JULS

Review Article

Virus-Like Particles as a CRIPSR-Cas9 Delivery Tool

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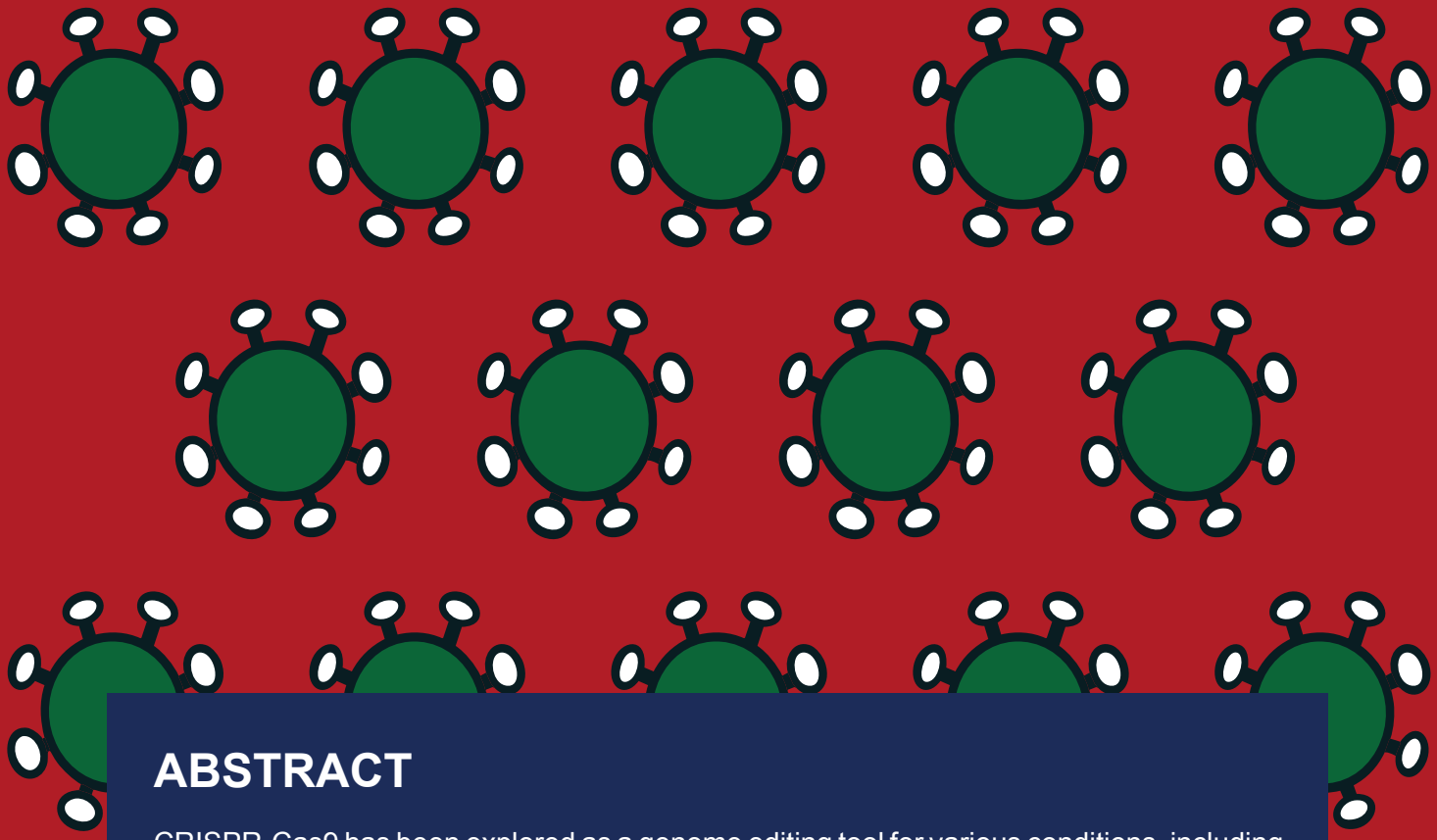

ABSTRACT

CRISPR-Cas9 has been explored as a genome editing tool for various conditions, including cancer and genetic diseases. By examining current research studies, clinical trials, and other literature reviews, here we discuss virus-like particles (VLPs) and their effective delivery of the molecular editing components, CRISPR-Cas9, to various target cell types. In this review, we first provide an overview of CRISPR-Cas9, including key clinical studies where this gene editing tool has proven to be successful, followed by an overview of VLPs, discussing both advantages and limitations. Finally, we highlight key preliminary studies where VLPs have been used specifically as gene editing delivery tools, and discuss how coupling these technologies will continue to positively influence the future of CRISPR-Cas9 genome editing in humans.

