1. Introduction

A major cause of morbidity and mortality around the world is infectious diseases. One of the major agents responsible for causing emerging infectious diseases is the virus [4]. Many diagnostic and therapeutic approaches have been developed till now for treating the viral diseases. But due to evolution, the genome of viral pathogens changes and in this way the treatment approach used for the same virus previously becomes ineffective.

CRISPR (Clustered Regularly Interspaced Short Palindrome Repeats) Cas (CRISPR-associated protein) system is natural defense mechanism of bacteria against pathogens. It uses guide RNA for specifically manipulating the specified region of genome. At target site, Cas enzymes introduce dsDNA break. PAM (Proteospecter Adjacent Motif) helps in recognition of target site [53]. PAM also helps distinguishing self and non-self-sequences [72]. If any mutation occurs in PAM region the ability of Cas enzyme to work properly will be compromised [54]. In this way, target pathogen will successfully escape from immune response of host [7, 53]. CRISPR provides application in introducing foreign DNA into host chromosomes as new spacer [3], this prevents the invasion of same pathogen in future.

There are six types of CRISPR systems. Different Cas proteins with crRNA are used for each class in CRISPR interference [116]. Type-I and type-III CRISPR systems uses complex of multiple Cas proteins for binding of crRNA and degradation of target sequence [106]. Type II CRISPR systems uses only one Cas protein for target recognition and cleavage with different nuclease domains: RuvC or HNH [38, 39, 54]. In Type II CRISPR systems, tracrRNA is small non-coding RNA required for maturation of crRNA [26].

The genes cas1 and cas2 are absent in type IV CRISPR/Cas systems, which are frequently not connected to CRISPR arrays. A significantly reduced large subunit (csf1) and two genes for Receptor Activity-Modifying Proteins (RAMP) from the Cas5 (csf5) and Cas7 (csf2) groups make up effector complex of type IV systems [71]. The presence of single effector protein termed Cas12 is the most important aspect of type V
CRISPR/Cas system [123]. The active endonuclease Cas12a in type V CRISPR/Cas system is able to perform targeted cleavage without the aid of an additional tracrRNA [127]. In the type VI CRISPR/Cas system, higher Eukaryotes and Prokaryotes nucleotide-binding (HEPN) domains constitute a distinguishing characteristic. It has been concluded that type VI systems are, in terms of substrate recognition, less strict than other types since they only target RNA, which has fewer negative side effects on the cell [16].

CRISPR/Cas system provides platform for genome imaging, editing genomes and epigenetic modulation. This technology allows the study of function of specific genes in disease onset and progression, activation or deactivation of cancer suppressor genes and oncogenes respectively [15, 29, 49, 113]. CRISPR/Cas9 based genome engineering is used for treating different diseases: sickle-cell anemia, viral infections, cancer, cystic fibrosis, muscular dystrophy, genetic, neurodegenerative, cardiovascular, and immunological disorders [5, 45, 70, 101, 120]. In this review CRISPR system application against viral diseases will be discussed. It involves CRISPR system for studying, detecting, and treating AIDS, COVID-19 and Herpes virus. The limitations and challenges of CRISPR/Cas system will also be discussed.

2. CRISPR/Cas system based genome editing

Recognition, cleavage and repair are three steps that make up CRISPR/Cas9 system for editing of genome [74]. Chosen sgRNA drives Cas9 and recognizes target sequence in gene of interest via its crRNA complementary base pair component. Without sgRNA, Cas9 protein remains dormant. A brief conserved DNA sequence downstream of cut site, PAM is made at a position 3 base pairs upstream from where the Cas9 nuclease generates double-stranded breaks (DSBs) [13]. PAM size varies based on species of bacteria. The Cas9 protein, most popular nuclease in genome editing tools, can identify PAM sequence at 5-NGG-3 (N can be any nucleotide base). When Cas9 locates target site with right PAM, it causes local DNA melting, which is followed by synthesis of an RNA-DNA hybrid. However, process through which Cas9 enzyme melts target DNA sequence is still not fully known. After that, Cas9 protein is activated to begin cleaving DNA. RuvC and HNH domains separate target DNA into its complementary and non-complementary strands, primarily causing blunt-ended double-strand breaks (DSBs). Host cellular machinery then fixes DSB (Fig. 1) [73, 53].

