Al-Rafidain J Med Sci. 2024;6(1):76-85.
DOI: https://doi.org/10.54133/ajms.v6i1.503

Online ISSN (2789-3219)

Review Article

The Applications of Genome Editing in the Management of Cancer: A Narrative Overview

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Received: 18 December 2023; Revised: 15 January 2024; Accepted: 19 January 2024

Abstract

Objective: To provide an overview of the status of applying genome editing, particularly CRISPR/Cas9, in the management of cancer. ***Method***: Several search tools were consulted in the preparation of this manuscript to obtain peer-reviewed articles using the given evaluation and selection criteria. ***Main points***: CRISPR/Cas9 and its associated variants stood out as the technology of choice for manipulating cancer cells and managing the disease. This genome-editing technology can positively contribute to the elucidation of the roles of cancer genes, establish animal models to study the disease, and therapeutically empower the development of next-generation immunotherapies. ***Conclusions***: The manipulation of the human genome using CRISPR/Cas9 to treat cancer has only recently begun. Several clinical trials are ongoing, and the results are eagerly awaited. In the meantime, improvements and advancements in genome editing are being developed at a rapid pace to take advantage of this evolving technology.

Keywords: Cancer, CRISPR/Cas9 gene-editing, genome-editing.

تطبيقات تحرير الجينوم في السيطرة على السرطان: نظرة عامة سردية

الخلاصة

الهدف: تقديم لمحة عامة عن حالة تطبيق تحرير الجينوم، ولا سيما كريسبر/كاس 9، في إدارة السرطان. **الطريقة**: تمت استقصاء العديد من أدوات البحث في إعداد هذه المخطوطة للحصول على مقالات تمت مراجعتها من قبل الأقران باستخدام معايير التقييم والاختيار المحددة. **النقاط الرئيسية**: برزت كريسبر / كاس 9 والمتغيرات المرتبطة بها كتقنية مفضلة لمعالجة الخلايا السرطانية والسيطرة على المرض. يمكن أن تساهم تقنية تحرير الجينوم هذه بشكل إيجابي في توضيح أدوار جينات السرطان، وإنشاء نماذج حيوانية لدراسة المرض، وتمكين تطوير علاجات الجيل التالي من العلاجات المناعية. **الاستنتاجات**: لم يبدأ التلاعب بالجينوم البشري باستخدام كريسبر/ كاس 9 لعلاج السرطان إلا مؤخرا. العديد من التجارب السريرية مازالت جارية، والنتائج منتظرة بفارغ الصبر. في غضون ذلك، يتم تطوير التحسينات والتطورات في تحرير الجينوم بوتيرة سريعة للاستفادة من هذه التكنولوجيا المتطورة.

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Article citation: Al-Janabi II. The Applications of Genome Editing in the Management of Cancer: A Narrative Overview. *Al-Rafidain J Med Sci*. 2024;6(1):76-85. doi: https://doi.org/10.54133/ajms.v6i1.503

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INTRODUCTION

Genome editing technologies have their roots in the 1970s, when scientists gained the ability to modify the DNA and study the influence that might have on genes with the hope of harnessing the outcomes for use in biotechnology and medicine [1,2]. Genome engineering made rapid advancements after this, enabling precise manipulation of the DNA in its genomic context in almost any living organism. Currently, several approaches can be employed for the precise and accurate editing of DNA sequences [3,4]. Three of these methods have become more popular: 1) zinc finger nucleases (ZFNs) [5], 2) transcription activator-like effector nucleases (TALENs) [6], and 3) CRISPR and Cas9 [7]. Of these three technologies, CRISPR-Cas9 proved to be the most popular, for the reasons outlined later, and will be the focus of most of the discussions that follow. All three technologies rely on producing a double-stranded cut in the DNA and the natural cell processes that rectify the cut. The use of ZFNs and TALENs relied on custom-designed proteins that could recognize and cleave specific DNA sequences. However, CRISPR-Cas9 is much more straightforward and has largely replaced the use of ZNFs and TALENs techniques because they are difficult and time-consuming to implement. This narrative review will briefly examine the role of genome editing in the management of cancer, with particular emphasis on CRISPR/Cas9 technology.

METHODS

A literature search was carried out for peer-reviewed articles using PubMed, Google Scholar, ResearchGate, Web of Science, SpringerLink and the Chinese database WanfangData, covering the period between September 2003 and July 2023. The

keywords and key phrases employed in the search were "CRISPR/Cas9 gene editing," "genome editing," and "cancer." The author evaluated and chose the selected hits while taking the article's citations and the journal's impact factor into account. Owing to the large number of hits resulting from the search, studies deemed to be insufficiently reflecting the narrative overview intended for the present work were excluded. Publications before September 2003 were only considered if the initial reading of the article suggested that they represented a significant contribution.

