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CRISPR-Cas technology and use in antiviral development

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ABSTRACT

Throughout history, viral diseases have periodically reached pandemic proportions and have had devastating effects on human history. With the advancement of science and technology, antivirals have been developed and continue to be developed in the fight against viral diseases. The difficulty in the development of antirival has tried to use new technologies in the development of antiviral. One of these new technologies is the CRISPR/Cas system. CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) defines a series of DNA sequences called clusters of regularly interspaced palindromic repeats, and CAS defines endonucleases that use CRISPR sequences as a guide to recognize and cut specific DNA chains related to the CRISPR region. While protein engineering systems defined before CRISPR/Cas systems can be off-target and cause undesirable results, the CRISPR/Cas system reduces this risk by Watson-Crick base pairing. In the fight against viral infections of humans and animals, vaccine protection methods are widely used due to the problems in developing antivirals. On the other hand, the difficulty of vaccination, inadequacies in long-term immunity and the emergence of new infections or epidemics due to mutational changes in viruses pave the way for developing new antivirals. This article emphasizes the history and working areas of CRISPR-Cas technology and the potential applications of this method in antiviral development for human and animal viruses.

Keywords: CRISPR-Cas technology, antivirals, antiviral development

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Introduction

CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) refers to a series of DNA sequences called clusters of regularly interspaced palindromic repeats, CAS refers to endonucleases that use CRISPR sequences as a guide to recognise and cut specific DNA strands related to the CRISPR site. Before the discovery of CRISPR, RNA-targeted nucleases, zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs) or self-directed meganucleases were widely used in protein engineering (Bogdanove and Voytas, 2011; Jinek et al., 2012 as cited in Gök and Tunalı, 2016). Although these methods are successfully applied, the proteins produced can sometimes cause off-target effects and toxic effects. On the other hand, CRISPR technology is based on simple Watson-Crick base pairing, which reduces the risks in different techniques. In the fight against viral infections of humans and animals, vaccine protection methods are widely used due to the problems in developing antivirals. On the other hand, the difficulty of vaccination, inadequacies in long-term immunity, and the emergence of new infections or epidemics due to mutational changes in viruses pave the way for developing new antivirals. In this article,

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developing new antivirals. In this article, the history and working areas of CRISPR-Cas technology and potential applications of this method in antiviral development are emphasized.

History of CRISPR-CAS technology

Firstly Ishino et al.(1987), while doing a gene sequencing study on *Escherichia coli*, noticed these gene regions repeating at certain intervals, but he could not make sense of these regions. In the following years, Mojica also noticed these repetitive gene regions in the 1990s and 2000 Mojica et al. named these regions SRSRs (Short Regularly Spaces Repeats) and focused his studies on SRSRs (F. J. Mojica et al., 1993, 1995, 2000).

Jansen et al. (2002) with Mojica et al. named these repetitive regions CRISPR. In this in-slico study, the genomes of more than 40 microorganisms were sequenced and as a result of these sequences, they found that CRISPR regions can be more than one and that there is a protein region following these CRISPR regions (CRISPR-Cas). Cas regions were found to bind to helicases, ligases and DNA.

In 2005, Mojica et al. proposed the idea that these repetitive regions are an immune site against phages in microorganisms (Mojica et al., 2005).

Various studies have been carried out around the world to find out the importance of CRISPR. As a result of these studies, it was discovered that these repetitive regions originated from bacteriophage. With this discovery, it started to be investigated whether CRISPR regions are related to the immunity of bacteria.

Barrangou et al., (2007) in their study on *Streptococus thermophilus*, as a result of their sequences, it was observed that as the number of CRISPR regions increased in the genome of the bacterium, the bacterium showed resistance to phages and did not die.

Since 2010, the basic mechanism of the CRISPR system has been investigated and revealed by various scientists. Doudna and Carpentier received the Nobel Prize in 2020 for their work on the mechanism of CRISPR, which largely revealed the functioning of the system. In almost the same period as Doudna and Carpentier, Siksyns also found the working principle of CRISPR and these three scientists received the Nanosience Prize (Cross Ryan, 2018; Nobel Prize, 2020).

During this period, studies have been carried out to reveal the CRISPR mechanism, defining the functional mechanisms of the CRISPR Type II system, the simplicity of this system for genome editing and the basic components. In a study on Streptococcus *thermophilus*, Cas9 was found to be the only enzyme in the cas gene clusters that enables the cutting of target DNA (Garneau et al., 2010).

Deltcheva et al. (2011) revealed trans-activating crRNAs (tracrRNAs). TracrRNAs are RNA hybrids formed from Cas9 and endogenous RNA III and are required for transcription of the CRISPR sequence into mature crRNAs in the CRISPR Type II system (a CRISPR system using Cas9).

