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Review

CRISPR/Cas9 technology: A promising gene-editing tool for the treatment of cancers

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Abstract

Clustered Regularly Interspaced Palindromic Repeats (CRISPR) technology is an innovative gene-editing technique that has emerged as a result of the development of genetic engineering. This technology has expanded the scope of oncology-related medical research and clinical applications. CRISPR is used in molecular techniques to decode genes and pathways, alter the expression of specific genes for therapeutic purposes, and comprehend the pathophysiology of cancer. If pre-clinical research with this technology is successful, it could lead to clinical trials and eventually be used in clinical therapy. To establish the CRISPR complex as a promising tool in oncology for effective clinical cancer therapy, a variety of CRISPR variants and applications, as well as numerous experimental techniques, are being developed at present. This review examines several CRISPR technology variations, their application in oncology, as well as the system's advantages and disadvantages in comparison to earlier gene-editing technologies. It also discusses the recently discovered capabilities of the technology and its potential future applications in oncology.

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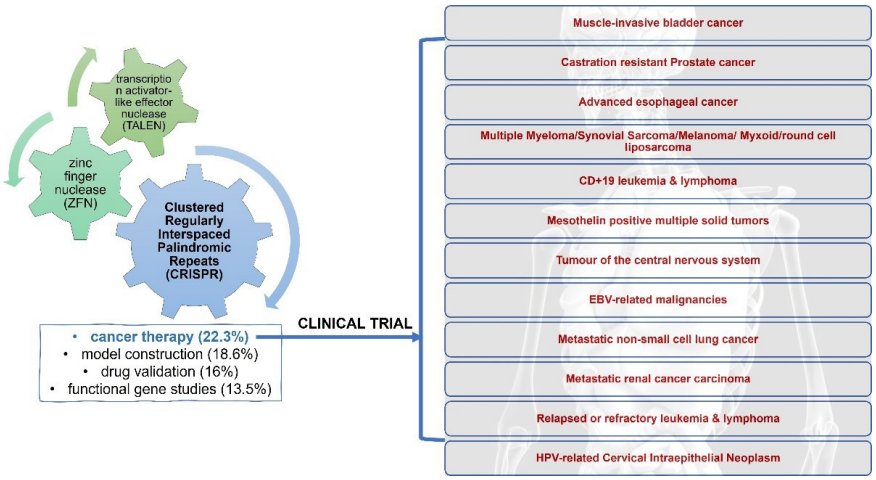

Graphical abstract This graphical abstract illustrates the application of Clustered Regularly Interspaced Palindromic Repeats (CRISPR) technology in the treatment of cancer.

1. Introduction

Clustered Regularly Interspaced Palindromic Repeats (CRISPR) is a new genome-editing technology that was discovered in the late 1980s as part of the adaptive immune system in bacteria and archaea. However, many aspects of this biological instrument, such as its molecular structure, functions, and mechanism, remained unknown for decades, resulting in a lack of appreciation. In the 1990s, research on CRISPR-associated genes focused on microbial genetics, such as in enterobacteria, M. tuberculosis, cyanobacteria, and S.pyogenes. In the early 2000s, researchers began to investigate the structure and components of CRISPR, specifically the Cas gene. By 2005, they had gained a better understanding of the molecular structure of CRISPR and its mechanism of action, which eventually led to its application in various medical fields, including oncology (1, 2).

This discovery of the CRISPR/Cas9 technology has revolutionised gene editing in the life sciences and medical research. Prior of its development, researchers employed other gene-editing technologies such as zinc finger nuclease (ZFN) and transcription activator-like effector nuclease (TALEN). ZFN are composed of dimerised zinc finger that recognise and bind to the target site, as well as a FokI cleavage domain that is linked to an array of these six zinc fingers. The cleavage domain of FokI induces double-strand DNA breaks that can be repaired by non-homologous end joining (NHEJ) (3). TALENs contain a DNA-binding domain and a FokI catalytic domain as well. One binding domain recognises one nucleotide of a target DNA sequence during the binding process, with two amino acid residues in the monomer being responsible for the binding. The FokI catalytic domains are located on opposite DNA strands at a sufficient distance to allow for dimerization. These FokI catalytic domains induce a double-strand DNA break that is repairable by NHEJ, resulting in small insertions or deletions (indels) (4, 5). The general structure of gene-editing technologies is illustrated in the **Figure 1**.