Zinc finger nucleases (ZNFs)

In the case of ZFNs, the approach involves putting together enough numbers of zinc finger proteins (ZFPs) to target a specific sequence within the genome. Each of these zinc finger proteins will target a desired triplet sequence of nucleotides (Figure 1).

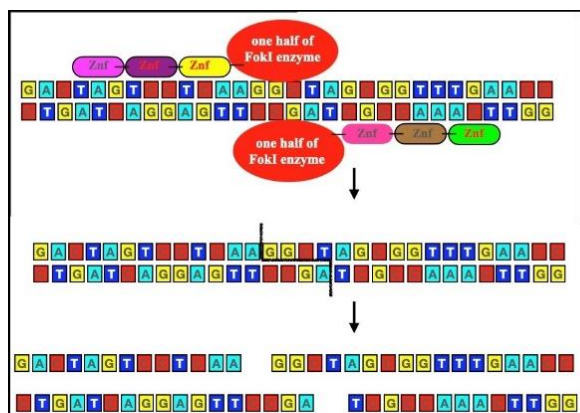

Figure 1: A schematic diagram of the principle involved in using zinc finger nucleases (ZNFs) in genome editing. FokI: a nuclease enzyme, A, C, G and T: DNA nucleotides.

The joining of several ZFPs will expand the accuracy and reduce the possibility of cutting the DNA off-target. The designed ZNF will then get joined to one of the two monomers of the non-specific nuclease FokI (an endonuclease from *Flavobacterium okeanokoites*) to form a construct that can bind to a specific target on one strand of the DNA [5,8]. When a similar but complementary construct is made, containing the second monomer of the FokI enzyme, this will bind with the complementary strand of the DNA. The two monomers of FokI will then come close to each other and dimerize to form an active enzyme capable of cutting the two strands of the DNA at the location specified (Figure 1).

Transcription activator-like effector nucleases (TALENs)

The TALENs are based on bacterial proteins that can recognize DNA [4]. These proteins were quickly adopted and made in a modular form that can recognize almost any sequence of DNA. While each of the zinc finger proteins can recognize a triplet sequence of nucleotides, a single TALE protein can recognize and bind to a single nucleotide (Figure 2). Assembling nine or more of these proteins in a modular structure greatly enhances the accuracy of targeting any specific sequence within the genome [6,8].

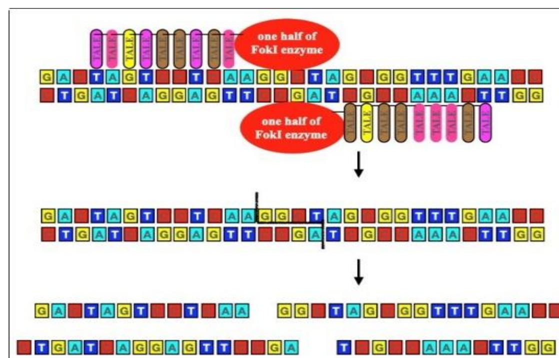

Figure 2: A schematic diagram of the principle involved in using transcription activator-like effector nucleases (TALENs) in genome editing. FokI: a nuclease enzyme, A, C, G and T: DNA nucleotides.

When the TALE protein group is joined with one of the FokI nuclease monomers, it can find its target sequence on a DNA strand and bind to it. This is similar to how ZFNs are put together. Once more, when the two monomers of FokI join together, they make an active enzyme that can cut the DNA into two strands at the right place (Figure 2).

Clustered regularly interspaced palindromic repeats (CRISPR) CRISPR-associated protein 9 (Cas9)

The CRISPR sequences were first discovered in 1987 in the bacteria *E. coli* [1,9]. Following that discovery, CRISPR sequences were also observed in archaea in 1993 reflecting their significance in these major domains of life [10]. However, due to the lack of sufficient sequence information, the utility of this finding remained unexplored until the mid-2000s. Meanwhile, various nuclease genes were found to be closely linked to the CRISPR locus and were collectively called CRISPR-associated genes (Cas genes) [11,12]. A lot of research has found that the spacer regions of the CRISPR loci are similar to the DNA sequences of bacteriophage and archaeal viruses. This led to the conclusion that the CRISPR system protects cells from viruses and plasmids from the outside [13–15]. Based on sequencing data, the CRISPR system is known to be present in around 90% of archaea and 40% of bacteria [8]. The idea that this natural immune mechanism could be applied, in some way, to alter the genome was soon to grip the imagination of various investigators. The CRISPR technology has revolutionized the field of genome editing, allowing for the precise manipulation of virtually any genomic sequence specified by a short guide RNA, thus enabling the elucidation of gene function [16]. Furthermore, the versatility of this gene editing method is such that the function of multiple genes could be interrogated at once to accelerate our understanding of the pathological processes involved [17]. CRISPR-associated gene 9 (Cas9) became the popular choice of nuclease, and the technology under the name CRISPR-Cas9 quickly gained acceptance as a leading genome-editing tool [2,7,18]. The wild-type Cas9 (wtCas9) endonuclease has six main domains

