, 2016). CRISPR locus in a bacterial genome provides evidence of immunization incidents thus been used for genotyping of *Mycobacterium, Corynebacterium, Streptococci, Escherichia, Legionella, Yersinia, Salmonella, Lactobacillus* and *Pseudomonas* (Barrangou and Doudna, 2016). Immune system components, are well established now for a decade has been spent in their exploration.

Among various applications of CRISPR/cas gene editing, the most out worthy and foremost achievements are generation of diseased models for study, treatment of genetically inherited disorders and the treatment of lethal infectious diseases (Guan et al, 2016).

Consecutive addition of invader derived sequences in CRISPR-locus might also provide evolutionary aspects of prokaryotic cell (Westra et al, 2014). As an immunity enhancement measure, CRISPR based editing has been reported in bacterial virulence regulation (Campylobacter jejuni), anti-sense CRISPR RNA for Listeria monocytogenes gene regulation, bacterial DNA repair, induction of dormancy upon phage infection, CRISPR mediated self-targeting leading to genome evolution (Westra et al, 2014). Elegant studies have shown therapeutic potential CRISPR/cas mediated genome editing in Hepatitis B virus (HBV) infection. Chronic HBV infections are incurable and finding the cure is a difficult challenge in antiviral drug research. However a practical approach has been achieved by targeting the cccDNA of HBV through CRISPR based designed guide RNA, inducing mutations (Seeger and Sohn, 2016).

Therapeutic potential of CRISPR/cas9 techno have been successfully observed in mammalian cells. Key focusing points in mammalian models were conditional knock out of genes in mouse and rabbits, drug targeting efficacy and validation through genomic screening, embryonic genome editing, alteration of T-cells ex-vivo (Blenke et al, 2016).

Single construct of RGN can attain required genes based manipulation, rendering mutations in desired strains thus avoiding the use of multigenerational mating between several strains (Harrison et al, 2014). Basis of immunological memory through CRISPR/cas adaptation could carry potential to store information in living organisms (Amitai and Sorek 2016). Combined activity of designed gRNA/cas9 and pharmaceutical product (Proprotein) has been reported to reduce the risk of cardiovascular disease through increased cholesterol levels. The study proposes that single gene (Pcsk9) disruption in hepatocytes through gRNA constructs delivered by adenovirus vector can reduce plasma cholesterol level up to 40% (van de Sluis, 2016).

A truly remarkable gene repairing potential of CRISPR/cas has been reported, according to which Mdx gene activity has been reverted back to functional form (Munshi, 2016). Epigenetic modifications are major gene expression controlling

element, and control on these modulations through CRISPR based editing is emerging as a novel tool to overcome certain pathophysiologies (Vojta et al, 2016).

Applications of CRISPR combined with pluripotent stem cell research are now being used to improve treatments of certain monogenic hereditary disorders and in study of synthetic biology, cancer research and gene therapy (Kim et al, 2017).

Mouse models have been used for genomic editing and proved successful in cardiomyopathy. Accurate and rapid single cardiac gene deletion with designed guide RNA in mice models, suffering from cardiac disorders, confirmed the effective of this method (Carroll et al, 2016). To date, the largest CRISPR mediated gene deletion has been achieved in Duchenne muscular dystrophy (DMD). DMD is a fatal genetic disorder, where there is progressive degeneration of muscles due to dysfunctioning of dystrophin encoding gene. With a single guide RNA, highly functional dystrophin protein has been restored in Pluripotent stem cells of patients suffering from DMD (Young et al, 2016). Injecting CRISPR/cas9 components in embryo at early developmental stages has provided the opportunity to eliminate consequences of genetic disorders in subsequent generations with permanent changes in genome (Savić and Schwank, 2016).

CRISPR/cas Editing Vs Cancer

Cancer biology has been extensively studied and still in process, as the curative measures have never reached its complete therapy. Cancer is basically a complex group of disorders, arose by alteration in DNA sequence ultimately leading to transformation of cell, growth of tumorigenic factors, spreading due to invasion and metastasis progressing the stages further (Sayin and Papagiannakopoulos, 2017). Genome editing has provided a tool to excise out altered and modified genes, which accelerate the growth of tumor.

In deletion mutations incorporated through CRISPR/cas based engineering has restored the genes that lost their functions (LOF genes) during tumori-genesis in cell lines, germ lines and somatic cells (Sayin and Papagiannakopoulos, 2017). Alteration of gene expression which have lost their function (e.g, p53) or gained function (e.g, over-expression of anti-apoptotic Bcl2) has been achieved through gene editing tool so as to enhance treatment of cancerous cells. Epigenetic modulations also play critical role in generation and progression of cancer status. Protein bringing about these modulations has been targeted through CRISPR/cas engineering in order to achieve the goal of cancer treatment (Sayin and Papagiannakopoulos, 2017).

In a study, lentiviral vector carrying designed gRNA and cas9 nuclease were injected in hematopoietic stem cells (HPSCs) of mouse, to modify the function of genes inactivated in myeloid malignancies (Wen et al, 2016). A novel approach relies on modifying the microenvironment of tumor cells through selective expression of genes required to antagonize the function of over expressed genes and gene products. In China and US, two clinical trials with CRISPR/cas9 based targeted therapy has been approved. Genome wide screenings studies based on CRISPR technology are now being used to generate gRNA libraries and to identify LOF genes contributing to tumorigenesis and metastasis (Barrangou and Doudna, 2016).

Future Directives with CRISPR/cas Genome Editing

It is stringent to play editing games at molecular levels of DNA and proteins and renders more baseline approaches in medical and therapeutic world. CRISPR mediated therapy have brought wide genetic screenings, covering a millennium and far-reaching advancing ethics in remedial approaches. There still room for improvement in CRISPR system after applications in microbial and mammalian gene editing. More effective and site directed constructs of gRNA and cas9 protein can pave ways of site directed delivery in some cell types. Further expansion in different aspects of this molecular editor may come up in near future that may provide cure to most fatal and incurable diseases and infections.

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