Sapranauskas et al. (2011) transplanted the CRISPR Type II region from *Streptococus thermophilus* into *Esherichia coli*, demonstrating that type II CRISPR is transferable and can be rearranged in different bacterial strains.

In 2013, the CRISPR-Cas9 system started to be used in genome editing with the successful use of Streptococus thermophilis and *Streptecocus pyogenes* for editing mammalian cells with CRISPR in two separate studies (Cong et al., 2013).

In their paper, Ran et al. (2013), showed that CRISPR could be used to identify the mammalian genome.

CRISPR-CAS system

In fact, CRISPR-CAS is a defence mechanism that bacteria and some archaea involved in fermentation etc. develop against phages coming from outside. Studies have shown that this defence mechanism is provided by CAS proteins in the bacterial genome. (van der Oost et al., 2009) (Bikard and Marraffini, 2012).

In short, Cas (CRISPR-associated protein) in the CRISPR-Cas system can also be called the adapted antiviral immune system of prokaryotes. Cas is widespread in archaea (~90%) but is also present in some bacteria (~50%) (Bayat et al., 2018).

CRISPR sequences are formed by the insertion of foreign genomes, foreign genome products, transplanted products of non-repeating sequences into repetitive sequences; certain parts of the genetic material of the phage infecting bacteria or archaea are integrated into CRISPR sites together with repetitive regions (Gök and Tunalı, 2016).

A bacterial or archaeal genome may contain different CRISPR regions. While the repeat sequences are around 21-48 base pairs (bp), the gaps, which vary from a few to several hundred, are 26-72 bp in length. At the 5' end of the first repeat sequence in the CRISPR region, there is a leader sequence rich in Adenine and Thymine. This leader sequence is approximately 550 bp in bacterial genomes. A genome may contain single or multiple CRISPR sites (Jansen et al., 2002; Pourcel et al., 2005; Rath et al., 2015; as cited in Kılıç Tosun and Kesmen, 2022).

The genomic components of the CRISPR system

are formed by trans-activated crRNA (tracrRNA), which is formed by short direct repeat sequences led by the cas protein. In between these repeat regions, gaps are formed from non-repeating regions. These spacer regions are mainly composed of invasive elements of the virus or plasmid. The CRISPR-Cas system provides the organism with resistance to foreign genetic material that has already been transferred to the CRISPR site in the organism's genome. (Bayat et al., 2018). This immune system consists of three stages:

1) Gap fragments obtained from exogenous nucleic acid are inserted into the CRISPR site and adaptation is achieved. The gaps to be inserted are determined by protospacer adjacent motifs (PAM) in the genome of the invading phage or plasmid. PAM is specifically recognised. These conserved sequences of 2-5 nucleotides of the genome of the invading microorganism are adapted to the CRISPR site by repeating genes in the spacer portion of the invading genome. The lack of PAM in the CRISPR region prevents the region from being cut, mutations in the PAM region can cause the invading microorganism to escape from CRISPR and thus from the host organism's immunity. (Jiang and Doudna, 2015)

2) The target region in the DNA of the invading microorganism is inserted into the CRISPR site and transcribed into pre-CRISPR RNAs, the transcribed pre-crRNA Cas endoribonucleases are converted into cr-RNAs corresponding to the invader genome, showing base pairing with the target sequences.

3) In the last step, nucleic acids belonging to the invader genome are targeted with crRNA, homologous sequences are cut with Cas nucleases, thus preventing the replication of viruses and plasmids. Nucleic acids of the invader genome are targeted according to the Watson-Crick base pairing principle (Gök and Tunalı, 2016)

Types of CRISPR system

The components of CRISPR are basically divided into two parts. The first is the Cas enzyme, which cuts the DNA strand at specific locations in the genome. The second is Guide RNA (gRNA), which drives the Cas protein to the target region of the genome.

The widely used classification was developed by Haft et al. in 2005 using the topology of the Cas1 phylogenetic tree on around 40 archaea and bacteria and CRISPR-Cas system typing on eight genomes. (Haft et al., 2005)

The names of the four core Cas genomes were proposed by Jansen et al. in 2002. The other two core Cas gene names Cas 5 and Cas 6 were added later. The names were proposed for genes encoding proteins unique to each of the eight genes. For example, a unique system found in *E. coli* was found and named cse1 (CRISPR system of *E. coli* gene number 1), cse2, cse3, cse4 and cse5 (elsewhere these *E. coli* genes have also been named casA, casB, casE, casC, casD, but this difference has caused confusion).

Although the diversity of Cas proteins, the presence of different CRISPR regions in a genome and the ability to switch between living organisms make classification difficult, according to the organisation of the CRISPR region and the content of Cas genes, the CRISPR-Cas system is basically divided into three main systems and 11 subsystems. Apart from these, there are also Unclassified CRISPR-Cas Systems.

