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Application of CRISPR-Cas9 technology in the treatment of Triple Negative Breast Cancer

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Abstract. Since its discovery in bacterial cells, the CRISPR-Cas9 system has gained popularity as a practical approach to modifying the genomes. The ability of CRISPR-Cas9 to perform precise gene editing made it a potent therapeutic option for cancer treatment. This article will provide a basic overview of the working mechanism of CRISPR-Cas9, the different variants of CRISPR systems, and the present application of CRISPR-Cas9 in cancer treatment. The role of CRISPR-Cas9 mediated gene therapy for triple-negative breast cancer (TNBC) – one of the most aggressive types of cancer – is investigated by focusing on multiple oncogenes, tumour suppressor genes, and chemoresistance genes that are aberrantly expressed in TNBC tumour cells. Possible sgRNAs targeting three oncogenes (MYC, CXCR4, YTHDF2) are designed, which can be inserted into CRISPR-Cas9 plasmid vector lentiviral v2. Different vector delivery methods are summarised, including physical, viral, and non-viral deliveries. Finally, CRISPR-Cas9-based cancer therapy is evaluated, existing obstacles that hinder the clinical application of this therapeutic are discussed, and plausible solutions are presented.

Keywords: CRISPR-Cas9, TNBC, gene therapy.

1. Introduction

In terms of frequency and death rate, cancer continues to be one of the most common diseases, raising serious concerns for the health and welfare of people everywhere. Along with lung cancer, colon cancer, and prostate cancer, breast cancer is one of the four types of cancer that are diagnosed most frequently worldwide. By 2024, more than 30,000 women in the US alone will have this illness; globally, the number is even more striking. [1]. Oncologists classified breast cancer based on the three types of molecular biomarkers expressed on the surface of tumour cells – progesterone receptors (PR), estrogen receptors (ER), and human epithelial growth factor receptor 2 (HER2). Using this classification principle, breast cancer is categorised into lumina A (ER+/PR+/HER2-), luminal B (ER+/PR+/), HER2 enriched (ER-/PR-/HER2+), and triple-negative breast cancer (ER-/PR-/HER2-) [2], as shown in Table 1.

Amongst all four subtypes, TNBC is the most aggressive subtype, with a poor disease prognosis, a high metastatic rate, and a low five-year survival rate. Conventional cancer treatment options such as surgical removal of tumour tissue or radiotherapy can have severe side effects. The absence of surface hormone receptors and HER2 proteins means that therapeutics targeting these receptor sites are

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ineffective in treating TNBC [3]. The low chemosensitivity of TNBC has made gene editing a potential therapeutic option. Gene therapy involves modifying the sequence of particular genes contributing to disease progression to restore a standard copy of the alleles. As a gene-editing tool with the most promising prospect, the CRISPR-Cas9-based therapy designed for TNBC treatment focuses on knocking out or inhibiting oncogenes and chemoresistance genes, activating tumour suppressor genes, repairing mutated genes, as well as enhancing the immunotherapy effectiveness [4].

CRISPR is an abbreviation for Cluster of Randomly Interspaced Short Palindromic Repeats. These peculiar sequences were first discovered in bacterial cells as a defence strategy against the invasion of phages. When a phage infects bacterial cells and injects its genetic material, the bacteria would randomly cut a short sequence of the phage DNA and insert it into the CRISPR sequence. Such acquired immunity enables the bacteria to obtain complementary mRNA molecules of encountered phages. This mRNA is called CRISPR RNA (crRNA). crRNA binds to targeted phage DNA; an associated Cas9 nuclease cleaves the viral DNA to inactivate the phages. By harnessing the power of this ingenious defence mechanism and replacing crRNA with self-designed mRNA sequences, CRISPR-Cas9 could have limitless applications in different research fields, including disease and cancer treatment.

Table 1. Classification of Breast Cancer.

