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Current Applications and Future Perspective of CRISPR/Cas9 Gene Editing in Cancer Therapy

Chenxi Xu

Hangzhou Xuejun High School, Hangzhou Zhejiang, 310000, China

ABSTRACT

Clustered Regularly Interspaced Short Palindromic Repeats/Clustered Regularly Interspaced Short Palindromic Repeats-associated 9 (CRISPR/Cas9) is one of the most popular gene-editing tools derived from bacterial adaptive immune systems in recent years. Cancer is a complex disease that is related with the accumulation of genetic mutation; it can be caused by physical, chemical, and biological factors and is almost impossible to cure. Due to enabling precise genomic modifications, CRISPR/Cas9 gene editing has revolutionized cancer therapy. Recent studies emphasize its multi-functional role in both gene correction and immune regulation, positioning it as a versatile tool against diverse malignancies. Through comprehensive literature research, analyzing, and discussion, this study explores the mechanisms of utilizing CRISPR/Cas9 gene editing to treat cancer, including traditional application that focuses on the intensity of gene expression and next generation technology that shifts the research subjects to primers and basepairs, the delivery ways, and some immunology-combined therapies. The study also points out the challenges that still need to be solved in the future. Considering safety, we should think about off-target and live-system-caused gene integration. And for validity, we should figure out what kind of cells can be edited, how efficient is the editing process, and how to deliver the system to the target in human's body. Through numerous propelling clinic trials, we can see that this newly-discovered gene editing method is bringing a brand new revolution in cancer treatment. CRISPR/Cas9 has the transformative potential to bridge the gap between the laboratory research and clinical application, and open a new era of cancer biology.

KEYWORDS

CRISPR/Cas9; Gene editing; Cancer treatment; Gene therapy; Immunotherapy

1. INTRODUCTION

ACRIPR/Cas9 has experienced decades of development to become today's useful tool. In 1989, Francisco Mojica first observed CRISPR sequence in Haloferax mediterranei. Its DNA fragment had multiple copies of repeated sequence that was approximately palindromic and separated by certain spacers. Using bioinformatics, till 2000, Mojica proved the appearance of CRISPR loci in 20 different microbes. Then within 2 years, researchers figured out the correlation between Cas gene and CRISPR sequence but still didn't know CRISPR's function. It was later in 2005, Alexander Bolotin first supposed CRISPR provides bacteria with immunity. And Philippe Horvath verified that CRISPR was an adaptive immune system in 2007 with his colleagues. They found that certain S. thermophilus strains' CRISPR system can integrate new phage's DNA into CRISPR sequence in order to defend the following phage's attack. These are the initial development of CRISPR. The monumental paper published by Jennifer A Doudna and Emmanuelle Charpentier in 2012 marked the beginning of regarding CRISPR/Cas9 system as a tool for gene editing. This study showed the potential of CRISPR/Cas9 system and was the foundation of following studies. In the field of gene editing, Feng Zhang was one of the pioneers for he successfully induce a precise cleavage at endogenous genomic

loci in human and mouse cells by Cas9 nucleases [1-5]. Nowadays, CRISPR/Cas9 system is broadly utilize to achieve precise gene editing in cells, plants, and animals. In particular, scientists have developed several gene therapy for the treatment of incurable diseases.

Recent advances and therapeutic applications of CRISPR/Cas9 has brought some new sights into medicine and biotechnology. In clinical therapeutics, CRISPR-based therapies have achieved milestones such as Casgevy, which is the world's first officially approved gene therapy designed for sickle-cell disease and transfusion-dependent beta-thalassemia. Beyond hematology, CRISPR is also advancing treatments for inherited retinal diseases. EDIT-101, a CRISPR/Cas9 gene-editing complex designed to treat Leber congenital amaurosis, has succeeded in clinical test to improve test takers' eyesights [6]. For neuromuscular disorders like Duchenne muscular dystrophy, CRISPR/Cas9 is also contributing to correct the mutations that are responsible for DMD [7]. More recently, first in vivo gene insertion clinical trial, ECUR-506, dosing infants reported no significant clinical safety concerns apart from asymptomatic transaminitis at four weeks. This gene editing therapy for ornithine transcarbamylas deficiency helped infants to remain within mean ammonia level. This means ECUR-506 does show certain safety and validity. In conclusion, CRISPR-based therapy is a biomedical research orientation with great potential. It shows great potential for curing these miscellaneous diseases. As for cancer, one of the most common severe diseases in the world, scientists have also gained some exciting progress.