Type I CRISPR-Cas System: Type I: CRISPR-Cas systems contain 6 subsystems from 1A to I-F. Type I-CRISPR-Cas system gene region, typically the cas3 gene. This gene synthesises a wide range of proteins with helicase DNAase activities. In addition, these genes probably also synthesise Cascade-like complexes of different composition. These complexes include proteins in the RAMP (receptor-activated regulatory protein) superfamily, including many Cas5 and Cas6 family proteins. Cas7 protein was also detected by HHPred method. The CRISPR-Cas function contained in these complexes includes large proteins such as Cse1 as well as small alpha-helicase proteins or subunits such as Cse 2. In the cascade complex there is a RAMP protein with RNA endonuclease activity, catalase activity, and a defined main enzyme activity that transcribes long gap- repeat segments into mature cr-RNA. Mostly catalytic RAMP proteins are peripherally encoded by the respective operon; Cas5 and Cas7 do not belong to RMAP families. However, subtype I-C (also known as Dvulg or CASS1) may be an exception for the RNAase activity of Cas5 and Cas7 (Makarova et al. 2011). Type-I CRISPR-Cas systems appear to target DNA. Target cleavage is mediated by Cas3-based HD nuclease activity. In several type-I CRISPR-Cas systems, the Cas4 domain RecB nuclease fuses with Cas1. Cas4 is potentially involved in cavity acquisition. (Makarova et al. 2011) Basically, in the type-I CRISPR-Cas system, cascade and Cas3 cut foreign DNA. mature crRNAs are produced from pre-crRNAs via Cas6. Cas6 performs the cutting of repeat portions of pre-cRNAs. (Gök and Tunalı , 2016)

Type II CRISPR-Cas System: Type II: CRISPR-Cas systems include 3 subsystems, II-A to II-C. Type II system is the system whose mechanism is the most researched and known in detail among CRISPR-Cas systems (Gök and Tunalı, 2016). Type II CRISPR-Cas systems include the "HNH"-type system (Streptococcus-like; also known as Nmeni subtype, Neisseria meningitidis serogroup A str. Also known as. Z2491, or also called CASS4,). It contains a very large

protein containing Cas9, which produces crRNA and cleaves target DNA. Cas1, Cas2 and Cas9 contain at least two nuclease domains. The RuvC-like nuclease domain is located near the amino terminus and the HNH (McrA-like) nuclease domain is located in the centre of the protein. The HNH nuclease domain contains abundant restriction enzymes and has endonuclease activity and is responsible for cutting target DNA (Makarova et al., 2011). The type 2 system cleaves the double formation between pre-crRNA and tracrRNA. The first cleavage occurs at repeat sites in the processing pathways of pre-crRNA. This cleavage is catalysed by hausekeeping. It is mediated by the double-stranded RNA-specific RNAase 3 in Cas9. (Deveau et al., 2008).

In the type II system, the endonuclease cas9 together with a non-coding RNA combines with CRISPR RNA (crRNA) to form a ribonucleoprotein complex that recognises and cuts the foreign genome. (Rath et al., 2015).

Type III CRISPR-Cas system: Type III: CRISPR-Cas systems contain 2 subsystems, III-A to III-B. The type III system contains polymerase and RAMP modules that perform cascade complex-like transcription by processing the gap- repeat complex. Ribonucleases identified in the type III system (except cas2 protein) are RAMP proteins. Type III systems contain at least two RAMPs in addition to the Cas6 protein. These additional RAMP proteins are involved in the trancription process. In many organisms, type III CRISPR-Cas operons lack the Cas1 - Cas2 gene pair. However, in most cases an additional CRISPR locus is present and this additional locus comes from Cas1 or Cas2. It is thought that these Cas1 or Cas2 genes existing in the relevant genome were added in trans. In type III CRISPR-Cas systems of *Staphylococcus epidermidis*, *Mycobacterium tuberculosis*, *Alorhodospira halophila*, it is suggested that there is a single CRISPR-Cas locus. It is suggested that this locus (polymerase-RAMP module) combines with Cas1 and Cas2 and takes part in the formation of new cavities with full function. The production of small mature crRNAs in the type 3 CRISPR-Cas system is based on cutting the repeated sequences of pre-crRNAs into crRNAs with the Cas6 nuclease family. The resulting crRNA forms a complex with Cmr/Cas10 or Csm/Cas10 proteins and the Cas protein in the complex cuts the invading genome.

