

# ***Research Advances in the Application of CRISPR/Cas9 Technology for Hepatocellular Carcinoma Therapy***

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**Abstract.** Hepatocellular carcinoma (HCC) is one of the most prevalent types of cancer in the world and is extremely invasive, with few available treatments as today. Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/CRISPR-associated protein 9 (Cas9), short for CRISPR/Cas9, is a gene editing tool, derived from the natural defence system of bacteria against exogenous viruses, that has recently been widely under research. It involves a guide RNA and Cas9 protein from the Cas family, and cleaves double stranded DNA to inhibit genes. CRISPR/Cas9 technology could have groundbreaking impacts into curing hepatitis B virus (HBV), the virus that stands as the most common cause of HCC. This paper reviews the mechanism and function of the CRISPR/Cas9 system in detail, suggesting some methods by which CRISPR/Cas9 can be used to deactivate genes (targets) that cause cancer. This review discusses the challenges of the CRISPR/Cas9 technology at present and suggests further research direction as it is ultimately an innovative approach that holds great potential for cancer treatment.

**Keywords:** CRISPR/Cas9, liver cancer, hepatocellular carcinoma, single guide RNA

## **1. Introduction**

Liver cancer is one of the most common cancers globally, ranking seventh in terms of prevalence. The primary types of liver cancer are hepatocellular carcinoma (HCC) and intrahepatic cholangiocarcinoma (ICC), with HCC accounting for over 90% of all cases. HCC is the third leading cause of death from cancer around the world, with its prevalence on the rise. However, HCC is unique among cancers. A recent analysis covering 17 types of human cancer has shown significant similarities in gene expression patterns, with the notable exception of HCC. This explains why treatments effective against other cancers often fail to improve outcomes for HCC patients. The major underlying risk factors for HCC include chronic liver damage caused by viral infections, cirrhosis, alcohol use, non-alcoholic fatty liver disease, and exposure to aflatoxins. Most HCC cases arise in cirrhotic livers. Around the world, the most frequent reasons are infections caused by the hepatitis B virus (HBV) and the hepatitis C virus (HCV). Even though many countries are seeing fewer cases of these infections because of vaccines, the number of people getting liver cancer linked to hepatitis C in the U.S. is still high. This is because there are a lot of people who have had the infection for many years and it's still affecting them. HCC is challenging to treat due to several factors, including its genetic diversity, underlying liver dysfunction in most patients, difficulty in

detecting early - stage disease, and the lack of effective treatments. At the time of diagnosis, only 30% of individuals with HCC are eligible for potentially curative procedures like liver transplantation. In general, classical chemotherapies and radiotherapies have either low efficacy or undesirable toxicity. Other treatment strategies include liver resection, transcatheter arterial chemoembolization (TACE), and molecular - targeted drugs such as sorafenib and lenvatinib [1].

Because of its elevated risk of reoccurrence and delayed detection, hepatocellular carcinoma (HCC) still has the second-highest mortality rate among malignancies, even with the availability of numerous therapeutic options. Therefore, improving the screening and management of HCC is crucial in today's context. In the last several years, advances in genetic research and gene therapy have emerged as potential alternatives to traditional treatment strategies, offering new hope for HCC management. Gene therapy is the process of repairing or replacing damaged genes in cells. A number of gene editing techniques could be used to treat HCC, suppressing oncogenes, activating tumor suppressors, and fixing mutations that promote HCC cell proliferation and survival. The capacity of CRISPR/Cas9 gene-editing technology to precisely target and modify particular genes inside the genome has recently attracted widespread attention. The CRISPR/Cas9 system, derived from the CRISPR family, is an adaptive immune system found in microorganisms that originates from bacterial defense mechanisms against viral infections. The method has been enhanced to serve as a diverse and potent instrument for gene editing, progressively supplanting outdated technologies owing to its comfort, affordability, and extensive applications. Because of its capacity to modify genes, CRISPR/Cas9 has the potential to transform the treatment of a number of disorders, including HCC. Furthermore, by combining "dead" Cas9 proteins with transcriptional activators or repressors, CRISPR can change gene expression without changing DNA sequences, enabling the activation or repression of transcription. CRISPR/Cas9 gene therapy for HCC has been the subject of numerous investigations, with encouraging findings. This technology exhibits promise as a biomarker for HCC as well as a treatment alternative [2].