From the most recent progress in CRISPR-based therapy to gene therapy for cancer, in order to focus on this topic, we do comprehensive literature research, analyze what did researchers did with CRISPR/Cas9 system, and discuss the common thoughts for applying CRISPR/Cas9 system into cancer treatment. CRISPR/Cas9 has become indispensable in oncology, offering tools to dissect tumor biology and develop precision therapies. Genome-wide CRISPR screens can detect gene that loses the function or gains the function [8]. The application of CRISPR-based therapeutic target screening is widely used in the development of cancer drugs. Its application in cancer immunotherapy and precision oncology is booming. In cancer immunotherapy, CRISPR engineers immune cells to enhance tumor targeting. Clinical trials using CRISPR-edited CAR-T cells show promise in treating refractory leukemia and solid tumors [9]. Additionally, CRISPR enables the creation of personalized cancer models, such as patient-derived organoids with tumor-specific mutations, to predict drug responses and study resistance mechanisms [10]. Clearly, CRISPR-based cancer study is very useful and there's still a great space in this field for following researchers to develop. Our study aims to introduce the recent mechanisms and progress, show the potential, and prospect for the future development.

2. OVERVIEW

The CRISPR/Cas9 system, a groundbreaking genome-editing tool, has opened new avenues for precision cancer therapy by enabling targeted gene knockdown or over expression (Fig.1). This technology leverages a single-guide RNA (sgRNA) to direct the Cas9 nuclease to specific DNA sequences, where it introduces double-strand breaks (DSB). For gene knockdown, these breaks are typically repaired through error-prone non-homologous end joining (NHEJ), leading to frameshift mutations that disrupt the function of oncogenes. Silencing these genes can induce apoptosis, inhibit tumor proliferation, or reverse therapy resistance. Conversely, gene over expression strategies employ a catalytically inactive Cas9 (dCas9) fused to transcriptional activators or utilize homology-directed repair (HDR) to insert therapeutic genes. This approach restores tumor suppressor functions, or delivers immune-modulatory molecules to stimulate anti-tumor immunity [11].

2.1. Traditional Applications of CRISPR/Cas9 in Cancer Therapy

Take MYC, a group of oncogenes, as an example of silencing genes to cure cancer. MYC's potential mechanism to induce cancer includes promoting cell reproduction, inhibiting cell death, regulating

metabolism, improving angiogenesis and regulating the forming of stem cells [12]. Besides these cell intrinsic effects, MYC is also proved to have the ability of changing the tumor microenvironment to implement immune evasion [13]. In the clinical experiment Phase Ib/2, Multicenter, Dose Escalation Study of DCR-MYC in Patients With Hepatocellular Carcinoma (NCT02314052), researchers aim to evaluate the safety and tolerability of a MYC targeted anticancer drug DCR-MYC, which is a novel synthetic double-stranded RNA in a stable lipid particle suspension that targets the oncogene MYC. In the study, patient groups (cohorts) will receive a single dose level of DCR-MYC; the dose level of DCR-MYC will be increased in subsequent cohorts. And researchers would record and analyze the number of patients with adverse events as a measure of safety and tolerability. From the posted results, the ratio of having adverse events is low, and the ratio of all-cause mortality is lowered. Thus, DCR-MYC do have certain safety and tolerance. Another type of oncogene, Bcl-2, can cause neoplasia via inhibiting apoptosis. In their article, Rita Nahta and Francisco J. Esteva mention that Bcl-2 antisense oligonucleotides can help treat breast cancer. Protein translation is inhibited by specifically hybridizing with the target mRNA through complementary base pairing. The mRNA in the generated heteroduplex is then cleaved by the endogenous endonuclease RNase H. According to known experiments, Bcl-2 knockdown mice appear to have prominent increase in apoptosis, which would be helpful to cure cancer [14].

As for cancer suppressor gene, p53 is a great example. Wild-type p53 maintains the stability of genome and prevents oncogenesis [15]. Its coding gene TP53's silenced form is very common in human cancers [16]. Naturally, we'd think of increase the expression of p53 to treat cancer. Mutant p53 may also develop the ability to promote cancer progression; this process is named gain-of-function (GOF) [17]. Now we have three main cancer treatments focusing on p53. The first is to suppress mutant p53. Since it relies on HSP90 to stabilize, HSP90 inhibitor provides the possibility to decrease mutant p53 to indirectly defense cancer [18]. The second is to turn mutant p53 into wild type. This tragedy shows thought of over expressing cancer suppressor gene. In 1999, Foster and colleagues discovered CP-31398, a compound that can change mutant p53 to wild type by stabilizing the DNA Binding Domain (DBD) in active conform [19]. Later, more compounds with such function have been identified. The third is to utilize the synthetic lethality caused by mutant p53. Scientists have proved that WEE1 kinase can stop the cycle of DNA damaged cell at G2 checkpoint by inactivating CDK1/Cyclin B complex, which is the key regulating factor of starting mitosis [20].