Non-homologous end joining (NHEJ) and homology-directed repair (HDR) pathways are used for repairing the DSBs caused due to Cas9 protein in the CRISPR/Cas9 process [68]. NHEJ participates in DSB repair throughout the whole cell cycle by joining DNA fragments via an enzymatic mechanism in external homologous DNA absence. Despite being most common and efficient cellular repair method, it is error-prone and can result in small random insertions or deletions at the cleavage site, which could cause frame shift mutations or premature stop codons [124]. HDR is incredibly precise and needs homologous DNA template usage. The late S and G2 phases of the cell cycle are when it is most active. A significant number of donor (exogenous) DNA templates bearing sequence

Fig. 1. CRISPR/Cas system mechanism of action
of interest are necessary for HDR in CRISPR gene editing. By inserting donor DNA template with sequence homology at anticipated DSB site, HDR carries out precise gene insertion or replacement [124].

3. CRISPR/Cas strategy against the viral diseases

CRISPR/Cas system provides application in:
1. Restricting viral entry in host: disease is caused only if the virus had got successful entry into host cell. If the receptors responsible for viral entry are modified, then viral entry can be prohibited. CRISPR/Cas9 helps in modification of viral receptors.
2. Editing the genome of virus for make it unable to replicate by introducing mutations in genes.
3. Target the latent infections and helps in their complete removal.

The contributions of CRISPR system against Coronavirus Disease-2019, Acquired Immunodeficiency Syndrome and Herpes virus will be discussed below.

3.1. COVID-19

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) closely resembles SARS-CoV-1 and World Health Organization named the disease as COVID-19 [42] due to its emergence in 2019. It is infection of upper and lower respiratory system. It has an animal origin first reported in Wuhan city of China where 102, 103 people were infected from this virus [14]. The virus spread extensively in the Wuhan region of China and then this disease spread worldwide.

The family of Coronaviruses is Coronavirinae and they are the largest group in Nidovirales [95]. There are four types of corona viruses based on their host: α and β (MERS-CoV, SARS-CoV-1 and SARS-CoV-2) are human pathogens while γ and δ are avian pathogens [132]. Seven coronaviruses have been discovered till now [24, 130] including SARS-CoV-1, MERS-CoV, OC43, 229E, NL63 and HKU1 [102] and seventh one is SARS-CoV-2.

SARS-CoV-2 contains single stranded genomic material (RNA) and is beta-coronavirus [118, 129] which size is approximately 29.9 kb [35]. Its genome encodes 16 nonstructural, 4 structural and 5 to 8 accessory proteins [118]. It contains replicate genes which encodes essential proteins [99, 105, 117] for nucleocapsid formation, viral replication, and spikes formation [24, 99, 105, 117]. Coronavirus spike proteins identifies ACE2 receptor in host and binds to it [25] and helps in fusing virus & host cell plasma membrane [97]. SARS-CoV-2 then enters the cell by endocytosis [61, 114] & releases its genetic material to be translated by host machinery and produces new viral particles [86].

CRISPR/Cas system against COVID-19

Coronaviruses are involved in respiratory diseases containing RNA as genetic material [67]. Therapies, drugs, and vaccines discovered to cure coronavirus focus on the inhibition of virus entry in cells [87]. Many diagnostic techniques are also developed but they have low accuracy & efficiency. CRISPR system on the other hand identifies and diagnoses the virus with higher efficiency. COVID-19 can be detected via CRISPR based technologies including: SHERLOCK, DETECTR and CREST [32]. CRISPR system also provides therapeutic approaches for COVID-19 targeting different regions of Coronavirus genome.

CRISPR based restriction of viral entry into host cell

The cells which contain ACE2 on their surface are highly targeted by SARS-CoV-2. ACE2 high expression is on kidney, lungs, and heart cells surface [28]. ACE2 helps in maintaining blood pressure [98]. It can be made target for preventing entry of SARS-CoV-2 into host cell but in such a way that its function is not disturbed. For this purpose, only its binding domain can be edited by use of CRISPR.

