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Applications of CRISPER/Cas9 as a Tool to study Human Diseases caused by Viruses: A review

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Abstract
CRISPR systems are a collection of adaptable gene-editing tools that perform a variety of ground-breaking tasks in a variety of application areas, including agricultural practices, the food industry, biotechnology, biomedicine, and clinical research. Particularly, the CRISPR/Cas9 technology has been widely and successfully used to combat human infectious viruses as the preferred new antiviral strategy. Human immunodeficiency virus (HIV), hepatitis B virus (HBV), human papillomavirus (HPV), and other viruses continue to pose a threat on a global scale and are likely to result in pandemics. The CRISPR/Cas9 system has already been modified to give host animals new antiviral powers by either changing the host genome or by specifically targeting viral intrinsic components in the form of DNA. This technology shows considerable potential for the treatment of human viral infectious illnesses, despite a number of restrictions and challenges that still need to be overcome. In this article brief biological overview of CRISPR/Cas9 systems and its applications to fight a variety of human infectious viruses was discussed.

Keywords: CRISPER/Cas9, Human Diseases, Viruses

Introduction
Numerous acute and chronic diseases, some of which result in grave situations like the most recent coronavirus disease 2019 (COVID-19) pandemic, are largely caused by viruses. Some of them, like herpes simplex viruses, only cause very modest illnesses. Human health and international stability are currently under risk from major viral infectious diseases like the human immunodeficiency virus (HIV), hepatitis B virus (HBV), and human papillomavirus (HPV) (Morens and Fauci, 2013) [17]. They unquestionably add to the socioeconomic strain on the global public health systems (Doerflinger *et al*., 2017) [19]. The three viruses stated above are more harmful to humans than other health-relevant infectious viruses, such as the herpes simplex virus; once contracted, they are also more challenging to treat due to lower success rates with medical treatment. Due to virus's excessive use of cellular resources and the development of latent viral repositories in hosts, treating viral diseases is a difficult job. Additionally, a lot of human viruses have the ability to create mutant strains that can escape and even travel between different species, causing pandemics (Parrish *et al*., 2008) [20]. Thus, a number of antiviral approaches have successively made their way into preclinical and clinical settings. These include synthetic drugs, medicinal herbs, animal-based medicines, specific antibodies drugs and genetically engineered drugs (Zündorf and Dingermann, 2000) [21]. A groundbreaking discovery in the fields of biomedicine and gene therapy, the clustered regularly interspaced short palindromic repeats (CRISPR)/Cas genome editing approach is one of the methods that shows tremendous potential for combating dangerous human pathogenic viruses.

A new avenue for gene therapy in biomedical research has become available since 2013 as a result of the effectiveness of genome changes made possible by the CRISPR/Cas9 machinery in established human cells (Mali *et al*., 2013) [34]. Nucleases are used to introduce site-specific DNA cleavages, and then the body's own repair mechanisms are used to close the DNA breaks. This process is known as gene editing. Several genomes engineering platforms, including mega nuclease (Maeder and Gersbach, 2016), zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and CRISPR/Cas nuclease systems (Hryhorowicz *et al*., 2017) [36], can cause exogenous DNA double-strand breaks in the genomes. The homology-directed repair (HDR) route with repair templates or the nonhomologous end joining (NHEJ) pathway without repair templates are then used to finish cell DNA repairs that were started by DNA lesions.

As of now, the CRISPR/Cas genome editing system has been developed as a reliable tool for targeted gene alterations in a wide variety of animal species, gut microbiota and their invasive viruses which result in changes in host-virus interactions. A novel class of therapy based on CRISPR/Cas9 editing technology is approaching the clinical phase for the treatment of viral infections as scientific interest in gene editing research rises (Hirakawa *et al*., 2020) [37].

CRISPR/CAS9 Machinery

The *E. coli* genome contains a variety of DNA repeat sequences known as CRISPR, whose function is undetermined and whose origin is unknown (Ishino *et al*., 1987) [22]. The prokaryotic species CRISPR/Cas complexes provide resistance to newly introduced genetic material, such as plasmids, phages, and viruses (Jinek *et al*., 2012) [23]. The CRISPR/Cas systems were first artificially split into three main categories, namely type I, type II, and type III, based on the Cas proteins amino acid sequences. Three further CRISPR/Cas systems (types IV–VI) have only recently been discovered across the bacterial genomes, of which type II and type V CRISPR/Cas systems are the same apparatuses that only contain a single subunit RNA effector (Cas9 and Cas12, respectively) (Khadempar *et al*., 2019) [24]. The type II CRISPR/Cas system, also referred to as CRISPR/Cas9, is composed of the endonuclease Cas9 and two short guide RNAs (gRNAs), transactivating CRISPR RNA (tacrine) and the CRISPR RNA (crRNA). (Saayman *et al*., 2015) [25], which is frequently utilized for modifying RNA-programmable genomes. To successfully perform its editing tasks in cell genomes, it only requires the optimization of Cas9 expression and the matched design of gRNA. According to Nishimasu *et al*. (2014) [26], in these types of nuclease systems, the gRNA directed Cas9 proteins to recognize and cleave target-specific DNA sequences with a short protospacer adjacent motif (PAM) of 17–20 nucleotides using the Watson–Crick base–pairing interactions.