Subtype	Surface biomarkers	Clinical feature
Luminal A	ER+/PR+ HER2+	50%-60% of breast cancer good disease prognosis
Luminal B	ER+/PR+ HER2-	10%-20% of breast cancer, faster cancer growth than luminal A
HER2 Enriched	ER-/PR- HER2+	15%-20%of breast cancer, over-expression of HER2 receptors
TNBC	ER-/PR- HER2-	10%-15% of breast cancer, the most aggressive subtype and worst disease prognosis

2. Mechanism of CRISPR-Cas9 system

2.1. CRISPR and gene editing

Zinc Finger Nuclease (ZFN) and Transcription Activator Effector Nuclease (TALEN) are two gene editing techniques extensively researched before CRISPR. Both use proteins that can attach to certain DNA regions. Nevertheless, these systems' design has several flaws, such as being rigid and time-consuming, so editing genomes with them is not a common practice. Gene editing is now much more affordable, practical, and targeted, thanks to the development of CRISPR-Cas9 [5].

An artificially created single guide RNA (sgRNA) that identifies the specific sequence and a Cas9 nuclease protein that cleaves the double-stranded DNA make up the CRISPR-Cas9 genome editing system. A trans-activating CRISPR RNA (tracrRNA) interacts with the 20 nt-long CRISPR RNA (crRNA) in the sgRNA by producing a hairpin structure, whereas the crRNA itself is complementary to the target DNA sequence. Cas9 nuclease initiates a double-strand break (DSB) 3nt upstream to a protospacer adjacent motif (PAM) sequence after this ribonucleoprotein complex has been guided to its target location. After that, the DSB can be repaired using either of two methods: homology-directed repair (HDR) or non-homologous end joining (NHEJ). The NHEJ repair pathway repairs DNA without a homologous DNA template; the predominant DNA recombination mechanism occurs throughout the cell cycle. Nonetheless, the lack of reference templates means that NHEJ is error-prone and usually introduces indel mutations to the original genome, resulting in the silencing of specific genes. The HDR pathway is more accurate as it repairs DNA using a homologous DNA template. Exogenous donor DNA can be added to the system, allowing sequences of interest to be inserted into targeted sites in the genome [5]. HDR pathway is only active at the S phase and G2 phase of the cell cycle. Various methods can be used to increase the efficiency of the HDR pathway, including inhibiting the function of critical enzymes in the NHEJ pathway, such as Ku protein or adding proteins that stimulate HDR activity, such as CtIP [6]. Figure 1 shows the working mechanism of the CRISPR Cas9 system.

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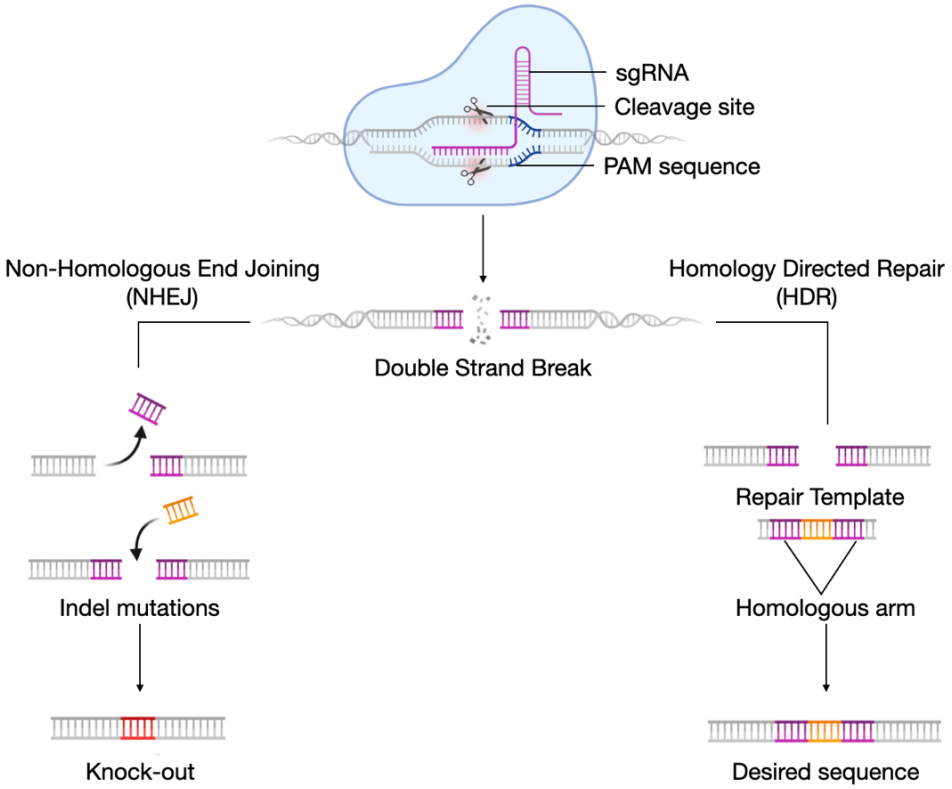