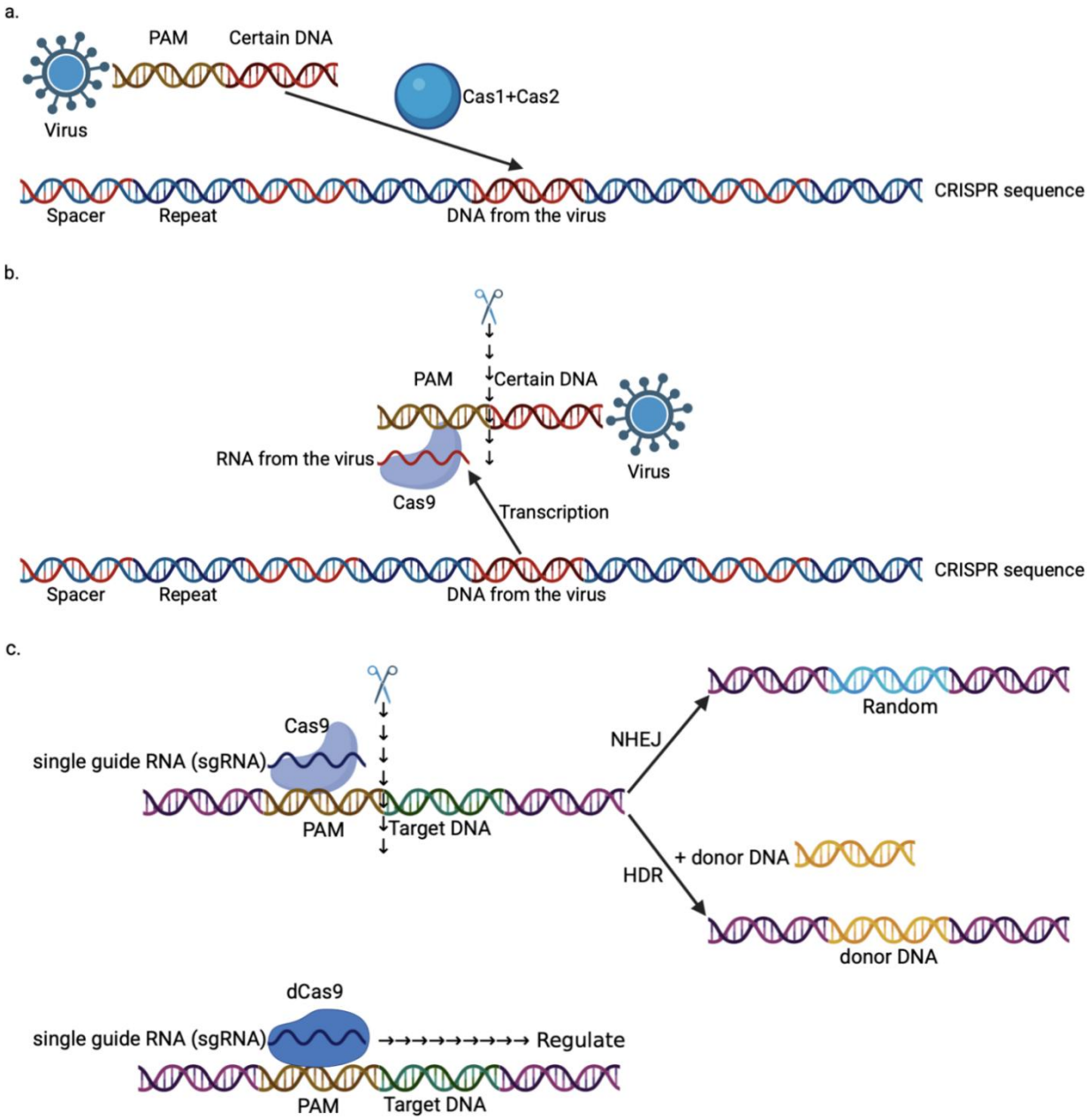

Figure 1. The original form of CRISPR system in bacteria and the adapted form for gene editing. a. Certain virus invading for the first time b. Certain virus invading for the second time c. Adapted CRISPR system

2.2. New Generation Application of CRISPR/Cas9 in Cancer Therapy

Those above are all traditional application of CRISPR/Cas9 in cancer treatment. Nowadays, scientists have developed new generation application of CRISPR/Cas9. Divided into 4 genres, new generation CRISPR/Cas9 application tries to solve the problem of delivery and combines gene editing with immunology.