## 2. Mechanism and development of CRISPR/Cas9 technology

Japanese researchers initially identified CRISPR in 1987, although at the time, its purpose was unclear. Researchers gradually discovered in 2007 that CRISPR is a sophisticated immune mechanism that microbes have evolved to protect themselves from the invasion of external genes. This technique creates an RNA-mediated nuclease to break exogenous genes after integrating segments of those genes into palindromic repeats. Based on the sequences encoding CRISPR-related proteins, additional study divided the CRISPR family into five or six kinds. In 2012, CRISPR/Cas9 was first employed as a powerful technique for modifying genes. The double-stranded combination of tracrRNA and crRNA was successfully combined by Jinek et al. to create single-stranded RNA known as single-guide RNA (sgRNA). This sgRNA has the ability to identify target genes and trigger the cleavage of double-stranded DNA by the Cas9 protein. Since then, researchers have advanced CRISPR/Cas9 technology in a number of noteworthy ways [3].

The mechanism of CRISPR/Cas9 technology is as follows. First, an exogenous gene is integrated into the CRISPR array. The foreign gene gets broken up into several DNA pieces, called protospacers by Cas1, Cas2, and Csn2 proteins, which are widely found in the CRISPR system, when it is introduced into the host by a phage. These protospacers are selected and integrated as new spacers, separated by repeat sequences, to form the CRISPR array. The selection of protospacers is primarily decided by brief segments of bases next to the target sequences, known as protospacer - adjacent motifs (PAMs). PAMs are specific to each CRISPR/Cas subtype and is a marker for foreign

gene sequences. This provides a means to identify and defend against similar future invasions by foreign genes [3].

Second, the CRISPR locus produces a tracrRNA-crRNA complex. A typical CRISPR locus consists of a trans-activating CRISPR RNA (tracrRNA) sequence, several Cas genes, a leader sequence, and the CRISPR array. TracrRNA, which is complementary to the repetitive sequences from the CRISPR array, is transcribed by the CRISPR system. In the meantime, repetitions and spacers are transcribed by the CRISPR array to create a crRNA precursor (pre-crRNA) that complements the foreign gene's target sequences. Rnase III and other nucleases break down the mature tracrRNA-crRNA complex, which is created when the precrRNA joins forces with tracrRNA, into single-stranded RNA. Lastly, the CRISPR/Cas9 system cleaves the target DNA to prevent the invasion of the exogenous genome. Cas9 is directed to the precise spot on the genome by single-guide RNA (sgRNA), which attaches to the target genes via complementary base pairing. Cas9 is able to precisely reach the right location on the genome because of this guidance. It is feasible to create a sgRNA that targets a particular gene by matching its sequence. Usually, these sgRNAs have a length of 20 nucleotides. Cas9 attaches itself to the sgRNA once it is within the cell. After that, this complex searches the genome for a complementary DNA sequence that matches the sgRNA. When a match is discovered, Cas9 cleaves the target DNA by causing a double-strand break. CRISPR/Cas9 is an effective gene editing technique because of its precise targeting mechanism, which may allow for the change of particular genes. The Cas9-sgRNA complex uses PAM sequences to locate target sites. Cas9 binding and DNA cleavage depend on the PAM sequence. In order to identify PAMs, the double-stranded DNA is cleaved three nucleotides upstream of the PAM, and the 20-nucleotide crRNA uses its spacer sequence to identify the particular target sequence. A different Cas9 nuclease domain (HNH or RuvC) cleaves each strand. This creates an opportunity for gene editing, allowing the insertion, repair, or replacement of genes. In eukaryotic cells, non-homologous end joining (NHEJ) is the primary method for repairing the cleaved DNA sequence. NHEJ rejoins the DNA ends after cleavage, often resulting in insertions or deletions that cause frameshift mutations and loss of gene function, thereby achieving gene knockout. NHEJ is the main repair pathway in most mammalian cells and tissues. Another repair method is homology-directed repair (HDR), which involves the recombination of an exogenous DNA template with the target site to introduce a target gene into the genome. This method is used for precise gene editing, such as gene knock-in or correction of mutations [4].