Figure 1. The working mechanism of the CRISPR-Cas9 system (created using Biorender).

2.2. Different CRISPR-Cas9 variants

By making modifications to the Cas9 nuclease protein, different CRISPR systems can be developed, each possessing unique functions. Wild-type Cas9 protein generates DSB and effectively knocks out or silences unwanted genes.

Cas9 nickase protein only creates single strand break (SSB) in the DNA chain; this mutant is produced by inactivating one of the cleavage domains (RuvC and HNH) of Cas9 nuclease. DSB can be generated using a pair of Cas9 nickase, reducing the possibility of off-target effects as the cleavage produces a long overhang instead of a blunted end. The length of the guide RNA is also doubled, allowing for a higher specificity [7].

Dead Cas9 (dCas9) is another Cas9 protein mutant in which loss of function mutations D10A and H840A inactivate the nuclease's RuvC and HNH nucleolytic domains. This dCas9 protein can be coupled with other functional proteins to acquire additional capabilities. One example is dCas9 combined with a transcription repressor, such as the repressive Krueppel-associated box (KRAB) domain. CRISPR interference (CRISPRi) is the name of this kind of CRISPR-Cas9 system that suppresses gene expression. Histone-modifying enzymes, including histone deacetylase and histone methyltransferase, are recruited by KRAB to inhibit expression. These enzymes alter epigenetic markers to prevent the transcription of genes. CRISPR activation (CRISPRa) uses dCas9 fused with transcription activators such as HSF1 and VP(16)n, which activates gene expression through histone acetylation and recruitment of transcription factors. dCas9 could also form a chimeric protein with enzymes cytidine or adenosine deaminase to enable the editing of a single base. Cytidine deaminase catalyses cytosine conversion to uracil, which the DNA repair system later repairs as thymine. Adenosine deaminase converts adenine to inosine, which polymerases read as a guanine base. The CRISPR base editor system allows transition mutation to be introduced to specific sites in the genome [8].

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Unlike the base editor system, which can only produce a limited number of base substitution mutations, the CRISPR prime editor system can overcome this problem by introducing longer sequences. This system consists of a Cas9 nickase fused with a reverse transcriptase domain that can synthesise DNA strands from an RNA template and a unique prime-editing guide RNA (pegRNA). In addition to a guide sequence complementary to the targeted DNA sequence, the pegRNA also contains an extended sequence of interest that would be incorporated into the genome. The guide sequence binds to targeted DNA to expose its complementary strand by the cleavage of Cas9 nickase. The unbound DNA acts as a primer for its elongation using pegRNA as a template – a process catalysed by reverse transcriptase. This method can quickly introduce the desired sequences into the genome [9]. Figure 2 shows the different variations of the CRISPR Cas9 system.