In order to treat patients, we need to achieve editing gene inside of human's body, which means designing an in vivo CRISPR system. Then comes the problem: how to safely and effectively deliver this system to the target position? The first technology exactly focuses on this problem. Liposomes represent one of the most widely studied delivery vehicles. These cationic lipid vesicles effectively encapsulate CRISPR components through electrostatic interactions, as demonstrated by recent studies. For instance, Daniel Rosenblum et al. designed sgPLK1-cLNPs—CRISPR-LNPs against PLK1, a typical kinase. Experiments proved that intracerebrally injecting a single dose of sgPLK1-cLNPs into

aggressive orthotopic glioblastoma can edit around 70% gene in vivo. It then inhibited tumor growth by 50% through tumor cell apoptosis, meanwhile improved survival by 30%. Scientists also engineered cLNPs to be antibody-targeted to reach disseminated tumors. They targeted sgPLK1-cLNPs to EGFR (Epidermal Growth Factor Receptor). This intraperitoneal injection can make itself be distributed to disseminated ovarian tumors, causing around 80% gene editing in vivo. Therefore, tumor growth was inhibited and the survival increased by 80% [21]. LNP (Lipid Nanoparticles) technology has also emerged as particularly promising due to its tumor-selective accumulation. The enhanced permeability and retention (EPR) effect allows ionizable LNPs to preferentially target tumor tissues [22]. Recently, a circular ADAR-recruiting RNA (Circ-arRNA) was designed to achieve in vivo RNA editing mediated repairing for the TP53-W53X nonsense mutation. Scientists used LNPs to encapsulate and deliver Circ-arRNA into triple-negative breast cancer TP53-W53X 4T1 cells and tumor-bearing mouse models. The result showed that the mutation correction efficiencies were 73.32% and 48.48% respectively [23]. The third solution would be lentiviral vectors. They carry exogenous items into target cells and offer distinct advantages for stable genetic modification. For example, genetically modified T cells provided brought us some good news in cancer immunotherapy. Scientists tried to generate cytotoxic T cells by transducting the tumor-specific TCR (T Cell Receptor) into the patient's own T cells. In the clinical trial, they used a lentiviral vector to transfer a peptide specialized TCR into the multiple myeloma patients' bodies, and out of 20 patients, 16 of them reported clinical responses, with minimal safety concerns [24]. Besides all of these three, we can also use oncolytic viruses as the delivery carrier. Oncolytic viruses are a type of virus that selectively infect and lyse cancer cells, while simultaneously activating the human immune system to fight against tumors. They target tumor cells through genetic modification or natural characteristics, having relatively little impact on normal cells. In a research, scientists generated a recombinant oncolytic herpes simplex virus type 1 (HSV-1) named SONC103, together with a CRISPR/Cas9 system. If it can reach cervical cancer cells, it would disrupt integrated HPV16 genes to inhibit tumor. Result showed that the experiment was quite a success. SONC103 successfully inhibited the expression of HPV16 oncogenes, therefore reducing cell proliferation and increasing apoptosis [25]. To make a brief conclusion, we now have several developing and promising solutions for the problem of delivering CRISPR system.