Unclassified CRISPR-Cas systems: Although most of the CRISPR-Cas systems found are classified up to subclasses, there are also systems that do not fit the existing classification. For example, the CRISPR-Cas system of *Acidithiobacillus ferrooxidans* needs a new class. And the name Type U was proposed by Makarova et al. This CRISPIR system was later referred to as the putative type IV CRISPR/Cas system in the 2015 paper by Makorova et al. In many bacteria and in the identified cpf1, the archival genome adjacent to Cas1, Cas2 and the CRISPR locus (e.g. cf. noncida Fx1 at the FNFX1 1431-FNFX1 1428 locus of Francisella) was named as the putative type V CRISPR/Cas system by Makrova et al. In the same paper, it was proposed to name CRISPR/Cas systems as Class 1 and Class 2. Again in 2015, in the classification of Class-2 CRISPR/Cas system by Shmakov et al. (2015) type II, type V were included in this class and the C2c2 gene locus synthesised by *Listeria seeligeri serovar* 1/2b str and expressed by *E. coli* was named as type 6 CRISPR/cas system and included in Class 2 CRISPR/Cas system. Class 1 CRISPR/Cas system type I, type III and type IV CRISPR/Cas system were also identified (Mohanraju et al., 2016).

CRISPR scans

In the development of antiviral agents, knowing the interactions, biology and reactions of the virus and host is important for the design of the studies to be carried out. At this point, CRISPR screening provides a great advantage for investigating the host and viral agent for these purposes. Genetic screening is one of the gold standards for finding host factors that restrict or promote viral infection. There are two types of advanced genetic screening. These are loss-of-function screening and gain-of-function screening. Loss-of-function screening is the most commonly used of these two screens (Puschnik et al., 2017; Chulanov et al.,2021). CRISPR screens are divided into 3 categories according to their mechanisms of action: CRISPRi, CRISPRa and CRISPR knockout (CRISPR-ko) screens (Chulanov et al. 2021). CRISPRi and CRISPR-ko screens are loss of function approaches. CRISPR-ko is classically based on the CRISPR-Cas9 system. This classical system, results in DBSs and indel mutations, or codons are converted into stop codons by cytidine-based regulators, enabling the production of truncated non-functional proteins (CRISPR-STOP or iSTOP approaches (Billon et al., 2017; Kuscu et al., 2017;as cited in Chulanov et al., 2021)

CRISPR screening is performed not only for the host but also for viral protein synthesis in viral replication. For example, Hoffmann et al (2021). utilised CRISPR analysis to examine the SARS-CoV-2 interactome in cells infected against COVID-19. For this, they specified 332 highly comprehensive, identified SARS-CoV-2 interactomes in the CRISPR-Cas9 library and designed targets. They stated that

thanks to the structure of the library, they screened on four related viruses. As a result of this study, they screened in HCoVNL63, HCoV-229E and HCoV-OC43 infection models to search for pan-coronavirus factors necessary for replication, and sterol regulatory element binding protein division activating protein (SCAP) was identified as the host factor important for the replication of all four coronaviruses. In healthy cells, SCAP regulates lipid and cholesterol haemostasis through secretion of binding proteins by sieving sterol regulation in the endoplasmic reticulum. It has been reported that SCAP may promote coronavirus infection by increasing SREBPs-dependent transport or cholesterol content in the cell membrane and increasing viral interaction.

Use of CRISPR-CAS Systems in antiviral field

The gene editing application of the CRISPR-Cas system was used in the development of antiviral therapies. It then revolutionized diagnostics as a gene detection system. At this point, CRISPR diagnostics enables accurate and rapid identification of any pathogen in clinical settings. CRISPR-Cas system is being studied for the development of new treatments against many viruses.

HIV and the CRISPR-Cas system

To date, the CRISPR-Cas system has been studied mostly for the treatment of HIV infections and today serves as an advanced treatment option. (Huang et al., 2017, 2022; Xu et al., 2019; Herrera-Carrillo et al., 2020 as cited in Kılıç Tosun and Kesmen, 2022)

In a study by Park et al. (2017) on HIV-1 (Human immunodeficiency virus-1), genome-wide CRISPR screening was performed to identify host factors and five factors, including HIV co-receptors CD4 and CCR5, were identified. Candidate pathways were validated by Cas9-mediated knockdown and antibody blockade in primary human CD4+ T cells.

Ophinni et al. (2018) reported that they inhibited HIV-1 replication by targeting Tat and Rev genes in HeLa cells with CRISPR/Cas9 vector.

In their 2016 study on HIV-1, Ueda et al. (2016) reported that the vector targeting the gag, pol and long terminal replication of HIV-1 with CRISPIR/Cas 9 was effective in the early stages of HIV-1 infection in the human T-cell line, but the vector was insufficient in the inhibition of wild-type (WT) HIV-1. They evaluated this inadequacy as a point to be considered when developing CRISPR-Cas system and treatment against HIV-1.

Xu et al. (2019) transplanted haematopoietic tissue and progenitor cells (HSPCs) with CCR5 protein with CRISPR/Cas9 system to an individual with acute lymphocytic leukaemia and HIV and treated lymphocytic leukaemia and HIV in the patient. In the study conducted by Jin et al. on HIV-1 in 2018, it was stated that they found two important genes (TSC1 and DEPDC5) that play a role in HIV-1 latency in their CRISPR screening study on HIV-1 latency. They stated that inactivation of TSC-1 or DEPDC5 genes increases reactivation in both T-cell line and monocyte cell line, and in general, both TSC1 and DEPDC5 agonists can be used in the development of new therapeutic approaches to activate HIV-1 latency.