Streptococcus pyogenes Cas9 (SpCas9) and *Staphylococcus aureus* (SaCas9) are the two types of natural CRISPR/Cas9 systems that are most frequently employed for study (Ran *et al*., 2013) [27]. Additionally, different bacterial species' Cas9 nucleases recognize various PAM sequences for finding targets (Lino *et al*., 2018). For example, SpCas9 uses "NGG" PAM as a binding target while SaCas9 uses "NNGRRT" PAM (Xie *et al*., 2018) [29]. The CRISPR/Cas9 system's application horizons in life science have significantly increased over the past few years, and it is currently at a stage of rapid development. CRISPR/Cas9 machinery has enormous potential for focusing on infectious viruses and eliminating their reservoirs to improve human health because it is an efficient, highly specific, and durable technique.

CRISPR/CAS9 Applications

Theoretically, the CRISPR/Cas9 technology can be used to edit the double-stranded DNA (dsDNA) of viral invaders in both in vivo and in vitro systems, in addition to targeting any specific nucleotide sequences in the human genome. Moreover, the Cas9 endonucleases can now target a variety of different genomic loci in a single cell thanks to the innovations of Cas9 outfitted with several single guide RNAs (sgRNAs) (Zhou *et al*., 2014) [30]. Additionally, the CRISPR/Cas system gains many other novel functions from the Cas9 variants and orthologs, such as targeted gene mutation, transcriptional activation and inhibition, epigenetic alteration, imaging of DNA loci, and single base mutation (Miller *et al*., 2020). Furthermore, any DNA or RNA virus having a DNA intermediary in its life cycle may theoretically be susceptible to viral eradications from cells using CRISPR/Cas9 technology (Mohammadzadeh *et al*., 2020) [32]. Therefore, the CRISPR/Cas9 technique with functional diversities shows great promise for focusing on various viral life cycle developmental stages and has the potential to facilitate an efficient and long-lasting genetic therapy against human viruses. This article will cover the CRISPR/Cas9-based antiviral strategy to manipulate the main human infectious viruses, including HIV, HBV and HPV.

Human immunodeficiency virus (HIV)

A viral communicable disease is the acquired immunodeficiency syndrome (AIDS), which is brought on by HIV infection. HIV, a significant global disease that mostly consists of HIV-1 and HIV-2, calls for cutting-edge therapeutic treatments. More than 36.7 million individuals worldwide are infected with HIV, and there are more than 5,000 new cases diagnosed every day, according to a recent UNAIDS report (Dash *et al*., 2019) [33]. HIV-1 differs from HIV-2 in that it is more transmissible and harmful in the human host (Campbell-Yesufu and Gandhi, 2011) [18]. Severe CD4+ T-cell depletion brought on by active HIV-1 replication in vivo eventually leads to the development of the so-called chronic illness of AIDS. Antiretroviral therapy (ART) and high active antiretroviral therapy (HAART) have been extremely effective in preventing the deadly AIDS epidemic and even saving lives (Lu *et al*., 2018) [16]. These HIV therapies, which aim to block different phases of the viral life cycle are however, unable to completely reverse the disease since HIV-1 has become permanently incorporated into the host DNA. In light of these findings, scientists have concentrated on treating AIDS using CRISPR/Cas9-based gene editing technologies in an effort to open up a wide range of new opportunities for HIV-1 prevention and treatment (Dampier *et al*., 2014) [13].

Since Cho and Ebina, respectively, announced the first two CRISPR/Cas9-based applications in the prevention of HIV-1 in 2013 (Ebina *et al*., 2013) [14], a plethora of studies using CRISPR/Cas9 technology as a technique for treating HIV-1/AIDS have been created quickly (Khalili *et al*., 2015) [12]. Targeting viral genomes and host genes have so far been the two main strategies for preventing HIV-1 infection. The C-C chemokine receptor 5 (CCR5) gene, C-C-C chemokine receptor 4 (CXCR4) gene, parvoviral DNA-encoding viral proteins, and the HIV 5′ and 3′ long terminal repeat (LTR) are the primary editing targets of CRISPR/Cas9 therapy (Liu *et al*., 2017) [10]. Despite the development of Cas9/multiplexed-sgRNA technology, there are yet no studies that specifically and jointly target the two coreceptor genes CCR5 and CXCR4 using CRISPR/Cas9 molecular scissors.

Although HIV was discovered over 30 years ago, there is still no effective anti-HIV vaccine on the market (Haynes, 2015) [11]. Since a decade ago, the "Berlin patient" has been widely acknowledged as the only case of HIV-1 cure (Gupta *et al*., 2019) [8], and the "London patient" is now likely to be the second (Gupta *et al*., 2020) [9]. Scientifically speaking, stem cell transplantation (SCT) is not a recommended treatment for HIV/AIDS. According to these two case reports, SCT was initially developed to treat cancer rather than HIV/AIDS in

the two patients. Fortunately, the inadvertent treatments do offer optimism for the potential application of individualized gene therapy for AIDS in the future.

