CRISPR/Cas9 Gene Editing in Hepatocellular Carcinoma: Current Progress and Future Perspectives

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Abstract: The use of CRISPR/Cas9 gene-editing technology to treat viral hepatitis and hepatocellular carcinoma (HCC) is examined in this paper. HCC, a deadly cancer with high incidence, poses significant challenges due to late diagnosis and aggressive progression. CRISPR/Cas9 offers promising avenues for treatment by targeting genetic mutations, oncogenes, and viral genomes. The technology's ability to disrupt HBV's cccDNA and silence HCV RNA highlights its potential in viral hepatitis therapy. In HCC, CRISPR/Cas9 can suppress tumor growth by correcting mutations in critical genes like TP53 and CTNNB1. Obstacles to clinical translation, however, include immunological reactions, off-target effects, and delivery efficiency. The document emphasizes the need for advancements in delivery systems, specificity improvements, and ethical considerations to unlock CRISPR/Cas9's full potential in precision medicine.

1 INTRODUCTION

Hepatocellular carcinoma (HCC) ranks among the most prevalent and lethal cancers worldwide, distinguished by its high incidence rate and unfavorable patient prognoses. According to the World Health Organization, liver cancer is the fourth leading cause of cancer-related mortality globally, with approximately 841,000 new cases and 781,000 deaths reported annually. In China, there are approximately 393,000 new cases and 369,000 deaths each year. Chronic infection with the hepatitis B virus (HBV) constitutes a major etiological factor for liver cancer. Additional significant risk factors encompass infection with the hepatitis C virus (HCV), cirrhosis, alcohol consumption, and non-alcoholic fatty liver disease. Notwithstanding considerable progress in surgical methodologies and the implementation of diverse therapeutic modalities—including hepatic resection, orthotopic liver transplantation, radiofrequency ablation, systemic chemotherapy, and molecularly targeted therapies, the overall prognosis for hepatocellular carcinoma (HCC) continues to be unfavorable. This unfavorable outcome is predominantly attributed to the tumor's intrinsic biological aggressiveness, characterized by rapid progression, high metastatic potential, and resistance to conventional treatments. Furthermore, epidemiological data consistently demonstrates that a substantial proportion of HCC cases are identified at an advanced clinical stage, often precluding curative intervention. Late diagnosis is frequently compounded by the asymptomatic nature of early-stage HCC and limitations in current surveillance protocols, particularly in high-risk populations with chronic liver disease. Consequently, these factors synergistically contribute to suboptimal long-term survival rates, underscoring the urgent need for refined diagnostic algorithms and innovative therapeutic paradigms in hepatocellular carcinoma management.

The advent of gene-editing technologies, particularly the CRISPR/Cas9 system, has introduced new avenues for the treatment of hepatocellular carcinoma (HCC). The CRISPR/Cas9 system, which is derived from the adaptive immune system of prokaryotic organisms, enables precise and targeted editing of specific DNA sequences within living cells. This technology holds the potential to correct genetic mutations, silence oncogenes, activate tumor suppressor genes, and even disrupt viral genomes, thus offering a powerful tool for the treatment of HCC.The CRISPR/Cas9 gene-editing platform constitutes a revolutionary molecular biology tool comprising two principal components: the Cas9

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endonuclease and a synthetic single-guide RNA (sgRNA). Through complementary base pairing, the sgRNA directs the Cas9 ribonucleoprotein complex to a precise genomic locus, where the endonuclease catalyzes the introduction of site-specific double-strand breaks (DSBs). These DSBs subsequently undergo repair via one of two primary cellular mechanisms: non-homologous end joining (NHEJ) or homology-directed repair (HDR). The error-prone nature of NHEJ frequently results in frameshift mutations through small insertions or deletions, thereby facilitating gene knockout. In contrast, HDR enables precise sequence modifications when a homologous donor template is provided, permitting targeted gene insertion or specific base-pair alterations. This dual-repair pathway paradigm underpins the remarkable versatility of CRISPR/Cas9 in facilitating diverse genetic modifications, ranging from loss-of-function mutations to precise gene activation or regulatory element introduction, thereby serving as a cornerstone technology in modern molecular biology and therapeutic development.