In another study on HIV-1 latency, Z. Li et al. (2020) used genome-wide CRISPRi screening to show that inhibition of FTSJ3, TMEM178A and NICN1 is effective. They stated that these genes stimulate RNA polymerase II-mediated transcription of HIV and increase its latency. In other immunoprecipitation experiments performed in the study, it was reported that depletion of TMEM178A and NICN1 increased poimerase II signalling in the HIV-1 envelope region, but not in the long term rpeat (LTR) region. Mandan et al. developed CRISPR/Cas9 vectors targeting two clinically important genes (B2M and CCR5) against HIV-1 in primary human CD4+ T cells and CD34+ hematopoietic system and progenitor cells (HSPCs) to develop CRISPR-Cas9 system against HIV-1, and it was observed that the vector targeting CCR5 was 30% effective in HSPC cells (between 22-44%); It was reported that the success of the vector targeting B2M varied between 7-48%. When HSPC cells with inactivated CCR5 protein were transplanted into mice, they reported that the transplanted HSPC clones were resistant to HIV1.

In their study on HIV-1, McLaurin et al. (2024) showed that the CRISPR/Cas9 system they developed against HIV-1 mRNAs reduced the neurocognitive effect of HIV-1 in invitro and in vivo conditions.

In their study on HIV-1 by Liao et al. (2015) targeted the repeat regions of the viral genome in HIV-infected CD41T cell culture with CRISPR-Cas9 and as a result, viral replication and latency decreased. At the same time, HIV reservoir was obtained in pluripotent stem cells and CRISPR/Cas9 vector targeting the repeat regions of the HIV genome was transferred and it was reported that the new reservoir cells formed as a result were immune to HIV.

Coronaviruses and the CRISPR-Cas systems

The study conducted by J. Wei et al. (2021) is to find therapeutic pathways for SARS-CoV-2, SARS-CoV-2, Middle East respiratory syndrome CoV (MERS-CoV), They performed genome-wide CRISPR screens in Vero-E6 cells containing bat CoV HKU5 and vesicular stomatitis virus (VSV)-containing Vero-E6 cells

expressing SARS-CoV-1 enhancement, and identified known SARS-CoV-1 receptors, including the ACE2 receptor and the protease Katepsin L.2 host factors, in addition to discovering pro-viral genes and pathways specific to the SARS lineage and pan-coronavirus, including HMGB1 and the SWI/SNF chromatin remodeling complex, and that HGM1 regulates ACE2 expression, and found that it is critical for SARS-CoV-1 and SARS-CoV-2 entry. It was reported by Daniloski et al. that depletion of RAB7A decreased the cell surface expression of ACE2.

Abbott et al. (2020) stated that they developed PAC-MAN (prophylactic antiviral CRISPR in human cells) strategy and investigated its antiviral activity on SARS-CoV-2 and live influenza A virus (H1N1) and that this strategy developed with Cas13d protein reduced the load of H1N1 virus in respiratory epithelial cells and that six of the crRNAs they developed were effective against SARS-CoV-2, and that this system is promising for the inhibition of pan-coronaviruses.

Cas13 is a Cas protein widely used in genome editing. It is used in the type VI CRISPR/Cas system. Since it targets RNA, it is especially used in viruses with RNA (Xie et al., 2021; Zhang et al., 2021 as cited in Kılıç Tosun and kesmen, 2022).

Arboviruses and the CRISPR-Cas systems

Ganaie et al.(2021) used a CRISPR/Cas9 library for the mouse microgial cell line BV-2 cell line for Rift Valley Fever Virus (RVFV) and identified lipoprotein receptor-related protein 1 (Lpr1) protein, heat shock protein (Grp94) and receptor associated protein (RAP) as possible antiviral targets; They stated that the RVFV genome binds specifically to the Lpr1 protein and when they transduced the line with lentivirus with a single-guided gRNA targeting the Lpr1 gene, they found that the remaining cells were resistant to RVFV. Thus, it was stated that Lpr1 protein is an important host factor against RVFV.

In the study on human orovirusvirus (HCMV), Wu et al., (2018). Showed that PDGFRα as a host-dependent factor is important for trimer-mediated entry of HCMV into the cell and trimer-mediated passage of HCMV from cell to cell and that pentamer-coated viruses have low efficiency in PDGFRRα-deficient cells by CRISPR tracking study. In another study on HCMV, they showed that OR1411 is an important co-receptor for the HCV pentameric complex, which is related to the sensitivity orovirlial cells to pentamer HCMV in OR1411 protein-mediated infection (Chulanov et al., 2021).