Today, CRISPR/Cas9 technology is used for much more than only inhibition of genes. In 2013, scientists modified the HNH and RuvC domains of the Cas9 protein to create an inactivated variant known as dead Cas9 (dCas9). While dCas9 can still bind to target DNA via sgRNA, it no longer cleaves the DNA. This inactivated form can be fused with activation domains (such as VP16, VP64, or NF- $\kappa$ B) to promote gene transcription or with repression domains (like KRAB or MIX1) to inhibit it. In 2016, Komor et al. developed a method for single-base DNA editing by fusing dCas9 with cytidine deaminase. This complex catalyzes the conversion of cytosine to uracil in specific DNA sequences through deaminase activity without needing to cut the double-stranded DNA. This approach enables base substitution without relying on HDR or NHEJ repair pathways. Further advancements were made by Perez-Pinera et al., who added VP16 to both the N-terminus and C-terminus of the dCas9 protein, making editing genes simultaneous more productive. Similarly, Bikard et al. constructed plasmids to target and edit multiple genes simultaneously. These studies demonstrate that CRISPR/Cas9 can edit multiple genes in the same cell or different cell types using a single medium. Researchers have also developed new CRISPR/Cas9 toolmodels with different additional functions. For example, Feng Zhang's team at MIT discovered Cpf1, a bacterial protein

that protects against viruses. Unlike Cas9, Cpf1 has its own RNase domain to process pre-crRNA, recognizes T - rich PAM sequences, and works with shorter sgRNAs without requiring tracrRNA. It also has a lower mismatch rate and edits DNA more precisely than Cas9 nickase. This discovery highlights the growing power and flexibility of CRISPR systems beyond the traditional Cas9. Overall, the CRISPR/Cas9 system is constantly evolving, with new innovations emerging regularly. These enhanced capabilities allow CRISPR/Cas9 to be used in a broader range of fields [4].

### 3. Applications of CRISPR/Cas9 technology in treatment of HBV and HCC

CRISPR/Cas9 technology holds immense potential for the detection and treatment of hepatocellular carcinoma (HCC), due to its ability to cause point mutations, inactivate multiple genes simultaneously, and induce chromosomal rearrangements. Chronic persistent infection with hepatitis B virus (HBV) is a leading cause of HCC, with approximately 30% to 40% of HBV carriers eventually developing the disease. Currently, no antiviral drug can completely eliminate HBV, making gene therapy a promising approach to lower the incidence of HCC. In Lin et al. initially showed in 2014 that CRISPR/Cas9 could cut particular HBV sequences, lowering the amount of surface antigen. Their findings demonstrated that the use of multiplex sgRNAs increased treatment efficiency and reduced the expression of HBcAg and HBsAg both in vitro and in vivo. Direct targeting and indirect targeting are the two primary approaches that CRISPR/Cas systems have traditionally been used to treat HCC. The main therapeutic targets for direct targeting are genes linked to HCC, such as tumor suppressor genes (TSGs) and oncogenes. CRISPR-mediated gene alterations can be used as an indirect strategy to improve the effectiveness of other treatments, including immunotherapy, anticancer medications, and other therapeutic agents or modifications. For example, a study shown that the suppression of ERK2 kinases by CRISPR/Cas improved the sensitivity of HCC cell lines to sorafenib, a multi-kinase inhibitor that is therapeutically advised for the treatment of HCC. Beyond sorafenib, other potential candidates for combination with CRISPR technology in HCC therapy include THZ1 (a CDK7 oncogene inhibitor), metformin, and additional agents [5].