2. Structure and Mechanism

CRISPR RNA (crRNA), trans-activatingcrRNA (tracrRNA), and the endonuclease enzyme are the three fundamental components of the CRISPR/Cas9 complex (Cas protein). The 20-nucleotide (nt) guide sequence in each crRNA determines the DNA target's specificity and recognition. A partial direct repeat

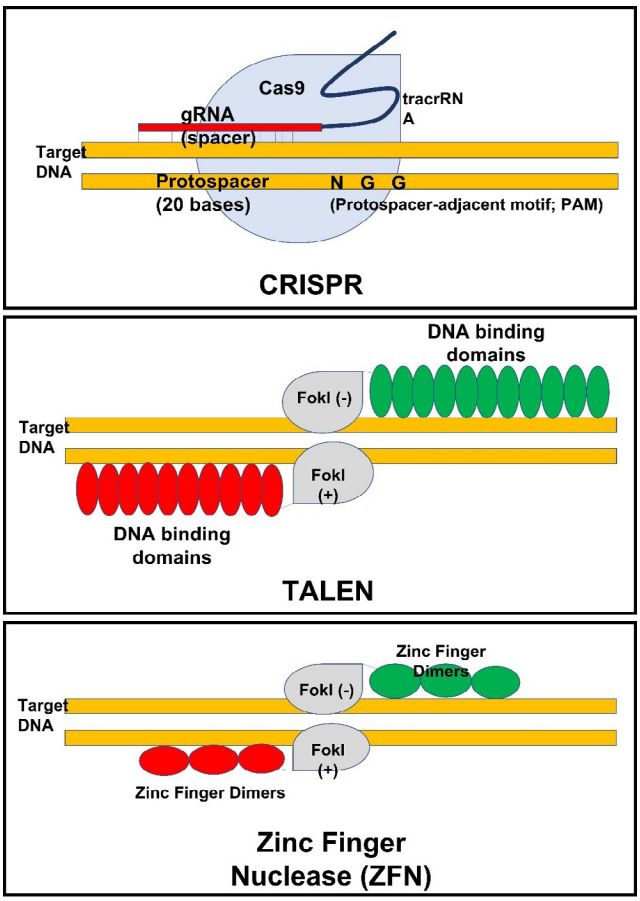

Figure 1. Gene-editing tools are structured similarly in their basic composition. The Clustered Regularly Interspaced Palindromic Repeats (CRISPR) complex consists of gRNA (spacer), tracrRNA, and Cas protein. In contrast, the transcription activator-like effector nuclease (TALEN) consists of DNA-binding domains and FokI as the catalytic domain, whereas the Zinc Finger Nuclease (ZFN) includes zinc finger dimers and FokI as the catalytic domain.

called a protospacer adjacent motif (PAM) sequence immediately precedes the 20-nt sequence and directs the Cas protein towards the DNA target. Each Cas protein type has its unique PAM sequence. is required for the maturation of precursorcrRNA (pre-crRNA), which enables the processing of the crRNA array into discrete units (6, 7). The characteristics of the Cas protein depend on the type of CRISPR/Cas system employed.

There are two primary classes of the system, as shown in **Figure 2.** Class I is distinguished by multiprotein effector complexes that are further subdivided into 12 subtypes and three types. Cas3 protein of type I contains a single-stranded DNA nuclease coupled to an ATP-dependent helicase, allowing it to unwind DNA/ DNA