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Published online on January 23, 2024

CRISPR-Cas9

CRISPR-Cas9, or clustered regularly interspaced short palindromic repeats and CRISPR-associated protein 9, is a genome-editing tool comprised of a single guide RNA (sgRNA) and an enzyme with nuclease activity. Based on complementarity, the sgRNA targets Cas9 to a specific DNA sequence inside the cell, where Cas9 then acts as a pair of molecular scissors, creating a double-strand break in the DNA[1] (Figure 1). When donor DNA is supplied along with the sgRNA and enzyme, cells will insert the donor DNA into the chromosome by homologous recombination, effectively altering the genome.

CRISPR-Cas9 genome editing is currently being explored as a treatment option for a variety of conditions, including genetic diseases and cancer. As a treatment for cancer, scientists can alter the genome to either help the immune system fight the cancer, or directly induce apoptosis in cancer cells. For example, by disabling the gene that encodes a protein called programmed death-1 (PD-1), which naturally inhibits T cell function, CRISPR-Cas9 can be used to reverse the effects of PD-1 and increase the efficiency by which T cells target and kill cancer cells. When Lu et al.[2] disabled PD-1 using CRISPR-Cas9 in a clinical trial involving 12 patients with non-small-cell lung cancer, they observed a median decrease in PD-1 expression on edited T cells by 46.3%, suggesting that this genome editing tool holds promise for improving the ability of T cells to kill cancer cells. CRISPR-Cas9 has also been used to disrupt polo-like kinase 1 (PLK1), a kinase that promotes cell cycle progression, that when absent, promotes cell apoptosis. When Rosenblum et al.[3] used lipid nanoparticles to deliver Cas9 and a sgRNA specific for PLK1 to mice containing glioblastoma or ovarian tumors, they observed a significant decrease in the overall tumor growth, as well as increased survival when compared to mice treated with negative control nanoparticles. Taken together, these data demonstrate that CRISPR-Cas9 may be a treatment option for a variety of cancers. Additional studies have been done that also indicate success with CRISPR-Cas gene editing tools for the treatment of colon[4] and bladder[5] cancers.

Researchers are also experimenting, and some have even proceeded to the clinical trial stage, using this technology to treat a small number of genetic diseases, including the blood diseases sickle cell anemia (also called sickle cell disease (SCD)) and β-thalassemia. Both of these diseases are caused by mutations in the hemoglobin β-subunit gene (HBB) that encodes for the β-chains of adult hemoglobin[6]. For example, patients with SCD have a point mutation that results in an amino acid substitution from a negatively charged glutamic acid to a hydrophobic valine. This single amino acid change causes hemoglobin molecules to stick together and polymerize, forming protein fibers within the red blood cells (RBCs) upon the release of oxygen into tissues[7], which leads to the formation of crescent-shaped RBCs that block small blood vessels. Due to the presence of γ-chains instead of mutated β-chains, fetal hemoglobin does not aggregate in patients with SCD. Therefore, when mutated and dysfunctional β-globin is replaced with γ-globin, blood diseases like SCD and β-thalassemia can be reversed. Recently, Dr. Frangoul and colleagues[8] used CRISPR-Cas9 to reactivate the production of fetal hemoglobin (γ-globin) in one patient with SCD and in another patient with β-thalassemia. To reactivate the production of γ-globin in adults, they targeted the BCL11A enhancer region with CRISPR-Cas9. BCL11A is a transcription factor that represses production of fetal hemoglobin (γ-globin) in RBCs. By editing the enhancer for BCL11A, repression of γ-globin is alleviated, and thus fetal hemoglobin can be re-expressed in adult RBCs. Following the introduction of CRISPR-Cas9 targeting the RBC-specific BCL11A enhancer into CD34+ stem cells, approximately 80% of the alleles were edited, fetal hemoglobin production was restored, and the adverse effects observed in patients with these diseases were reversed[8]. Although this study had a small sample size, the implications for CRISPR-Cas9 as a therapeutic approach to curing genetic blood diseases is significant.