Below are some detailed mechanisms by which CRISPR/Cas9 can be applied in the treatment of HBV and HCC. A crucial target for gene therapy to limit the spread of HBV, which can lead to HCC, is covalently closed circular DNA (cccDNA). Replication of HBV is closely linked to intracellular cccDNA. The circular, partially double-stranded HBV DNA enters the hepatocyte and moves to the nucleus, where it either becomes cccDNA or is incorporated into the host genome. This cccDNA assembles into a mini-chromosome, serving as the transcription template for viral mRNA synthesis. The The progenome RNA (pgRNA), which is the reverse transcription of the terminally redundant viral mRNA, is subsequently used by the virus to replicate. This pgRNA can be translated into viral proteins like polymerase, surface antigen (HBsAg), core protein (HBcAg), and hepatitis B virus X protein (HBxAg). These proteins are critical for the assembly of new virus particles. Viral envelope proteins and heparan sulfate proteoglycans on the surface of liver cells interact to cause HBV infection. Polymerase chain reaction (PCR) analysis is used to evaluate the relative amounts of HBV pgRNA and HBsAg mRNA, and cccDNA levels and total HBV DNA concentration variations are tracked. Thus, HBV replication and the ensuing carcinogenesis can be efficiently inhibited by targeting HBV cccDNA and its intermediates utilizing CRISPR/Cas9 technology. The CRISPR/Cas9 system can break the genome of virion DNA, resulting in either intentionally induced gene mutations or damaged DNA double strands that are prone to error-prone repair. This results in the loss of cccDNA biological activity or even degradation due to frameshift mutations, reducing its cellular content. Consequently, cccDNA cannot be reverse - transcribed into viral mRNA,

preventing the synthesis and packaging of viral proteins into new virion particles, thereby achieving the goal of curing HBV [5].

CRISPR/Cas9 can also be used for immunotherapy. One new development in cancer immunotherapy is CAR-T cell treatment. By deleting genes that block immune response receptors, CRISPR/Cas9 can improve the body's immune response to tumors in HCC therapy. T cells are extracted from the patient's body and modified with "combat equipment" and "GPS navigation" to quickly identify and eliminate cancer cells. The patient is subsequently given these altered T cells again. The third generation of CAR-T treatment has demonstrated exceptional effectiveness in treating multiple myeloma, non-Hodgkin lymphoma, acute myeloid leukemia, chronic myeloid leukemia, and certain solid malignancies. One particular instance is the alteration of CAR-T cells and gene editing of PD-L1 or PD-1 as workable approaches to cancer immunotherapy. Programmed death-1 (PD-1) is expressed by T cells and B cells, whereas PD-L1 is expressed by certain tumor tissues. The tumor-elimination effect can be weakened and autoimmune responses can be disrupted by the binding of PD-L1 and PD-1. Genetically altered T cells known as chimeric antigen receptor-modified T cells (CAR-T) are better able to identify and combat cancer cells. They generate an immunological response by identifying and binding to tumor-associated antigens using particular extracellular single-chain variable fragments (scFv). In The first human trial employing CRISPR/Cas9 to modify the PD-1 gene in immune cells for tumor research was started in October 2016 by Professor You Lu's team. CRISPR/Cas9 deactivated the PD-1 gene sequence in immune cells isolated from patients with metastatic non-small cell lung cancer (NSCLC). To strengthen the immune response against cancer cells, the altered immune cells were reinjected into the patients after being expanded in vitro. This method provides a model for comparable HCC therapies. Additionally, basic research on PD-L1 in HCC has produced pertinent findings. Guo et al. investigated the effects of chimeric antigen receptor-modified T cells (CAR-T) on HCC progression. They discovered that the protective effect against tumors of CAR-T cells with PD-1 expression suppression is considerably longer. They were able to modify the PD-1 gene using CRISPR, which increased the immunotherapeutic effectiveness of CAR-T cells. This study showed that PD-1 alteration can greatly enhance CAR-T cell therapeutic efficacy in the treatment of HCC [6].