Recent research has shown promising potential for the CRISPR/Cas9 gene-editing system in treating hepatocellular carcinoma (HCC). For instance, scientists have used this system to target and disrupt the covalently closed circular DNA (cccDNA) of the hepatitis B virus (HBV). This cccDNA is essential for the virus to stay in the host and has a major impact on the development of hepatocellular carcinoma. The CRISPR/Cas9 system can specifically cut cccDNA, which helps to inhibit viral gene expression and reduce the amount of viruses in infected cells. Also, CRISPR/Cas9 has been used to target oncogenes and tumor suppressor genes in HCC cells, like TP53, CTNNB1, and MYC, which are often changed in liver cancer. By fixing these changes or turning off the overactive oncogenes, CRISPR/Cas9 can slow tumor growth and make existing treatments work better. Furthermore, CRISPR/Cas9 has demonstrated potential in overcoming drug resistance in hepatocellular carcinoma (HCC). For example, studies have identified genes such as PHGDH and HK1, which contribute to resistance to sorafenib and regorafenib, respectively. By knocking out these genes using CRISPR/Cas9, researchers have increased the sensitivity of HCC cells to these drugs, thereby improving therapeutic outcomes. Also, CRISPR/Cas9 has been used to target long noncoding RNAs (lncRNAs) that are important for HCC progression and metastasis. For example, removing lncRNAs like SNHG9 and RP11-156P1.3 has been proven to restrain the proliferation, migration, and invasion of HCC cells. Although CRISPR/Cas9 has shown great results for HCC treatment, its clinical use is challenged by delivery efficiency, off - target effects, and immune responses. Creating effective and safe delivery systems (such as viral vectors and nanoparticles) is key to translating CRISPR/Cas9 technology into clinical practice. Meanwhile, enhancing the specificity and minimizing off—target effects of CRISPR/Cas9 are vital for guaranteeing the safety and effectiveness of this gene—editing technology.

2 THE DEVELOPMENT AND APPLICATION OF CRISPR-CAS9 TECHNOLOGY

CRISPR-Cas9 technology, discovered in 2012 by Jinek et al. in the paper “A programmable dual-RNA–guided DNA endonuclease in adaptive bacterial immunity” published in Science, has rapidly become a core tool in gene editing (Jinek et al. 2012). Rooted in the adaptive immune system of bacteria, it harnesses guide RNA (gRNA) to target specific DNA sequences with remarkable precision for gene editing. This technology has not only demonstrated its prowess in basic research but also holds great promise in clinical applications. In basic research, CRISPR-Cas9 allows scientists to delve into the functions of genes by selectively modifying or deleting them. For instance, as described in various research studies similar to the concept presented in an overview of the evolution of CRISPR/Cas9 and its potential in HCC therapy, in model organisms like drosophila and mice, the researchers can use this technology to create gene knock-out or knock-in, observing the resulting phenotypic changes to understand the roles of specific genes in development, metabolism, and disease mechanisms (Wei et al. 2019). This has significantly advanced our understanding of biological processes at the genetic level. Clinically, the potential of CRISPR-Cas9 is vast. In cancer treatment, it can be used to target and disrupt oncogenes or repair mutated tumor - suppressor genes in cancer cells. For example, in melanoma, although a specific melanoma - related paper is not cited here, many studies in the field of cancer treatment, such as a summary of written works on utilizing CRISPR/Cas9 gene therapy for hepatocellular carcinoma treatment, explore the use of CRISPR-Cas9 to target key genes

in cancer cells (Wu et al. 2020). Knocking out genes associated with drug resistance can enhance the sensitivity of tumor cells to chemotherapy. In the context of genetic diseases, such as sickle cell anemia, while no specific paper is directly referenced, numerous research efforts in the area of gene - editing for genetic diseases aim to correct the mutated gene responsible for the disease using CRISPR-Cas9 technology, offering hope for patients who previously had limited treatment options.

