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Epigenome Editing for Treatment of Genetic and Infectious Diseases

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Abstract

Epigenome editing is fast becoming a promising technology for use in not only basic research but also various medical fields. Its applications are being studied for treatment of various diseases, including cancer, genetic diseases, and infectious diseases. Viral infections are known to cause epigenetic modifications in the host. Therefore, epigenome editing is a promising therapeutic approach which also has the capability to downregulate receptors for pathogen to enter the host. However, addressing the challenges of drug delivery systems is an essential and critical component for the success of these approaches. In this review, we briefly introduce the distinctive differences between genome editing and epigenome editing, highlight the challenges in application of the epigenome-editing approach to infectious diseases, and briefly outline the recent advances and challenges in drug delivery systems that are essential for therapeutic applications of epigenome editing. While further advances in delivery systems are needed, epigenome editing could be a potentially new generation of treatments and open new avenues for treating infectious diseases, in addition to genetic and other diseases.

Keywords: Epigenome editing; Genetic disease; Infection; Drug Delivery System (DDS)

Abbreviations: DTX3L: Deltex E3 Ubiquitin Ligase 3L; HDAC7: Anti-Histone Deacetylase 7; HDGF : Hepatoma-Derived Growth Factor; PRDM1: PR/SET Domain 1; PRMT1: Protein Arginine N-Methyltransferase 1; FOXO1: Forkhead Box O1; RPE65: Retinal Pigment Epithelium-specific 65 kDa Protein; SMN1: Survival of Motor Neuron 1

Introduction

Infectious diseases are the leading cause of death worldwide [1]. Despite significant advances in the research and treatment of infectious diseases over the years, their control and eradication face enormous challenges, and significant efforts are being made to overcome these diseases by establishing new therapeutic interventions. The recently developed genome-editing technology has the potential to be a breakthrough in disease treatment because it can permanently repair errors in disease-causing genes at the genomic level [2]. Thus, genome editing is considered beneficial for future clinical applications in genetic diseases and cancers. Research on the application of genome editing to infectious diseases, such as Coronavirus Disease 2019 (COVID-19), Human Immunodeficiency Virus (HIV), and Human Papillomavirus (HPV) [3], is also progressing [4]. However, practical clinical applications still face challenges because genetic repairs cannot fail *in vivo*; unintended genomic DNA mutations in patients caused by repair failures would be semi-permanent, causing unforeseen harm to their health. Thus, although genome editing is a promising and attractive technology, securing its clinical application through technological advances that specifically repair the genomic DNA of interest is essential. Considering this challenge, the clinical application of genome editing methods for safe *in vivo* treatment of human diseases is expected to take a long time. However, it has a promising potential for *ex vivo* treatments; in cells that can be cultured *in vitro* (*ex vivo*), such as blood cells, genome editing technology can ensure the exclusive growth of cells that have undergone DNA, and only those that have been successfully repaired would be returned to the patient. Therefore, *ex vivo* genome editing is currently considered to face a lower hurdle in terms of its clinical application than compared to *in vivo*.

In contrast to genome editing, epigenome editing does not permanently alter genomic DNA. Epigenome editing is a method that temporarily regulates the expression levels of target genes. Rapid advances in epigenome editing technology are driving research aimed at treating a variety of diseases, including genetic and infectious diseases. Different molecular platforms for epigenome editing have been developed, including a system of Zinc Finger Proteins (ZFs), Transcriptional Activator-Like Effectors (TALE), and Clustered Regularly Spaced Short Palindromic Repeats (CRISPR) and CRISPR-related (Cas) proteins [4]. These EpiEffectors act as custom DNA-Binding Domains (DBDs) that are fused to epigenetically modified domains to manipulate epigenetic marks at specific sites in the genome. Addition and/or removal of epigenetic modifications may reconfigure the local chromatin structure and cause long-term alterations in gene transcription. The manner in which the chromatin state is altered depends on the EpiEffector used [5,6]. For example, the use of transcriptionally active domains, such as VP64, relaxes the chromatin structure and activates the transcription of target genes. In contrast, domains with transcriptional repression capabilities, such as the KRAB domain, condense the chromatin structure and suppress the transcription of target genes. As the effect of epigenome editing is characterized as transient (reversible), theoretically, it can temporarily control the expression of target genes, increasing or decreasing the dose or duration of their expression and subsequently their respective protein expression, depending on the disease symptoms in the patient, similar to that with conventional drug treatments. Among the various diseases that may be treated using epigenome editing, infectious diseases are prominent. This method is currently attracting global attention and has potential applications in the development of drugs for the treatment of infectious diseases in the future [7,8]. Viral infection modifies the host epigenome to benefit the virus, favoring the pathogen and modulating host defense mechanisms to influence the innate immune system through the resistance and training of macrophages. Viral infection causes epigenetic changes in multiple host genes, some of which affect the severity of the infected host, leading to poor clinical outcomes. Several strategies involving epigenome editing have been proposed [7,9]. One strategy is to modify the levels of such host genes,

