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CRISPR technology: new paradigm to target the infectious disease pathogens

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Abstract. – OBJECTIVE: Infectious diseases are one of the prime causes of death worldwide. An innovative sequence specific editing technology "Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)" has been tested on a broad range of microorganisms to target and destroy invading foreign DNA to human cells or tissues. This study aimed to discuss the mechanism and therapeutic usage of CRISPR/Cas9 genome editing technology in the management of various infectious disease pathogens.

MATERIALS AND METHODS: We conducted a broad search of the English-language literature in "PubMed" using the search terms "CRISPR", "Cas-9", "Genome editing", "Gene therapy", "infectious disease pathogens". All the articles were reviewed and required information was recorded.

RESULTS: CRISPR technology is used to modify and modulate the gene expression in biomedical research and therapeutic development. This technology facilitates the understanding of fundamental biology and broadens the horizon of treatments of germ-laden conditions.

CONCLUSIONS: The applications of CRISPR technology are widely established in the diagnosis and treatment of various bacterial, viral, fungal and parasitic infectious diseases. CRISPR technology is a simple, efficient and tested on a broad range of microorganisms to rectify disease-associated genetic defects and destroy invading foreign DNA to human cells or tissues.

Key Words
CRISPR, Cas-9, Genome editing, Gene therapy, Infectious disease pathogens.

Abbreviations

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR); Deoxyribonucleic acid (DNA); Ribonucleic acid (RNA); Zinc-finger nucleases (ZFNs); Transcription activator-like effector nucleases (TALEN); Primary duck hepatocyte (PDH); Duck hepatitis B virus (DHBV); Covalently closed circular DNA (cccDNA); Single guide RNA (sgRNA).

Introduction

Infectious diseases are a common cause of death around the globe despite remarkable advancements in diagnostic and therapeutic technologies. In developing countries, they are heading as the top most sources in children demises[1]. A rising number of hospitalizations due to malignancy, diabetes mellitus and geriatric problems are because of multidrug resistant organisms, and highlight the economic accountability of public health system across the globe[2].

A large number of people are affected by life-threatening persistent viral infections in developing countries[2,3] and health community is unable to combat many microbial diseases because of their frequent epidemic occurrence and particular specification. Therefore, numerous pathogenic disorders are still without proper therapeutic remedies and remain a serious threat due to specific nature of the causative microorganism and lack of fundamental healthcare facilities to the population especially in the disadvantaged group. Thus, substantial efforts are required to develop new or alternative modern treatment modalities to be deployed for their prevention. Presently, an innovative sequence specific genome editing technology such as "Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)" has been tested on a broad range of microorganisms to rectify directly disease-associated genetic defects, and target and destroy invading foreign DNA to human cells or tissues in a simple, efficient, and selective manner.

Genome Editing Technologies

The genome editing is a technique to remove, add, or alter the genetic material to reshape the organism's deoxyribonucleic acid (DNA). It is a

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relatively new procedure to put down such pathogenic conditions[4]. The current advancement in the genome editing technologies is based on the "CRISPR-associated RNA-guided endonuclease Cas9" permitting the organized examination of genome functions. Cas9 can be guided to specific locations within complex genomes by a short RNA search string[5]. CRISPR is an advanced form of the former method to remold genomes at a particular site and has proven very effective to control many infectious conditions[6-8].

CRISPR Cas-9 Technology

CRISPR/Cas9 is a most efficient, widely used flexible and unique genome editing system around the world. Degrading of targeted bacterial nucleic acid with type II Cas system was also manipulated by this procedure as a clinical trial, which demanded customization of the sgRNA to produce Double Strand Breaks (DSBs) at the user designated position by translating the genetic codes of the pathogens[9,10]. This technique, having a wide range of diagnostic applications, is trouble free and almost four times more effective than the previous gene translation tools including ZFN, TALEN[11]. Its mechanism was demonstrated AND later the construction of its vectors and DNA transformation of the target gene were expressed by Hatada and Horii[12]. It has been used to target genes in mice, rabbits, zebra, bacteria, and viruses. Even now in humans[13], it has modified the genes in both pathogens and host cells[6].