However, CRISPR-Cas9 technology's use faces many difficulties. Off - target effects are a major concern, as they can cause unintended mutations in non - target genes. These off - target mutations may lead to unforeseen consequences, including the activation of oncogenes or disruption of normal cellular functions. Immune responses are another obstacle. The human body may recognize the introduced Cas9 protein or delivery vectors as foreign, triggering an immune reaction that can neutralize the treatment and even cause adverse effects. These challenges are discussed in documents like Cutting-edge nano-theranostics of CRISPR-Cas for viral hepatitis and hepatocellular carcinoma and “Description of CRISPR/Cas9 development and its prospect in hepatocellular carcinoma treatment” (Wei et al. 2019, Amjad et al. 2024). To address these issues, researchers are actively developing more precise gRNA design methods. By optimizing the gRNA sequence, scientists aim to improve its binding specificity to the target DNA, reducing the likelihood of off - target effects. Additionally, the development of Cas9 protein variants with lower immunogenicity is underway. These engineered Cas9 proteins are designed to evade the immune system's detection, enabling more effective in vivo applications. In tandem with these efforts, delivery systems that combine nanotechnology are being refined. Lipid - nanoparticle - based delivery systems, for example, are being optimized to enhance the stability and targeting of CRISPR-Cas9 components in the body, ensuring that the technology reaches its intended target cells while minimizing side effects. As described in cutting-edge nano-theranostics of CRISPR-Cas for viral hepatitis and hepatocellular carcinoma, these progress are essential for CRISPR-Cas9 technology to be applied successfully (Amjad et al. 2024).

3 THE USE OF CRISPR-CAS9 IN MANAGING VIRAL HEPATITIS

Viral hepatitis, especially that caused by HBV and HCV, poses a serious threat to global public health as it can result in severe liver diseases and cancer. The CRISPR-Cas9 gene - editing system presents new treatment options for viral hepatitis. For HBV, cccDNA is the key therapeutic target. It has four long open reading frames that code for proteins crucial for the virus to copy itself. Once HBV infects liver cells, its double-stranded DNA moves into the nucleus to form cccDNA, which is essential for making new viruses and maintaining long - term infection. As mentioned in the review "Advanced Nanotheranostics of CRISPR Cas for Viral Hepatitis and Hepatocellular Carcinoma", multiple CRISPR/Cas9 systems have been created to target the stable parts of cccDNA in lab and animal studies. For example, in some studies, CRISPR/Cas9 - mediated editing achieved a significant decrease in cccDNA levels in HBV - infected cells, inhibiting viral replication and potentially reducing the risk of tumorigenesis associated with HBV infection (Bai et al. 2020). For HCV, which copies itself in the cell fluid, the main method is to target its RNA. When HCV RNA gets into liver cells, it keeps making the virus. Studies, like those using the FnCas9 system, have shown that CRISPR/Cas9 can block HCV RNA. In one experiment, the FnCas9 system with special RNA - targeting guides was able to lower HCV protein levels by more than 50%, showing that CRISPR—Cas9 could be a promising way to treat HCV—related viral hepatitis (Bai et al. 2020).

However, treating viral hepatitis involves not only clearing the virus but also repairing liver damage and modulating immune responses. The immune system's response to the virus and the CRISPR - Cas9 system itself is a crucial factor. The introduced CRISPR/Cas9 components may trigger immune reactions, which could potentially lead to cell death or other negative consequences. As mentioned in "Advanced Nanotheranostics of CRISPR Cas for Viral Hepatitis and Hepatocellular Carcinoma", pre - existing immunity to Cas9 proteins, such as SaCas9 and SpCas9, has been detected in a significant portion of the population, which may impact the effectiveness of CRISPR - based therapies (Jinek et al. 2012). Therefore, the application of CRISPR - Cas9 in viral hepatitis treatment must be integrated with other therapeutic

approaches, such as antiviral medications and immune modulators. Conventional antiviral agents like reverse transcriptase inhibitors and RNA interference technology have been used to combat viruses in the liver. Combining these with CRISPR - Cas9 could enhance the overall therapeutic effect. For instance, some research has looked into using mixed treatment methods to focus on various parts of the virus's life cycle and the host's immune reaction (Wu et al. 2020).