Additional approaches have utilized the CRISPR-Cas system to treat genetic blood disorders, such as one study that engineered inducible pluripotent stem cells (iPSCs) to grow cells and organoids for the correction of hemophilia A[9]. It will be interesting to observe if this treatment approach will be applicable to other diseases as well, even those in which multiple genes are responsible. For example, the currently incurable disease, hereditary spherocytosis, is a common form of inherited anemia where individuals produce sphere-shaped RBCs. This disease can be caused by genetic mutations in five genes: ANK1, SLC4A1, SPTA1, SPTB, and EPB42[10]. Although ANK1 encodes for the ankyrin-1 protein, which contributes to the structure and flexibility of RBCs, these other genes also have important functions that, when mutated, may contribute to the observed hereditary spherocytosis phenotypes. Over the past several years, there have been great advances in the CRISPR technology so that it is now possible to multiplex[11], or perform genome modifications to multiple genes simultaneously. Multiplexing is essentially achieved by supplying cells with a Cas enzyme and multiple sgRNAs, each targeting different genes, which can be accomplished by several different methods. In a recent study, Campa and colleagues[12] simultaneously targeted and edited 25 genes by supplying cells with a CRISPR array and Cas12a enzyme, providing direct evidence that multi-gene editing therapies for humans are on the horizon.

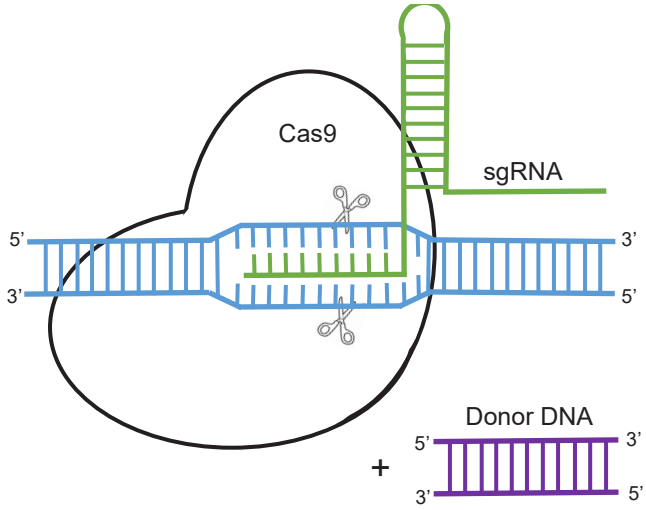

Figure 1. CRISPR-Cas9 Gene Editing. During CRISPR-Cas9 gene editing, the single guide RNA (sgRNA, green) directs the Cas9 enzyme to the target DNA (blue) based on complementary base pairing between the sgRNA and the target DNA. Using its nuclease activity, Cas9 makes a cut on both strands of the target DNA. A DNA repair mechanism inside the cell called non-homologous end-joining (NHEJ) will repair the breaks, and if a piece of donor DNA is supplied to the cells along with Cas9, it can be integrated into the region of target DNA containing the double-strand break by homologous recombination. The resulting target DNA is edited.

The implications of treating a polygenic hereditary disease will likely extend to a myriad of conditions where mutations in multiple genes are involved, such as type 2 diabetes[13].

Due to the success of CRISPR-Cas9 gene editing in humans, it is essential to explore the ways in which these molecular tools can efficiently be delivered to cells. There are currently several methods used to deliver gene editing tools that can be separated into three main categories: physical (e.g. electroporation), viruses/virus-like particles (e.g. lentivirus particles), and nonviral delivery methods such as gold particles[14]. We will examine the use of virus-like particles (VLPs) for the delivery of CRISPR-Cas, after first providing an overview of VLPs and discussing both the advantages and potential limitations of VLPs as delivery tools.

Virus-like Particles (VLPs)

VLPs are self-assembling macromolecular structures containing a capsid, or protein shell made of viral proteins, but are incapable of causing infections due to the lack of a viral genome. Although they can be found naturally, VLPs are commonly synthesized for a variety of biological applications[15], made by expressing the capsid proteins of a virus in cells, where the proteins then self-assemble into intact virus particles lacking a genome (Figure 2). As an empty shell made of viral capsid proteins, VLPs have several characteristics that enable them to serve as a specific and efficient delivery agent to human cells, when loaded with biological molecules, such as DNA, RNA, or proteins.

Specificity

Viral capsid proteins bind to specific cellular receptors, and this binding event initiates entry of the virus into a host cell. Not all host cells contain the appropriate receptor for capsid protein binding, and therefore, viruses and VLPs alike exhibit specificity, which is one advantage of using VLPs as a molecular delivery tool. For example, the Hepatitis E virus (HEV) discriminately targets the liver. When the major capsid protein of HEV is used to assemble VLPs, genetic material can be encased within the capsid and specifically delivered to liver-derived cells, where the genes are then expressed[16].