Delivery is a technological problem. To make more innovation on the application of CRISPR/Cas9, scientists think about utilizing it to carry out immunotherapy. One very famous example is ICI with CRISPR/Cas9. ICI stands for Immune Checkpoint Inhibitors. This is a very new therapy acts on the checkpoint molecules on immune cells. By inhibiting the interaction of these molecules, it enhances the immune system's ability to recognize and attack tumor cells. Human's immune system is pretty complex and can be generally divided into two category: one is innate immunity: human was born with it and it works to defend every kind of pathogen; the other one is adaptive immunity: it's gained only after human's body once met that pathogen and it's specific to every pathogen. For innate immunity, macrophage cells and natural killer (NK) cells are very important. For adaptive immunity, T cells are very important. Cluster of differentiation 47 (CD47), a trans-membrane protein, is frequently over-expressed on many kinds of tumor cells. It can trigger the "don't eat me" signal when together with signal regulatory protein α (SIRPα) to the macrophages, and inhibit phagocytosis. PD-1, a protein located on T cels, can accept the message of terminating immune reactions carried by PD-L1. In ICI therapy, studies cutting both the PD-1/PD-L1 and CD47-SIRPα signal pathways off have been tried. Already developed BsAbs (bispecific antibodies) blocking dual checkpoint CD47 and PD-L1 can help us learn how T cells and bone marrow cells are specifically regulated by dual congenital and adaptive checkpoint mAbs (monoclonal antibodies). The results proved their potential of improving prognosis through simultaneously targeting PD-L1 and CD47. Regulating "eat me" and "don't eat me" signals at the same time can trigger Treg (regulatory T) cell exhaustion in the TME (Tumor Micro Environment) and therefore possibly treat solid tumors [26]. As for NKG2D (Natural Killer Group 2D), it is a kind of receptor of the NKG2 family and can activate the killing function in immune cells. Among all the selective inhibitors, Periostat, also named Doxycycline, a kind of

inhibitor of MMPs (matrix metalloproteinases) that can cleave the extracellular domain of MICA/B, which are ligands for the NKG2D receptor), is the only one undergoing clinical trials for different tumors. Periostat benefits to increasing NK cell-mediated cytotoxicity by regulating the increase in NKG2DL (NKG2D ligand) cell surface expression. Some drugs, for example histone deacetylase (HDAC) inhibitors or bortezomib, along with conventional cancer treatments like radiation therapy, chemotherapy, or immunotherapies, together with NKG2D-blockade therapies, have already shown a prominent upregulation of NKG2D ligands on cancer cells, through activating NKG2D+ effector cells that kill the tumor [27].

Besides ICI, there're still several therapy focusing on human's immune system. For example, T Cell Receptor-T cell therapy (TCR-T cell therapy) treats cancer by adding new genes into normal T cells to make it express TCR that specifically recognize tumor cells' MHC molecules, then kill the tumor cells. The procedure would be take out patient's T cells, edit them, and inject them back to the body. CRISPR/Cas9 is an indispensable part in the treatment process, because without it, we can't modify T cells. In a recent phase 1 clinical trial about epithelial cancers, scientists conducted a first-in-human experiment of HPV-16 E7 targeted TCR-T cells to test the treating effect (NCT02858310). In 6 of 12 patients reported obvious regression of tumor, along with objective clinical responses. Notably, these 6 patients included 4 of the 8 anti-PD-1 refractory patients. However, this treatment can stick in resistance mechanisms because of critical components loss in interferon response and antigen presentation pathways, therefore lead to different reactions in different part of the tumor [28].

Just like TCT-T cell therapy, CAR-T therapy also based on the function of T cells. CAR stands for Chimeric Antigen Receptor, a structure that can navigate the T cell to certain location. With a designed CAR, T cells can be navigated to tumor cells to kill them. The procedures are almost the same to TCR-T cell's: first take out patient's T cells, then edit them, finally inject the cells back to patient's body. But there are a bit difference. Though these two both modify patients' T cells in vitro, then inject the cells back, CAR-T depends on human-designed CAR structure that can only recognize the antigen on tumor cell's surface. But for TCR-T cells, they can recognize the antigen delivered by tumor cell's MHC molecule, which means it can not only recognize the antigen on tumor cell's surface, but also hundreds of thousands of antigen inside of the tumor cell. This advantage makes TCR-T cell therapy more suitable for solid tumor's treatment. Nowadays, UCAR-T (Universal CAR-T) therapy is developing rapidly. Universal means that the injected CAR-T cells are all the same from a healthy donor's T cells for every patient, but not specially produced from patient's own T cells for each person, which is a great progress compared to the conventional operation. Also, the injected cells can be derived from any species, no need to be confined to human cells. Recently, Yongxian Hu et al. reported CTA101, a universal CD19/CD22 dual-targeted CAR-T cell that disrupted T-cell receptor alpha chain (TRAC) and CD52 by CRISPR/Cas9. This showed a CR (Complete Response) rate of 83.3% (5/6) without neurotoxicity, dose-limiting toxicity, GVHD (graftversus host disease), or other adverse events concerned with genome editing [29]. Different from UCAR-T, ACAR-T (Allogeneic CAR-T) confines that injected CAR-T cells must come from human. UCART19 is the first use of allogeneic CAR-T in humans. In 2020, two Phase I trials reported monumental results. They were all about using UCART19 in relapsed/refractory B-cell acute lymphoblastic leukemia (R/R B-ALL) patients, and there were 7 children and 14 adults taking part in the trial. The data showed 67% promising complete response (CR/CRi) rate, while treatment-related deaths also existed [30].