Additionally, achieving efficient delivery and specific targeting of CRISPR - Cas9 to the liver remains a key focus of current research. Delivery vectors need to overcome several barriers, including the large size of CRISPR/Cas9 cargos, degradation by nucleases in physiological fluids, crossing cell membranes, and potential degradation in endosomes and lysosomes. Viral vectors, including adenovirus and adeno-associated virus (AAV), have served as delivery tools for CRISPR/Cas9 systems to the liver. But they have flaws, such as limited packaging ability, possible integration into the host genome, and immune response - triggering potential. Liposome - or lipid - based nanoparticles, polymer - based nanoparticles, inorganic nanoparticles, cell - derived nanoparticles, and peptide/protein - based nanoparticles are being developed as non - viral vectors. These non - viral vectors have advantages such as lower immunogenicity and flexible universality, but they also face challenges like lower delivery efficiency. Researchers are exploring ways to optimize these delivery systems, for example, by modifying the physicochemical properties of nanoparticles, adding targeting ligands, and improving endosomal escape mechanisms (Bai et al. 2020, Amjad et al. 2024).

4 THE APPLICATION OF CRISPR-CAS9 IN THE TREATMENT OF HEPATOCELLULAR CARCINOMA

Hepatocellular carcinoma (HCC) is one of the most common cancers globally and a major public health concern due to its high incidence and death rates.CRISPR-Cas9 technology has emerged as a transformative tool in HCC treatment by enabling precise targeting of oncogenes, tumor suppressors, and critical signaling pathways. For instance, CRISPR-mediated knockout of PHGDH (phosphoglycerate dehydrogenase) disrupts serine biosynthesis, leading to oxidative stress and enhanced sensitivity to sorafenib in HCC cells. This approach has demonstrated efficacy in inhibiting tumor growth both in vitro and in murine xenograft models (Amjad et al. 2024). Similarly, targeting CTNNB1 (β-catenin) using CRISPR-Cas9 disrupts the aberrant Wnt/β-catenin signaling pathway, effectively reducing cell proliferation and tumorigenesis in preclinical models. Restoring TP53 function via CRISPR editing also shows promise by reactivating cell cycle control and apoptosis, thereby HCC progression.

Beyond direct gene editing, CRISPR-Cas9 is being leveraged to reshape the tumor microenvironment. For example, editing DUSP4 reactivates the ERK/MAPK pathway, sensitizing HCC cells to tyrosine kinase inhibitors and potentially augmenting immunotherapy outcomes. Genome-wide CRISPR screens have also identified novel targets such as ADAMTSL3, whose inactivation suppresses HCC proliferation and metastasis, highlighting its potential as a therapeutic candidate. Additionally, CRISPR-based modulation of long noncoding RNAs (lncRNAs) like SNHG9 and CASC11 has been shown to disrupt oncogenic pathways, underscoring the versatility of this technology in addressing HCC's complex genetic landscape (Lu et al. 2021). However, translating these findings into clinical applications faces significant challenges. The inherent genomic heterogeneity of HCC necessitates delivery systems capable of achieving precise and targeted editing while minimizing off-target effects. Recent advancements in nanotechnology, such as charge-reversing lipid nanoparticles, show promise in enhancing CRISPR-Cas9 delivery to hepatic tumors. Furthermore, integrating multi-omics data with CRISPR screening platforms could enable personalized treatment strategies by identifying actionable genetic alterations and predicting therapeutic responses. Addressing these challenges will be critical to unlocking the full potential of CRISPR-Cas9 in overcoming HCC's therapeutic resistance and improving patient outcomes.

5 DELIVERY SYSTEMS FOR CRISPR-CAS9 TECHNOLOGY

The effective application of CRISPR-Cas9 technology relies on robust delivery systems to ensure its precise localization and release within target cells or tissues. Current delivery systems are mainly divided into viral and non-viral vectors. Viral vectors, including AAVs and lentiviruses, provide high efficiency and sustained expression but risk triggering immune responses and integrating into the host genome. For example, AAV vectors have been widely used in hepatic targeting due to their natural tropism for hepatocytes, allowing effective CRISPR-Cas9 delivery to treat viral hepatitis and HCC (Kong et al. 2021). However, their limited packaging capacity (<5 kb) restricts the delivery of large genetic payloads, and pre-existing immunity to AAV serotypes in humans may reduce therapeutic efficacy. Lentiviruses, while capable of integrating transgenes into the host genome, pose oncogenic risks due to random integration events, necessitating careful design to avoid insertional mutagenesis.