Ease of Modification

VLPs are easily editable. A single framework can be modified and used for other applications through methods such as exchanging viral envelope proteins or packaging different RNAs, ribonucleoproteins (RNPs), or proteins inside the VLPs[17]. There are two basic methods used to introduce biological molecules into VLPs: chemical conjugation and genetic fusion, each of which provides flexibility. The ability to be easily modified limits both the time and costs associated with designing and developing each VLP from scratch. Repurposing VLPs is successfully being used for a variety of applications, including vaccine development. For example, modification of the hepatitis B virus

surface antigen is being used to generate numerous vaccines to combat infectious diseases, ranging from hepatitis B to malaria[18-19].

Dose and Cytotoxicity

Current research shows that additional advantages of using VLPs are lower effective dosage and reduced cytotoxicity. VLPs can reduce the drug dosage needed for effective cancer treatment. One team of researchers found that VLPs loaded with the chemotherapy drug, doxorubicin (DOX), killed hepatocellular carcinoma cells (HCCs) 20 times more effectively than free DOX, with little harm to normal liver cells[20]. Another group of researchers demonstrated that using VLPs more than doubled the effectiveness of an in vitro caspase treatment for killing PC3 prostate cancer cells[21].

Limitations

Although VLPs hold great potential, they do come with limitations; namely, they can be unstable in conditions of fluctuating pH and temperature. Several studies suggest that VLPs are stable at temperatures up to approximately 50°C, thus human body temperatures are low enough that denaturation of VLPs is not a concern[22-25]. However, it has also been documented that the structural stability of VLPs changes considerably at pH levels that would not be unusual in various parts of the body[26], with a maximum stability in the range pH 3-7 for certain VLPs[22]. Experiments should be conducted to determine how to effectively modify VLPs to avoid stability pitfalls. A current approach researchers are taking to overcome the pH limitation is producing VLPs in specific formulations of buffered solutions[27]. Fiedler and colleagues[28] conducted a mutagenesis study in which they manipulated the primary protein structure of VLP coat proteins to observe impacts on stability, discovering that disulfide linkages were of central importance. Further research will help determine optimal conditions that promote structural stability for various types of VLPs.

Immunogenicity

One aspect of VLPs that has both advantages and disadvantages depending on the application, is their ability to induce an immune response. It has been shown that VLPs have an inherent ability to cause an immune response dependent on their size and the immunogenicity of the capsid proteins[29], and that VLPs are efficient activators of antigen-presenting cells (APCs), and promote B cell (antibody), T helper cell, and cytotoxic T cell responses against VLP-associated antigens[30-31]. Therefore, VLPs have been tested in a variety of vaccine studies, including those used for infectious disease prevention against various families of viruses[31] those used for allergy immunotherapy[24], and those used for treating various types of cancers[32].

Further support of the benefits of immune responses against VLPs comes from research on chimeric VLPs, which are those that contain additional molecules from a given pathogen. For example, Wang and colleagues[33] created chimeric influenza VLPs (cVLPs) consisting of the influenza matrix M1-derived protein and enteropathogenic Salmonella flagellin fused to