In-silico study, Pedro Tanaka et al. evaluated the impact of replacing particular residues of amino acid in ACE2 alpha-helix, which are important in SARS-CoV-2 viral binding, using techniques for protein modeling. To interact with the virus, they created a CRISPR/Cas9 model system that destabilizes a portion of the ACE2 protein (N-terminal alpha-helix amino acid residues Phe28, Lys31 and Tyr41). The destabilizing effect of the edited alterations preserved the helicoidally structure at the ACE2 side while weakening interface connection between SARS-CoV-2 S protein and ACE2 alpha-helix [104]. Most important amino acid residues for ACE2 catalytic action, Arg273, His505, His345, Arg169, Arg514, and Tyr515, are preserved while also maintaining the structural shape of this system [44].

PAC-MAN strategy for treating COVID-19

PAC-MAN is a therapy for treating coronavirus based on CRISPR technology developed by Abbott et al. [1, 122]. PAC-MAN reorients Cas13d system capacity to cut RNA of coronavirus in humans and thus inhibiting its life cycle. This CRISPR/Cas13d system is extracted from Ruminococcus flavefaciens XPD3002 [60]. Cas13d calls up crRNAs containing 22 nucleotides-spacer seq. crRNA helps the Cas13d proteins in degrading the target viral genome [11, 39, 54]. Cas13d PAC-MAN strategy minimizes the replication and expression of coronavirus by its action of cleaving SARS-CoV-2 RNA genome. It thus results in degradation of viral mRNAs that can produce viral essential proteins [94] (Fig. 2).
Abbott et al. reported two conserved regions in SARS-CoV-2 important for its functioning and replication: Nucleocapsid and RdRP genes [1]. They target these genes and constructed crRNA library. They tested 40 designed crRNAs on human lung epithelial A549 cells. For this purpose, proteins of SARS-CoV-2 were tagged with fluorescent reporter genes and then transfected them to lung epithelial cells under study along with crRNA and Cas13d. They reported that many crRNA effectively worked in reducing the formation of mRNA from RdRP and nucleocapsid genes with 85% and 70% reduced GFP signal, respectively [1].

Coronavirus NSP1 to NSP16 proteins can be targeted by CRISPR/Cas13 in treatment. Polyprotein encoding ORF1a/b plays role in pathogenesis of SARS-CoV-2 could be a target for CRISPR. gRNA have been designed for targeting the regions of ORF1a/b coding peptides [1, 79].

In treating the COVID-19 pandemic, the suggested CRISPR-based antiviral medicines show considerable promise. However, the justification for the effective use of antiviral CRISPR therapeutics in humans is solely based on an in vitro trial. However, the main obstacle preventing clinical use is the transport of CRISPR components into patients. Even though current delivery techniques like polymer-based, liposomal, and adeno-associated virus (AAV) vectors seem doable, they are only somewhat effective [111]. Engineering Cas13 nuclease so that it can adhere to viral particles and deliver CRISPR components along with virus into host cell is one possible method to achieve selective and effective delivery. Once virus, that possesses its own shredding machinery (CRISPR/Cas), reaches cell, the CRISPR system can quickly target viral RNA and cleave it before Viral RNA is replicated or translated. Ironically, the virus itself may be employed to help distribute information selectively and effectively [76].

ABACAS

ABACAS is a therapeutic strategy against virus in which Cas13 fuse with specific AB (antibody) of S protein of SARS-CoV-2 helping in delivery of Cas13 in infected cells. Detection of SARS-CoV-2 S-protein could be made by using ABACAS fused antibody that helps in delivery of virus along with Cas13 in cells being infected [56, 125]. RNA of virus is degraded when it is exposed to ABACAS in infected cell (Fig. 3). In this way off-tissue effects are reduced by this approach due to target delivery of viral RNA by CRISPR/Cas13. This strategy also helps in preventing viral entry into cell.
ery by virus. CR3022, LY-COV555 and REGN-COV2 are ideal for ABACAS development because they bind SARS-CoV-2 S protein tightly. The efficiency of CR3022 was tested on 293F cell lines by Joyce et al. [56].