Additionally, CAR-T cells with antigen alterations that target the AFP gene in AFP-positive liver cancer cells were employed by Liu and colleagues. The findings demonstrated a significant in vivo inhibition of tumor growth, offering a viable targeting approach for CRISPR-based applications. By increasing effectiveness, decreasing toxicity, and lowering expenses, CRISPR/Cas technology can prospectively further CAR-T immunotherapy and provide synergistic "1 + 1 > 2" benefits. Ex vivo trials have actively investigated CAR-T cell therapy in conjunction with CRISPR/Cas editing, which has shown potential for enhancing the treatment of HCC. Furthermore, by deleting gene segments that encode human leukocyte antigen I (HLA-I) on T cells, CRISPR/Cas9 can remove the possibility of xenograft rejection. This strategy might make it possible to treat several patients across species boundaries using universal CAR-T cells, improving utilization efficiency and broadening therapeutic applications [6].

It is known that the TP53 gene is frequently mutated in many cancers, including liver cancer. In normal liver cells, the p53 protein plays a crucial role in maintaining genomic stability. When DNA damage occurs, p53 prevents the cell cycle from progressing beyond the G1/S checkpoint, allowing time for repair. If the damage cannot be repaired, p53 promotes apoptosis to prevent the propagation of damaged cells. In liver cells with TP53 mutations, however, the cell cycle becomes uncontrolled, leading to unchecked cell division even under adverse conditions. This uncontrolled growth can ultimately result in the development of liver cancer. Given the critical role of TP53 in tumor

suppression, the Cas9 protein may be able to precisely target and fix the mutant nucleotide in the TP53 gene if a single-guide RNA (sgRNA) that detects the protospaceradjacent motif (PAM) of the TP53 gene is created. Through the restoration of the p53 protein's normal activity, this targeted repair may be able to slow the growth of liver cancer. It might be feasible to restore TP53's native function using gene-editing technologies, which would stop liver cancer from spreading. Another crucial tumor treatment method is the introduction of suicide genes, which cause cell death in addition to correcting TP53 mutations. Researchers can find key factors or blockers of liver tumor growth, such as long non-coding RNAs (lncRNAs), by using CRISPR-mediated genome-wide screening. By targeting and modulating these factors, it may be possible to create successful strategies to inhibit or diminish their activity, thereby treating patients with liver cancer [7].

#### 4. Current challenges and future prospects of CRISPR/Cas9 technology

The off-target effect has been identified as the most significant issue with the CRISPR/Cas9 technology. The majority of researchers think that the sgRNA's guide sequence, which is complementary to the 20 nucleotides upstream of the PAM in the CRISPR/Cas9 system, is the main factor influencing target gene recognition. But sometimes, among the billions many base pairs in the genome, the intended sgRNA might not be able to find and completely pair with the target region. As a result, Cas9 may potentially cleave sites that are not completely complementary to the sgRNA, producing off-target effects. Unwanted editing at offtarget locations can negatively affect the lifespan of cells and other biological functions, as well as significantly reduce the effectiveness of gene editing. As anticipated, there is a strong correlation between specificity and sgRNA length. Compared to TALEN, non-specific complementary sequences and off-target effects are inclined to take place in CRISPR/Cas9 because sgRNA only includes 20 complementary nucleotides, another gene - editing tool used for HCC treatment. TALEN's designed sequence contains 30 to 40 nucleotides, which enhances its specificity. However, plainly lengthening the complementary sequence in sgRNA is not a viable solution. Research has found that only the gene sequence of 14 nucleotides—comprising 12 nucleotides of sgRNA and 2 nucleotides of PAMs—decides the target site for the Cas9 nuclease. Longer sgRNAs and prolonged complementary areas actually decrease on-target editing efficiency, according to additional research. On the other hand, shortened sgRNAs can lessen off-target effects without compromising the effectiveness of gene editing. The simplest technique to investigate nonspecific binding with engineered sgRNAs is genome-wide homology sequencing. However, because of its high resource requirements and time-consuming nature, this approach is not feasible for basic research. Furthermore, the ability of Cas proteins to recognize particular PAM sequences limits their use. For instance, PAMs having NGG nucleotides are uniquely recognized by *S. pyogenes* Cas9 (SpCas9). This limitation still limits the application of CRISPR/Cas9, despite the fact that NGG sequences are very common in the human genome [8].