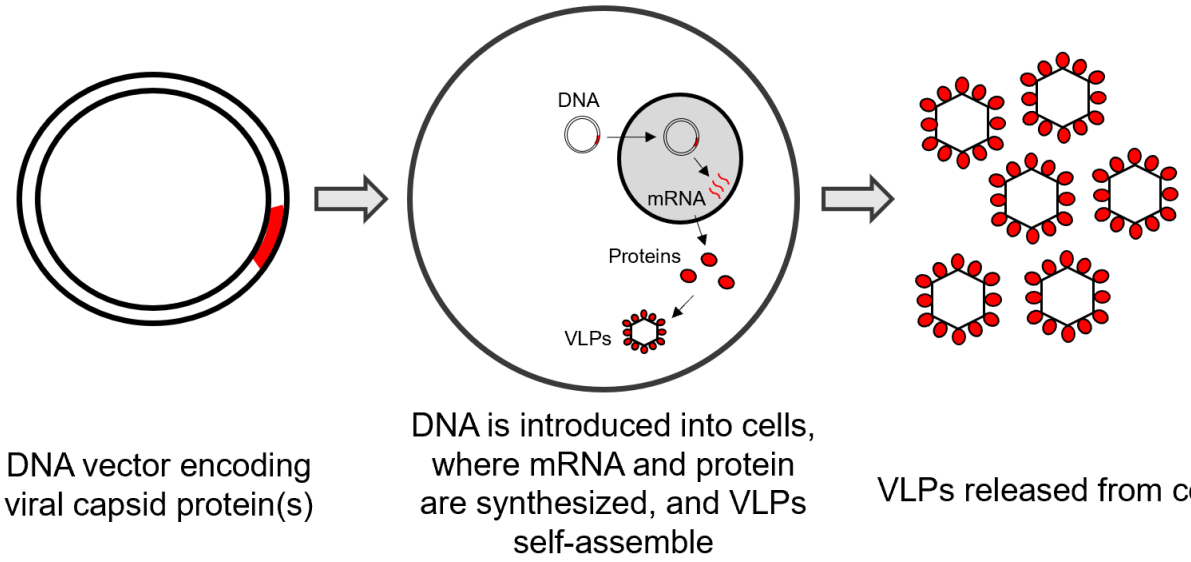

Figure 2. Generation of Virus-like Particles (VLPs) *in vitro*. A DNA vector encoding the viral capsid protein(s) is introduced into cells, where mRNA and protein synthesis occur, followed by VLP self-assembly. VLPs are then released from the cell, where they can be purified from the cell culture supernatant and used for further studies.

influenza hemagglutinin (HA), making it membrane anchored. In an in vivo mouse experiment, they found that the cVLPs induced higher neutralization and HA inhibition antibody titers than control influenza VLPs containing only M1 and HA proteins. The increased immune response observed with the cVLPs in this study was presumably due to the presence of additional epitopes provided by the flagellin protein that served as an adjuvant by delivering signals through toll-like receptor 5 (TLR5). Other studies have also clearly demonstrated that chimeric VLPs are able to generate heightened immune responses, where antibody production is an essential step to this process[34-35]. Taken together, these studies support the benefit of immune responses derived against VLPs, especially when the VLPs are used as vaccines.

Although there are clear advantages of VLP-induced immune responses, one major concern is the production of neutralizing antibodies, which could be problematic when VLPs are used for specific and targeted delivery of biological agents, including but not limited to, genome editing tools requiring more than one dose. For example, if a patient mounts an immune response against a primary dose of VLPs, it is possible that antibodies produced by the patient could effectively neutralize a subsequent dose and prevent the VLPs from binding to their appropriate cellular receptor. This type of response could ultimately prevent VLP delivery to cells upon second and subsequent doses. Typically, neutralizing antibodies are a cause for concern when large tissues and organs are involved, and thus where a single dose is not likely to be effective[36]. Therefore, it will be important to determine the effects of neutralizing antibodies on the overall outcome of VLP drug and genome editing tool delivery.

VLPs for the Delivery of CRISPR-Cas9

Despite the limitations and due to the many advantages for biomolecule delivery, VLPs have been effectively utilized for CRISPR-Cas9 genome editing in a handful of studies. A literature review by Lyu and Lu (2022)[37] summarizes all 17 different studies performed to date that utilized VLPs to deliver gene editing tools in vitro or in vivo with cells or mice, respectively, indicating that human trials at this point in time are limited, but will undoubtedly be an important area of focus in the field of genome editing within the near future. To provide some perspective on this growing area of research, here we review several notable studies where researchers have used VLPs to deliver molecular components required for CRISPR-Cas genome editing specifically highlighting recent work where either Nanoblades or Lentivirus-VLPs have been successfully implemented to deliver either RNAs encoding Cas9 and the sgRNA or RNPs containing the Cas9 protein and sgRNA complex.

Nanoblades

Nanoblades are mouse leukemia virus (MLV)-VLPs used to deliver CRISPR-Cas9 that contain Cas9-sgRNA RNPs[38]. Current research has indicated that nanoblades have lower off-target edits and higher editing efficiency than other CRISPR delivery methods, including electroporation and DNA transfection. For instance, Mangeot and colleagues[38] transduced multiple cell lines and difficult to transfect primary cell types of both mouse (bone marrow) and human (iPSCs and stem cells) origin, with nanoblades loaded with Cas9-sgRNA RNPs designed to target a single gene. When they compared off-target edits and overall editing efficiency to standard biomolecule delivery methods, they observed approximately 5% lower off-target editing in HEK293T cells transduced with Cas9-sgRNA-containing nanoblades than in cells that underwent a standard transfection protocol. As compared to cells that underwent electroporation, they observed much higher genome editing efficiencies in both cell lines and primary cells transduced with nanoblades. It is evident that nanoblade delivery shows much promise for editing primary cells that are notoriously difficult to transfect without affecting their ability to differentiate, and thus appears to be a remarkable tool for ex vivo genome editing.

Since the original and seminal study where nanoblades were developed for genome editing in various difficult-to-transfect mouse and human primary cells, another research group used nanoblades to knock out the androgen receptor (AR) and the cystic fibrosis transmembrane conductance regulator (CTFR) genes in mouse and human organoids[39]. Notably, the use of nanoblades resulted in editing of up to 80% of cells within the organoids, with no off-target effects or cytotoxicity.