SHERLOCK

SHERLOCK (Specific High-Sensitivity Enzymatic Reporter unLOCKing) is FDA approved detection method [40] introduced to detect COVID-19 with sensitivity of 10–100 copies/μl of input [128]. This technique is based on CRISPR Cas13 of type VI. It includes combined action of RT-RPA and Cas13a nuclease activity [41]. crRNA-Cas13a complex bind & then cleave specific target (RNA) along with non-target fluorescent reporter coupled RNA. As a result of cleavage fluorescence is produced that enables real time & rapid virus detection [119].

CREST

CREST (Cas13-based, rugged, equitable, scalable testing), developed by Rauch et al. is used for SARS-CoV-2 detection. This involves utilizing Cas13 detection along with PCR, transcription, and then final detection of positive results by using fluorescence detector. It targets viral RNA with LOD 10 copies/μl [88].

DETECTR

Cas12 based DETECTR (DNA endonuclease-targeted CRISPR trans reporter) detects SARS-CoV-2. It also uses RNA sample of patient. Its principle is based on RT-LAMP [82], that amplifies RNA in sample extracted from oropharyngeal or nasopharyngeal. Reporter molecule cleaves from the target coronavirus sequence followed by its recognition by Cas12. The target is then cleaved by Cas12. The overall procedure takes half an hour for completion. In this time, RT-LAMP reaction takes 20 minutes at 62°C temperature followed by Cas12 detection requiring 10 min at 37°C and final visualized or detection of virus on dipstick. Detection of Nuclease protein and Envelope protein genes is required for positive test output to confirm infection caused by SARS-CoV-2. Detection limit for DETECTR is 10 copies/μl [10].

3.2. AIDS

AIDS is an immune system suppressing disease caused by Human Immunodeficiency Virus infections [9] transmitted by vertical transmission, sex, and intravenous injections [23, 46].

HIV-1 & HIV-2 are two types of HIV, causing AIDS, having numerous similar characteristics [81]. Pathogenicity and transmissibility of HIV-2 is less in comparison to HIV-1. The RNA genomic size of HIV-1 is 9.8 kb. LTRs present at the edges of genome are responsible for coding viral proteins functional in viral replication and invasion: gag, vif, pol, vpu, env, rev, vpr, tat, ASP and nef [12, 69].

HIV-1 invades immune cells, and its receptor is CD4 on which it binds with the help of gp120 envelope protein. CCR5 or CXCR4 are the co-receptors with which virus interacts in progressing disease. Main target host cells are monocytes, astrocytes, dendritic cells, T cells, microglial cells and macrophages. Viral life cycle is intricate due to which it is difficult to eliminate virus completely (Fig. 2). Active and latent infections are caused because of entry of HIV-1 [17]. In case of active infection, provirus keeps on replicating and host cell buds to produce progeny virions.

Latent infections are arbitrated by environment of chromatin [37], integration sites of HIV-1 provirus [103], RNA interference [92] and transcription factors [63]. As a result of latent infection reservoirs of resting infected astrocytes [77], microglial cells [78], T helper cells [21] and macrophages [98] are formed. It is difficult for antiviral drugs to reach these reservoirs which are in brains [36], gastro-intestinal tracts and lymphoid tissues [21].

CRISPR/Cas system based therapeutic & diagnostic approach against AIDS

HAART is used for treating AIDS inhibiting replication of Human Immunodeficiency Virus [96]. Highly active antiretroviral therapy (HAART) is medical regimen used for treatment and control of human immunodeficiency virus type 1 (HIV-1). It contains a variety of drugs from the antiretroviral groups [33]. Although latent viral reservoirs can be reduced in number by highly active antiretroviral therapy (HAART), they cannot be completely eliminated in HIV-1/AIDS patients. With the potential to treat HIV-1/AIDS, the CRISPR/Cas9 system has just been developed. To diminish HIV-1 infection and eradicate the provirus, it could be used to target cellular co-factors or the HIV-1 genome. It can also be employed to promote transcriptional activation of latent virus in latent viral reservoirs for eradication. [71, 51]. CRISPR technology is being used for inactivating viral genome by gRNAs with specific viral genes or LTR promoter site as a target [131].