Hepatitis B virus (HBV)

HBV is still considered to be a serious health issue based on the estimated 350–400 million chronic HBV carriers worldwide (Seo and Yano, 2014) [7]. It is a hepatotropic DNA virus that replicates by reverse transcription in host hepatocytes at the stage of RNA intermediates (Locarnini *et al*., 2013) [6]. In chronic HBV infectors, liver cirrhosis and liver cancer can arise rather frequently. Eight genotypes (A-H) of the HBV genome have been determined taxonomically; between any two of them, there are approximately 8% nucleotide changes (Sunbul, 2014) [5]. Given the low likelihood of sustained viral response (SVR) or cure in HBV-infected individuals, novel and more potent HBV treatment regimens must be developed. The CRISPR/Cas9 technology is developing quickly, opening up possibilities for novel methods to the diagnosis, prophylaxis, and therapy of HBV-related viral illnesses. As is well known, the main challenge to the complete eradication of chronic hepatitis B (CHB) in spite of current antiviral therapies such nucleoside analogues (NAs) and interferon-alpha (IFN-a) is the survival of covalently closed cyclic DNA (cccDNA) of HBV (Emery and Feld, 2017) [15]. Gene therapies currently offer great promise for entering clinical applications after overcoming several technical difficulties and have emerged as a promising prospective treatment for HBV infections, particularly in efficiently targeting cccDNA.

Numerous studies have used specially designed Cas9/sgRNA (or Cas9/multiplex gRNA) combinations to alter just one locus (which is typically in the conserved region of the HBV genome) in order to successfully inhibit viral replication and production (Liu *et al*., 2016) [38]. Many research groups have worked on the applications of CRISPR/Cas9 for the simultaneous targeting and cleavage of several functional loci (e.g., surface antigen region, X gene, reverse transcriptase (RT) gene, and epitomal cccDNA) in HBV genomes via cell cultures or mouse models (Ramanan *et al*., 2015). In addition to the CRISPR/Cas9 system itself, numerous other studies involving the fusion of CRISPR/Cas9, and additional techniques (such as various molecules or inhibitory systems) have been created with the aim of eradicating HBV genomes (Wang *et al*., 2017; Zheng *et al*., 2017) [10, 40]

Human papillomavirus (HPV)

The Papovaviridae family of tiny double-stranded DNA viruses, or HPVs, has about 150 different varieties that have been found so far. According to Ebrahimi *et al*. (2019) [3], the HPV genome is about 8 kbp in length, contains nine or ten open reading frames (ORFs), eight early viral regulatory proteins (E1–E8), and two late capsid proteins (L1 and L2). The significance of HPVs in relation to human diseases and public health must be underlined because they exhibit epithelia tissue tropism, sexual transmission and carcinogenic property (Moens, 2018) [1]. According to Gupta and Mania-Pramanik (2019) [8], persistent high-risk type HPV infection, such as HPV-16 and HPV-18, is strongly linked to the onset of cervical cancer in women. Additionally, HPV can cause genital warts, head and neck malignancies, and additional types of anogenital cancer in both men and women (Chen *et al*., 2018) [2]. Due to the virus's capacity to reduce activity in a host cell in order to evade host immune surveillance, there is currently no clinical treatment for HPV infection that can produce a satisfactory result (Lee, 2019) [4]. As a result, it is very challenging to remove a viral genome from an infected host cell in a latency state.

According to the findings, the CRISPR/Cas9 strategy offers a great deal of potential for advancement as a clinically useful treatment for disorders linked to HPV. Because it can fill in the technological gaps in pharmacological therapy when a vaccination is already available, CRISPR technology represents a revolutionary approach for treating both HBV and HPV. To enhance the therapeutic effects, CRISPR-related technologies still need to be developed.

Conclusion

Within the infectious life cycle, virus-host interaction is a variable and ongoing process. The existence of numerous challenging viruses has prompted the ongoing improvement of anti-virus strategies. Future applications of CRISPR/Cas-based genetic targeting technologies offer a different treatment option for diseases caused by viruses. The CRISPR/Cas9 technique has so far shown promise in treating a wide range of human diseases, including cancers, viral infections, and genetic disorders. This technique is growing more potent and has already been extensively used in research on preventing and combating additional human infections in addition to the viruses mentioned above.

Additionally, CRISPR/Cas9 technology has been used to study the biological mechanisms underlying viral carcinogenesis as well as to treat viral infections. In conclusion, further work on creating CRISPR/Cas systems will increase the set of tools available to us, allowing us to better comprehend the intricate biological processes connected to hosts and viruses. Before being used in clinical settings, CRISPR/Cas9 will need to undergo significant refinements and advancements for gene treatments.

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