MLV is a retrovirus, and the tropism of these viruses can be modified by a process called pseudotyping, or where the envelope glycoprotein of the natural virus is exchanged for that of another, promoting the attachment to a different cell type. Such modifications allow viruses and VLPs to be targeted to specific cell types, and have been used to increase the efficiency of cell-specific targeting by nanoblades[40]. Variations of the original MLV-VLP nanoblades have also been generated that extend beyond pseudotyping, including engineering modifications that promote a higher efficiency of RNP packaging and genome editing at target sites, while displaying less off-target effects[41]. When these engineered nanoblades were introduced into mice via a single injection, gene editing of target cells within the brain, liver, and retina was observed. It will be exciting to see the implications of this study as they relate to the future of in vivo delivery of gene editing tools in humans.

Lentivirus (LV)-VLPs

While many conditions are caused by mutations on a single gene, there are other conditions such as hereditary spherocytosis that are polygenic and will require a system capable of delivering tools to simultaneously edit mutations on multiple genes. Through their research, Lu and colleagues[42] observed that LV-VLPs were able to more effectively edit multiple genes in a human cell line with a singular dose, than when they targeted individual genes. When comparing VLPs to electroporation, they observed a higher on-target editing rate with LV-VLPs with a much lower concentration of Cas9. They also found that the ratio of on-target edits to off-target edits was significantly higher with LV-VLPs than with electroporation, suggesting that these virus particles show promise as a multi-gene editing delivery vehicle[42]. Additional studies also show high potential of LV-VLPs for CRISPR delivery to establish gene knockout cell lines[43-44] and the simultaneous delivery of CRISPR-Cas9 and transgenes[45]. Hamilton et al.[45] treated primary human T cells with a LV-VLP engineered to contain a Cas9-sgRNA RNP, a chimeric antigen receptor (CAR) transgene, and an HIV-1 envelope glycoprotein to specifically target CD4+ T cells. Upon delivery of this engineered LV-VLP, CD4+ T cells were selectively edited in a mixed population of cells such that the cells expressed the CAR. These data suggest that LV-VLPs may be a promising future method for generation of CAR-T cells in patients with various types of blood cancers.

Conclusion

CRISPR-Cas9 is one of a handful of gene editing technologies, but what sets it apart from alternative genome editing tools is the relatively lower cost[46-47], making it a more accessible treatment option as compared to expensive pharmaceuticals. The intent of this article is to highlight the importance of the CRISPR-Cas9 gene editing technology, and the potential VLPs have in revolutionizing the way CRISPR-Cas9 is delivered to cells. Our hope for the future is that researchers will continue to identify and develop treatment options for a number of conditions using these methods that enable higher targeting of intended cells, higher gene editing rates, and lower off-site editing at a more affordable price. We are excited for a future where biotech and healthcare companies invest in research involving CRISPR-Cas9 and VLPs as delivery tools, gaining more opportunities for long-term profit, and decreasing patient costs, while improving the overall quality of life for many.

COMPETING INTERESTS

No competing interests declared.

ACKNOWLEDGEMENTS

We would like to extend a special thanks to the Talaria Summer Institute, through which our initial introduction and continued collaboration would not have been possible.

Received: June 6, 2022
Accepted: December 5, 2022
Published online: January 23, 2024