To test the effectiveness of lentivirus-mediated intravenous delivery of CRISPR in editing HIV-1 genome from circulating peripheral blood mononuclear cell engrafts, Bella et al. used the NRG mouse model, which has multiple immunodeficiencies that are primarily caused by the absence of T, B, and NK cells [84]. Targeted area of the viral genome, which was located inside the HIV-1 LTR, was excised from human peripheral blood mononuclear cells (PBMCs) placed in NRG mice spleens via lentivirus-mediated delivery of a multiplex of guide RNAs and Cas9 endonuclease. After being treated
with lentiviral vectors producing Cas9 and gRNAs, Sanger sequence analysis of the viral DNA confirmed the editing and removal of the proviral DNA fragment from the viral genome at the expected places [6].

**CRISPR based restriction of viral entry into host cell**

To eliminate host cell permeability to be infected by HIV-1, its receptors are targeted by CRISPR. Cocchi et al. reported that the viral entry can be prohibited by blocking or altering the receptors responsible for HIV-1 entry into host cell that are CD4 receptor and two co-receptors: CXCR4 & CCR5. We cannot alter CD4 receptor because it plays a necessary role in immune system but co-receptors can be edited. CRISPR/Cas9 has been widely used in disrupting CCR5 and CXCR4 expression. Cho et al. reported that CRISPR/Cas9 could be used to silence CCR5 receptor gene in HEK-293 T-cells by transfecting sgRNAs and Cas9 [20]. Bitti et al. stated that if a person has missing homoyzous 32 base pair in gene of CCR5 (CCR5132) shows a natural resistance against R5-tropic HIV-1 [8]. When CCR5 is being inactivated by CRISPR the viral envelope is susceptible to mutation that will result in shift of the usage of viral receptor from CCR5 to CXCR4 [34, 109].

Li et al. disrupted CCR5 expression by combining sgRNAs that target the fourth exon of CCR5 with adenovirus-delivered CRISPR/Cas9. In TZM-BL cells, two particular sgRNAs can generate more than 60% cutting efficiency. The outcomes were also confirmed in human T cell lines and Chinese hamster ovary (CHO) cell lines. Finally, they delivered the CRISPR/Cas9 system to human CD4+ T cells using a chimeric Ad5/F35 adenovirus vector in order to mute CCR5 expression, which protected cells from HIV-1 infection with high efficiency and little off-target consequences [64]. Meanwhile, numerous studies have documented CRISPR/Cas9-induced CXCR4 gene disruption. With the use of two sgRNAs that selectively target conserved CXCR4 regions and CRISPR/Cas9 supplied by lentivirus, Hou et al. successfully disrupted CXCR4 expression in human CD4+ T cells and rhesus macaque CD4+ T cells [101]. The CXCR4 [48] co-receptor or other replication factors of virus can be targeted by CRISPR strategy [52, 83].

**CRISPR based genome editing virus**

Ebina et al. used CRISPR technology for HIV-1 infection prevention for the first time in 2013. They used CRISPR for setting viral LTRs as target and thus suppressing the gene expression in cell line of immortalized T lymphocytes [31]. They used CRISPR/Cas9 to inhibit Human Immunodeficiency Virus-1 provirus replication and transcription. For this purpose, they targeted the NF-κB binding cassettes in TAR sequence of R region and LTR sequence of U3 region [31].

Hu et al. worked to remove genome of HIV-1 by using CRISPR/Cas9 technology in 2014. Cas9-gRNA was used by Hu et al. for targeting the conserved spots in HIV-1 LTR U3 region with no off-target editing and small geno-toxicity. They inactivated the replication and gene expression of virus in cell lines that were latently infected by HIV-1. The studied cell lines include microglial, pro-monocytic and T cell line [50].

In the past few years, CRISPR/Cas9 technology has been widely used in HIV/AIDS research employing experimental, laboratory-adapted HIV-1 strains due to its simple, high-efficiency, and limited off-target effect properties [30]. CRISPR/Cas9 systems are used for effective elimination of HIV-1 proviruses by targeting the viral genomic regions like: LTR region and several genes and proteins (Rev, Pol, Env, and Gag). Some of the studies based on the targeted viral regions for the elimination of HIV-1 by targeted delivery of CRISPR system via transfection and lentiviruses are explained in Table I.