called RECI, RECII, Bridge Helix, Pam-interacting, HNH and RuvC [19,20]. The RuvC and HNH domains are the most important nuclease domains that contribute individually and separately to the double-strand break. Deactivating one or both domains does not affect the other's function, enabling the creation of a nickase (nCas9) or deactivated Cas9 (dCas9). The D10A mutation in Cas9 (aspartic acid change to alanine in position 10) turns off the RuvC domain. This leaves an endonuclease, now known as nickase, that can only cut the target strand. However, the H840A mutation (a change from histidine to alanine at position 840) in the Cas9 domain makes a nickase that cuts strands that are not its target. Additionally, mutations in both domains, D10A in RuvC and H840A in HNH, lead to the creation of deactivated Cas9 (dCas9). More importantly, all forms of Cas9 (wtCas9, nCas9 and dCas9) can be fused to other functional proteins, such as DNA methyltransferase, to generate new functions for CRISPR/Cas9 genome editing tools [21]. To understand the usefulness of CRISPR-Cas9 in genome editing, we must look at the process by which this system provides adaptive immunity to bacteria and archaea. Following exposure to foreign DNA from viruses or plasmids, short fragments of their genetic material are integrated into the CRISPR locus within the host chromosome. The CRISPR locus is made up of several repeat elements with spacers in between where the foreign DNA fragment is incorporated (Figure 3).

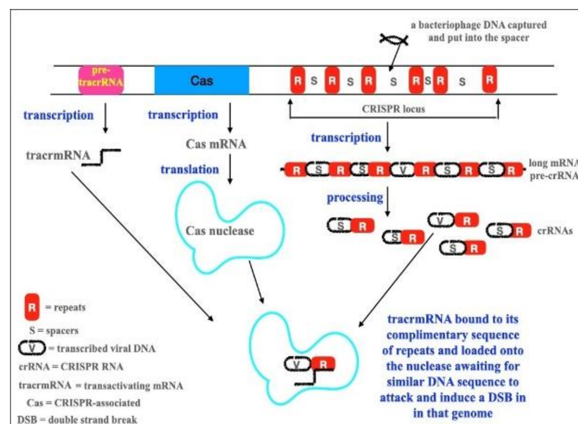

Figure 3: An illustration of the CRISPR-mediated immunity in bacteria.

Upstream of the CRISPR locus is located the CRISPR-associated gene assembly encoding the all-important nuclease enzymes. Next is the transactivating RNA (tracrRNA) gene, which encodes the unique noncoding RNA with homology to the repeat sequences. Transactivating RNA (tracrRNA) is necessary for the maturation of crRNAs. Although there are several types of CRISPR systems, type II employs a single DNA endonuclease in the form of Cas9 (also referred to as SpCas9, first identified from *Streptococcus pyogenes*) to recognize and cleave dsDNA, and this type is the most widely adopted [19,22-24]. Once the foreign DNA is joined to the spacer, it will be copied along with the other spacers and repeats to make a long pre-crRNA (Figure 3). The tracrRNA will be copied separately and will attach to the pre-crRNA so that it can mature properly and be cut by the RNase III enzyme. The viral DNA guide sequence is cut down to 20 nucleotides (20 nt) by a group of unknown enzymes. Then, the assembly of tracrRNA-crRNA will turn on Cas9 nuclease to cut the foreign complementary sequence of DNA. For efficient binding and DNA cleavage, the target sequence must be flanked on the 3` side by a protospacer adjacent motif (PAM) [17, 20–25]. Different Cas enzymes recognize different PAM sequences, which are usually 2–6 nt in length. The most common spCas9 enzyme (simply known as Cas9) only recognizes NGG, where N represents any nucleotide and G represents guanine [19,23]. Following the binding to the target location, Cas9 will then cut the individual strand at three base pairs upstream (on the 3` side), which confers specificity and limits the targeting requirement for genome editing. There are two main pathways for fixing the double-strand breaks (DSBs) that the enzyme Cas9 introduces. The first and most common pathway is called non-homologous end-joining (NHEJ). It is a pathway that is prone to mistakes and can be used to change genes by adding or removing parts (called indels). The second but less commonly used repair pathway is called homology-directed repair, which is a more precise process that can be utilized for the introduction of specific gene alterations [24]. The typical method of creating a synthetic sgRNA is to combine the functions of crRNA and tracrRNA into a single guide RNA in order to use the adaptive immunity that bacteria and archaea have as a genome editing tool (Figure 4) [19].