REFERENCES

1. Doudna, J. A. & Charpentier, E. The new frontier of genome engineering with CRISPR-Cas9. Science 346, 1077 (2014).
2. Lu, Y. et al. Safety and feasibility of CRISPR-edited T cells in patients with refractory non-small-cell lung cancer. Nat. Med. 26, 732–740 (2020).
3. Rosenblum, D. et al. CRISPR-Cas9 genome editing using targeted lipid nanoparticles for cancer therapy. Sci. Adv. 6, eabc9450 (2020).
4. D'Antonio L, Fieni C, Ciummo SL, et al. Inactivation of interleukin-30 in colon cancer stem cells via CRISPR/Cas9 genome editing inhibits their oncogenicity and improves host survival. J. for ImmunoTher. of Cancer. 11, e006056 (2023). https://doi:10.1136/ jitc-2022-006056.
5. Su, G. et al. A synthetic targeted RNA demethylation system based on CRISPR□Cas13b inhibits bladder cancer progression. Clin. and Transl. Med., 12, e734 (2022).
6. Carlice-dos-Reis, T. et al. Investigation of mutations in the HBB gene using the 1,000 genomes database. PLoS ONE 12, e0174637 (2017). https://doi: 10.1371/journal.pone.0174637
7. Henry, E. R. et al. Allosteric control of hemoglobin S fiber formation by oxygen and its relation to the pathophysiology of sickle cell disease. Proc. Natl Acad. Sci. 117,15018-15027 (2020).
8. Frangoul, H, et al. CRISPR-Cas9 Gene Editing for Sickle Cell Disease and β-Thalassemia. NEJM 384, 252-260 (2020).
9. Son, J. et al. Therapeutic correction of hemophilia A using 2D endothelial cells and multicellular 3D organoids derived from CRISPR/Cas9-engineered patient iPSCs. Biomaterials, 283, 121429–121429 (2022).
10. He, B.-J., Liao, L., Deng, Z.-F., Tao, Y.-F., Xu, Y.-C., & Lin, F.-Q. Molecular genetic mechanisms of hereditary spherocytosis: current perspectives. Acta Haematologica 139, 60-66 (2018).
11. McCarty, N. S., Graham, A. E., Studena, L., & Ledesma-Amara, R. Multiplexed CRISPR technologies for gene editing and transcriptional regulation. Nat. Comm. 11, 1281 (2020). https://doi.org/10.1038/s41467-020-15053-x
12. Campa, C. C., Weisbach, N. R., Santinha, A. J., Incarnato, D., & Platt, R. J. Multiplexed genome engineering by Cas12a and CRISPR arrays encoded on a single transcript. Nat. Meth. 16, 887-893 (2019).
13. Ayeleso, T. B., Ramachela, K., & Mukwevho. E.