2.3. Challenges to Complete

The clinical translation of CRISPR/Cas9 systems for tumor therapy confronts four intricately interconnected biological challenges that demand urgent multidisciplinary solutions. These hurdles—immunogenicity, macroscopic off-target effects, microscopic on-target inefficiency, and delivery toxicity—collectively represent the critical path bottlenecks preventing CRISPR from realizing its transformative potential in oncology. Each challenge manifests complex biological mechanisms requiring tailored intervention strategies spanning several different subjects.

Immunogenicity reduction is an important task for applying CRIPSR systems in human, as bacterial-derived Cas9 proteins function and are recognized as potent antigens. In a 2019 study, scientists hypothesized that there are already preexisting adaptive immune responses to the Cas9 orthologs, SaCas9 and SpCas9, derived from S.aureus and S.pyogenes, respectively, since these two orthologs are the most commonly used ones and these two bacteria infect human very frequently. They used enzyme-linked immunosorbent assay to test whether there are anti-Cas9 antibodies in human serum, and detected 78% and 58% of donors carrying antibodies for SaCas9 and SpCas9, respectively. Also, scientists found that there are anti-SaCas9 T cells in 78% of donors, and there are anti-SpCas9 T cells in 67% of the donors. To confirm that these T cells are specific to Cas9, they implemented a series of experiments: isolation, expansion, antigen restimulation, and then demonstrate a Cas9-specific cytokine response. With all these results together, scientists successfully demonstrate that for SaCas9 and SpCas9, it's prevalent to have antigen-specific T cells. This finding—both humoral and cell-mediated adaptive immune responses have already preexisted in human bodies—should be taken into serious consideration when applying CRIPSR/Cas9 system to clinical trials [31].

The second challenge involves minimizing off-target editing. Off-target effects mean that CRISPR components erroneously disrupt non-tumor tissues, potentially leading to adverse outcomes. In their 2023 essay, a group of scientists concluded four types of solutions to off-target effects. First, for Cas9 improvement, high-fidelity SpCas9 mutants like eSpCas9, SpCas9-HF1, and hypaCas9 are engineered via structure analysis to reduce non-specific binding; variants such as evoCas9 are screened through high-throughput methods, while paired Cas9 nickases or Cas9 homologs with rare PAMs are adopted to lower off-target docking. Second, regarding sgRNA improvement, optimal sgRNA sequences are screened first, with length extended with 5'-GG for T7 transcription or truncated by 2-3bp at the 5' end or chemical modifications like MP, BNA/LNA, deoxyribonucleotides added to boost specificity, though this is limited to synthetic RNAs. Third, DSB-independent editing uses base editors ABE/CBE for single-nucleotide conversion without DSBs or dCas9-based epigenetic editors to modulate gene expression, avoiding permanent DNA edits but requiring epigenome off-target checks. Finally, delivery optimization prefers RNP electroporation, mRNA delivery via electroporation or liposomes, or LNPs for transient editor expression to reduce off-target risks, while AAVs are avoided as they cause long-term expression and accumulated off-target mutations [32].

At the microscopic level, enhancing on-target editing precision remains critical, as even correctly localized CRISPR machinery frequently fails to induce functional edits. The first-in-human trial of CRISPR/Cas9 gene therapy (EBT-101-001) for HIV, presented at the 2024 International AIDS Conference, underlines CRISPR/Cas9's "microscopic imprecision and on-target editing failure". Applied via AAV9 intravenously to 6 patients split into two different concentration dose groups, it targeted latent HIV in CD4+ T cells. While no off-target DNA damage or serious adverse events occurred, 4 patients who paused antiretroviral therapy (ART) had HIV RNA rebound. Only 1 saw a ~16-week delayed rebound and reduced HIV reservoir. Notably, 2 subtype B-designed gRNAs had mismatches with some patients 'virus sequences, undermining efficacy. One patient couldn't pause ART due to low CD4+ counts, and another hadn't hit the 12-week ART pause milestone. Post-dose follow-up ranged from 10 weeks to 2 years, yet most still showed therapy ineffectiveness—clearly exemplifying CRISPR/Cas9's flaws: poor precision across HIV subtypes and frequent on-target editing failure, failing to control HIV in most patients.