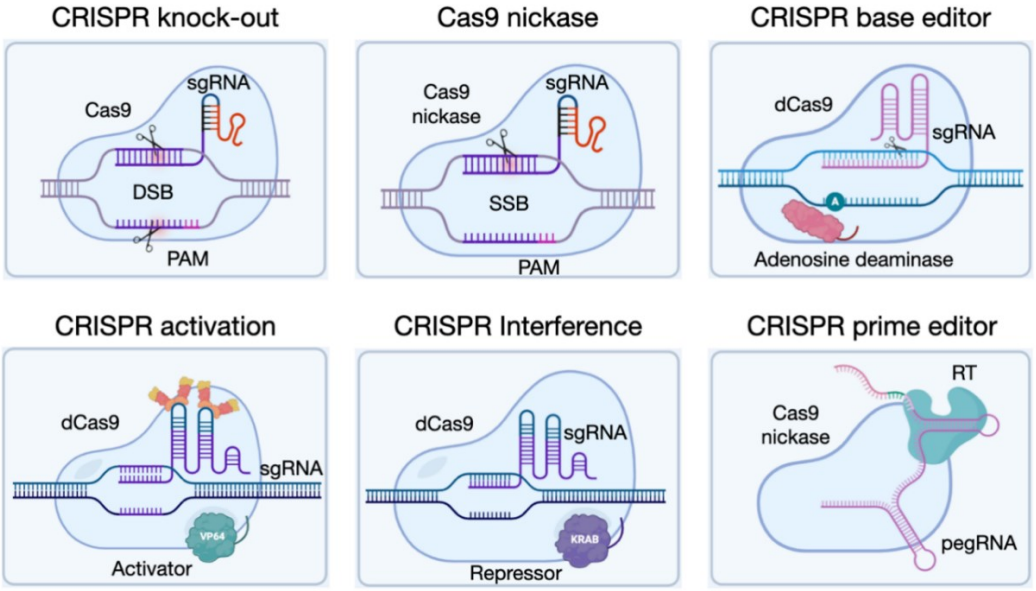


Figure 2. Different variations of the CRISPR-Cas9 system (created using Biorender).

3. Current application of CRISPR-Cas9 in cancer treatment

3.1. CRISPR-Cas9 targeting tumor genes

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The most straightforward method of using CRISPR-Cas9 in cancer therapy is to target genes that are abnormally expressed in tumour cells. The gene expression profile is distinct for each type of cancer, and overexpression of oncogenes or loss-of-function mutations of tumour suppressor genes often contribute to the pathogenesis of cancer. CRISPR technology can be precisely designed to target specific oncogenes related to tumour cell survival, growth, proliferation, migration, metastasis, and apoptosis evasion, effectively reducing tumour size and delaying disease progression. CRISPR-Cas9 can also repair mutated tumour suppressor genes to reactivate their ability to regulate the cell cycle and induce apoptosis, hindering the development of tumours [10].

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3.2. CRISPR-Cas9 enhancing immunotherapy

Enhancing the effectiveness of existing immunotherapies like CAR-T is another use for CRISPR-Cas9. T cells may be able to express chimeric antigen receptors (CAR) on their cell surface by CRISPR-Cas9-based genome editing. The body's immune system can target tumour cells selectively thanks to these uniquely made receptors that specifically target tumour signals that are only present on tumour cells. Modifying the degree to which specific genes are expressed in T cells may also improve their capacity to combat cancer cells. For instance, raising PD-1 expression in T cells has been found to strengthen CAR-T efficacy [10].

3.3. CRISPR-Cas9 and anti-cancer drug delivery

CRISPR-Cas9 could also be used to precisely deliver anti-cancer drug molecules because of their ability to distinguish cancer cells from healthy cells using the guide sequence. By designing guide RNA that only targets cancer genes and coupling the CRISPR-Cas9 complex with toxins or anti-cancer drugs, this method can be used to destroy tumour cells effectively [10].

4. Triple Negative Breast Cancer (TNBC)

4.1. CRISPR-Cas9 mediated oncogene knock-out

Tumor cells can have their oncogenes knocked out using the CRISPR-Cas9 system. Due to poor DNA repair at the cleavage location, the double-strand break (DSB) caused by the Cas9 nuclease would result in gene silence. TNBC oncogene knockout prevents tumour cell development and metastasis by suppressing cell survival and proliferation.

A proto-oncogene called MYC is overexpressed in TNBC and various other malignancies. About 30 to 50 percent of TNBC exhibits MYC overexpression [3]. Basic helix-loop-helix (bHLH) transcription factors are encoded by the MYC gene, and the MYC protein combines with MYC-associated factor X (MAX) to create a heterodimer that enhances the expression of several downstream gene targets that control the course of the cell cycle [11]. Therefore, the downregulation of the MYC gene by the CRISPR-Cas9 system may reduce tumour cell growth and proliferation.