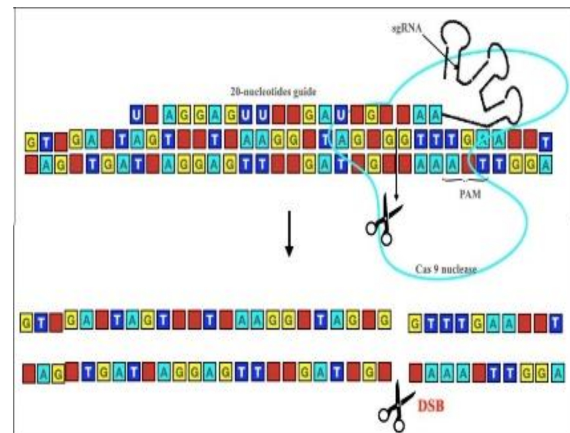

Figure 4: The basic construction and function of CRISPR-Cas9 and the creation of a targeted double strand break. sgRNA: single guide RNA, PAM: protospacer adjacent motif, DSB: double strand break.

This sgRNA is usually 20 nucleotides long and can tell the Cas9 endonuclease to make a double-stranded break in the right place on the genomic sequence. The double-stranded break is frequently repaired by the more error-prone NHEJ method, which does not require a template but can lead to gene disruption due to deletions, insertions or substitutions. Alternatively, in the presence of a donor template, the error-free HDR repair pathway can be activated to create the desired outcome, such as gene knock-in, mutation, correction or deletion (Figure 5). When the DSB is fixed with the error-prone NHEJ, most of the results

are mutants, with only a few accurate background HDR corrections.

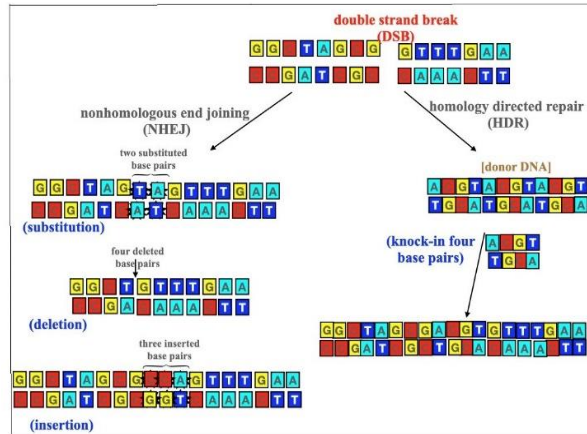

Figure 5: The mechanisms employed by cells to repair DNA double strand breaks.

Furthermore, out of the very small number of HDR corrections, significant proportions are likely to be in one allele, leaving the second allele with random mutations. Also, HDR happens in a smaller window of the G2/M cell cycle compared to the G1/S phase for NHEJ, which makes this repair pathway less useful [26].

Refinements and modifications to the basic CRISPR/Cas9 editing

While the genome editing outlined in this review represents the basic approach, there are nevertheless several modifications designed to further enhance the capabilities of the technology. The most prominent of these editing enhancements are mentioned below:

A. The use of multiple sgRNAs

Multiple sgRANs can be used with CRISPR/Cas9 technology. These sgRNAs can be fused into a single vector to perform multiplex genome editing [27,28].

B. The use of a different nuclease

In addition to SpCas9 from *Streptococcus pyogenes,* numerous other bacteria and archaea have Cas enzymes that are suitable for gene editing in mammalian cells [29]. Prominent among these are SaCas9 (from *Staphylococcus aureus*), Cas12a (from *Acidaminococcus* and *Lachnospiraceae),* and Cas13a (from *Leptotrichia shahii*). Cas12a, unlike Cas9, which produces a blunt cut, is characterized by its staggered cut and has only one nuclease activity, the RuvC domain, while Cas13a is an RNA-editing enzyme [30,31]. In general, the different Cas enzymes usually have their own features and sequence recognition, enabling the targeting of wider genomic loci [32,33]. Furthermore, the size of the individual Cas enzyme can vary, allowing for more efficient vector packaging, as in the case of SaCas9 being smaller than SpCas9 [34,35]. Certain other Cas enzymes can catalyze the maturation of their guide RNA, as in the case of Cas12a, thus enhancing multiplex editing, while the targeting of RNA instead of DNA by Cas13a provides an alternative way to manipulate gene expression [36,37].