In their study on Zika virus (ZV), dengue virus (DENV) and West Nile virus (WNV), Richardson et al. found that the functional gene pair between IFI 6 (encodes IFN-α inducing protein) is important for the inhibition of replication of Flaviviruses by CRISPR screening and showed that IFI 6 inhibits the replication of viruses invitro with the CRISPR-Cas system (Richardson et al., 2018).

van Diemen et al. (2016) has been shown that when Epstein-Barr virus (EBV) remains latent in cells, the latent EBV genome can be edited with the CRISPR-Cas system.

Lin et al. (2017) reported that in their CRISPR screen for host factors for Dengue virus (DENV), they identified the oligosaccharyltransferase (OST) complex as an essential host factor for DENV infection. However, the STT3B-associated OST subunit MAGT1 is also required for DENV propagation. MAGT1 expression requires STT3B and a catalytically inactive STT3B also enables MAGT1 expression, supporting the hypothesis that STT3B serves to stabilise MAGT1 in the context of DENV infection. Since cells expressing an AXXA MAGT1 mutant were unable to support DENV infection, and found that the oxidoreductase CXXC active site motif of MAGT1 is required for DENV propagation; cells expressing single cysteine CXXA or AXXC mutants of MAGT1 were able to support DENV propagation. Using the engineered peroxidase APEX2, they demonstrated the proximity between MAGT1 and NS1 or NS4B during DENV infection. They stated that these results revealed that the oxidoreductase activity of STT3B-containing OST is required for DENV infection and that this could guide the development of antiviral agents targeting DENV.

Labeau et al. (2020) similarly used CRISPR scanning to identify the host factor for DENV and identified two endoplasmic reticulum-resistant dolichol-phosphate mannose synthase (DPMS) complex subunits, DPM-1 and 3. They also found that DPMS complexes are important in regulating viral RNA replication and supporting the stability of folding of viral structural proteins.

Enteric viruses and CRISPR-Cas systems

Orchard et al., (2019) stated that they found 49 genomes that would prevent murine norovirus proliferation in human cells in their CRISPR-Cas scan. Hosmillo et al. (2019) stated that they identified G3BP1 as an important host factor for human noravirus and murine noravirus as a result of their CRISPR screening study. They identified G3BP1 protein as an important host factor for VPg-dependent translation of noravirus.

Ding et al. (2018) reported that STAG2 is an important part of the cohesin complex, an important nuclear protein complex that coordinates the sister chromatid during cell division and is an important

element of the replication of human rotavirus (HRV) in the cell.

Other viruses and the CRISPR-Cas systems

In a study on human papillomavirus (HPV) conducted by Kennedy et al. in 2014, the effects of vectors prepared with Streptococus pyogenes Cas9 protein targeting E6 and E7 genes of HPV-16 and HPV-18 were investigated and HeLa and SiHa cell cultures were used for this purpose. As a result, it was reported that the designed vector did not affect the E6 gene of HPV-18, HPV-16 affected the E6 gene and both viruses affected the E7 gene at a significant level (Kennedy et al., 2014).

Zhen et al. (2014) conducted invitro (SiHa cell line was used) and in vivo (nude mice were used) studies on HPV and found that CRISPR-Cas9, which they developed to target the E6 and E7 trancrept of HPV-16, significantly reduced the proliferation of HPV-16 both invitro and in vivo.

In the article written by Y. Wei et al. (2022) various studies for the treatment of HPV with CRISPR/ Cas9 technology are seen.

In a study conducted by Chou et al. (2016) on John Cunningham Polyomavirus (JCPyV), it was reported that CRISPR/Cas9 targeted the non-coding control region and the late open reading frame in the genome of JCPyV and observed that the administration of JCPyV-specific single-guide RNA Cas9 protein before or after infection significantly reduced virus replication and protein formation. In 2015, in a study on John Cunningham Poliomavirus, the N-terminal region of the T-antigen gene was targeted for CRISPR-Cas9 and it was reported that plasmid-mediated mutation in this region inhibited viral replication invitro (Wollebo et al., 2015).

In the study conducted by Roehm et al.,(2016) on Herpes Simplex Virus-1 (HSV-1) in 2016, it was reported that the virus developed a guide-mediated CRISPR-Cas9 system targeting the genome associated with the ICP0 protein of the virus and provided InDel mutation to the exon 2 region of the ICP0 genome and reduced the infection in an invitro environment. When this system for the ICP0 gene was combined with the version for ICP4 or ICP27, it was observed that the infection was completely eliminated.

In the study conducted by Das et al. (2020) , it was found in CRISPR screening that gangliosides are an important endosomal receptor for semi-enveloped or naked (non-enveloped) Hepatitis A Virus (HAV). This has revealed a point that can be used for antiviral strategies against HCV.