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including DTX3L, HDAC7, HDGF, PRDM1, PRMT1, and FOXO1 [9]. Another strategy involves prevention of specific viral infections. Theoretically, a method aimed at a transiently reducing the expression of the receptor(s) responsible for pathogen entry into the cells would suppress viral infections, and epigenome editing is a candidate method for reducing the expression of viral receptors. For example, epigenetic modifications to inhibit the SARS-CoV-2 receptor Angiotensin-Converting Enzyme 2 (ACE2) have been proposed and research in this direction is ongoing [9,10]. From this perspective, the identification of viral receptors for each pathogen is extremely important, and research on receptor identification is expected to attract increasing attention and accelerate in the future. Another method involves modifying viral DNA; reducing the levels of important viral DNA, such as the covalently closed circular DNA (cccDNA) of the Hepatitis B Virus (HBV), would be beneficial [11]. Thus, the development of novel therapies using epigenome editing in the field of infectious diseases is expected to attract attention as an important strategy in the future. While companies conducting research on epigenome editing are marking preclinical progress, the development of molecular tools and delivery systems for this technique remains important for its continued clinical application [5,12]. Ongoing disease-specific application studies include genetic and infectious diseases, such as Facioscapulohumeral Muscular Dystrophy (FSHD) (Epicrispr Biotechnologies, Inc., CA, USA) and HBV infection (Chroma Medicine, Inc., MA, USA). The race to develop technologies that actively turn epigenome editing on and off has also begun, promising critical innovations for clinical use.

Despite the progress in research on epigenome-editing enzymes, many challenges remain. Because the gene sizes of the EpiEffector and Cas proteins are generally large, the gene sizes of all epigenome-editing enzymes tend to be large [5]. To overcome this problem, attempts have been made to reduce the gene size of the EpiEffector and Cas proteins. Smaller Cas proteins have also been reported, including Cas12j (also known as CasPhi) [13], Cas12f [14], Cas13bt [15], and Cas13ct [15]. Downsizing the genes involved in epigenome editing continues to be an important research area because genome size may be a rate-limiting factor for epigenome editing using viral vectors. However, the most attractive approach to overcoming size limitations is to use or develop delivery systems suitable for large molecules to avoid specificity-related issues.

Literature Review

Importance of Drug Delivery Systems (DDSs)

As is the case for all existing drugs, the method used for the delivery of a drug to its target site is a critical factor in epigenome editing. In particular, the delivery of epigenome-editing enzymes, which exhibit dramatically increased target specificity in the cell nucleus, to target cells is particularly important for enhancing therapeutic efficacy. This section briefly outlines a few DDSs that have attracted considerable attention in recent years.

Adeno-Associated Viruses (AAVs)

Viral vectors have long been studied as DDSs; however, recent studies have focused on methods using nonpathogenic AAVs [16,17]. AAVs are present in multiple serotypes, and different serotypes are more likely to infect different organs. For example, AAV5 is more likely to be taken up by the lung tissue, AAV9 by the cranial nerve tissue, and AAV11 by the kidneys. An advantage of AAVs is that, unlike other viral vectors, they cannot be incorporated into the genome. Thus, AAVs may be administered into the blood, and many nucleic acid medicines may use AAVs for DDS in the future. AAVs have been used in gene therapy for several rare diseases, and its applications are likely to expand in the future and eventually include epigenome editing. However, a limitation of AAVs is that they can only carry small amounts of DNA, up to approximately 4.7 kb. Various methods, including the use of dual vectors, have been used to increase the amount of DNA that AAVs can carry [18]. Although not pathogenic, the frequent use viral vectors, such as AAVs, as DDSs for epigenome-editing drugs often triggers innate and cell-mediated immune responses, creating safety issues that may limit long-term outcomes. In contrast, non-viral vectors as DDSs are safer and may mediate the transient expression of epigenome-edited drugs, leading to reduced off-target efficacy. Another advantage of non-viral DDSs is that they are not hampered by immunogenicity related to the size of the nucleic acid or protein payload, innate immunity, or long-term epigenome-editing enzyme expression.