Delivery is also a classical challenge being studied for decades. Current delivery strategies aim to improve the safety of delivery systems. Exposing to viral vectors too long after injection can lead to various risks, including mutation, off-target cleavage, and latent in vivo immune responses. o avoid these risks, scientists thought of using mRNA in the form of RNP (Ribonucleoprotein) to replace the role of plasmids. This can make the viral vectors stay for shorter time in the host, therefore avoid off-target effect and also increase efficacy at the same time. Non-viral vectors, though, face multiple hurdles: poor accumulation in target tissues, phagocyte clearance during delivery, protease/nuclease

degradation, cytoplasmic movement issues, and nuclear delivery difficulties. Researchers have done much to boost their target accumulation. One way uses EPR (enhanced permeability and retention) effects to gather nanocarriers in tumors; physical factors like radiation, ultrasound, etc., can enhance EPR. Developing targeted, smart vectors, for example aptamer-modified for active targeting also helps. NLSs, recognized by Importin α, start cargo transport to the nucleus, so adding NLSs aids efficient nuclear delivery [33].

3. CONCLUSION AND OUTLOOK

CRISPR/Cas9 has achieved notable progress in tumor therapy (Fig.2). It allows for precise gene editing, with applications spanning from traditional gene knockdown/overexpression to innovative combinations with immunotherapies such as ICI, TCR-T, and CAR-T. Clinical trials have shown encouraging results: for example, CRISPR-edited CAR-T cells targeting CD19 have led to partial remission in patients with refractory B-cell leukemia, and in solid tumor trials, edits to PD-L1 genes have increased tumor sensitivity to immunotherapy. Universal CAR-T products like CTA101, which utilizes CRISPR to disrupt TRAC and CD52 genes, have reported considerable complete response rates in cases of relapsed acute lymphoblastic leukemia. Yet, there are still challenges. The immunogenicity of Cas9 proteins, particularly SpCas9, can trigger preexisting IgG or T-cell responses in a significant portion of patients, reducing editing efficiency or causing systemic inflammation. Off-target editing, though lessened by newer systems, still happens—genome-wide studies have detected unintended mutations in some non-target loci in edited cell lines. Delivery remains a major obstacle: viral vectors carry insertion risks, while non-viral systems often have low accumulation in tumor tissues due to endosomal trapping and enzymatic degradation. Since more scientific progress has been made, there are already prospective solutions for these challenges. High-fidelity Cas9 mutants like eSpCas9 and Cas9-HF reduce off-target cleavage to a great extent by stabilizing on-target binding. Optimized sgRNAs with modified nucleotides enhance specificity and resistance to nucleases. For delivery, RNP-based mRNA systems can shorten vector residence time, lowering integration risks. Utilizing the EPR effect via appropriately sized nanocarriers boosts tumor accumulation, while aptamer modifications enable active targeting, and NLS tags improve nuclear entry efficiency. To address immunogenicity, Cas12a or Cas14 orthologs with lower cross-reactivity, or humanized Cas proteins engineered via epitope masking, are being tested in preclinical models.