Animal Viruses and CRISPR-Cas systems

Marek and CRISPR-Cas Systems: Zhang et al. (2019) showed that the viral gene phosphoprotein 38 (pp38) is important in the latent/litic phase transitions of Marek's disease virus (MDV). It was emphasised that proliferation increased when pp38 was transfected with CRISPR/Cas9. This finding suggests that pp38 is a potential target for antiviral drugs to be developed against Marek.

In the study conducted by Luo et al. (2020) on MDV-1 , it was stated that virus-encoded micro-RNAs (miRNAs) play an important role in the latency, replication, etc. phases of herpesviruses, and they stated that if the Meq-cluster miRNAs of MDV-1 were interfered with CRISPR-Cas9, the replication of the virus decreased. They stated that deletion of miRNAs in the middle-cluster increased viral replication.

In the study conducted by Senevirathne et al. (2021), it was reported that when the pp38 gene of MDV was targeted with CRISPR-Cas9 using Salmonella spp. as a plasmid, the effectiveness of the plasmid in the spleen was seen between 1.7-13%, 1.8-8% in the spleen, and the highest effect was seen in chickens treated with plasmid treatment before MDV infection. This suggests that CRISPR-Cas9 application may be an effective treatment option against MDV when given according to the course of the disease.

In the study conducted by Teng et al. (2023) , CIRISPR-Cas9 study was performed with hybridoma technology on the Meq gene, which is effective in the oncogenic feature of MDV, and as a result of the study, 5 Meq-deleted hybridoma MDV-1 were obtained.

In a study conducted by Li et al. (2020) , they used MDV as a vector to create a vaccine against Reticuloendotheliosis virus (REV) and showed that this recombinant strain developed using CRISR-Cas9 significantly reduced the REV load.

Similarly, Liu et al. (2020) programmed MDV with CRISPR-Cas9 as a target for avian leukosis virus subgroup J (ALV-J) in 2020 and showed that the MDV strain obtained was effective in the resistance of the host cell against ALV-J and emphasised that MDV could be an important vector in CRISPR-Cas technology.

Bovine herpes virus (BHV) and CRISPR-Cas systems

In the study conducted by Dai et al. (2022), they stated that when they interrupted the UPL-41 protein of the BHV with CRISPR-Cas9 technology, they found that the proliferation of the virus in the host cell decreased. It has also been tried to develop a vaccine for BHV using CRISPR-Cas technology. In the study conducted by Ma et al. (2023), BHV-1's glycoprotein I, glycoprotein E, TK gene and UL-23 genes were targeted with CRISPR-Cas to create a vaccine strain

and Pseudorabies virus (PRV) was also included in the study and it was stated that CRISPR-Cas9 technology could be effective in the development of multivalent vaccines.

In their study conducted by Zhao et al. (2022). showed that BHV can be used as a vector for developing a vaccine for rabies virus. For this, they added rabies virus glycoprotein-g (RABVG) to BHV-1 virus by interfering with CRISPR-Cas technology. They stated that they observed that the recombinant BHV-1 had a protective effect against severe fatal infection in mice after 20 passages.

Yu et al. (2024) also studied gene editing on BHV and pseudo rabies virus (PRV) with CRISPR-Cas9 system in their study in 2024. In the study, thymidine kinase (TK) gene of PRV or glycoprotein I (gI) and glycoprotein E (gE) of BHV were targeted. With this approach, recombinant TK-/eGFP+ PRV and gIgE-/ eGFP+ BHV-1 mutants were generated and then characterised and their invitro and invivo biological activities were examined. As a result, it was reported that alpha herpes virus, including PRV and BHV-1, can be rapidly edited using the CRISPR/Cas9 approach and may contribute to the development of animal herpes virus vaccines.

Canin distemper virus (CDV) and CRISPR-Cas systems

In the study conducted by Cai et al. (2019) it was suggested that cell lines with mavs (mitochondrial antiviral signalling) activity produced with CRISPR-Cas technology could be used in the development of CDV vaccine.

Gong et al. (2020) reported that they developed a highly efficient recombinant canary pox virus containing CDV virus-like particles (VLPs) called "ALVAC CDV-M-F-H/C5-" with CRISPR/Cas9 technology, which enabled simultaneous expression of matrix (M), H and F genes.

Gradauskaite et al. (2023) stated in their study in 2022 that LPR6 is an important receptor for CDV and will be important in the development of attuned vaccines. They stated that when they silenced the LPR6 receptor in cells with CRISPR-Cas9, they eliminated cell entry in multiple cell lines and lost infectivity in LRP6KO cells pseudotyped with CDV-OP envelope glycoproteins after transfer to recombinant viral particles and vesicular stomatitis virus (VSV) and that the study identified LRP6 as the long-sought cell entry receptor of CDV OP in multiple cell lines.