Non-viral vectors, including liposomes, polymer nanoparticles, and inorganic nanomaterials, are characterized by low immunogenicity and high customizability, though their delivery efficiency is comparatively lower. Lipid-nanoparticle (LNP) systems, for instance, have been optimized to encapsulate CRISPR-Cas9 components and enhance cellular uptake through endosomal escape mechanisms (Yang et al. 2020). These LNPs can be surface-functionalized with ligands targeting specific cell surface receptors, such as asialoglycoprotein receptors in hepatocytes, to improve tissue specificity. Polymeric vectors, such as polyethyleneimine (PEI), offer tunable physicochemical properties but often require further modification to reduce cytotoxicity and improve transfection efficiency. Emerging inorganic nanomaterials, including gold nanoparticles and mesoporous silica, have shown promise in improving intracellular delivery through their ability to protect nucleic acids from degradation and facilitate endosomal escape. To address the limitations of traditional delivery systems, researchers are developing innovative platforms with spatiotemporal control. Extracellular vesicles (EVs), such as exosomes, have emerged as natural nanocarriers due to their low immunogenicity and endogenous targeting capabilities. EVs derived from mesenchymal stem cells (MSCs) have been engineered to carry CRISPR-Cas9 components, demonstrating efficient delivery to hepatic tissues in preclinical models (Yang et al. 2020).

6 CLINICAL TRANSLATION AND ETHICAL ISSUES OF CRISPR-CAS9 TECHNOLOGY

The clinical translation of CRISPR-Cas9 technology is a hot topic and major challenge in the field of gene editing. Despite significant progress in laboratory research, numerous obstacles remain in clinical applications. First, making sure CRISPR-Cas9 is safe and works well in humans is key. It needs careful testing in clinical trials to check for side effects and long - term results. In sickle cell disease (SCD) treatment, CRISPR-Cas9 has potential by turning on fetal hemoglobin (HbF) production. But studies found it can cause gene - editing errors and DNA rearrangements, especially in SCD samples. So, a full safety check is needed (Li et al. 2022). Secondly, ethical considerations surrounding CRISPR-Cas9 technology have generated extensive debate. Gene editing could lead to genetic variations and raise concerns related to germline editing, which may affect future generations. The international community is divided on the appropriate scope of CRISPR applications, with some advocating for a regulatory framework that accommodates all types of human genome editing, including germline editing. Additionally, developing sound regulatory policies and ethical guidelines to ensure the rational application of this technology is another problem that needs to be addressed. By May 2018, at least 15 clinical trials using CRISPR for various diseases were underway globally. In China, genes of at least 86 people were altered in such trials. This highlights the need for strong regulations to oversee CRISPR applications (Hsu et al. 2014).

Moreover, the clinical application of CRISPR-Cas9 technology also faces challenges in optimizing delivery systems for efficient gene editing while minimizing impact on non-target tissues. Enhancing editing precision and reducing off-target effects are critical research focuses. Despite these challenges, CRISPR-Cas9 shows great potential in treating various diseases, such as precisely repairing gene mutations for genetic disorders or modulating gene expression for complex diseases. With technological advancements and deeper understanding of gene editing mechanisms, CRISPR-Cas9 is poised to play

a larger role in clinical medicine, offering new therapeutic options and hope for patients.

7 CONCLUSION

CRISPR-Cas9 technology, from bacterial immune systems, has transformed gene editing and shows promise in treating diseases like viral hepatitis and HCC by enabling precise DNA changes. However, the transition from laboratory research to clinical application is complex and multifaceted, requiring careful navigation of technical, ethical, and regulatory challenges. In the realm of viral hepatitis, CRISPR-Cas9 has demonstrated remarkable potential. For HBV, targeting the covalently closed circular DNA (cccDNA) represents a critical advancement, as this viral component persists in infected cells and hinders cure. Studies have shown that CRISPR-Cas9 can effectively reduce cccDNA levels, inhibit viral replication, and potentially decrease the risk of HCC development. Similarly, for HCV, RNA-targeting approaches using CRISPR-Cas9 have shown the ability to silence viral RNA and reduce viral protein expression. These applications highlight the versatility of CRISPR-Cas9 in combating viral infections that pose significant global health burdens. The treatment of HCC has also been transformed by CRISPR-Cas9 applications. By targeting oncogenes such as PHGDH and CTNNB1, researchers have successfully suppressed tumor growth and enhanced the efficacy of existing therapies. The technology's ability to disrupt drug resistance mechanisms, such as those involving HK1, further underscores its therapeutic potential. Additionally, the modulation of long noncoding RNAs and tumor microenvironment components demonstrates the comprehensive approach CRISPR-Cas9 offers in addressing the complex genetics of HCC.