**Targeting latent infections by CRISPR system**

CRISPR/Cas9 strategy was used to target provirus LTR in infected (HIV-1) T cell lines and then treating it with TNF-α [131]. Sequencing showed that target sites

<table>
<thead>
<tr>
<th>CRISPR-Cas system used</th>
<th>Delivery method for CRISPR components</th>
<th>Targeted regions</th>
<th>Cell line or organism studied</th>
<th>Targeted locus</th>
<th>Efficiency</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>SpCas9</td>
<td>Transfection</td>
<td>LTR (U3 region)</td>
<td>293T, Jurkat &amp; Hela</td>
<td>293T, Jurkat &amp; Hela</td>
<td>30–90%</td>
<td>[104]</td>
</tr>
<tr>
<td>SpCas9</td>
<td>Transfection</td>
<td>LTR (U3 region)</td>
<td>CHME5, TZM-BI, &amp; U937</td>
<td>101–127</td>
<td>312–341</td>
<td>30–90%</td>
</tr>
<tr>
<td>SpCas9</td>
<td>Lentivirus</td>
<td>LTR (R region)</td>
<td>293T-CD4-CCR5, hPSC, &amp; 293 Primary T cells</td>
<td>293T-CD4-CCR5, hPSC, &amp; 293 Primary T cells</td>
<td>64–486</td>
<td>485–507</td>
</tr>
<tr>
<td>SpCas9</td>
<td>Transfection</td>
<td>Rev (the second exon)</td>
<td>JLat10.6</td>
<td>JLat10.6</td>
<td>8513–8532</td>
<td>30%</td>
</tr>
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<td>Lentivirus</td>
<td>Gag/ Pol/ Rev/ Env</td>
<td>SupT1</td>
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<td>8497–8525</td>
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<tr>
<td>SpCas9</td>
<td>Lentivirus</td>
<td>LTR (U3 and R region)</td>
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<td>J.Lat FL &amp; SupT1</td>
<td>300–408</td>
<td>463–482</td>
</tr>
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Table I: CRISPR/Cas systems for elimination and targeting HIV-1 provirus
were mutated that causes the lowered replication and expression of virus [131].

When various sites of Human Immunodeficiency Virus-1 genome is targeted then deletion and interference of un-integrated part of proviral genome can be increased by using CRISPR technology [66]. When using two sgRNAs together for targeting various genomic regions of HIV-1, the escape and replication of virus can be prevented [62].

Wang G et al. reported that CRISPR editing by sgRNA causes the target site mutations in HIV-1 provirus resulting in its inactivation [126]. Although sgRNA causes inactivation of viral genes but some viruses escape from being cleaved by sgRNA. These viruses can be made less infectious by using CRISPR/Cas9 approach [62]. In presence of specific stimuli, the resting HIV-1 infected cells will become activated and undergo replication and starts infecting the neighboring cells. This will cause the re-formation of latent reservoirs and thus these reservoirs are the most important challenge for potent cure of AIDS.

The mutations caused by CRISPR/Cas9 differs from that of antiviral drugs [110]. Targets of CRISPR system in viral genome, which were poorly, conserved showed insertions or deletions by action of Cas9 while substitutions were observed in highly conserved regions. gRNA directs the Cas9 to target site on HIV DNA. Upon recognition Cas9 introduces dsDNA break at distance of three nucleotides from PAM. HIV DNA will then be repaired by host cell DNA repair mechanisms that results in mutations: nucleotide deletion, insertion, or substitutions at the site where Cas9 has cleaved. These mutations can either activate or inactivate the viral replication. Sometimes these mutations result in preventing recognition of HIV DNA by gRNA and virus will escape from the treatment [65].

3.3. Herpes Virus

Herpesviridae is a virus family that includes DNA viruses infecting birds, reptiles, and mammals. They are also capable of causing latent infections. There are three families of herpes viruses on basis of the sequence and organization of their genome: α, β, and γ herpes viruses. There are eight Herpes viruses that infect humans including: HSV-1, HSV-2, VZV, HCMV, HHV-6A, HHV-6B, HHV-7, EBV, and KSHV [108]. Their genetic material constitutes linear dsDNA having sequence of 125 to 295 kilo base pairs. When linear DNA of herpes virus enters nucleus of host it becomes circularized [85]. Genome of Herpesvirus encode approximately 70 to 200 ORF [100].