C. Base editing

As many human genetic diseases, including certain cancers, are associated with point mutations, refinements to the basic gene editing technology allowed the creation of base editing [38,39]. Base editing does not require a double-strand break or a donor DNA template for repair. If you want to change a single base or a group of bases, you need a deaminase enzyme and a nuclease-defective Cas9 with the D10A mutation, which changes aspartate at position 10 of the protein to alanine. An adenine base editor changes an A.T. base pair to a G.C. base pair, and a cytosine base editor changes a C.G. base pair to a T.A. base pair. Together, these two types of editors cause all four possible transition mutations and target most disease-associated SNPs [38]. Scientists have deactivated the Cas9 nuclease, allowing it to only bind to the target, and at the same time, they fused a base converter to the Cas9, which allows CRISPR/Cas to convert a specific base from one to another [40,41]. The nickase (nCas9) enzyme has mostly taken the place of this type of deactivated Cas9, which is also called "dead Cas9." A mutation can disable either the RuvC domain through the D10A mutation or the HNH domain through the H840A mutation, which helps fix the DNA strand that wasn't changed after the nick. To date, two major classes of base editors have been developed and applied in cancer biology and treatment. 1) CBEs, and 2) ABEs. CBE catalyzes the conversion of C.G. to T.A. (40), whereas ABE catalyzes the A.T. to G.C. conversion (41). Using different Cas variants, CRISPR/Cas base editors can be used to target about 95% of pathogenic transition mutations deposited in ClinVar [42,43].

D. Prime editing

The more recently introduced prime editing technology allows all 12 possible base-to-base conversions, 4 transitions and 8 transversions, as well as insertions and deletions [44]. The nuclease-defective enzyme usually has an H840A mutation, which means that the histidine at position 840 of the protein is changed to alanine. It is then joined with an engineered reverse transcriptase (RTase) enzyme and paired with an extended prime editing guide RNA (pegRNA) that both tells the RTase enzyme where to go and what to change. Scientists made a special pegRNA that attaches to the target site and helps nCas9-RTase find the cut. They then use the pegRNA to make a new strand of DNA that replaces the target sequence [39]. The pegRNA also contains a primer-binding sequence (PBS) that hybridizes with the 3`end of the nicked target DNA strand to form a primer-template complex [43]. Thus, prime editing serves as a search and replace genome sequence tool without creating a double-strand break or needing donor DNA. Since the corrected sequence can be designed into the pegRNA, prime editing can be used to not only correct any point mutation but also generate small insertions and/or small deletions in specifically targeted DNA

locations in a precise manner. Prime editing has the capability of correcting SNPs, inserting up to 45 bp, deleting up to 80 bp, and claiming to correct up to 89% of all known human pathogenic variants (refer to Figure 6) [39,44].

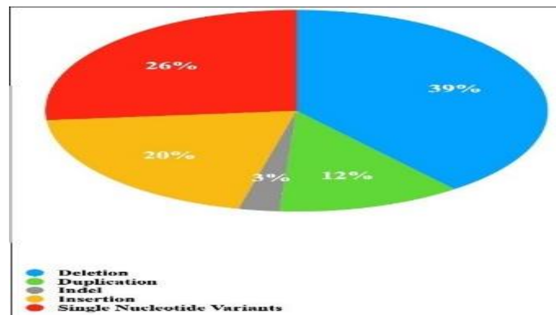

Figure 6: Human pathogenic variants of cancer germline susceptibility genes (7613 variants). Data extracted from NCBI ClinVar (last accessed on 29/10/2023).

Designing a suitable pegRNA can be more challenging than designing sgRNA, although several computational programs have been developed for public use [18,45,46].

E. The use of CRISPR/Cas9 to influence gene regulation

CRISPR/Cas system can be quickly modified to target gene regulation by fusing dCas9 with gene regulators such as transcription factors, gene inducers or repressors to create CRISPRr or CRISPRa editors [47]. Unlike Cas9, Cas13 is an RNA-targeting CRISPR protein that lacks a DNase domain. Thus, CRISPR/Cas13 targets bind to and cleave an RNA substrate instead of a DNA sequence, which therefore does not lead to any DNA sequence change and can be considered an important epigenetic regulator like microRNAs and siRNAs [21,48]. In 2017, Abudayyeh *et al*. were able to successfully delete several genes linked to cancer, such as KRAS and CXCR4, in human cells. This suggests that CRISPR/Cas13 can be used in cancer gene therapy at the post-transcription level [49]. Like Cas9, Cas13 can also be modified and deactivated to form dCas13, which does not affect the Cas13 binding function and offers new editing tools [50].

CRISPR/Cas9 genome editing in the management of cancers

CRISPR/Cas9 gene-editing technology has shown great promise and potential in various applications within the management of cancers (Figure 7) [51–54]. In the next sections, we summarize the various ways gene editing can be utilized in the management of cancer.

Gene editing of cancer cells

Gene-editing by CRISPR/Cas9 can be used to directly modify the DNA of cancer cells by targeting specific genes associated with cancer development or progression. By creating a gRNA that specifically targets the gene of interest, the Cas9 enzyme can be told to cut DNA at a certain spot when connected to that gRNA.