Despite these advances, several challenges must be addressed for successful clinical translation. Off-target effects remain a primary concern, as unintended edits can lead to serious consequences, including carcinogenesis. Immune responses to CRISPR-Cas9 components, particularly Cas9 proteins, may limit therapeutic efficacy and cause adverse reactions. The development of more specific guide RNAs, immune-evasive Cas9 variants, and advanced delivery systems is crucial to mitigate these issues. Delivery systems represent another critical area of research. Viral vectors, while efficient, face limitations such as immunogenicity and packaging capacity. Non-viral vectors, like lipid nanoparticles and polymers, have less immunogenicity but need optimization to enhance delivery efficiency. The integration of nanotechnology and spatiotemporal control mechanisms, such as extracellular vesicles, holds promise for enhancing targeted delivery and reducing off-target impacts. Ethical and regulatory considerations are equally important. The potential for germline editing and unintended genetic variations has sparked intense debate, with diverse opinions on the appropriate scope of CRISPR applications. The establishment of robust regulatory frameworks is essential to ensure the safe and ethical use of this technology, particularly as clinical trials involving CRISPR-Cas9 continue to expand globally.

CRISPR-Cas9 technology leads in precision medicine, creating unique chances to treat once - hard - to - manage diseases. The path forward requires a balanced approach that fosters innovation while addressing safety, ethical, and regulatory concerns. Future research should prioritize the development of more precise editing tools, efficient delivery methods, and comprehensive ethical guidelines. By doing so, the scientific community can harness the full potential of CRISPR-Cas9 to deliver effective therapies and improve outcomes for patients with viral hepatitis, HCC, and other debilitating conditions.

REFERENCES

Amjad, E., Pezzani, R., Sokouti, B. 2024.A review of the literature on the use of CRISPR/Cas9 gene therapy to treat hepatocellular carcinoma. Oncology Research 32(3):439-461.

Bai, Q., Shao, J., & Cao, J., et al. 2020. Protein kinase C-α upregulates sodium channel Nav1.9 in nociceptive dorsal root ganglion neurons in an inflammatory arthritis pain model of rat. Journal of Cellular Biochemistry 21(1):768-778.

Hsu, P. D., Lander, E. S., & Zhang, F. 2014. Development and applications of CRISPR-Cas9 for genome engineering. Cell 157(6):1262–1278.

Jinek, M.,Chylinski,K.,& Fonfara,I.,et al. 2012. A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. Science 337(6096): 816-821.

Kong, H., Ju, E., & Yi, K. 2021. Advanced Nanotheranostics of CRISPR/Cas for Viral Hepatitis and Hepatocellular Carcinoma. Advanced Science 8(24): e2102051.

Li, C., Zhan, W.,&Yang, Z. 2022. Broad neutralization of SARS-CoV-2 variants by an inhalable bispecific single-domain antibody. Cell 185(8):1389–1401.

Lu, J., Ding, Y., & Zhang, W., et al. 2021. SQSTM1/p62 Knockout by Using the CRISPR/Cas9 System Inhibits Migration and Invasion of Hepatocellular Carcinoma. Cells 12(9):1238.

Wei, L., Lee, D., & Law, CT. et al. 2019. Genome-wide CRISPR/Cas9 library screening identified PHGDH as a critical driver for Sorafenib resistance in HCC. Nature Communications 10(1): 4681.

Wu, X., Ma, W., & Mei, C., et al. 2020. Description of CRISPR/Cas9 development and its prospect in hepatocellular carcinoma treatment. Journal of Experimental & Clinical Cancer Research 2020:39(1):97.

Yang, Y. C., Chen, Y. H., & Kao, J. H. 2020. Permanent Inactivation of HBV Genomes by CRISPR/Cas9-Mediated Non-cleavage Base Editing. Molecular Therapy Nucleic Acids 20:480–490.