Aqueous-methanol extracts of
orange-fleshed sweet potato (Ipomoea batatas) ameliorate oxidative stress and modulate type 2 diabetes associated genes in insulin resistant C2C12 cells. Molecules 23, 2058 (2018). https://doi.org/10.3390/molecules23082058
14. Lino, C. A., Harper, J. C., Carney, J. P., & Timlin, J. A. Delivering CRISPR: a review of the challenges and approaches. Drug Delivery 25, 1234-1257 (2018).
15. Shirbaghaee, Z., & Bolhassani, A. Different applications of virus-like particles in biology and medicine: vaccination and delivery systems. Biopolymers 105, 113–132 (2016).
16. Lee, E. B., et al. Liver-specific gene delivery using engineered virus-like particles of Hepatitis E virus. Scientific Reports 9, 1616 (2019). https://doi.org/10.1038/s41598-019-38533-7
17. Zeltins, A. Construction and characterization of virus-like particles: a review. Mol. Biotechnol. 53, 92–107 (2013).
18. Qian, C. et al. Recent progress on the versatility of virus-like particles. Vaccines 8, 139 (2020). https://doi.org/10.3390/vaccines8010139
19. Datoo, M. S., et al. Efficacy of a low-dose candidate malaria vaccine, R21 in adjuvant matrix-M, with seasonal administration to children in Burkina Faso: a randomised controlled trial. The Lancet 397, 1809–1818 (2021).
20. Ashley, C. E., et al. Cell-specific delivery of diverse cargos by bacteriophage MS2 virus-like particles. ACS Nano 5, 5729–5745 (2011).
21. Kaczmarczyk, S. J., Sitaraman, K., Young, H. A., Hughes, S. H., & Chatterjee, D. K. (2011). Protein delivery using engineered virus-like particles. Proc. Natl Acad. Sci. 108,16998-17003 (2011).
22. Ausar, S. F., Foubert, T. R., Hudson, M. H., Vedvick, T. S., & Middaugh, C. R. Conformational stability and disassembly of Norwalk virus-like particles: effect of pH and temperature. J. Biol. Chem 281 19478–19488 (2006).
23. Caldeira, J. C. & Peabody, D. S. Thermal stability of RNA phage virus-like particles displaying foreign peptides. J. Nanobiotechnol. 9, 22 (2011). https://doi.org/10.1186/1477-3155-9-22
24. Dai, S., Wang, H., & Deng, F. Advances and challenges in enveloped virus-like particle (VLP)-based vaccines. J. Immunol. Sci. 2, 36-41 (2018).
25. Simon, C., et al. Disulfide linkage and structure of highly stable yeast-derived virus-like particles of murine polyomavirus. J. Biol. Chem. 289, 10411–10418 (2014).
26. Samandoulgou, I., Fliss, I., & Jean, J. Zeta potential and aggregation of
virus-Like particle of human norovirus and feline calicivirus under different physicochemical conditions. Food Environ. Virol. 7, 249–260 (2015).
27. Hashemi, K., et al. Optimizing the synthesis and purification of MS2 virus like particles. Sci. Rep. 11, 19851 (2021).
28. Fiedler, J. D., et al. Engineered mutations change the structure and stability of a virus-like particle. Biomacromolecules 13, 2339–2348 (2012).
29. Mohsen, M. O., Gomes, A. C., Vogel, M., & Bachmann, M. F. Interaction of viral capsid-derived virus-like particles (VLPs) with the innate immune system. Vaccines, 6, 37 (2018). https://doi.org/10.3390/vaccines6030037
30. Cubas, R., et al. Virus-like particle (VLP) lymphatic trafficking and immune response generation after immunization by different routes. J. Immunother. 32, 118–128 (2009).
31. Zepeda-Cervantes, J., Ramirez-Jarquin, J. O., & Vaca, L. Pattern recognition receptors (PRRs) from dendritic cells (DCs): toward better engineering of VLPs. Front. Immunol. 11, 1100 (2020).
32. Caldeira, J. C., Perrine, M., Pericle, F., & Cavallo, F. Virus-like particles as an immunogenic platform for cancer vaccines. Viruses 12, 488 (2020). https://doi:10.3390/v12050488
33. Wang, B. Z., et al. Incorporation of membrane-anchored flagellin into influenza virus-like particles enhances the breadth of immune responses. J. Virol. 82, 11813-11823 (2008).
34. Lv, L., et al. Production and immunogenicity of chimeric virus-like particles containing the spike glycoprotein of infectious bronchitis virus. J. Vet. Sci. 15, 209–216 (2014).
35. Kazaks, A., et al. Production and purification of chimeric HBc virus-like particles carrying influenza virus LAH domain as vaccine candidates. BMC Biotechnol. 17, 79 (2017). https://doi.org/10.1186/s12896-017-0396-8
36. Taha, E. A., Lee, J. & Hotta, A. Delivery of CRISPR-Cas tools for in vivo genome editing therapy and challenges. J. Controlled Release 342, 345-361 (2022).
37. Lyu, P. & Lu, B. New advances in using virus-like particles and related
technologies for eukaryotic genome editing delivery. Int. J. of Mol. Sci., 23(15), 8750 (2002). https://doi.org/10.3390/ijms23158750
38. Mangeot, P.E., et al. Genome editing in primary cells and in vivo using viral-derived Nanoblades loaded with Cas9-sgRNA ribonucleoproteins. Nat. Commun. 10, 45 (2019). https://doi.org/10.1038/s41467-018-07845-z
39. Tirolle, V. et al. Nanoblades allow high-level genome editing in organoids. bioRxiv, (2022). https://doi.org/10.1101/2022.08.04.502859
40. Gutierrez-Guerrero, A. et al. Baboon envelope pseudotyped “nanoblades” carrying Cas9/gRNA complexes allow efficient genome editing in human T, B, and CD34+ cells and knock-in of AAV6-encoded donor DNA in CD34+ cells. Frontiers in Genome Editing 31, 604371 (2021).
41. Banskota, S. et al. Engineered virus-like particles for efficient in vivo delivery of therapeutic proteins. Cell 185, 250-265 (2022).
42. Lu, Z., et al. Lentiviral capsid-mediated Streptococcus pyogenes Cas9 ribonucleoprotein delivery for efficient and safe multiplex genome editing. CRISPR J. 4, 914-928 (2021). https://doi.org/10.1089/crispr.2020.0106
43. Beaufils, M. et al. Development of Knock-Out Muscle Cell Lines using Lentivirus-Mediated CRISPR/Cas9 Gene Editing. J. of Visualized Experiments (2022). https://doi.org/10.3791/64114

44. Lin, S., Haga, K., Zeng, X., & Estes, M. Generation of CRISPR-Cas9-mediated genetic knockout human intestinal tissue-derived enteroid lines by lentivirus transduction and single-cell cloning. Nat. Protocols 17, 1004-1027 (2022).
45. Hamilton, J. et al. Targeted delivery of CRISPR-Cas9 and transgenes enables complex immune cell engineering. Cell Reports 35, 109207 (2021).
46. Rahim, J., Gulzar, S., Zahid, R., & Rahim, K. A systematic review on the comparison of molecular gene editing tools. Int. J. Innovat. Sci. and Res. Technol. 6, 829-836 (2021).
47. Memi, F., Ntokou, A., & Papangeli, I. CRISPR/Cas9 gene-editing: research technologies, clinical applications and ethical considerations. Sem. Perinatol. 42, 487-500 (2018).