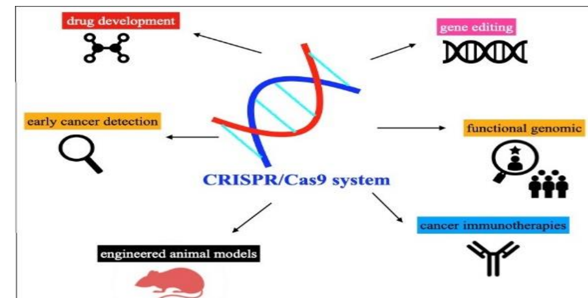

Figure 7: Applications of the CRISPR-Cas9 technology in the management of cancer.

This double-strand break triggers the cell's common repair mechanism, the NHEJ, to cause gene disruption that might lead to loss of function. However, under certain circumstances and when a suitable donor template is provided, the repair can proceed by HDR, leading to potentially precise and useful edits. Both outcomes can be exploited to disrupt or modify the cancer genes, potentially inhibiting tumor growth or enhancing the sensitivity of cancer cells to therapy. Gene editing also holds promise for developing personalized cancer treatments. Somatic mutations in the *TP53* gene, a well-known tumor suppressor, are commonly associated with an increased risk of cancer and can be seen in about half of all cancer cases, such as breast, bladder, head and neck, lung and ovarian cancers [55,56]. Furthermore, inherited mutations in the *TP53* gene, such as in Li-Fraumeni syndrome, are also known to be associated with cancer predisposition [56]. In most cases, only one nucleotide is mutated, leading to a single amino acid substitution in the p53 protein [57]. These mutations often result in a non-functional protein with impaired transcriptional and tumor suppressor activity. In addition to this loss of function, the mutant p53 protein may also promote cancer cell division through epigenetic regulations affecting late-stage and hard-to-treat cancers such as cancers of the pancreas, breast, brain, esophagus, head and neck [58]. It would be good to edit the TP53 gene so that pathogenic mutations can be reversed back to the wild-type (wt) state [59]. It was suggested by Chira *et al*. that HR could be used to replace a fully functional wild-type copy of p53 with a mutant copy that doesn't work, which would restore normal expression of the p53 protein [60]. Zhan and his colleagues reported a tool that specifically eliminated p53-deficient cells [61]. The authors constructed a p53 genetic sensor that detected the cellular expression of wt p53. The sensor was combined with diphtheria toxin (DT) using CRISPR/Cas9 to kill tumor cells that don't have p53. Potentially, such sensor-associated genetic tools could be used as anti-tumor agents targeting cells that do not express wt p53. It is crucial, given the high mutation rate of the *TP53* gene, to understand how CRISPR/Cas9 editing of this gene might influence any potential therapeutic benefit. The TP53 protein function is delicately balanced, and it is vital to understand its actual cellular status for successful

editing. *KRAS* is another frequently mutated oncogene in cancer, and its mutations represent important therapeutic targets. CRISPR/Cas9 system can recognize a specific DNA sequence and make a double-strand cut, enabling the editing of the gene. Kim *et al*. used a reporter system to find gRNAs that only bind to mutated KRAS genes and not the wild-type (wt) [62]. Because it's hard to tell the difference between indels made at the mutant allele and those made at the wt allele, we needed a substitute NHEJ reporter system that lets us test the activity of gRNA. For subsequent studies, the researchers selected three guide RNAs: two with high targeting selectivity (35T9P17 and 38A6P17) and one with low targeting selectivity (35A9P17) for mutant *KRAS.* Having identified suitable gRNAs, they were then employed to evaluate whether their delivery, in conjunction with Cas9, would influence survival, proliferation and tumorgenicity *in vitro* using cultured cells and in *vivo* monitoring of tumor growth [62]. Kim *et al*. results showed that doxycycline-inducible expression of gRNA in *KRAS*-mutant cancer cells transduced with a lentiviral vector encoding Cas9 disrupted the mutant *KRAS in vitro* and *in vivo,* leading to the inhibition of cancer cell proliferation [62]. Such an effect was not observed in cells containing the wild-type *KRAS*.