The human epithelial growth factor receptor protein, EGFR, is another gene type abundantly expressed in TNBC cells. The PI3K/AKT/mTOR cell proliferation pathway and the MAPK cell survival pathway are overactivated when this receptor is amplified [11]. The CRISPR-Cas9 system may target EGFR to suppress the activation of various signalling pathways by EGFR proteins.

Researchers have also examined YTHDF2, UBR5, ITGA9, and CXCR4/CXCR7 as possible oncogenes. The chemokine CXCL12 can activate the G-protein coupled receptors (GPCRs) CXCR4 and CXCR7, which in turn causes angiogenesis, migration, and cell proliferation. Abnormal expression of these receptors in TNBC tumour cells is linked to a high risk of tissue invasion and metastasis. Both single knock-out and co-knock-out of these two genes using CRISPR-Cas9 would significantly hinder tumour development and metastasis [12]. Nuclear phosphoprotein UBR5 plays a vital role in the immune evasion of TNBC tumour cells [13]. CRISPR-mediated suppression of the UBR5 gene is linked to reduced tumour metastasis and enhanced apoptosis [4]. ITGA9 is expressed at a significantly higher level in TNBC compared to other subtypes of breast cancer. It has been discovered to regulate the Wnt/β-catenin pathway, which promotes tumour metastasis and epithelial-mesenchymal transition (EMT) of cancer cells. Reduced ITGA9 expression by CRISPR-Cas9 could restrict the development of TNBC via increasing β-catenin degradation [14]. YTHDF2 is an RNA-binding protein that may be a candidate oncogene for MYC-elevated TNBC therapeutic design, as downregulation of YTHDF2 would induce cellular apoptosis [15].

4.2. CRISPR-Cas9 mediated tumor suppressor gene activation

The CRISPR-Cas9 system can also target tumour suppressor genes, usually mutated or repressed in cancer cells. This CRISPRa system uses dCas9 protein fused with transcription activators such as VP64

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to increase the expression level of targeted genes. Activation of tumour suppressor genes would improve the disease progression of TNBC via various mechanisms.

The most frequently mutated tumour suppressor gene in TNBC is the P53 gene, with mutations identified in 65% to 80% of TNBC cases. P53 protein is an essential regulatory element in cell cycle progression, DNA repair, and programmed cell death via apoptosis. Malfunctional P53 protein dramatically contributes to the tumorigenesis of TNBC by disrupting the normal cell cycle and allowing tumour cells to divide uncontrollably [16]. Repairing aberrant P53 genes and activating silenced P53 genes in cancer cells may be a possible treatment for TNBC.

Another example of a tumour suppressor gene that can be re-activated in TNBC cells is PTEN. PTEN protein can act as an inhibitor of PI3K, thereby suppressing the PI3K/AKT/mTOR signalling pathway. PI3K catalyses the conversion of phospholipid PIP2 to PIP3, while PTEN reverses this process by favouring the formation of PIP2. As the PI3K/AKT/mTOR signalling pathway results in cell growth and proliferation, inhibiting this cascade allows PTEN to reduce tumour development. Using dCas9 fused with transcription activators, the PTEN gene can be expressed to inhibit tumour cell growth [17].

4.3. CRISPR-Cas9 in Combating Chemotherapy Resistance

In addition to silencing oncogenes and activating tumour suppressor genes, CRISPR-Cas9 could also target genes responsible for the chemoresistance of TNBC tumour cells. One of the significant challenges of cancer treatment is drug resistance, and the chemosensitivity of tumour cells determines the effectiveness of chemotherapy. Unfortunately, TNBC tumour cells develop resistance to a range of chemotherapy drugs, including taxanes and anthracyclines [18].

MALAT1, a long non-coding RNA (lncRNA) that controls cellular gene expression, is one gene that has been found to contribute to the chemoresistance in TNBC. TNBC cells' heightened expression of MALAT1 is linked to their resistance to doxorubicin and paclitaxel, two prominent chemotherapy medications that target this malignancy. Therefore, CRISPR-Cas9-mediated MALAT1 deletion would increase the effectiveness of chemotherapy by sensitising TNBC cells to these two medications [4].