Lipid Nanoparticles (LNPs)

Depending on the size of the hydrophilic head group relative to the hydrophobic tail, lipids form different structures such as micelles, liposomes, and LNPs. The US Food and Drug Administration (FDA)-approved LNPs have four basic ingredient variations: Cationic or ionic lipids, cholesterol, helper lipids, and Polyethylene Glycol (PEG) lipids. Most intravenously administered LNPs accumulate in the liver. Specifically, many LNPs are coated with ApoE lipoproteins in the bloodstream, which are mediated by ApoE-LDL receptor interactions, leading to their uptake by hepatocytes [19]. Therefore, LNPs are commonly used to deliver therapeutic cargo to the liver. Although LNPs were originally optimized to transport siRNA, major advances in LNP formulations have enabled the efficient encapsulation and delivery of mRNA; Anderson et al. optimized the lipid formulations for mRNA delivery [20]. Lipids and LNPs are currently used DDSs for many drugs, including mRNA vaccines, owing to their highly efficient introduction [19,21,22]. However, native LNPs are more likely to be taken up systemically owing to a lack of transport direction that may cause unexpected side effects, such as inflammation [23-25]. To avoid this, arbitrary ligands may be added to LNPs [26] so that only cells with ligand receptors can take up the LNPs, providing a direction for drug delivery. Although LNPs are easy to prepare, they are prone to triggering inflammation in the body or accumulating in certain organs and have stability issues. Therefore, it may be necessary to devise measures to address these issues such as administering them directly to the organ being treated rather than into the blood.

Gold Nanoparticles (AuNPs)

AuNPs have attracted considerable attention as DDSs [27]. AuNPs are known for their relative ease of synthesis, high cellular uptake, biocompatibility, and hydrophilicity [28, 29]. Non-immunogenicity and low toxicity are the major advantages of AuNPs. Furthermore, once AuNPs reach their target sites, they can migrate externally or release payloads upon internal stimulation, which is another major advantage of AuNPs. This process is influenced by many factors, including particle size, shape, surface charge, and functionality. Depending on these characteristics, AuNPs are taken up *via* passive uptake, pinocytosis, phagocytosis, receptor-mediated endocytosis, or non-specific receptor-independent endocytosis [30-32]. As an example of active targeting, AuNPs conjugated to anti-CD133 monoclonal antibodies serve as nanocarriers for cancer cell targeting [33]. Thus, AuNPs bound to ligands, such as monoclonal antibodies and peptides, may interact with cell surface receptors and enable the specific endocytosis of target cells. While there is a need to improve bioavailability and solubility, minimize toxicity, and protect therapeutics from degradation, the targeted delivery of epigenome-editing tools using AuNPs may improve drug efficacy as

they are transported to specific targeted tissues. Currently, AuNPs are only used experimentally to transport genome- and epigenome-editing enzymes. For example, the combination of cationic arginine AuNP and Cas9 preformed Ribonucleoprotein (RNP) caused indels in up to 30% of HeLa cells *in vitro* [34]. Moreover, AuNPs packaged with RNPs and donor DNA were reportedly injected intramuscularly in a mouse model of Duchenne muscular dystrophy to achieve genetic correction of the dystrophin gene [35]. In a mouse model of fragile X syndrome, intracranial administration of AuNP carrying a packaged RNP that targeted the hypermetabolic Glutamate Receptor 5 (mGluR5) gene reduced the symptoms of fragile X syndrome [36]. Although the results of AuNP application are still experimental, further developments are expected.

Protein-based microdroplets

The excellent biocompatibility, biodegradability, low immunogenicity, and self-assembling properties of proteins make them an attractive class of materials for application in DDSs [37]. However, the stable, controllable, and uniform formation of protein nanoparticles, which is key to successfully delivering cargo such as pharmaceuticals into cells, has been difficult to achieve using conventional methods. To address this problem, droplet microfluidics has been employed in the formation of protein nanoparticles, taking advantage of the property of rapid and continuous mixing within microdroplets to produce highly monodispersed protein nanoparticles [38]. Currently, there are only little reports on the use of protein nanoparticles as DDSs; however, they are expected to be safe because they are composed of solely proteins [39]. Besides microfluidics, the use of methods such as spray-drying techniques to form micro-sized particles, over the years has added to the knowledge of protein formulations. Therefore, once established, protein-based microdroplet technology would have wide-spread application with potential for significant development. As epigenome-editing agents based on TALE and ZFs can be synthesized as proteins, microdroplets may be a better way to deliver these agents. Further developments are expected in this area.

Other methods

Nonviral vectors are an alternative to viral vectors [40]. Virus-Like Particles (VLPs) have been studied for drug delivery applications. Unlike traditional delivery systems such as viral vectors, VLPs lack a viral genome. Instead, viral capsid proteins that retain their icosahedral or helical capsid structures are used as non-viral carriers [41].