Detection of cancer

The use of CRISPR for targeted enzymatic digestion can be harnessed as a diagnostic tool in cancer. Well-known markers for the diagnosis of cancer are the microsatellites, which are short tandem repeats (STRs) in the noncoding DNA. High-throughput sequencing of the digested (fragmented) DNA can yield an accurate and sensitive detection method for a variety of cancers [2,24]. Similar platforms to those employed for the diagnosis of SARS-Cov-2 infections have also been used to identify cancer-associated mutations in tumor biopsies. The nuclease enzyme Cas9 can be converted to Cas9 nickase by mutating one of its two main domains. This produces cuts on only one strand of the dsDNA at a specific site. Such precise recognition and cleavage activities have been used for targeted amplification [63]. The enzyme Cas12 can find specific spots on ssDNA and dsDNA and cuts the target (cis) and non-target (trans) DNA. On the other hand, Cas13 can find its target ssRNA and cuts any ssRNA [63]. With their unique collateral cleavages, Cas12 and Cas13 have been used for nucleic acid amplification. The first reported CRISPR method for signal amplifications used Cas13. It was called SHERLOCK, which stands for "specific high-sensitivity enzymatic reporter unlocking" [64]. While working on something else, some others created DETECTR (a DNA endonuclease-targeted CRISPR trans reporter) [65] and HOLMES (a one-hour, low-cost, multipurpose, highly efficient system) that use Cas12 to boost signals [66].

Functional genomics

The CRISPR/Cas9 technology can enable researchers to perform large-scale functional genomic screens to identify cancer genes and their pathways. Through the systematic editing of genes in cancer cells, one can assess the impact of gene loss or alterations on cellular behavior, drug response and cancer progression. This can lead to the identification of new therapeutic targets. For large-scale CRISPR-Cas functional screening, lentiviral libraries of sgRNAs are used. Usually, 3–10 sgRNAs are used for each gene [67–71]. The cells that have Cas9 and the sgRNA library are then put through the desired phenotypic selection and high-throughput DNA sequencing to find out which sgRNAs were increased or decreased during the treatment. Three types of genome-scale CRISPR-Cas screening have been used to identify essential genes in various cancer cell types: knockout (CRISPRn-targeting DNA with a catalytically active Cas9 to generate a double strand break), inhibition (CRISPRi-fusing transcriptional repressors to catalytically inactivated Cas9 to decrease the expression of an RNA), and activation (CRISPRa-fusing transcriptional activators to catalytically inactivated Cas9 to increase the expression of an RNA) [70,71]. Functional screening with CRISPRn introduces deletions and mutations that shut down targeted genes. This process keeps going until it stops. This screening strategy can, therefore, generate homozygous knockout phenotypes at high frequency in most cells. The CRISPRi screening doesn't need frameshift and can help find new drugs because decreasing gene expression is more like the effects of a small-molecule inhibitor than completely deleting genes [72]. The CRISPRa screening assesses gene targets whose over-expression, through transcriptional activation, leads to a given phenotype [67,73]. The application of CRISPR-Cas to study functional genomics can identify cancer growth advantages (or disadvantages) and look for proliferation, resistance or sensitivity to therapies.

Engineering animal models

CRISPR/Cas9 allows scientists to create genetically engineered animal models of cancer that closely mimic the human condition. By introducing specific mutations associated with cancer development, researchers can gain a better understanding of the underlying mechanisms, test potential therapies and evaluate the effectiveness of treatment strategies. The technology has revolutionized the engineering of transgenic animal models. The generation of animal models can be achieved in two ways: a) through the traditional, and more resource-intensive, embryonic stem cell (ESC) manipulation; and b) through the microinjection or electroporation of zygotes. In addition to mice, CRISPR-Cas editing has been employed in the generation of transgenic rats, dogs, monkeys and primates to accelerate cancer drug development and test the efficacy and safety of therapeutics [74].

CRISPR in cancer immunotherapies

One form of immunotherapy that has generated substantial excitement in the field of cancer therapeutics is called CAR-T cell (chimeric antigen receptor-T cell) therapy. This therapy uses modified T