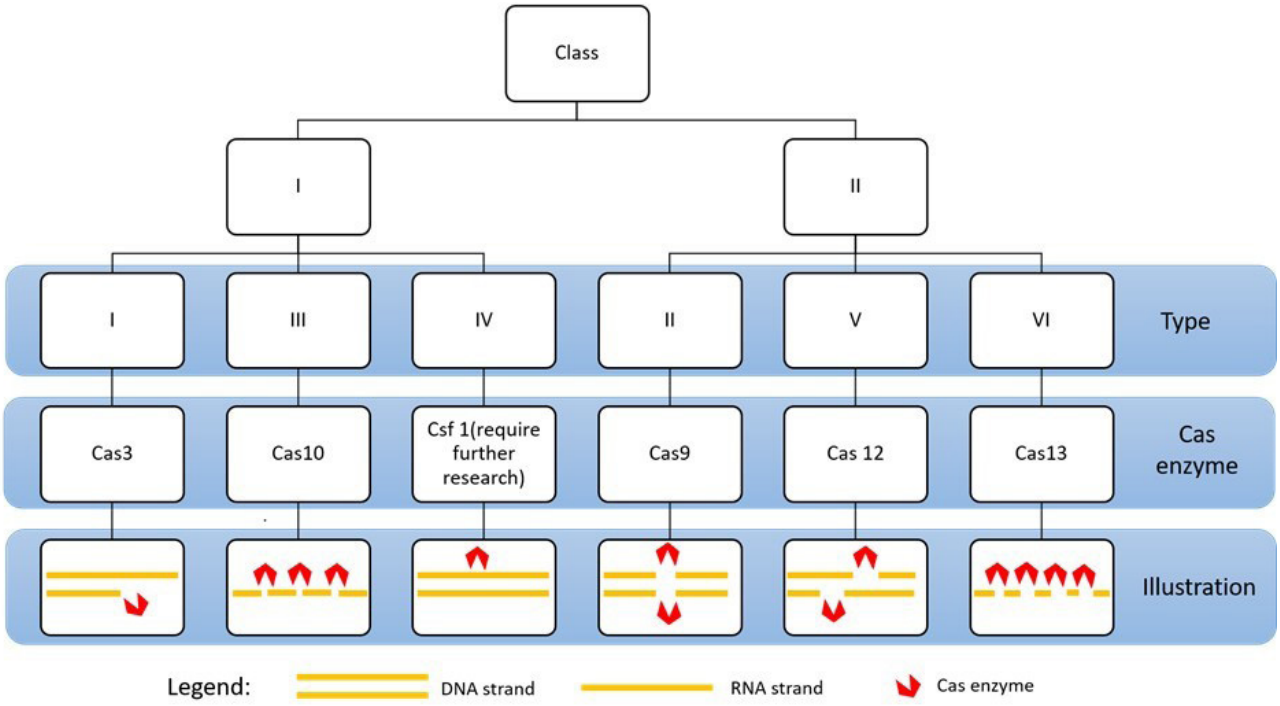

Figure 2. The CRISPR/Cas system is an innovative gene editing tool that can be divided into two main classes: Class I and II. Class I is distinguished by multiprotein effector complexes, which can be further subdivided into type I, type II, and type IV, and utilise Cas3, Cas10, and Csf1 enzymes. Class II consists of three types: type II, type V, and type VI, and uses Cas9, Cas12, and Cas13 enzymes with distinct activities that can target RNA or DNA strands. This figure depicts the relevant Cas enzymes and their respective catalytic mechanisms.

or RNA/DNA duplexes (8). Cas3 exonuclease cleaves multiple sites of the single DNA strand in the R-loop structure, resulting in a single-strand break in the protospacer region, according to the proposed mechanism.

In system type III, Cas10 is utilised, which operates through a slightly different mechanism. Multiple Cas proteins assemble with crRNA into Csm (type III-A) or Cmr (type III-B) effector complexes, which scan for the complementary target sequence and coordinate the degradation of transcriptionally active DNA. CRISPR/Cas3 is then reconfigured for the cleavage of the second DNA strand (8). In system type III, Cas10 is utilised, which operates through a slightly different mechanism. Multiple Cas proteins assemble with crRNA into Csm (type III-A) or Cmr (type III-B) effector complexes, which scan for the complementary target sequence and coordinate the degradation of transcriptionally active DNA. The recognition of the invasive transcript by the Csm/Cmr complex via base-pairing between the crRNA and the transcript leads to the RNA cleavage by the Csm3/Cmr4 subunits, while simultaneously activating the single-stranded deoxyribonuclease (ssDNase) activity of the Cas10 subunit (HD domain).

This also activates Csm6, which degrades ssRNA effectively (9). In addition, Cas10 has demonstrated its dynamic ability to distinguish between self- and non-self RNA targets. Cas10 undergoes rapid conformational fluctuations on foreign RNA targets but remains locked and static on self-RNA at the molecular level (10).

Ozcan et al. have shed light on the processing and multisubunit effector complex formation of the type IV, class I CRISPR/Cas system in Aromatoleum aromaticum, which is still under investigation (11). This study identified a complete type IV Cas gene locus as well as an adjacent CRISPR array that contains an additional type I-C system on its chromosome, suggesting an evolutionary link between the two types. The type IV CRISPR system has been shown to involve multiple crRNA-guided effector complexes, and a Cas6 variant (Csf5) has been identified as the source of crRNAs in type IV CRISPR-ribonucleoprotein (crRNP) complexes. Recombinant production and purification of the type IV Cas proteins, as well as electron microscopy, have revealed the function of Csf2 as the helical backbone of type IVcrRNPs, which include Csf5, Csf3, and a large subunit (Csf1) (11). However, additional research is required to investigate

the system's function, mechanism, and other aspects.