PAPR1 is also a gene that could be targeted to increase the chemosensitivity of TNBC cells. PAPR1 is responsible for repairing damaged DNA with single-strand break (SSB); it is a synthetic lethal partner of a gene frequently mutated in breast cancer – BRCA1. Synthetic lethality is when the combined mutation of two genes has a fatal effect on cells, while a single mutation in either gene does not result in cell death. PAPR inhibitor drugs use the synthetic lethality interaction between PAPR1 and BRCA1 to treat TNBC. Using a similar mechanism, the deletion of PARP1 by CRISPR-Cas9 could also increase the sensitivity of TNBC cells to standard chemotherapy drugs such as doxorubicin, docetaxel, and gemcitabine [4]. Table 2 shows the Potential target genes of the CRISPR-Cas9 system in TNBC treatment.

Table 2. Potential target genes of CRISPR-Cas9 system in TNBC treatment.

Gene	Function in tumour cell	TNBC Expression	CRISPR-Cas9 Approach
MYC	bHLH transcription factor that promotes cell growth and proliferation	Overexpressed in 30%-50% of TNBC	Knock-out or suppression
EGFR	Human epithelial growth factor Receptor that promotes cell survival and proliferation	Overexpressed in 50% of TNBC	Knock-out or suppression
CXCR4/CXCR7	G-protein coupled receptors that Promote cell migration and angiogenesis.	Overexpression in TNBC	Knock-out or suppression
UBR5	Nuclear phosphoprotein that contributes to immune evasion	Overexpression in TNBC	Knock-out or suppression

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Table 2. (continued).

ITGA9	Integrin proteins that promote tumour metastasis and EMT	Overexpression in TNBC	Knock-out or suppression
YTHDF2	RNA binding protein that is linked to cell survival and proliferation	Overexpression in TNBC	Knock-out or suppression
P53	Malfunctioned P53 protein disrupts DNA repair and cell cycle	Mutated or suppressed in TNBC	Repair or activation
PTEN	Malfunctioned PTEN protein fails to Inhibit Pl3K/AKT/mTOR pathway.	Mutated or suppressed in TNBC	Repair or activation
MALAT1	Long non-coding RNA (lncRNA)that regulates gene expression	Overexpression in TNBC	Knock-out or suppression
PARP1	Poly (ADP-ribose)polymerase 1 that repairs tumour DNA and Contribute to cell survival.	Overexpression in TNBC	Knock-out or suppression

5. Proposed sgRNA and plasmid design

A software named Benchling was used to design single guide RNA (sgRNA) for three selected oncogenes – MYC (ENSG00000136997), CXCR4 (ENSG00000121966), and YTHDF2 (ENSG00000198492), as shown in Figure 3. The genomic sequences of these three genes found in the GRCh38_EnsembleRelease_104 genome were imported into Benchling, with all possible transcripts annotated. A region that targets all versions of transcripts was selected, and the 20nt long sgRNAs adjacent to the PAM sequence of 5' NGG 3' were automatically generated by the program. The cut position, strand (+ or -), guide sequence, PAM site, and on-target and off-target scores were listed. sgRNAs were selected based on their on-target score; three sgRNAs with the highest on-target score that can target all possible transcripts were chosen.

LentiCRISPR v2 (Addgene plasmid # 52961) was used as the CRISPR vector plasmid to deliver to tumour cells. The filter region in the original plasmid vector can be replaced with the guide sequence using the two BsmBI restriction enzyme cutting sites. Once the plasmid vector is successfully constructed using restriction cloning, primer pairs hU6-F (5'-GAGGGCCTATTTCCCATGATT-3') and LKO.1 5' (5'- GACTATCATATGCTTACCGT-3') can be used to verify the sequence. The sgRNA sequences, PAM sites, and on-targets score targeting all three oncogenes were summarised in Table 3.