cells that express tumor-targeting receptors and have shown promise in the treatment of various cancers [75,76]. T cells are the backbone of this therapy and are currently produced by collecting these cells from the patient and engineering them in the laboratory to produce proteins on their surface called chimeric antigen receptors (CARs). These designed proteins recognize and bind to specific proteins (antigens) present on the surface of the targeted cancer cell. After the engineered T cells are produced, they are expanded and re-infused back into the patient to fight the malignancy. The first generation of CAR-T cells targeted CD19, an antigen that B cells and related cancer cells express. There are currently six CAR-T cell therapies that the FDA has approved that target either CD19 or BCMA, one of the two B-cell antigens. However, despite the excitement surrounding the introduction of CAR-T cell therapy, its cost remained prohibitive in many cases (around half a million US dollars for a single treatment). Additionally, the poor quality and quantity of these cells hinder efforts to obtain T cells from patients. This led to reviewing the source of the T cells and investigating the possibility of collecting these cells from healthy donors instead of patients (allogenic, off-the-shelf CAR-T cells). Ren *et al*. suggested the use of the combined lentiviral delivery of CAR and the transfer by electroporation of CAS9 mRNA and gRNAs targeting the endogenous TCR, B2M and PD-1 simultaneously to generate allogenic (universal) CAR-T cells [77]. The following two examples were selected to represent the application of CRISPR/Cas9 gene-editing technology in this growing area of CAR-T cell therapeutics for cancer. To generate universal CAR-T cells, multiple genes need to be eliminated. They need to get rid of the T-cell receptor (TCR) to stop graft versus host disease (GvHD) and the human leukocyte antigen class I (HLA-I) to lower the immunogenicity for this plan to work. In this first example, Liu *et al*. developed a protocol to generate CAR-T cells with either a) two genes disrupted (*TRAC* and *B2M*) or b) three genes disrupted (*TRAC*, *B2M* and *PD-1*) and tested these allogenic T-cells for their toxicity and anti-tumor activity *in vivo* and *in vitro* [78]. The authors concluded that these universal T-cells are safe and have acceptable side effects, so they could progress to clinical trials. The second example comes from the results of a clinical trial that came out in 2020. The goal of the trial was to see if editing T-cells from people with advanced, refractory cancers using multiplex CRISPR/Cas9 was safe and possible [79]. In this clinical trial, three patients were used: two with advanced refractory myeloma and one with metastatic sarcoma. The experimental investigation was designed to replace the endogenous TCR and PD-1 with a synthetic TCR transgene specific for the peptide SLLMWITQC in NY-ESO-1 and LAGE-1 (cancer testis antigen-CTA) to improve the function, persistence and safety of the engineered T cells [79]. The T cells were isolated from the patient's blood and the CRISPR/Cas9 ribonuclear protein complex loaded with three sgRNAs was edited into these T cells to produce edits of the following genes: *TRAC*, *TRBC1*, *TRBC2* and *PDCD1* (encoding PD-1). The T cells were then transduced with a lentiviral vector to express the CTA before being returned to patients. The results of this trial demonstrated the safety and feasibility of using CRISPR-Cas9 for the multiplex genome engineering of T cells. In summary, using CRISPR/Cas9 to modify the genome of T cells can improve their tumor recognition and killing abilities and enhance the effectiveness of cancer immunotherapies. This includes knocking out genes that inhibit immune responses or introducing genes that enhance T-cell function. A selection of clinical trials for cancer treatment where CRISPR/Cas9 was used for editing is shown in Table 1.

Table 1: A selection of clinical trials employing CRISPR/Cas9-edited cells for the treatment of cancers

Clinical trial number	Intervention used	Cancer type	Status
NCT05643742	Allogenic CAR-T cells CD19-directed	B-cells malignancies	Ongoing
NCT05795595	Allogenic CAR-T cells CD70-directed	Solid tumours	Ongoing
NCT04502446	Allogenic CAR-T cells CD70-directed	B-cells malignancies	Ongoing
NCT04426669	TIL-inhibited Intracellular immune checkpoint CISH	Metastatic gastrointestinal cancers	Ongoing
NCT04438083	Allogenic CAR-T cells CD70-directed	Renal cell carcinoma	Ongoing
NCT04244656	Allogenic CAR-T cells BCMA-directed	Multiple myeloma	Ongoing
NCT02793856	Autologous T cells *PD-1* knocked out	Metastatic NSCLC	Completed
NCT03081715	Autologous T cells *PD-1* knocked out	Advanced esophageal cancer	Completed
NCT05812326	Allogenic CAR-T cells *PD-1* knocked out, MUC1 directed	Breast cancer	Completed

CAR-T cells: chimeric antigen receptor T cells, CD followed by number: proteins (cluster of differentiation) on the surface of immune cells, CISH: cytokine inducible SH2-containing protein, BCMA: B-cell maturation antigen, PD-1: also called programmed cell death 1 protein (PCDP1), NSCLC: non-small cell lung cancer and MUC1: mucin 1.

Conclusion

The ability to edit the genome of human cells has revolutionized and accelerated our ability to manage cancer. It was a lot easier to change the genome when people knew that an adaptive immune system used by some bacteria and other microorganisms could be taken over and used to change almost any part of the genome precisely. The idea was further developed and refined to arrive at the CRISPR/Cas9 technology and its various arms. CRISPR/Cas9 genome editing is now widely used in various applications in the management of different cancers, including the elucidation of the role of genes in the progression of cancer, the efficient generation of animal models and organoids, and the creation of next-generation immunotherapies. Despite its success stories, the CRISPR/Cas9 system continues to have limitations. The concerns are mainly focused on the unwanted alterations caused by double-strand breaks and the possible immune response caused by the continued expression of Cas9 [24]. Further future refinement of the CRISPR/Cas9 technology could see solutions to these limitations. CRISPR/Cas9 genome editing is here to stay and will continue to bring expected rewards from the many ongoing clinical trials.

Conflict of interests

No conflict of interests was declared by the author.

Funding source

The author did not receive any source of fund.

Data sharing statement

Supplementary data can be shared with the corresponding author upon reasonable request.

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