Latent infection development in permissive cells is one of the characteristics of these viruses. Viruses are difficult to be detected by immune system of host during resting stage because protein expression of virus is prohibited during latency [43]. When the resting virus got signals, they start replication again. HSV-1 causes herpes simplex keratitis, cold sores and corneal blindness while HSV-2 causes genital ulcers [91].

CRISPR based strategy against Herpesvirus

Herpes viruses cause many diseases and some of them lack symptoms. For treating the Herpesvirus-based diseases treatments involve using nucleoside analogs. These analogs can limit the replication of virus by restricting the activity of polymerase [75] but due to negligible effectiveness against latent viruses [22] they are to be replaced by some effective treatment strategies. Along with these issues’ viruses develop nucleoside analog resistance, such as against ganciclovir and acyclovir [22]. CRISPR/Cas9 is effective in providing protection to cell against infection and removing latent virus from the infected cell. This technology is also easy to use. It also allows the formation of mutant herpes virus to treat existing ones [93, 121].

CRISPR/Cas9 system for Herpesvirus-Host interaction studies

CRISPR/Cas9 is used in studying the role of different factors involved in controlling viral progression. Human antiviral factor IFI-16 binds DNA of virus and facilitates the antiviral response [27]. IFI-16 plays role in regulating induction and transcription of IFN-β. Diner BA et al and Johnson KE et al used CRISPR/Cas9 for determining IFI-16 role in HSV infection control. They generated IFI-16 knockouts [27, 55]. They reported that IFI-16 lowers gene expression of HSV-1 and prevent the attachment of transcription factors with promoters of HSV-1. Thus, ultimate result is the inhibition of viral replication and it might have played role in modification of histone [27, 55].

Anti HSV gRNAs against Herpesvirus

Besides editing the viral genome and constructing mutants of virus, CRISPR provides therapeutic approaches too to kill HSV within host body. anti-HSV gRNAs for targeting viral genome can cause change in specific genes making it non-functional. PC Roehm et al. and Van Diemen et al. reported in their studies that anti-HSV-1 sgRNAs can restrict the replication of virus in both human & non-human cell lines [90, 108]. PC Roehm et al. performed tests on human oligodendrogliaoma cells to check the effectiveness of anti-ICP0 sgRNAs that were designed by them against HSV-1. ICP0 is a viral protein responsible for viral replication and gene expression. They reported that these gRNAs had effectively reduced the viral replication lowering the load of HSV-1 to ~ 10-folds [90].
**Multiple gRNAs strategy and latent Herpesvirus**

Van Diemen et al. used CRISPR/Cas9 lentiviruses for inhibition of HSV-1 replication in cells of human lung fibroblast (MRC5). When one gRNA was used for inhibition of viral replication, viral titer lowered up to 4-log but resistant virus outgrowth was still present. Mutations were displayed at target site of gRNA by variants that results in making these variants resistant to be edited by CRISPR/Cas9. But if two gRNAs are used instead of one, multiple sites of viral genes can be targeted. In this way the chances of virus to become resistant is reduced resulting in complete removal of viral particles [107].

When the genome of HSV-1 is in its resting state in MRC5 infected cells, Van Diemen et al. reported that CRISPR/Cas9 was unable to target their genome while it targeted the genomes of viruses that produced from the re-activated HSV-1. They concluded that Cas9 activity might be interfered by resting genome of HSV-1 [107]. Latent genomes of HSV-1 are in inactivated state having close association with nucleosome [80]; nucleosome presence inhibits the binding and functioning of Cas9 at target site [19, 47].

Herpes viruses enter the cell and its dsDNA serve as target for CRISPR/Cas9. sgRNA induces viral gene disruption while multiple gRNAs result in viral genome fragmentation. If repair template is present, then mutation is inserted in herpes virus (Fig. 4).