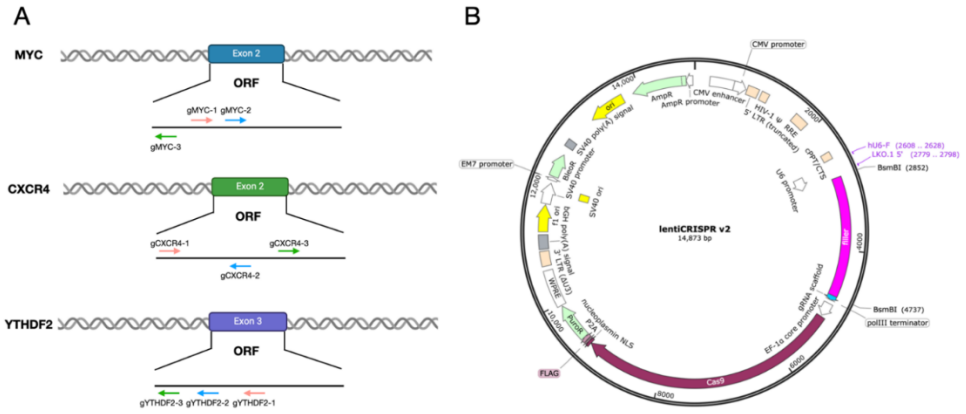


Figure 3. Schematic representation of gRNA and plasmid. A) gRNA location on exons of three targeted oncogene B) map of lentiCRISPR v2 plasmid.

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Table 3. sgRNA design.

Gene	sgRNA	Guide sequence	PAM	On-targeted score
MYC	gMYC-1	5'GCCGTATTTCTACTGCGACG3'	AGG	73.1
	gMYC-2	5'CTGCTCGCCCTCCTACGTTG3'	CGG	68.3
	gMYC-3	5'GCTGCACCGAGTCGTAGTCG3'	AGG	66.9
CXCR4	gCXCR4-1	5'CACTTCAGATAACTACACCG3'	AGG	77.2
	gCXCR4-2	5'AGCCAGGATGAGGATGACTG3'	TGG	74.6
	gCXCR4-3	5'CAAGTGGATTTCCATCACCG3'	AGG	72.3
YTHDF2	gYTHDF2-1	5'ATTGCATCTGGTAGGAAGTG3'	GGG	70.9
	gYTHDF2-2	5'AGAATAGGAGAAGCCAATGG3'	AGG	70.2
	gYTHDF2-3	5'GGGTAAGTAGGAATCTGACA3'	TGG	69.6

6. Vector delivery methods of CRISPR-Cas9

Optimising the transport of CRISPR technology, initially in vitro and then in vivo, including people, is one of the primary obstacles that therapeutic advancements in this technology must overcome. Since its beginnings, attempts have been made to provide CRISPR components, and several strategies have been developed. Physical, viral, and non-viral distribution are the categories into which the CRISPR system's delivery technique can be separated based on its nature [3].

Microinjection, hydrodynamic caudal vein injection (HTVI), electroporation, ultrasonic microbubble, and laser are examples of physical techniques. These techniques have the advantages of being inexpensive, easy to use, and very efficient. However, they have drawbacks, such as being time-consuming. While electroporation can result in cell damage and apoptosis due to high pressure, microinjection can be challenging. Lastly, there is no specificity in HTVI. It has been noted that adverse effects on the kidneys and liver may occur [14].

The two delivery systems most commonly employed for the treatment of breast cancer or triple-negative breast cancer (TNBC) are viral and non-functional. Non-viral vectors are used in the non-viral CRISPR delivery techniques. They have a cheap cost, a safety profile, and excellent delivery and editing efficiency. However, when given in vivo, some are rapidly eliminated, and some may be immunogenic and need a complicated preparation procedure. Polymers, lipid-based nanoparticles (NPs), gold NPs, other inorganic NPs, exosomes, cell-penetrating peptides (CPPs), and DNA nanoclews are examples of non-viral CRISPR carriers. Regarding polymer nanoparticles, their key attributes—long blood circulation, high drug encapsulation, strong biocompatibility, and relatively simple functionalisation—make them a promising and effective delivery vehicle. Over the years, lipid-based delivery systems have gained a lot of value, primarily due to their capacity to cross complex physiological barriers, ease of synthesis, affordability, and ease of large-scale manufacturing. Lipid nanoparticles can be classified as solid lipid nanoparticles, liposomes, niosomes, and nanostructure lipid carriers, depending on the synthesis technique and their physical and chemical characteristics [19].