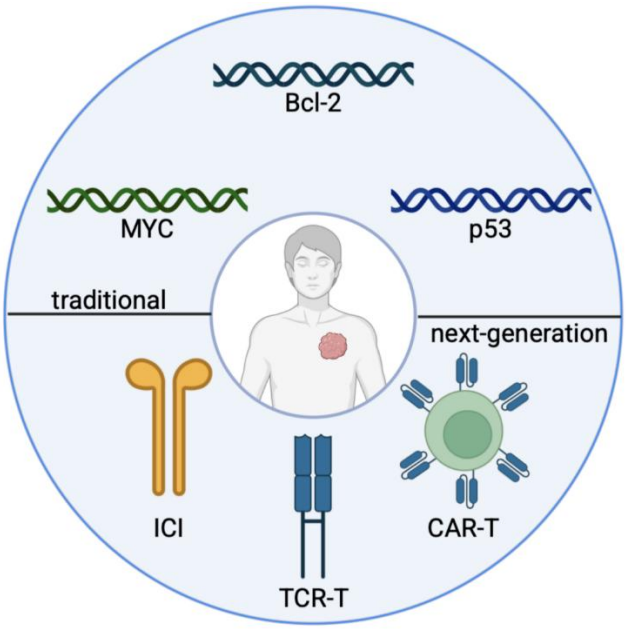

Figure 2. Traditional and next generation cancer therapies

This study systematically reviews mechanisms from traditional gene editing to next-gen immunotherapy combinations, integrating basic theory with clinical cases. By analyzing delivery strategies across viral/non-viral platforms and immunology interactions, it provides a comprehensive overview that bridges laboratory research—such as optimizing editing efficiency in cell lines—and clinical translation, offering actionable insights for researchers designing trials or refining delivery systems. This study can help researchers get a brief understanding of rapid-developing CRISPR/Cas9 technology. Also, this study might be helpful to help researchers find latent research orientations, and inspire more scholars, more medical companies to devote their wise into this field. The future of CRISPR/Cas9 in tumor therapy is promising. With the advancement of single-base editing and prime editing technologies, its precision will further improve, minimizing on-target errors. In the coming years, more phase III trials may result in approvals: CAR-T products edited via CRISPR could enter routine clinical use, and in vivo delivery systems may become feasible. Combined with modalities like radiotherapy or targeted drugs, it will revolutionize oncology, enabling personalized edits for driver mutations and offering long-term remission for patients with previously untreatable tumors.

ACKNOWLEDGEMENT

As I complete this paper focusing on the application of CRISPR/Cas9 technology in cancer treatment, I find myself overwhelmed with gratitude, eager to express my sincere thanks to those who have supported me throughout this journey. Among them, my instructor, Shaohua Hu, stands out as a pivotal figure, whose selfless guidance has been the cornerstone of this research.

The topic of this paper, exploring how CRISPR/Cas9 technology can be harnessed to revolutionize cancer treatment, is both cutting-edge and complex. When I first proposed the general direction, I was merely driven by a vague interest in gene editing and its potential in medical science, with little clarity on how to translate this interest into a structured research paper. It was Shaohua Hu who stepped in, not as a paid advisor—for all this guidance has been entirely voluntary—but as a dedicated mentor genuinely invested in my growth. From the very beginning, he played a crucial role in shaping the project: after listening to my initial thoughts, he helped refine the research direction, making the research more feasible and targeted. What followed was his meticulous work in helping me outline the paper; he sat with me for multiple discussions, breaking down the paper into logical sections—from the introduction to CRISPR/Cas9 technology, to its current applications in cancer therapy, to existing challenges and future prospects—ensuring that each part flowed seamlessly into the next. This initial framework provided me with a clear roadmap, without which I would have undoubtedly wandered in disorganized research.

The process of completing the paper was not without its difficulties, and at every hurdle, Shaohua Hu was there to offer guidance. The first major challenge arose during the literature collection phase. As an high school student, I lacked experience in academic research: I struggled to find relevant, high-quality papers, often getting lost in a sea of unrelated articles on gene editing, and even when I managed to locate some papers, I found it hard to grasp their core arguments due to the complex scientific terminology. Understanding my struggle, Shaohua Hu taught me some practical skills like using academic database PubMed and choosing authoritative journals. With these skills, I was able to collect over 30 papers and news reports, laying a solid foundation for the paper.

The next challenge came during the writing process. My academic writing skills were not that good: my initial writings were like simply listing scientific concepts and experimental results, but lacked the variation of expression. Shaohua Hu pointed out this question after reading my first draft. He also spent time discussing the structure with me, suggesting that I rearrange some sections to present the information in a more logical order, starting with the basics of CRISPR/Cas9, then moving to its applications, and finally addressing challenges. Following his advice, I revised the draft repeatedly—three full revisions in total—and with each version, the paper became clearer and more polished. Additionally, Shaohua Hu carefully guided me on the paper format, ensuring that the citation style,

headings, and reference list all adhered to the required academic standards, which was crucial for the paper's professionalism.

It is important to note that this research is entirely the product of collaborative efforts between Shaohua Hu and me, with no other individuals or institutions involved in its completion. The division of labor was clear: I took responsibility for selecting the initial topic, collecting and organizing the literature, and drafting and revising the manuscript, while Shaohua Hu provided indispensable guidance in refining the research direction, outlining the paper, offering comments for revision, and ensuring the paper's format was correct. Throughout the process, his guidance was always voluntary.

In conclusion, Shaohua Hu's expertise, patience, and selflessness have not only helped me complete this research on CRISPR/Cas9 in cancer treatment but have also taught me valuable research skills and instilled in me a deeper appreciation for academic rigor. This experience will undoubtedly inspire me to continue exploring the fascinating field of medical science in the future.

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