Equine arteritis virus (EAV) and CRISPR-Cas systems

de Wilde et al. (2018) investigated the proliferation of EAV, human coronavirus 229E (HCoV-229E), and betacoronavirus Middle East respiratory syndrome coronavirus (MERS-CoV) by deleting the Cyclophilin A (CypA) receptor in Huh7 cells with CRISPR-Cas9 . As a result of the research, they stated that the proliferation of EAV and MERS-CoV in CypA deleted cell lines decreased around 3log.

Equine Herpesvirus (EHV) and CRISPR-Cas Systems

In the study conducted by Hassanien et al. (2024), they developed single-guide RNAs targeting ORF30, ORF31, ORF74 and ORF7 regions of EHV with CRISPR-Cas9 and stated that sgRNAs targeting ORF30 and ORF7 showed synergistic effect in reducing viral replication of EHV.

Feline leukoma virus (FeLV) and CRISPR-Cas systems

Helfer-Hungerbuehler et al. (2021) investigated the infection-reversing effect of the cat's immune system using CRISPR/Cas9-assisted gene therapy and evaluated different adeno-associated vectors (AAVs) for their ability to provide gene regulation. The CRISPR -Cas system was transferred into cat cells and then the efficiency of the CRISPR/SaCas9 system to target different regions of the FeLV provirus was investigated, for which nine natural AAV serotypes, two AAV hybrid strains and Anc80L65, an AAV ancestor predicted by Silico, were tested for their potential to infect different viruses. They reported that the CRISPR/SaCas9 system was used to target selected FeLV provirus regions, followed by T7-validated endonuclease 1 (T7E1) and Truncation of Indels (TIDE) analysis, showing that the gag and pol regions had the highest percentage (up to 80%) of non-homologous end joining (NHEJ) in the conserved region. Subsequent transduction experiments using AAV-DJ confirmed indel formation and showed a significant reduction in FeLV p27 antigen for some targets. Targeting of FeLV provirus was effective when using the CRISPR/SaCas9 approach invitro, while the means to overcome infection in vivo should be further investigated.

Feline calicivirus (FCV) - feline herpesvirus (FHV) and CRISPR-Cas9 system

Studies on FCV and FHV are more oriented towards viral diagnosis and viral screening.

In the study conducted by Huang et al. (2022), they stated that they developed a CRISPR-Cas13a and RPA reaction-based analysis for FCV detection. The recombinant plasmid they designed targeted the ORF1 gene of FCV. They stated that the positive detection rate of the FCV-Cas13a test they developed was higher than RT-PCR and showed that CRISPR-Cas systems can also be used in viral diagnosis.

In a study conducted by Fang et al. (2023) , they reported that they developed a nucleic acid detection system called 4 thermostatic steps (4TS) for the diagnosis of respiratory disease agents, including FCV and FHV, using the CRISPR-Cas12 system.

Poxvirus and CRISPR-Cas systems

In their publication by Siegrist et al. (2020) is showed that vaccine strains developed by using vaccinia irus (VACV) as a vector and targeting E3L, I2L, A17L genomes against orthopoxviruses with CRISPIR-Cas9 reduced CPEs.

Ohlson et al. (2023) reported that AAA ATPase and SPATA5 are important host factors for these virus groups as a result of CRISPR-Cas screening of poxviruses and flaviviruses.

Singh et al. (2023) developed a Cas12a nuclease-based assay associated with clustered, regularly spaced short palindromic repeats to detect monkey poxvirus (Mpox), They stated that they identified Mpox-specific conserved sequences that differ from all viruses present in the genus Orthopoxvirus by a single nucleotide polymorphism (SNP) and used this SNP in our assay to specifically distinguish mpox virus from other related orthopox viruses with a detection limit of 1 copy/µl in 30 minutes. They stated that this region may provide practicality in cases where the detection of Mpox virus needs to be sensitive and specific. This suggests that SNP may be a key point for the antiviral agent that can be developed against Mpox virus in the future.

In the study conducted by F. Zhao et al. (2023) , they stated that they developed a recombinase polymerase amplification (RPA)-coupled CRISPR-Cas12a study for the detection of Mpox virus and used 6DR and E9L, which are important for orthopoxviruses, and N3R and N4R gene regions specific for Mpox in the experiment. This suggests that these regions may be key in the development of tests for antiviral agents that can be developed against Mpox virus and other orthopoxviruses in the future.

Conclusion

The ability to develop CRISPR/Cas vectors for various stages of viral replication, including the latent stage, or to develop CRISPR/Cas vectors against various stages of viral replication, both in terms of its usefulness in revealing the virus-host relationship and in terms of guiding the development of antivirals for various stages of viral replication, including the latent stage, paves the way for the development of antivirals and paves the way for the development of antivirals, which are more difficult to develop than other antimicrobial agents due to technical conditions.

CRISPR/Cas studies have started to be used in the production of viruses to be used in vaccine development as well as antiviral development studies. With the transition from the laboratory-scale production stage to the high-scale production stage, an important stage will be passed in the fight against human viral diseases and animal viral diseases.

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