Lastly, the viral CRISPR delivery methods use viral vectors, including lentiviruses, adenoviruses, and adeno-associated viruses. Their significant advantage is the high efficiency of gene editing. However, viral vectors can elicit oncogenic effects, trigger immunogenic responses, and lead to insertional mutations [3]. Table 4 shows the vector delivery methods of CRISPR Cas9.

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Table 4. Vector delivery methods of CRISPR-Cas9.

Delivery method	Subtype	Advantages	Disadvantages
Physical delivery	Microinjection Electroporation HTVI Ultrasonic microbubble Laser	High efficiency, low cost, simple operation	Time-consuming, non-specific, cell damage, side effects in liver and kidney
Viral delivery	Lentivirus Adenovirus Aadeno-associated virus	High efficiency of gene editing	Potential oncogenic effects, potential immune response
Non-viral delivery	Polymer Lipid-based nanoparticle Gold nanoparticle Inorganic nanoparticle Exosome Cell-penetrating peptide DNA nano clew	High delivery, editing efficiency, low cost, safety profile	Quick clearance, potential immune response, complex preparation method

7. Evaluation of CRISPR technology

Gene therapy based on CRISPR-Cas9 offers several benefits over conventional cancer therapies, including chemotherapy, radiation, and surgery. The long-term cure for cancer is provided by CRISPR-Cas9 technology, which addresses the illness at the genetic level in contrast to traditional therapies. This system is far from ideal, though. The off-target effect occurs when the sgRNA identifies additional genes with sequences identical to the target DNA and is one of the most often discussed issues with CRISPR-Cas9. There has been an attempt to reduce off-target effects, including employing different CRISPR-Cas9 variations, such as paired Cas9 nickase and more cautious guide sequence selection, to reduce the risk of damaging mutations with unknown implications. The specificity of vector delivery is another challenge before CRISPR-Cas9 may be applied in therapeutic settings. Ensuring that the plasmid is exclusively transferred to the tumour and not healthy cells is crucial. This might be accomplished by including vector antibodies specific to tumour antigens in the delivery. In conclusion, even if CRISPR-Cas9 has certain benefits over other conventional treatments, several issues must be resolved before it can be used in TNBC patients.

8. Conclusion

The advent of CRISPR-Cas9 technology has provided a novel landscape for tumour therapy. By using CRISPR-Cas9 to knock out and silence overexpressed oncogenes, repair mutated tumour suppressor genes, and increase the chemosensitivity of cancer cells, tumour growth and development can be significantly inhibited.

CRISPR-Cas9-based gene therapy has numerous advantages over traditional cancer treatments such as radiotherapy, chemotherapy, and surgeries. Unlike conventional therapeutics, CRISPR-Cas9 technology targets cancer at the genetic level, a long-term solution to this disease.

Nonetheless, this system is far from perfect. One of the most widely discussed concerns of CRISPR-Cas9 is its off-target effect when the sgRNA recognises other genes with similar sequences to the target DNA. Off-target effects have a high possibility of introducing deleterious mutations that have unpredictable consequences, some of which might be detrimental and result in the death of normal

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somatic cells or even encourage cancer progression. Multiple attempts have been made to minimise the off-target effect, including more careful selection of guide sequence and using other CRISPR-Cas9 variants such as paired Cas9 nickase. Another obstacle that needs to be overcome before CRISPR-Cas9 can be used clinically is improving the current vector delivery method. The most widely applied delivery methods - physical, viral and non-viral all have certain limitations, including low uptake efficiency, immune response stimulation, and a lack of delivery specificity. It is pivotal to ensure that the plasmid is only delivered to tumor cells instead of normal healthy cells. Add antibodies that only recognise tumour antigens to the delivery vector may achieve this. However, it remains a challenge to identify unique tumour surface markers only found on the membrane of specific tumour cells.

Therefore, although significant progress has been made in the lab regarding the CRISPR-Cas9-based gene therapy, further research and improvements are still necessary for this technology to move from bench to clinic, and its effectiveness in treating specific cancers - such as needs to be more closely evaluated before in vivo CRISPR-Cas9 gene editing can be conducted on patients.

Authors Contribution

All the authors contributed equally and their names were listed in alphabetical order.

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