The CRISPR/Cas system of class 2 employs single-protein effectors (12) and is subdivided into three types: type II, type V and type VI. Cas9 acts as an endonuclease in the type II system of class 2 to generate two blunt cuts that result in a double-strand DNA (dsDNA) break (13). Cryo-EM structures have uncovered coordinated domain movements that regulate Cas9 DNA cleavage. Cas9 adopts the 'checkpoint' conformation (state I) in its pre-catalytic state, with the HNH nuclease domain located far from the targeted DNA. Nevertheless, upon successful recognition of the PAM and DNA target sequence in the presence of Mg2+, the complex is activated and the HNH nuclease domain undergoes a dramatic 34-swing towards the cleavage site. This is then followed by REC2 disorder, the ordering of REC3 loops that bind to the distal PAM, and interaction between the RuvC domain and the longer distal portion of the DNA duplex. During catalysis, the active site of the HNH domain cuts the sense strand, whereas the active site of the RuvC domain cuts the antisense strand. After post-catalysis, the HNH remains bound to the sense strand and the active site of the RuvC domain is located close to the antisense strand. The HNH domain dissociates from the cleaved sense strand and becomes disordered in state III (product complex), REC2 is ordered, and the system returns to state I. In the meantime, REC3, RuvC, and nucleic acids continue to interact (14).

Additionally, in the class II type V system, Cas12 (also known as Cpf1) serves as the active Cas protein in the complex and presents an alternative to Cas9. However, Cas12 possesses distinct characteristics, such as the absence of trans-activating crRNA, the ability to target T-rich motifs, generating sticky-ends double-strand breaks, and its applicability for both RNA processing and DNA nuclease activity (15). Structurally, the complex consists of only two components: crRNA and Cas12, the Cas effector protein. The crRNA contains a seed region that interacts with the target DNA, a spacer that recognizes the target sequence for endonucleolytic activity by Cas12, and a pseudoknot structure configured from the direct repeat segment of the pre-crRNA, which is crucial for Cas12 recognition. In its mechanism, the Cas12-crRNA complex recognizes a protospacer sequence downstream of the PAM (5'-TTTN-3'). During the process, the RuvC and Nuc domains of Cas12 cleave the non-sense and sense strands, respectively, leading to a staggered double-strand break (15).

In class II's type VI, Cas13 acts as the endonuclease component of the complex. Cas13 has four subtypes (A-D) and structurally contains two enzymatically different ribonucleases. One ribonuclease is responsible for processing pre-crRNA to form mature Type IV interference complexes, while the other ribonuclease degrades and cleaves the targeted messenger RNA of the host (16). The cleaved mRNA is then processed non-specifically, resulting in collateral damage (2, 17).

When the Cas protein cleaves DNA, it activates either non-homologous end joining (NHEJ) or homology-directed repair (HDR). NHEJ can result in insertion or deletion (Indels) mutations, which can result in frameshift mutations and gene knockdown or knockout. In contrast, HDR utilises a single-stranded oligonucleotide (ssODN) for repair, reducing the likelihood of mutations (6). This mechanism can also be used to repair mutated DNA sequences and introduce specific nucleotide sequences into a targeted DNA site, making it an essential tool for medical and life sciences research.

3. Advantage and Disadvantage

Compared to previous gene-editing methods, the CRISPR/Cas system has been reported to possess numerous advantageous features that can contribute to experimental benefits. One of these advantages is the simplicity of target design. Genomic targets can be designed using a 20-nt sequence that is complementary to the target sequence. Additionally, the CRISPR/Cas technology exhibits high editing efficiency, facilitating efficient genome editing in diverse cell types and organisms in both in vitro or in vivo research (6). Moreover, the CRISPR/Cas complex is capable of multiplexed mutations, enabling mutations to be introduced in multiple genes. This is achieved by transfecting multiple guide-RNAs (gRNA) targeting different genes into cells or organisms (18).

Another recently discovered advantage of the CRISPR/Cas system is its ability to cut and splice whole chromosomes precisely. Prior genetic engineering techniques were incapable of dealing with lengthy stretches of DNA, making precise cutting and pasting difficult and leaving unwanted DNA at the splicing site. In addition, traditional editing tools were unable to join large DNA segments. However, a research team recently demonstrated that they could modify CRISPR to cut long stretches of DNA precisely, without leaving

scars, and use a modified version of a well-known enzyme tool, lambda red recombinase, to reattach the ends of the original chromosome without the removed DNA segment. They also successfully fused the two ends of the removed DNA part. (19). Moreover, CRISPR operated using Cas3 was recently proven to become a "DNA shredder." Segments of DNA ranging from 300 to 100,000 base pairs long were deleted in human embryonic stem cells and HAPI cells using this DNA shredder. This tool can be used to shred long sequences of non-coding DNA, especially DNA that does not synthesize proteins and whose function is unknown. This would assist researchers in exploring this large segment of DNA for any links with specific diseases (20).