Scientists have significantly improved the CRISPR/Cas9 system from a number of perspectives in order to solve the serious problem of off-target consequences. As noted above, Ran et al. made significant strides in 2013 in their efforts to mutate the Cas9 protein. To produce a Cas9 nickase, they inserted mutations into the Cas9 protein's RuvC or HNH domains. The Cas9 nickase cuts one DNA strand under the guidance of sgRNA, supplying an appropriate repair template for the HDR process that follows. Two specially created sgRNAs can efficiently lengthen the corresponding sequence when double-stranded DNA cleavage is needed, improving specificity. High-fidelity SpCas9 (SpCas9-HF1) was created by Kleinstiver et al. in 2019; whole-genome sequencing cannot reveal its off-target rate. This variant enables non - repetitive sequence gene editing in human cell lines. Compared to wild - type SpCas9, SpCas9 - HF1 demonstrated superior performance in editing

85% of the sgRNAs tested in their study. In 2018, a further restriction pertaining to the identification of particular PAM sequences was resolved. In order to enhance Cas9's recognition capabilities and reducing the likelihood of off-target effects in intended goodsresearchers altered SpCas9 to create xCas9, which can recognize PAM sites with sequences NG, GAA, and GAT [8].

Another significant concern is the potential immunogenicity of CRISPR/Cas systems. These systems might be perceived by the body as foreign substances when they are delivered, which may trigger anti-Cas antibodies and Cas-specific cellular reactions as well as immunological responses. These immune responses may result in cell death or other adverse effects, which would ultimately lead to the failure of CRISPR/Cas editing. Severe immunological reactions and graft-versus-host rejection are also possible. More than 70% of humans have antibodies against the popular CRISPR proteins SaCas9 and SpCas9, according to Porteus' group. Given that these antibodies are ready to target foreign proteins and cause inflammation or even death, this study raises questions for CRISPR-based therapeutic trials. 85% of healthy participants have preexisting immunity to SpCas9, according to later research. These findings highlight the difficult issue of immunogenicity in CRISPR/Cas editing. Furthermore, it is still exceedingly difficult to deliver CRISPR/Cas9 into the human body. Hydrodynamic tail vein injection, used in mouse models of HCC for cancer treatment research, is not feasible for human applications. However, recent advancements in materials science offer potential solutions. For example, Wang et al. created a mature delivery agent, PEGlipid/AuNPs/Cas9-sgPlk-1 (LACP), by compressing Cas9 sequences and sgRNA plasmids into gold nanoparticles and encasing them in a lipid shell. The Cas9 sequence and sgRNA can be released into the cell nucleus by targeting this delivery agent with a laser's thermoelectric effect. This approach shows promise for delivering CRISPR/Cas9 into target tumor cells in the human body, despite the fact that it is still in the preclinical stage and that further research is needed to determine how well it delivers the protein [9].

CRISPR/Cas9 technology is likely to continue evolving and improving rapidly in the future. It offers new treatment mechanisms and holds great potential for improving the efficacy of HCC treatment, as well as building on previous versions of gene-editing technology. Even though immunogenicity and off-target effects are still challenges, these could be solved with the aid of alternative engineering techniques. To uncover answers, scientists are working hard. Numerous researchers are investigating the potential of CRISPR/Cas as a treatment for viral infections, including HCC, due to its ability to cleave, eliminate, or inactivate hepatitis viruses and genes linked to HCC. Research on CRISPR-based therapies and diagnostics is just getting started. To overcome these constraints, the next generation of CRISPR/Cas9 systems for HCC therapy must incorporate developments in cellular biology, immunology, materials science, and genomic analysis. Control, safety, and accuracy are necessary for successful clinical translation. To get over these obstacles, the scientific community must work together to improve technical aspects and create strict ethical and regulatory frameworks. For CRISPR to become a dependable, broadly used therapeutic strategy, advancement in these areas will be crucial [9].