Discussion

Electroporation is another delivery method that is based on physical mechanisms. Briefly, this method uses an applied electrical pulse to create small pores in the phospholipid bilayer of cellular membranes, temporarily increasing cellular permeability and allowing drug entry. Electroporation is more useful for *ex vivo* delivery than *in vivo* [42,43].

It can also be administered directly to an affected area (organ) such as a specific area of the brain. In case of neurodegenerative diseases, it is possible to administer the drug to the cerebrospinal fluid, which is a highly invasive method; however, it is superior in terms of reliably delivering the drug to the affected area. In such cases, LNPs or AuNPs can be used as carriers to deliver the drug directly to the affected area, such as a specific region of the brain. In contrast, diseases that can be treated *in vitro*, such as blood diseases, can be transduced using highly efficient transduction methods, such as electroporation and LNPs. The advantage of *ex vivo* methods is that large gene sizes can be introduced. Another major advantage is that the treated cells can be returned to the patient after its therapeutic effects have been confirmed. However, there was no complete DDS in any case reported so far. Because of the advantages and disadvantages of each method, it is necessary to determine the characteristics of each DDS and use them differently (Table 1).

Tools	Advantages and disadvantages	References
Electroporation	Applicable only *in vitro*, still a challenge *in vivo* (Disadvantage).	[42,43]
Adeno-Associated Virus (AAV)	By using different serotypes, they can target specific organs. Dual-vector systems have been devised to increase the amount of DNA carried by AAVs. AAV2, AAV5 and AAV9 are FDA-approved (Advantage) as follows: Recombinant AAV2 delivers RPE65 to treat patients with RPE65-mutation-associated retinal dystrophy. Recombinant AAV5 delivers F9 to treat patients with certain kinds of Hemophilia B. Recombinant AAV9 delivers SMN1 to treat certain types of patients with spinal muscular atrophy.	[17,18]
Gold Nanoparticles (AuNPs)	AuNPs are among the FDA-approved metallic nanoparticles and have shown great promise in a variety of roles in medicine (Advantage).	[27,44,45]
Lipid Nanoparticles (LNPs)	LNPs are approved by the US FDA (Advantage). Among the FDA-approved nanoparticle drugs, polymeric (29%), liposomal (22%) and lipid-based (21%) drugs are the most common. However, native LNPs are more likely to be taken up systemically owing to a lack of transport direction that may cause unexpected side effects, such as inflammation (Disadvantage).	[19-25,46,47]
Protein nanoparticles	Their excellent biocompatibility, biodegradability, low immunogenicity, and self-assembling properties make them an attractive class of material for therapeutic applications such as DDS. There is an example of an FDA-approved protein nanoparticle drug (Advantage). Abraxane is a protein nanoparticle drug approved by FDA that enables the delivery of paclitaxel by albumin.	[37,38]
Others	These include viral vectors, such as herpes simplex virus (HSV)-mediated delivery, and non-viral vectors, such as virus-like particles (VLPs). An HIV vaccine made from VLPs, has contributed to significant development, accelerating research into protein nanoparticles for clinical use (Advantage). Direct injection, such as stereotaxic injection into the specific brain regions, is possible. Recombinant HSV-1 delivers GM-CSF to treat patients with certain unresectable melanoma lesions (FDA-approved) (Advantage).	[40,41]

Table 1: Administration (delivery) tools for epigenome editing.

Conclusion

Epigenome editing alters the expression of proteins encoded in DNA by adding or removing molecular markers from DNA or histone proteins without altering the DNA sequence itself. Furthermore, epigenome editing does not simply introduce or remove genetic functions but allows for the fine control of gene expression. In other words, epigenome editing provides a dimmer switch rather than an on/off switch. Moreover, epigenome editing does not cut the DNA; therefore, the risk of off-target effects is significantly reduced. Finally, in multiplexed applications, epigenome editing avoids the introduction of multiple breaks in the genome, thereby increasing the potential for treating complex diseases without threatening genome stability. For *in vivo* applications, the relatively large size of epigenome editing may require the identification and use of different properties for each DDS. Alternatively, new delivery methods may need to be developed for *in vivo* delivery depending on the target tissue. Basic understanding of the effectiveness of epigenome editing in disease treatment is rising, and related preclinical trials have been initiated. Thus, after DDSs have been elucidated, they can be used in clinical trials. Currently, newly developed technology is expected to be applied to genetic diseases and cancer; however, infectious diseases are also expected to become important target diseases in the future.

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