The primary challenge associated with the CRISPR/Cas technology is its potential for off-target effects, whereby mutations are introduced at unintended sites due to similarities in genomic sequence homology. Several bioinformatics websites have been created to predict and score potential off-target sites, thereby assisting researchers in identifying and scanning for these locations (6, 21). Off-target effects can lead to altered gene function and genomic instability, impeding future clinical applications of the technology. The advantages and disadvantages of CRISPR/Cas technology is summarised in **Figure 3**.

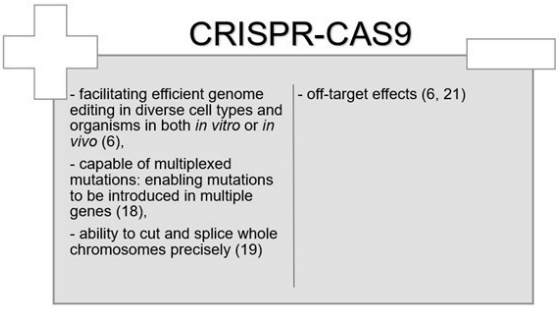

Figure 3. Compared to earlier gene-editing techniques, the CRISPR/Cas system reportedly possesses a number of advantageous characteristics that can contribute to experimental benefits as listed in the Figure. However, the technology has been linked to off-target effects, which are regarded as a disadvantage.

4. Therapeutics application of CRISPR/Cas System

CRISPR/Cas system has been utilized extensively in medical oncology research due to its sophisticated features. According to X. Tian et al, a significant proportion of CRISPR-associated publications focused on various aspects of cancer research, such as cancer therapy (22.3%), model construction (18.6%), drug validation (16%), and functional gene studies (13.5%) (22). Since its early discovery, many oncological studies have utilized the CRISPR/Cas system as a biological tool to identify oncogenes and cancer mediators (23). Therefore, the genetic understanding gained through the use of CRISPR/Cas technology has opened up new possibilities for clinical medicine.

The clinical application of CRISPR/Cas system began a decade after its discovery, with various approaches and strategies developed for oncological use. In 2013, two research teams demonstrated the use of CRISPR/Cas-mediated genome editing in mammalian cells. The first team engineered two different type II CRISPR/Cas systems, which accurately cleaved genomic loci of human and mouse cells using short RNAs. They also converted Cas9 into a nicking enzyme to promote homology-directed repair with minimal mutagenic activity (24). The second group engineered the type II bacterial CRISPR system to work with gRNA in human cells, including in 293T, K562, and induced pluripotent stem cells (25). These pioneering studies laid the foundation for further pre-clinical research in genetics and the functions of genes.

Building on the promising results of pre-clinical studies, the CRISPR/Cas system has been applied to clinical oncology. In 2016, a group of Chinese researchers conducted the first human CRISPR trial on metastatic non-small cell lung cancer, which is resistant to currently available treatments such as chemotherapy and radiation therapy. The team extracted immune T-cells from the patients' blood and used CRISPR/Cas9 technology to knock out the PD-1 gene, which typically regulates a cell's ability to trigger an immunogenic reaction. The genetically-engineered T-cells were then reintroduced into the patient's bloodstream. The trial is currently in clinical phase 1 (NCT02793856) (26, 27).

Following the successful first trial of knocking out the PD-1 gene in metastatic non-small cell lung cancer using CRISPR/Cas, subsequent trials targeting the same molecular target in T-cells are being conducted for bladder (NCT02863913) (28), prostate (NCT02867345) (29), and renal cancers (NCT02867332) (30). In addition, a phase II clinical trial (NCT03081715) utilising PD-1 knockout in T-cells is currently underway for esophageal cancer (31).