Since the delivery system is one aspect that has yet to be sufficiently developed, future research should focus on improving the safety and feasibility of the CRISPR/Cas9 system. Targets ought to be precisely located for lesion sites, enabling CRISPR/Cas therapeutic editing to be performed without endangering cells or organs that are not the intended targets. It could be required to target several targets in order to gain increased efficacy. Crucially, for CRISPR/Cas editing at different sites, a delivery mechanism that is safe, effective, and targeted is necessary. Transient CRISPR/Cas expression and nanoparticles with adequate encapsulating capacity and acceptable biocompatibility are the main requirements for safety. Safety is of utmost importance to ensure that this technology

can be implemented as a large-scale treatment strategy. Optimizing the CRISPR/Cas system itself and putting delivery mechanisms in place that speed up endosomal escape and nuclear localization can increase efficiency. The specificity, stability, and safety of delivery vectors must be enhanced. With a focus on reducing immunogenicity, future studies are probably going to concentrate more on biocompatible materials, targeted ligands, and controlled release mechanisms. Since the majority of research is currently pre-clinical, future efforts must involve planning thorough clinical studies to evaluate the efficacy, safety, and viability of CRISPR/Cas9 for HCC. To record long-term results, possible adverse effects, and enhancements in quality of life, a methodical methodology is required. Significant genetic heterogeneity is seen in HCC patients, which increases the efficacy of individualized gene therapy. To accomplish this, further research is required on thorough genetic screens and the creation of quick and affordable CRISPR toolkits customized to each patient's unique profile [10].

## 5. Conclusion

Researchers see potential the CRISPR/Cas9 gene-editing technology because of its low experimental cost, straightforward construction procedure, and exceptional work efficiency. The use of CRISPR/Cas9 in cancer research has increased as its variety of applications has broadened. This is partially due to the fact that ongoing innovation and change have greatly decreased the initial off-target effect. The discovery of CRISPR/Cas9 technology is of utmost importance and urgency with respect for the need for an effective treatment strategy for HCC. New developments in CRISPR/Cas9 technology boost hopes for innovative HCC treatment approaches, even if the majority of CRISPR/Cas9-related treatment strategies are still in the experimental stage. In terms of technology, CRISPR/Cas9 has a lot to offer. High targeting ability and numerous editable locations are achieved by the system's straightforward vector creation. The method mediates Cas9-mediated DNA cleavage by simply creating a CRISPR sgRNA with hundreds of nucleotides that are identical to the target DNA sequence. CRISPR/Cas9 can fix damaged genes through the introduction of new gene codes, which may be able to cure some genetic illnesses and increase the effectiveness of cancer treatments. Moreover, CRISPR/Cas9 can be used to discover and manipulate specific genes, serving as a versatile tool for exploring disease physiology and diagnosis. Thus, CRISPR/Cas9 has significantly transformed basic science, yielding impactful results and paving the way for the development of new treatment methods.

Future advancements in the fascinating subject of liver-associated disease diagnostics and treatment, especially for viral hepatitis and hepatocellular carcinoma (HCC), are anticipated thanks to next-generation CRISPR/Cas-based technologies. The conversion of CRISPR/Cas technology into precise diagnostics and efficient treatments, however, still faces several obstacles. It's critical to strike a balance between editing effectiveness and any negative effects. To advance the theranostics of viral hepatitis and HCC, significant work will be needed to create strategies for CRISPR-associated design and delivery in light of the identification of new markers and targets. It is anticipated that more CRISPR - based technologies will progress to clinical trials and be implemented on a larger scale over the next few decades, ultimately benefiting a vast majority of HCC patients.

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