Infections *vs.* Cas9 Technology

Bacterial "CRISPR Cas-9" is the investigative medium to manipulate genetic characteristics associated with infections. This way it plays a critical role in adaptive immunity against certain bacteria by framing a resistance to invading bacterial DNA[9]. This system is particularly applied for determination of genome structure and acts as powerful and commanding prophylactic and therapeutic antimicrobial approach. Therefore it is successfully used to limit the production of new infectious particles from human cells and destroy the pathogens[14]. It is a novel diagnostic and therapeutic intervention as its mechanism includes description of genes specifically potentially harmful genetic codes[6].

Mycobacterium Tuberculosis (TB)

In spite of clinical success in treating tuberculosis, it remains a major killer with 1.7 million deaths per annum globally. Tuberculosis is one of the top 10 causes of death in the world mainly in the low and middle income developing countries[15].

Scholars[16,17] succeeded to achieve transcriptional repression of targeted TB genes by employing CRISPR interference. It inhibits the expression of essential genes based on enzymatically inactive Cas9 protein with RNAs CRISPR; therefore, it is the most powerful tool in treating the multidrug resistant conditions[16,17]. Its mechanism of action involves inhibition of growth, changes in susceptibility to small molecules inhibitors and disruption of normal cell morphology[16].

Viral Infections and CRISPR-Cas 9 Technique

Hepatitis B virus (HBV) is one of the most common infectious diseases and is a leading public health problem worldwide. Globally, approximately 257 million people are living with hepatitis B virus infection. In 2015, viral hepatitis caused 1.34 million deaths[18]. Advancement in immunization and novel HBV antivirals are extremely dynamic in preventing infection and suppressing viral replication. However, still the clinicians experience definite therapeutic challenges in curing the viral infections as these causative agents rely on host proteins for their replication. They are of lack of their own cellular metabolism, thus may precipitate further complications[4]. CRISPR-Cas 9 system is an effective technique to target invading viruses and foreign DNAs. It has been proven a therapeutic tool in producing intended and promising results by its illumination of specific DNA sequence to impede hepatitis B virus replication. Also, the disruption of closed circular DNA has been observed *in vitro* and *in vivo*[7,8] .

This technique has shown a targeted inhibition of duck hepatitis B virus (DHBV) DNA especially cccDNA when primary duck hepatocyte (PDH) was infected with DHBV. Two sgRNAs, sgRNA4 and sgRNA6, played a significant role in hampering of DHBV total DNA. Its function is further speeded up by adding entecavir (ETV) and this combined technology has a greater role in culture medium of the victimized pathogen[19].

The "CRISPR/Cas9 technology" has vast ability to modify the genome and brought about a new age of gene therapies to manage the diseases. "CRISPR/Cas9 system" can be used to inhibit HBV replication and gene expression both *in vitro* and *in vivo*, and becomes a novel therapeutic approach for HBV infection. "CRISPR/Cas9" based therapy and RT inhibitors were used to achieve the highest rates of viral response. "CRISPR/Cas9 technologies" offer high hope to remedy the chronic HBV infection[20]. "CRISPR Cas9" gene editing system precisely targets the conserved regions of the HBV genome and it is considered as a novel therapy for HBV. These results in viral suppression provides a promising tool for eradicating the virus[20]. Although, safety and an efficient delivery of the system keep remaining obstacles.

Kennedy et al[21,22] demonstrated an active inhibition of HBV DNA production and cccDNA growth *in vitro* models of chronic HBV infection. Seeger and Sohn[23] found that infections could be inhibited up to eight-fold by HBV-specific guide RNAs. Liu et al[11] showed that HBV-specific gRNA/Cas9 could inhibit the replication of HBV of different genotypes both *in vitro* and *in vivo*.