The CRISPR/Cas9 system is increasingly being investigated for designing clinical oncology

therapeutics, particularly chimeric antigen receptor T-cell therapy (CAR-T). In this strategy, genetically modified T cells express chimeric antigen receptors, which aid in directing them to surface cancer antigens. Kymriah and Yescarta, which use CD-19 directed genetically modified autologous T cells to treat patients with acute lymphoblastic leukaemia (ALL) and certain types of non-Hodgkin lymphoma, have been approved by the FDA (32, 33). In addition, researchers from the University of Pennsylvania conducted the first-ever human trial to examine the effect of HLA-A*0201-restricted NY-ESO-1-redirected T cells with CRISPR-edited endogenous T cell receptor and PD-1. Multiple myeloma, synovial sarcoma, myxoid/round cell liposarcoma, and melanoma were the targets of the experiments (NCT03399448) (34). Nevertheless, the project was concluded in 2020. The CRISPR/Cas9 system has also demonstrated promise as a targeted immunotherapy treatment for acute myeloid leukaemia. CD33, a lineage-specific myeloid antigen, was targeted by CD33-targeted CAR-T cells or the ADC Gemtuzumab Ozogamicin in conjunction with transplantation of hematopoietic stem cells engineered to eliminate CD33 expression using the CRISPR-Cas9 system. The transplantation of CD33-ablated human stem/progenitor cells (HSPCs) with CD33-targeted immunotherapy resulted in leukaemia clearance without myelosuppression, as demonstrated by the engraftment and recovery of multilineage descendants of CD33-ablated HSPCs. This strategy may be applicable to additional cancers and antigens (35). Moreover, the CRISPR/Cas9 system has been used to generate gene-disrupted allogeneicCD19-directed BB CAR-T cells, also known as UCART019. Combining lentiviral delivery of CAR with CRISPR RNA electroporation to simultaneously disrupt endogenous TCR and B2M genes (NCT03166878) achieved this result (NCT03166878) (36). Consequently, a clinical trial was conducted to assess the efficacy of UCART019 in patients with relapsed and resistant CD19+ leukaemia and lymphoma. This trial is currently in clinical phase I/II. CRISPR/Cas9 has additional clinical applications in oncology for the treatment of relapsed or resistant leukaemia and lymphoma. Using CD20 or CD22 as a target, CAR-T cells are modified to recognise and kill CD19-negative malignant cells (NCT03398967) (37). Using the CRISPR/Cas9 system, the programmed cell death-1 (PD-1) gene was disrupted in primary human T cells. By interacting with its ligand on tumour cells, the PD-1 gene is known to inhibit CAR-T cells, which may hinder the ability of chimeric antigen receptor (CAR) T cells to eliminate solid tumours. Reducing the PD-1 high population had little effect on CAR-T cell proliferation, but significantly increased cytokine production and cytotoxicity against PD-L1-expressing cancer cells in vitro, according to the study. CAR-T cells with PD-1 disruption demonstrated superior tumour control and relapse prevention in vivo compared to those lacking PD-1 antibody blockade (34). This has led to a phase 1 clinical trial (NCT03545815) (38) to investigate CRISPR-Cas9-mediated PD-1 and TCR gene-knocked out mesothelin-directed CAR-T cells in patients with mesothelin-positive multiple solid tumours.

In addition, the CRISPR/Cas9 method has been validated for identifying Neurofibromatosis type 1 (NF1) individuals with heightened discomfort. The CRISPR/Cas9 technology and cationic polymer were utilised in this instance to induce allele-specific C-terminal truncation of neurofibromin, a Nf1 gene-encoded protein. Their biological model, the rat with truncated neurofibromin, revealed an increase in voltage-gated calcium (particularly N-type or CaV2.2) and sodium (specifically tetrodotoxin-sensitive) currents in dorsal root ganglion neurons, resulting in increased nociceptor excitability and behavioural hyperalgesia. Researchers discovered that the cytosolic regulatory protein collapsin response mediator protein 2 (CRMP2) controls the channel's function and binds to the targeted C-terminus neurofibromin. The inhibition of CRMP2 phosphorylation by (S)-lacosamide normalised channel current densities, excitability, and hyperalgesia caused by neurofibromin truncation using the CRISPR/Cas9 system. This has revealed the protein partners responsible for NF1 discomfort and suggests CRMP2 as a potential therapeutic target (39). In addition, the CRISPR/Cas9 technology has been employed to produce a bank of induced pluripotent stem cells (iPSC) in children with Neurofibromatosis type 1-related central nervous system tumours (NCT03332030) (40). Using the CRISPR/Cas9 technology, isogenic NF1 wild-type (NF1+/+), NF1 heterozygous (NF1+/-), and NF1 homozygous (NF1-/-) iPSC lines were generated from individual patients. Stem cells obtained from these people will provide crucial insight into the mechanism responsible for tumour growth and central nervous system symptoms, hence expediting the identification of therapeutic targets.