Replication of herpes virus in lytic and latent infection is reduced by using CRISPR/Cas9 as reported by Diemen et al [107]. They determined efficiency of CRISPR against HSV, EBV, and HCMV. They designed sgRNAs for targeting EBV latent and lytic infection. Viral miR-BART6, smiR-BART5, and miR-BART16 were set as target of designed sgRNAs for models with latent infections. Transduction of gastric carcinoma cells latently infected by EBV was made to check the efficacy of CRISPR/Cas9 based sgRNAs. Viral EBNA1 and EBV Ori were set as target of designed sgRNAs for models with latent infections. In this way they successfully edited and downregulated the miRNAs, antigens, and Ori under study. In the same way, gene editing by CRISPR showed viral infections suppression [107].

Wang and Quake designed sgRNAs for targeting specific genomic regions of EBV that are responsible for transformation, structure, and latency of virus. They used this designed CRISPR/Cas9 strategy to treat EBV latently infected cells derived from patients. They successfully removed genome of EBV and decreased load of virus in quarter and half of cell respectively [115].

EBV genome EBNA1, W repeats and OriP regions were targeted by Yuen et al. sRNAs were designed by them. Copy number of EBV genome was reduced up to 50%. EBV latent infected cells were not affected by this suppression but it produced effect in other infected cells [126].

4. Limitations of CRISPR technology

The features of CRISPR/Cas enable its use in diagnostic and therapeutic. Tools of CRISPR as are no doubt simple to use, efficient and cheap as compared to other gene editing technologies but there are some limitations too (Fig. 5).

With its advantages of being safe, effective, and straightforward to utilize, CRISPR/Cas9 technology has been widely applied to cure human disorders such as...
CRISPR/CAS SYSTEM: AN EFFECTIVE TOOL AGAINST PATHOGENIC DISEASES

as Parkinson’s disease (PD) Duchenne muscular dystrophy (DMD) and HIV/AIDS [18]. However, various restrictions must be taken into account while planning clinical trials. Potential off-target effects, which could result in significant gene changes and chromosomal translocations, are a big worry [59]. By using ChIP-seq, some researchers demonstrated that the off-target cleavage caused by Cas9 was significantly less than that caused by ZFNs, TALENs, and homing endonucleases [30]. By putting the Cas9 gene under the control of a minimum HIV-1 promotor, which is activated by viral transcriptional activator Tat, Kaminski et al. modified the CRISPR/Cas9 system. With this method, Cas9-expressing HIV-1 infectious cells are created, while difficulties brought on by unnecessarily high Cas9 expression in cells are diminished [58]. If not, the off-target effects can be reduced by directly delivering Cas9 RNPs rather than expressing plasmid in target cells. RNP treatment has been demonstrated to cause cytotoxicity in cells by inducing innate immune responses in some cells. Inhibiting innate immune responses and reducing cell mortality can be accomplished by chemically creating sgRNA and using phosphatase to remove its 5’-ppp [58].

How to successfully transfer this big complex into cells that are HIV-1-infected is still another significant hurdle in the application of CRISPR/Cas9. Adenoviral, lentiviral, and adeno-associated viral vectors are the main delivery vehicles, according to prior publications [112]. Since adenoviral vectors can incorporate large DNA fragments and their immunogenic effects in clinical trials have improved, they can be employed in a variety of CRISPR/Cas9 systems. However, production of recombinant adenoviral vectors may still be a significant drawback [2].

5. Conclusion

Traditional therapies used to treat viral diseases focus on boosting the immune response of the patient. So that their immune system can determine the viral proteins and prohibit them from entering the cells and controlling the spread of disease. CRISPR/Cas9/12/13 systems serve as a potential tool against the viral pathogens. They introduce dsDNA breaks followed by its repairing and editing through host repair machinery. CRISPR systems are new in therapeutics and got attention after its first usage in 2013 by Ebina et al. [31]. When combined with other treatment strategies it increases treatment efficiencies. When pathogenic viruses are studied, viruses of Coronaviridae, Retroviridae and Herpesviridae family are said to be belonged to highly lethal ones. These viruses are targeted by CRISPR/Cas13/9/12 that inhibits the viral proteins and ORF. Among them it also provides platform to study new viruses, their pathogenicity, and treatments for their infections. Where there are advantages of CRISPR systems it has some challenges including its safe delivery into host body and less effectivity due to some viral proteins.

References


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