Zhen et al [24] found that HBsAg level in mouse serum was dropped by CRISPR/Cas9. The system also significantly inhibits the HBV DNA levels and HBsAg. Similarly, it is also reported that "CRISPR/Cas system" can be used in inhibiting the cccDNA and viral replication in pre-cccDNA-transfected Huh7 cells. Ramanan et al[8] showed that "CRISPR Cas system" targeting conserved regions of HBV, causes strong inhibition of virus replication both *in vitro* and *in vivo*. Karimova et al [7] demonstrated that "CRISPR/Cas9" system can disrupt both HBV cccDNA and integrated HBV sequences in "HeLa and HEK293 cell lines". These studies demonstrated the usefulness of the CRISPR/Cas9 system in destroying HBV cccDNA and therapeutic potential of CRISPR/Cas9 in acute and chronic HBV infection.

Chronic infections with hepatitis-C virus (HCV) account for the majority of cases of liver fibrosis, liver cirrhosis and hepatocellular carcinoma. It is estimated worldwide that 110-180 million people have chronic HCV, about 80% of people have developed chronic infections when infected with HCV [25-27]. Price et al [28] examined the impact of HCV UTR disruption on virus replication with the "CRISPR/Cas9" system and showed 40% reduction in the translation of HCV proteins. Ren et al[29] have also successfully applied the "CRISPR/Cas9" screening for Hepatitis C. They found that "CRISPR/Cas" systems have highly promising findings and the recent results become the basis of chronic hepatitis B and C therapy[30]. Moyo et al [31] reported that targeting RNA from HCV with "CRISPR/Cas" have therapeutic utility. Pre-clinical findings show that "CRISPR/Cas technology" has a potential role to manage HCV infection.

Herpes Simplex Virus

Herpes simplex virus is carried by most of the adult human population causes widespread lifelong infections and treating the herpes infections remains a major challenge. Van Diemen et al [14] established the "CRISPR/Cas9" system to target viral genetic elements and found an effective retraction of "HCMV" and "HSV-1" repetition by targeting the gRNAs to viral genes. Targeting HSV-1 with multiple gRNAs fully eliminated the production of infectious particles from the human cells. They concluded that "CRISPR/Cas9" system can effectively target the herpes virus genomes. It is an effective prophylactic and therapeutic anti-viral strategy that could be used to impair the viral replication and clear the dormant virus infections.

Malaria

Approximately 200 million cases of malaria infections were reported in the last few years, nearly half of the world's population is at a risk of malaria infection[32]. Techniques to eradicate the malaria infections with CRISPR technology are already under investigation. A mosquito strain incorporating a synthetic system called a "gene drive" that passes a malaria-resistance gene on to the mosquitoes' offspring was measured by applying the new technology to manipulate three different genes that confer a recessive female-sterility phenotype upon disruption. Mosquito population was suppressed by introducing the gene driven with the help of this technique to the levels that do not support malaria transmission[33-34]. Infectious diseases are the greatest global challenge for the health officials as these diseases are multi-factorial and frequently attack the vitamin deficient children[35] in the developing world. However, CRISPR technology is the new hope to fight against the infectious diseases.

Conclusions

Basic biomedical sciences to translational medicine are furnished with CRISPR technology. This technology has been tested on a broad range of microorganisms to rectify directly disease-associated genetic defects, and target and destroy invading foreign DNA to human cells or tissues, in a simple, efficient and selective manner. The current applications of CRISPR technology are widely discussed in the treatment of various bacterial, viral, fungal and parasitic infectious diseases. It is a cost effective technique to target host pathogens that could interrupt the therapeutic remedies of a number of infections. CRISPR technology is widely used, even if it has certain limitations in its application, since it is potentially effective. CRISPR technology is easy to use in translating the causative genetic code and correct the gene defects, thus making its application as a demanding therapeutic tool in the present era.

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Conflict of Interest

Authors declare no conflicts of interest.

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