The CRISPR/Cas9 system has demonstrated its

therapeutic potential for cervical cancer caused by high-risk human papillomavirus (HPV). Researchers designed the system to target HPV16-E7 DNA in HPV positive cell lines, successfully disrupting HPV16-E7 at specific sites, inducing apoptosis, and inhibiting growth in HPV positive SiHa and Caski cells. No disruption of the target was observed in HPV negative C33A and HEK293 cells. Additionally, E7 DNA disruption resulted in downregulation of E7 protein and upregulation of the tumor-suppressor protein pRb (41).

In addition, the CRISPR/Cas9 system has been used to target another HPV gene called E6 oncoprotein, which promotes the degradation of the host tumor-suppressor gene p53, resulting in the development of tumours. This gene is constitutively expressed in tumour tissue but absent from normal tissue. Three high-risk HPV-positive cervical cancer cell lines (HeLa, HCS-2, and SKG-I) were transfected with Cas9 to establish cell lines that continuously expressed Cas9, followed by the introduction of an adeno-associated virus (AAV) vector carrying a gRNA targeting E6 into the cell lines. Simultaneously, AAV-sgE6 was injected directly into the subcutaneous tumour of an in vivo mouse model of cervical cancer. In a concentration-dependent manner, AAV-sgE6-transduced cells displayed decreased expression of E6, increased expression of p53, increased apoptosis, and decreased growth. In contrast, subcutaneous tumour growth was significantly suppressed in vivo in a mouse model of cervical cancer, and no adverse events were observed (42). Currently, a phase 1 clinical trial utilising CRISPR technology to knockout HPV16-E6/E7 or HPV18-E6/E7 is being conducted (NCT03057912) (43).

In an effort to improve adoptive cellular therapies for Epstein-Barr virus-associated gastric cancer, the CRISPR/Cas9 system has also been used to disrupt PD-1 on human T cells (EBVaGC). EBVaGC is characterised by a high lymphocyte infiltration and amplification of immune-related genes, including PD-L1. A study demonstrates that CRISPR/Cas9-mediated PD-1 disruption in cytotoxic T-lymphocytes (CTLs) can increase the immune response to the EBV-LMP2A antigen and the cytotoxicity of CTLs against EBV-positive gastric cancer cells. In an EBVaGC xenograft mouse model, these PD-1-disrupted CTLs exhibited a remarkable antitumor effect when combined with low-dose radiotherapy. This breakthrough will eliminate T-cell tolerance in EBVaGC adoptive cell therapy (44).

The next step was a clinical trial (NCT03044743) designed to assess the safety of using PD-1 knockout EBV-CTL cells derived from autologous sources to treat EBV-positive advanced-stage malignancies (45). In this study, patients undergo four cycles of cell therapy while their safety, clinical response, biomarkers, and immunological markers are monitored.

5. Future Perspective of CRISPR technology in Oncology

CRISPR/Cas technology has become a powerful gene-editing tool for researchers in both fundamental and clinical research. Its applications have now expanded to clinical trials, offering promising therapeutic benefits in clinical oncology. However, the author believes that several aspects need to be considered during pre-clinical and clinical studies. During the pre-clinical phase, most studies using CRISPR/Cas technology focus on identifying and targeting molecular markers such as mutated oncogenes or genes that can be manipulated for therapeutic purposes. To ensure the success of future clinical research, researchers must have a clear understanding of the purpose of their research, comprehend the molecular aspects of the studied subject in vitro and in vivo, and assess its potential for future clinical applications. The targeted gene's role and function must also be thoroughly understood for normal physiological and pathological conditions. In the clinical phase, safety, efficacy, and effectiveness, as well as side-effect and toxicology evaluations, are paramount. It is essential to evaluate both the short and long-term effects of the treatment carefully. As previously stated, the Cas protein originates from bacteria, which may cause an immune response when introduced into the human body (46). Additionally, sgRNA from an exogenous source may be recognized as a foreign body by immune cells, such as monocytes and macrophages, leading to clearance or removal by the immune system (47). Another crucial issue is the delivery system required to transport the gene-editing complex into the human body, which can be broadly classified into physical, viral, and non-viral delivery methods based on the research objectives and targets (48, 49). However, concerns arise when viral delivery systems, such as lentiviral systems, are used to transport the gene-editing complex due to their potential to induce severe immunogenic reactions. Studies have shown that direct lentivector injection can potently induce B- and T-cell immunity in transgenic mice

Table 1. The following is a list compiled from the ClinicalTrials.gov website of clinical trials employing CRISPR technology for medical interventions (as of September 2019).

Clinical Trial Identification	Clinical Application	Target site	Editing Strategy	Study Phase
NCT02863913	Muscle-invasive bladder cancer	PD-1	PD-1 knockout in human T-cell	I
NCT02867345	Castration resistant Prostate cancer	PD-1	PD-1 knockout in human T-cell	I
NCT03081715	Advanced esophageal cancer	PD-1	PD-1 knockout in human T-cell	I
NCT03399448	Multiple Myeloma/Synovial Sarcoma/Melanoma/ Myxoid/round cell liposarcoma	PD-1 TCR	PD-1 & TCR knock-out	I
NCT03166878	CD+19 leukemia & lymphoma	TCR B2M	TCR & B2M knockout	I / II
NCT03545815	Mesothelin positive multiple solid tumors	PD-1 TCR	PD-1 & TCR knock-out in CAR-T cells	I
NCT03332030	Tumour of the central nervous system	NF1	NF1 gene fixation in stem cell	_
NCT03044743	EBV-related malignancies	PD-1	PD-1 knockout in human T-cell for adoptive cellular therapy	I / II
NCT02793856	Metastatic non-small cell lung cancer	PD-1	PD-1 knockout in engineered T-cells	I
NCT02867332	Metastatic renal cancer carcinoma	PD-1	PD-1 knockout in engineered T-cells	I
NCT03398967	Relapsed or refractory leukemia & lymphoma	CD19 & CD20 or CD22	CD19 & CD20 or CD22 were edited	I / II
NCT03057912	HPV-related Cervical Intraepithelial Neoplasm	HPV16-E6/E7, HPV18-E6/E7	HPV16-E6/E7 or HPV18-E6/E7 knockout	I

with human carcinoembryonic antigen-expression tumors, and toll-like receptor 3 and 7 have been identified as causing immunogenic reactions in ex vivo and in vitro studies as a result of HIV-1 lentiviral vectors (50, 51). Furthermore, the possibility of the lentiviral vector randomly integrating into the host genome and causing insertional mutagenicity is another concern (52). This could impact the expression of other genes, altering the nature and originality of the host cells. However, these delivery system issues can be addressed by using alternatives, such as integrase-deficient lentiviral vectors or nanoparticles like magnetic nanoparticles (53) and bioreducible lipid and messenger nanoparticles (54). Regulations governing gene-editing research are essential for ensuring the technology's safety and proper application. In 2018, the World Health Organization (WHO) called for the formation of a gene-editing expert panel as a WHO advisory committee in order to develop global standards for the governance and oversight of human genome editing. In July2019, WHO solicited input and perspectives from all relevant stakeholders on the scientific, ethical, social, and legal challenges associated with human genome editing (both germline and somatic) (55). Through their bioethics and biosafety acts, numerous nations, including Germany, South Korea, France, and others relevant stakeholders on the scientific, ethical, social, and legal challenges associated with human genome editing (both germline and somatic) (56). However, some nations have adopted a

different strategy. In 2016, UK scientists were granted permission to edit the genomes of human embryos for research purposes. Japan has also permitted gene-editing in human embryos (57), and Japan is expected to follow suit (58). In the United States, the FDA has not yet announced its oversight of clinical applications of the CRISPR-Cas system (59). As previously mentioned, the CRISPR/Cas system has significant potential for clinical oncology, allowing researchers to manipulate specific genes for therapeutic purposes. This could aid in the development of novel cancer treatments, whether through the direct targeting of dysregulated genes or the genetic modification of immune cells for cancer immunotherapy. CRISPR/Cas therapy can be applied as the main conventional treatment or as a complementary therapy to established cancer treatments such as chemotherapy. This approach could also indirectly improve current treatments, especially in cases of cancer resistance. A major benefit of combining therapies is the potential to reduce the dosage of chemotherapeutic drugs, resulting in lower side-effects for cancer patients while maintaining the same efficacy, efficiency, and effectiveness of treatment. Hence, the author believes that regulation is necessary for gene-editing research, particularly for the CRISPR/Cas system, but it should not impede scientific progress. In the context of CRISPR/Cas system application in oncology, a thorough evaluation must be performed to ensure that the benefits of using the technology in clinical settings outweigh the drawbacks.

6. Conclusion

It has been demonstrated that the CRISPR/Cas system is superior to other gene-editing techniques. Nevertheless, there are several areas that require improvement, particularly in addressing the issue of off-target effects. Overcoming these limitations will allow the CRISPR/Cas system to become a crucial tool in molecular research and clinical applications, particularly in clinical oncology.

Conflict of interest

The authors declare that they have no conflict of interest.

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